X Congreso Nacional de Biología Molecular y Celular de Hongos

27-31 de octubre de 2013

Oaxaca, Oaxaca

Sede: Hotel Victoria

X Congreso Nacional de Biología
Molecular y Celular de Hongos
P r o g r a m

Oaxaca, Oax. October 27 – 31, 2013

Sunday, October 27	
12:00 – 18:00	Registration
14:00 – 17:00	Lunch
18:00 – 18:30	Opening Ceremony
18:30 – 19:30	Opening Talk. Chair: Alfredo Herrera Estrella *Emily McClung* **Restos arqueológicos de plantas en la reconstrucción de subsistencia, ritos y el manejo de ecosistemas prehispánicos.** Instituto de Investigaciones Antropológicas, UNAM
19:30 – 21:30	Welcome Cocktail
Monday, October 28	
7:00 – 9:00	Breakfast
9:00 – 10:00	Plenary Talk I. Chair: Edgardo Ulises Esquivel *Rosa Mouriño* **Keeping the shape. Endocytosis and hyphal morphogenesis.** Departamento de Microbiología, CICESE. Ensenada
	Plenary Session I. Fungal Cell and Developmental Biology Chair: Sergio Casas Flores
10:00 – 10:30	**Dissecting the epigenetic mechanisms of blue light perception in the filamentous fungus *Trichoderma atroviride*.** *Sergio Casas-Flores*, Mayte Guadalupe Cervantes-Badillo, Edith Elena Uresti-Rivera, Macario Osorio-Concepción, and Gema Rosa Cristóbal-Mondragón Division de Biología Molecular, IPICYT
10:30 – 10:45	**The NADPH oxidases and tetraspanin PLS-1 in fusion and cell differentiation in the fungus *Neurospora crassa*.** *María del Sol Hernández Galván*, Nallely Cano Domínguez, Olivia Sánchez and Jesús Aguirre Instituto de Fisiología Celular, UNAM
10:45 – 11:00	**The role of chitin synthases in development and morphogenesis of *Neurospora crassa*.** *Rosa A. Fajardo-Somera*, Jönhk Bastian, Robert Roberson, Özgür Bayram, Gerhad H. Braus and Meritxell Riquelme CICESE

11:00 – 11:15	**Role of the photoreceptors BLR1 and BLR2 in the response to mechanical injury in *Trichoderma atroviride*.** *José Manuel Villalobos-Escobedo*, Nohemí Carreras-Villaseñor and Alfredo Herrera-Estrella Fungal Development and Genetic Expression Laboratory. LANGEBIO
11:15 - 11:30	**Analysis of the hda1 disruption on telomere length maintenance in *Ustilago maydis*.** *Denisse Cisneros-Ramírez,* Estela Anastacio-Marcelino, Candelario Vázquez-Cruz, Reynaldo Galicia-Sarmiento, Ma. Patricia Sánchez-Alonso Instituto de Ciencias, BUAP
11:30 – 11:45	**GRB and APS1 are components of the Microtubule Organizing Centers of *Neurospora crassa.*** *Olga Alicia Callejas Negrete*, Rosa María Ramírez Cota, Michael Freitag, Robert W. Roberson, Salomón Bartnicki García and Rosa R. Mouriño Pérez Departamento de Microbiología, CICESE
11:45 – 12:00	Coffee Break
	Plenary Session II. Comparative and Functional Genomics Chair: Elva Aréchiga
12:00 – 12:30	***Ustilago maydis* adaptation to alkaline pH, trough Rim101 regulation.** Franco-Frías E, Ruiz-Herrera J, *Aréchiga-Carvajal ET* Universidad Autónoma de Nuevo León
12:30 – 12:45	**Suboptimal response to stress underlies adaptation by gene inactivation.** *J Abraham Avelar Rivas*, Luis Díaz & Alexander de Luna LANGEBIO CINVESTAV-IPN. Unidad Irapuato
12:45 – 13:00	**Functional genomics of *Bjerkandera* sp. UAMH8525: Bio-prospecting of a new glycosyl hydrolase.** *Ramón Alberto Batista García*, Claudia Martínez Anaya, Jorge Luis Folch Mallol Centro de Investigación en Biotecnología, UAEM
13:00 – 13:15	**Horizontal gene transfer of a caleosin gene from host to pathogen in pathosystem *Phaseolus vulgaris*/*Colletotrichum lindemuthianum.*** *Saúl Fraire-Velázquez*, Erick Saúl Leandro-Pérez, Lenin Sánchez-Calderón, Alejandra Alcalá-Ramírez, César Díaz-Pérez Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas
13:15 – 13:30	**Analysis of the oligomeric organization between paralogous proteins in *Saccharomyces cerevisiae.*** *Geovani López*, Mariana Duhne, Héctor Quezada, Alicia González Manjarrez Instituto de Fisiología Celular, UNAM

13:30 – 13:45	**Systematic identification of subtelomeric silencing pathways in *Saccharomyces cerevisiae.*** *Alejandro Juárez-Reyes*, Jhonatan Hernández-Valdés & Alexander de Luna. CINVESTAV, IPN
13:45 – 15:00	Lunch
15:00 – 17:30	Poster Session Odd Numbers
17:45 – 18:45	Plenary Talk II. Chair: Alfredo Herrera Estrella *Jesús Aguirre Linares* **Cell Differentiation and Resilience in Fungi.** Instituto de Fisiología Celular, UNAM
18:45 – 19:00	Coffee Break
	Plenary Session III. Biochemistry and Signal Transduction Chair: Alejandro de las Peñas
19:00 – 19:30	**Secondary metabolites in the fungal pathogen *Candida glabrata.*** *María Guadalupe Gutiérrez Escobedo* División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, AC.
19:30 – 19:45	**Functional analysis of the NADP- Dependent glutamate dehydrogenase (NADPH-*Kl*Gdh1) of *Kluyveromyces lactis.*** *José Carlos Campero-Basaldua* and M. Alicia González Manjarrez Instituto de Fisiología Celular, UNAM
19:45 – 20:00	**Proteomic analysis of signaling pathways mediated by the heterotrimeric Gα protein Pga1 of *Penicillium chrysogenum.*** *Ulises Carrasco Navarro*, Francisco José Fernández Perrino, Horacio Reyes Vivas, Francisco Fierro Fierro Universidad Autónoma Metropolitana. Unidad Iztapalapa
20:00 – 20:15	**Morphological changes in the *Candida albicans* ultrastructure caused by the transglutaminases in competitive inhibitor cystamine.** *Elizabeth Reyna-Beltrán*, Labra-Barrios ML, Luna-Arias JP. CINVESTAV-IPN. Unidad Zacatenco
20:15 -20:30	**Search S6 ribosomal protein not associated with ribosomes in nuclei of *Saccharomyces cerevisiae.*** *Reynaldo Tiburcio-Félix,* Samuel Zinker-Ruzal CINVESTAV-IPN. Unidad Zacatenco

20:30 – 20:45	**Mycoviral presence on *Isaria fumosorosea* isolates from Mexico.** *Judith Castellanos Moguel*, Viridiana Mendoza Álvarez, Nancy Romero Martínez, Estela Tavares Macías, Zyania Pérez Juárez Universidad Autónoma Metropolitana. Unidad Xochimilco
20:45 – 21:00	***Saccharomyces cerevisiae ALT2*: A bioinformatics approach to uncover its function.** *José María Uriel Urquiza García*, Alicia González Instituto de Fisiología Celular, UNAM
21:00	Art Auction. “Noel Cayetano. Contemporany Art”
Tuesday, October 29	
7:00 – 9:00	Breakfast
9:00 – 10:00	Plenary Talk III. Chair: Jorge Luis Folch *Salomón Bartnicki García* **The hyphal morphogenesis conundrum: navigating the fine line between fact and speculation** Centro de Investigación Científica y de Educación Superior de Ensenada
10:00 – 11:00	WORKSHOP I. Selected Posters
11:00 – 11:15	Coffee Break
	Plenary Session IV. Biotechnology I Chair: Jorge Luis Folch Mallol
11:15 – 11:45	**Analysis of *Trichoderma atroviride* strains that express a laccase from *Pycnoporus sanguineus*.** *Jorge Luis Folch Mallol,* Balcázar López Edgar, Méndez Lorenzo Luz Helena, Esquivel Naranjo Edgardo Ulises, Ayala Aceves Marcela, Herrera Estrella Alfredo Centro de Investigación en Biotecnología, UAEM
11:45 – 12:00	**Endophytic fungi associated with *Taxus globosa* Schltdl. with potential anticancer taxol production.** *Claudia López Sánchez*, Felipe de Jesús Palma Cruz and Lucia Martínez Martínez Instituto Tecnológico del Valle de Oaxaca
12:00 – 12:15	**Analysis of the fungal community associated with litter from two species of oak.** *Jesús Andrei Rosales-Castillo*, Gerardo Vázquez-Marrufo and Felipe García-Oliva Centro de Investigaciones en Ecosistemas**,** UNAM

12:15 – 12:30	**Specific and fast molecular detection of several common species of *Candida* in human infections: *Candida albicans, Candida tropicalis* and *Candida parapsilosis*.** *Cesia Janell Hernández Howell*, Alba Saucedo Fuentes, María del Rosario Baltazar Lara, Alejandro De Las Peñas Nava, Irene Castaño Navarro Division de Biología Molecular, IPICYT
12:30 – 12:45	**Heterologous expression in *Pichia pastoris* of transcription factors PcYap1, RsmA and AtfB of *Penicillium chrysogenum*.** *Wylma D. Pérez Pérez*, Ulises Carrasco Navarro, Javier Barrios González, Ma. Concepción Gutiérrez Ruiz, Francisco Fierro Fierro Universidad Autónoma Metropolitana, Unidad Iztapalapa
12:45 – 13:00	**Analysis and characterization of the *YLR177W* gene overexpression in *Saccharomyces cerevisiae* during Agave juice fermentation.** *Naurú Idalia Vargas Maya*, Gloria Angélica González Hernández, Araceli López Andrade, Adriana García Tapia and Juan Carlos Torres Guzmán División de Ciencias Naturales y Exactas. Universidad de Guanajuato
13:00 – 14:00	Plenary Talk IV. Chair: Lina Raquel Riego Irene Castaño **How does the opportunistic pathogen *Candida glabrata* avoid sexual reproduction?** Departamento de Biología Molecular, IPICYT
14:00 – 16:00	Lunch
	FREE AFTERNOON
Wednesday, October 30	
7:00 – 9:00	Breakfast
9:00 – 10:00	Plenary Talk V. Chair: Wilhelm Hansberg *Michael Feldbrügge* **Intracellular trafficking along microtubules in fungi.** Heinrich-Heine University Düsseldorf
	METHODS
10:00 – 10:13	*James González* **PICH protocol a key tool to analyze promoters.** Instituto de Fisiología Celular, UNAM

10:13 – 10:26	*Alfredo Herrera* **RNAseq strategies for transcriptome analyses.** LANGEBIO - CINVESTAV-Unidad Irapuato
10:26 – 10:40	*Nancy Coconi Linares* **A Novel and Highly Efficient Method for Genetic Transformation of Fungi Employing Shock Waves.** CINVESTAV-Unidad Irapuato
10:40 – 10:45	Questions and comments
10:45 – 11:00	Coffee Break
	Plenary Session V. Comparative and Functional Genomics Chair: Melina López-Meyer
11:00 – 11:30	**PvLOX2 silencing on mycorrhiza induced resistance in common bean.** *Melina López-Meyer*, Arlene Mora-Romero, Francisco Quiroz-Figueroa, Sergio Medina-Godoy, Carlos Calderón-Vázquez, Ignacio Maldonado-Mendoza, Analilia Arroyo-Becerra Instituto Politécnico Nacional CIIDIR Unidad Sinaloa
11:30 – 11:45	**Role of the small RNAs synthesis machinery on the antagonistic capacity of *Trichoderma atroviride*.** *Emma Beltrán-Hernández*, Molina-Torres Jorge, Herrera-Estrella Alfredo LANGEBIO CINVESTAV-IPN. Unidad Irapuato
11:45 – 12:00	**Immune sensing of *Sporothrix schenckii* and *S. brasiliensis* by human mononuclear cells.** *Héctor M Mora-Montes*, José A. Martínez-Álvarez, Ivonne S. Fernández-Ham, Luis A. Pérez-García Departamento de Biología, División de Ciencias Naturales y Exactas, Campus Guanajuato, Universidad de Guanajuato
12:00 – 12:15	**Arsenic transport and speciation in *Glomus intraradices* by molecular and synchrotron radiation spectroscopic analysis.** *Ignacio Eduardo Maldonado-Mendoza*, M.C. González-Chávez, B. Miller, K. Scheckel and R. Carrillo-González Departamento de Biotecnología Agrícola, Instituto Politécnico Nacional, CIIDIR-Sinaloa
12:15 – 12:30	**The 4-phosphopantetheinyl transferase of *Trichoderma virens* plays a role in activation of plant immunity through volatile organic compound emission.** *Hexon Ángel Contreras-Cornejo*, José López-Bucio, Alfredo Herrera-Estrella and Lourdes Macías-Rodríguez IIQB, Universidad Michoacana de San Nicolás de Hidalgo

12:30 – 12:45	**Cytokines profile during the innate and the inter-phase innate-adaptive immune response in murine pulmonary histoplasmosis induced with fungal mycelial phase propagules.** *Jorge Humberto Sahaza*, Pérez-Torres Armando, Taylor María Lucia Facultad de Medicina, UNAM
12:45 – 13:15	Bussiness Session
13:15 – 15:00	Lunch
15:00 – 17:00	Poster Session Even Numbers
	Plenary Session VI. Biotechnology II Chair: Marcela Ayala
17:15 – 17:45	**Fungal oxidoreductases applied to bioremediation: opportunities and challenges.** *Marcela Ayala Aceves* Departament of Celular Engineering and Biocatalysis. Institute of Biotechnology, UNAM
17:45 – 18:00	**An acidic MES buffered media prevents alkaline hydrolysis of cephalosporin C in *Acremonium chrysogenum* pacCC fermentation cultures.** *Alberto Cristian López Calleja*, Francisco Fierro Fierro, Octavio Loera Corral, Francisco José Fernández Perrino Universidad Autónoma Metropolitana. Unidad Iztapalapa
18:00 – 18:15	**High-Level Expression of Manganese Peroxidase, Lignin Peroxidase, and Versatile Peroxidase in Ligninolytic Fungus *Phanerochaete chrysosporium.*** *Nancy Coconi-Linares*, Elizabeth Ortíz-Vázquez, Francisco Fernández, Achim M. Loske, Miguel A. Gómez-Lim CINVESTAV Unidad Irapuato
18:15 – 18:30	**Gene cloning and expression of a fungus carbohydrate esterase of *Bjerkandera adusta* in *Pichia pastoris*, and evaluation of its effect on lignocellulosic material degradation.** *Laura I. Cuervo Soto*, Jorge Luis Folch Mallol Centro de Investigación en Biotecnología, UAEM
18:30 – 18:45	**Esterase prospecting for pesticide degradation through the screening of a DNA library from activated sludge.** *Ayixon Sánchez Reyes*, Ramón Batista García1, Víctor González, Soledad Juárez and Jorge Luis Folch Mallol Biotechnology Research Center. Laboratory of Molecular Biology of Fungi, UAEM

18:45 – 19:00	**Antifungal activity of silver nanoparticles against *Candida albicans*.** *Ernestina Castro-Longoria*, Roberto Vázquez-Muñoz, Miguel Avalos-Borja Departamento de Microbiología, CICESE
19:15 – 20:15	WORKSHOP II. Selected Posters
20:15 – 21:15	Plenary Talk VI. Chair: Alicia González *James Broach* **Transcriptional Regulation of the Yeast Stress Response through a Dynamic Interplay of Signaling Networks.** Department of Biochemistry and Molecular Biology, Penn State University
21:15 – 21:45	Final Announcements and Closing Ceremony
22:00 – 24:00	DINNER GUELAGUETZA

All oral presentations will be held in the Mitla Room

All poster presentations will be held in the Tennis Area

Odd Poster Presentation: Monday October 28th

Tennis Area

Even Poster Presentation: Wednesday October 30th

1.	**Establishment of *Fusarium solani* biofilm and differential protein expression analysis in two differentials states of growth.** *Rosa Paulina Calvillo Medina*, María de los Ángeles Martínez Rivera, Aida Verónica Rodríguez Tovar and Victor M. Bautista de Lucio. Instituto de Oftalmología Fundación Conde de Valencia, Escuela Nacional de Ciencias Biológicas, IPN
2.	**Proteolitic activity comparision among entomopathogenic fungi *Isaria fumosoreosea* isolates from Mexico.** *Anaid Penelope Solís-Hernández*, Judith Castellanos-Moguel. Universidad Autónoma Metropolitana
3.	**Analysis of *GDH3* glucose-repression in *Saccharomyces cerevisiae*.** *Maritrini Colón-González*, Brisa Aranzazú Campos-Oliver, Cristina Aranda-Fraustro and Alicia González-Manjarrez. Instituto de Fisiología Celular, UNAM
4.	**Cloning of *cat-1* and expression in *E. coli*.** *Isareli Cruz Cruz*, Wilhelm Hansberg Torres. Instituto de Fisiología Celular, UNAM
5.	**Study Molecular and Biochemical characterisation of gene ICL from S. *schenkii*.** *Laura Cecilia González Sánchez*, José Bernardo Héctor Escobar Henrriquez, José de Jesús Daniel López Muñoz, María Teresa Croda Todd, Francisco Solís Páez, Claudia Belen Ortega Planell. Facultad de Bioanálisis, Universidad Veracruzana
6.	**Functional Divergence of Genes Implicated in the Leucine Biosynthesys in *Sacharomyces cerevisiae* and the Ancestor Type Yeast *Kluyveromyces lactis*.** *Mijail Lezama* and Alicia González. Instituto de Fisiología Celular, UNAM
7.	**Studies on functional divergence between *Saccharomyces cerevisiae ALT1* and *ALT2* using *Kluyveromyces lactis KlALT1* and *Lachancea Kluyveri* SkALT1 as ancestral type.** *Ximena Martínez de la Escalera Fanjul,* Maritrini Colón González & Alicia González. Instituto de Fisiología Celular UNAM
8.	***Ustilago maydis* as model of Ustilaginales to evaluate antifungal effects with chitosan and derivatives.** *Dario Rafael Olicón Hernández*, Ana Niurka Hernández Lauzardo, Guadalupe Guerra Sánchez. Escuela Nacional de Ciencias Biológicas, IPN
9.	**Purification of hetero-oligomers of the isozymes GDH1, GDH3 of *S. cerevisiae* and GDH1 of *S. kluyveri*.** *Edson Robles*, Mirelle Flores, Zeeshan Mutahir, Birgitte Munch-Petersen, Manuel Soriano, Jure Piskur, Alicia Gonzalez. Institute of Cell Fiosiology, National Autonomous University of Mexico/Molecular Cell Biology Unit, Lund University
10.	***Ustilago maydis* has two plasma membrane H^+-ATPases related to fungi and plants.** *Leobarda Robles Martínez,* Juan Pablo Pardo, Guadalupe Guerra Sánchez. Escuela Nacional de Ciencias Biológicas-IPN
11.	**Purification and characterization of Alt1 and Alt2 of *Saccharomyces cerevisiae*: Study of functional divergence in metabolism of alanine.** *Rojas- Ortega Eréndira*, Gonzalez-Manjarrez Alicia. Instituto de Fisiología Celular, UNAM

12.	**Immunogenicity of the cell wall of *Sporothrix brasiliensis* and *S. globosa*.** *Estela Ruiz-Baca*, Gustavo Hernández-Mendoza, Mayra Cuéllar-Cruz, Conchita Toriello, Norma Urtiz-Estrada, Aurora Martínez-Romero, Maribel Cervantes-Flores, Gerardo Alfonso Anguiano-Vega, Everardo López-Romero. Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango
13.	**Functional characterization of proteins encoded by *LkLEU4* and *LkLEU4BIS* of *Lachancea kluyveri* as a model of duplicated genes in ancestral type yeast that were selectively retained.** *Yusvel Sierra* Gómez; Mijaíl Lezama Barquet; Geovani López Ortiz; Alicia González Manjarrez. Instituto de Fisiología Celular, UNAM
14.	**Biochemical characterization of the recombinant catalase-peroxidase from *Neurospora crassa* and four monofunctional mutants.** *Vanessa Vega García*, Wilhelm Hansberg Torres. Instituto de Fisiología Celular, UNAM
15.	**Role of PARP/PARG [(Poly-ADP-ribosyl) polymerase/glycohydrolase] in the pathogenic fungus *Fusarium oxysporum f.*sp. *lycopersici*.** *Carlos A. Araiza-Cervantes*, Nancy E. Lozoya-Perez, María Isabel González Roncero, Guadalupe Martínez Cadena, Georgina E. Reyna López. División de Ciencias Naturales y Exactas, Universidad de Guanajuato
16.	**Is the histidine kinase important for *Candida glabrata*?** *Natalee Carapia-Minero*, María de los Ángeles Martínez Rivera, Néstor Octavio Pérez Ramírez y Aída V. Rodríguez-Tovar. Escuela Nacional de Ciencias Biológicas, IPN
17.	**ROS and cAMP signalling in *Neurospora crassa* conidation.** *Sammy I. Gutiérrez Terrazas*, Wilhelm Hansberg. Instituto de Fisiología Celular, UNAM
18.	**Role of G-protein heterotrimeric α subunit in glucose sensing and cell differentiation in *Y. Lipolytica*.** *Huerta-Oros Joselina,* Sifuentes-Gaspar Evelyn, Jiménez-Salas Zacarías, Campos-Góngora Eduardo. Universidad Autónoma de Nuevo León
19.	**The contribution of transcription factors NapA, SrrA and AtfA to the antioxidant response and cell differentiation in *Aspergillus nidulans*.** *Ariann Mendoza*, Fernando Lara, Olivia Sánchez y Jesús Aguirre. Instituto de Fisiología Celular, UNAM
20.	**Roles of the MAPK cascade components NRC-1 and STE50 in *Neurospora crassa*.** *Miguel Ángel Sarabia Sánchez*, Wilhelm Hansberg Torres. Instituto de Fisiología Celular, UNAM
21.	**Site-directed mutagenesis of the *Acremonium chrysogenum aga1* gene to obtain constitutively active and inactive Aga1 Gα subunits.** *Eduardo Zúñiga León*, Francisco Fierro Fierro, Jessica Y. Cruz Ramón, Francisco José Fernández. Universidad Autónoma Metropolitana
22.	**Growth of filamentous fungi on dibutyl phthalate and toxicity of its breakdown products shown on the basis bacterial growth.** *Ahuactzin Pérez M*, Rodríguez-Pastrana BR, Soriano-Santos J, Díaz-Godínez G, Díaz R, Téllez-Téllez M and Sánchez C. Universidad Autónoma de Tlaxcala
23.	**qPCA: a scalable assay to measure the perturbation of protein–protein interactions in living cells.** *Francisco Torres-Quiroz*, Luca Freschi, AlexandreDubé & Christian R Landry. Instituto de Fisiología Celular, UNAM
24.	**A role for Hof1p and the Dbf2p/Mob1p Mitotic Exit Network kinase as a sensor for the mitochondrial inheritance checkpoint.** *Leonardo Peraza Reyes*, Pallavi Srivastava, David Crider, Istvan R. Boldogh and Liza A. Pon. Instituto de Fisiología Celular, UNAM

25.	**Isolation and identification of fungi able to degrade polyurethane.** *Joyce Álvarez-Barragán*, Guillermo Aguilar-Osorio, Hermilo Leal-Lara, Martín Vargas-Suarez, Herminia Loza-Tavera. Facultad de Química, UNAM
26.	***In silico* analysis of two new thermostable fungal xylanases (GH11) domains.** *Zazil Yadel Escalante García*, Anne Gschaedler, Enrique Herrera, Lorena Amaya Delgado. CIATEJ AC.
27.	**Heterologous expression of the enzyme 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGR) from *Candida glabrata.*** *Dulce María-Andrade Pavón*, José Antonio–Ibarra and Cesar Hugo-Hernández- Rodríguez, Lourdes-Villa Tanaca. Escuela Nacional de Ciencias Biológicas, IPN
28.	**Effect of Chitosan Films and Thyme Essential Oil on Mycobiota and Biological Quality in Stored Corn.** *María Guadalupe Arguijo Pérez*, María Cristina Julia Pérez Reyes, Gabriela Sánchez Hernández, Patricia Susana Miranda Castro. FES-Cuautitlán, UNAM
29.	**Bioleaching of Cr (VI) contaminated effluent and soil by fungi.** *Juan José Becerra Rodríguez*, Georgina E. Reyna López. División de Ciencias Naturales y Exactas, Universidad de Guanajuato
30.	**Molecular identification of lead-tolerant rhizosphere fungi isolated from a polluted site.** *Alma Delia Burciaga Monge*, Francisco Javier Zavala Díaz de la Serna, Guadalupe Virginia Nevárez Moorillón, María del Rosario Peralta Pérez. Facultad de Ciencias Químicas Universidad Autónoma de Chihuahua
31.	**Development of a methodology for the molecular detection of *Sclerotium cepivorum* in soils, based on the amplification of internal transcribed spacer sequences (ITS).** *Alejandra Cortez Pérez*, Ramón Gerardo Guevara González. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro
32.	**Popcorn irradiation with UV-C light to control the nature mycobiota.** *Anaid García Trejo*, María Cristina Julia Pérez Reyes, Claudia Hernández Aguilar, Flavio Arturo Domínguez Pacheco, Gabriela Sánchez Hernández, Carmen Liliana Rodríguez Páez, Ernesto Moreno Martínez. Facultad de Ciencias, UNAM
33.	**Mycelial growth and hyphal structure of edible mushrooms grown on di (2-ethyl-hexyl) phthalate.** *Ángel González Márquez*, José Luis Torres García, Miriam Ahuactzin Pérez, Gerardo Díaz Godínez, Rubén Díaz Godínez, Carmen Sánchez Hernández. Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala
34.	**Production of extracellular xylanase produced by *Wickerhamia* sp.** *Zahuiti Hernández-Montañez*, Daniel Chávez-Blancas, Eliseo Cristiani-Urbina, Griselda Chávez-Camarillo**.** ENCB-IPN
35.	**Evaluation of *Lentinula edodes* and *Pleurotus ostreatus* extracts as bioactive compounds antibacterial.** *Perla Xochitl Hernández-Ramírez*, Armando Zepeda-Bastida, Deyanira Ojeda-Ramírez, Oscar Arce-Cervantes, Leticia-Romero Bautista and Maricela Ayala-Martínez. Universidad Autónoma del Estado de Hidalgo
36.	**Degradation of ligno cellulose by acellulolytic strains (C-) of *Pleurotus ostreatus.*** *Hermilo Leal Lara*, Hilda Heredia Caamaño y Rebeca Ramírez Carrillo. Facultad de Química, UNAM
37.	**Production of lipolytic activity by the thermophilic fungus *Chaetomium* sp. using different carbon sources.** *Brenda Anabel López- Ruiz*, Alejandro Santiago- Hernández, María Eugenia Hidalgo- Lara. CINVESTAV, IPN

38.	**Isolation, identification and phenotypic characterization of yeast obtained from mine tailings of Zacatecas, Mexico.** *Zeltzin Magaña-García*, Lily Xochitl Zelaya-Molina, Alfredo Patiño-Siciliano, Lourdes Villa-Tanaca, César Hernández- Rodríguez. Escuela Nacional de Ciencias Biológicas, IPN
39.	**Heterologous expression of a lipase gene from the basidiomicete fungus *Bjerkandera adusta* UAMH 8525 in *Pichia pastoris*.** *Catalina Morales-Herrera*, Ayixon Sánchez-Reyes, Laura I. Cuervo-Soto y Jorge L. Folch-Mallol. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos
40.	**Functional analysis of *arg2* gene encoding the small subunit of carbamoyl-phophate synthetase and its usefulness as a selection marker in *Trichoderma atroviride*.** *Omar Nieves Ugalde*, Fidel Landeros Jaime, José Antonio Cervantes Chávez y Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro
41.	**Production of INVA and INVB invertases from *Zymomonas mobilis* in *Pichia pastoris*, under the control of AOX1 and GAP1 promoters.** *Ara Itzel Pérez de los Santos-Mondragón*; Ángeles Calixto-Romo; José Alejandro Santiago-Hernández, Florina Ramírez-Vivez, Víctor Eric López y López and María Eugenia Hidalgo-Lara. CINVESTAV, IPN
42.	**Sanitary Quality of Nixtamalizated Masses with Nejayote.** *María Cristina Julia Pérez Reyes*, Ninel Amparo Flores Saldaña, Priscilla Villagran Ortíz, Gabriela Sánchez Hernández, Yazmín Yael López Ramírez y María del Carmen Valderrama Bravo. FES-Cuautilán, UNAM
43.	**Transformants of *A. rouxii*, an option for increase tyrosinase activity.** *Angelica Patricia Ramos Puebla,* José Francisco Fierro Fierro*, Horacio Reyes* Araceli Tomasini Campocosio. Universidad Autónoma Metropolitana, Unidad Iztapalapa
44.	**Biodegradation of lignocellulosic materials by solid state cultures of two strains of the fungus *Penicillium*.** *Eduardo Rodríguez-Bustamante*, Andrea S. Callejas-Iberri, Santiago J. Carreto Castro, Luis Miguel Gallardo-Roldán y Roberto Arreguín-Espinosa. Instituto de Química, UNAM
45.	**Functional analysis of *pyr*4 gene encoding orotidine-5'-monophosphate decarboxylase and its usefulness as a selectable marker in *Trichoderma atroviride*.** *Erick Rojas Espinosa*, Fidel Landeros Jaime, José Antonio Cervantes Chávez y Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro
46.	**Study of phenoloxidases, proteases and peroxidases in *Rhizopus oryzae* ENHE.** *Ruiz Badillo Arianna*; Fernández Perrino Francisco José1; León Santiesteban Hécto Hugo; Tomasini Campocosio Araceli. Universidad Autónoma Metropolitana-Iztapalapa
47.	**Extracellular β-glucosidase activity from the thermophilic fungus *Corynascus sepedonium*.** *Lourdes Viridiana Soto-Robles*, Alejandro Santiago-Hernández, María Eugenia Hidalgo-Lara. CINVESTAV, IPN
48.	**Heterologous expression of a protein with amorphogenetic activity on cellulose from B*jerkandera adusta* in A*rabidopsis thaliana* plants.** *Irán Tapia Vázquez*, Ramón Suárez Rodríguez and Jorge Luis Folch Mallol. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos
49.	**Radial growth rate, biomass and cellular ultrastructure of the vegetative phase of fungi filamentous grown on di octil phthalate isolated from a paper industry.** *José Luis Torres García*, Adriana Madrid Ramírez, Miriam Ahuactzin Pérez, Gerardo Díaz Godínez, Rubén Díaz Godínez, Carmen Sánchez. Universidad Autónoma de Tlaxcala

50.	**Analysis of the overexpression of the *TRX1* gene and the deletion of *ATH1* gene in the production of dry active yeast.** *Alfredo Valadez Cedillo*, Gloria Angélica Gonzáles Hernádez, Adriana García Tapia, Araceli López Andrade, Juan Carlos Torres Guzmán. División de Ciencias Naturales y Exactas. Universidad de Guanajuato
51.	**Hyphomycetes endophytes of *Taxus globosa* Schltdl. antagonists of *Fusarium* sp. and *Alternaria* sp. phytopathogenics of *Capsicum annuum* L.** *Romalda Vásquez Gutiérrez*, Felipe de Jesus Palma Cruz, Lucia Martínez Martínez and Claudia López Sánchez. Technologic Institute of Oaxaca., Autonomus University Benito Juárez of Oaxaca
52.	**Regulation and function of NADPH oxidases in polarized growth and cell fusion in *Neurosporacrassa.*** *Cano-Domínguez Nallely*, Ernestina Castro-Longoria, AlexanderLichius and Jesús Aguirre. Centro de Investigación Científica y de Educación Superior de Ensenada
53.	***"In vivo"* analysis of the transcriptional expression of the telomerase reverse-transcriptase gene in *Ustilago maydis.*** *José Alfredo Jiménez-Valdéz*, Pavel Sierra-Martínez, Candelario Vázquez Cruz, Estela Anastacio-Marcelino, Ma. Patricia Sánchez-Alonso. Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla
54.	**Growth of the colony of *pleurotus ostreatus* grown on di (2-ethylhexyl) phthalate: fractal dimensión and biochemical analysis.** *Nerit Montiel Martínez*, José Luis Torres García, Brenda H Camacho Díaz, Gustavo F Gutiérrez López, Rubén Díaz Godínez, Maura Téllez Téllez, Gerardo Díaz Godínez y Carmen Sánchez. Universidad Autónoma de Tlaxcala
55.	**Diagnosis and molecular characterization poisoning using gender *amanita* ITS3 E ITS4 sequence of *candida* sp.** *Diana Matías Pérez*, Marytrini García Ramírez, Iván Antonio García Montalvo, Gabriel Mayoral Andrade, Eduardo Pérez Campos**.** Laboratorio de Patología Clínica "Dr. Eduardo Pérez Ortega"
56.	***Neurospora crassa* catalase-3 catalytic and C-terminal domains: an *in vitro* assay.** *Teresa Nava Ramírez,* Pablo Rangel Silva, Wilhelm Hansberg**.** Instituto de Fisiología Celular, UNAM
57.	**The chromatin modifying enzymes HOS-2 and SET-5 are involved in development and response to light in *Trichoderma atroviride*.** *Macario Osorio Concepción*, J. Sergio Casas Flores. Instituto Potosino de Investigación Científica y Tecnológica AC.
58.	**The adenylate-forming enzyme AfeA regulates asexual development in *Aspergillus nidulans.*** *Olivia Sánchez*, Gabriela Soid-Raggi and Jesús Aguirre. Instituto de Fisiología Celular, UNAM
59.	**Growth of the mycelial and reproductive phases of *pleurotus ostreatus:* biochemical analysis and cellular ultrastructure.** *José Luis Suárez Segundo*, José Luis Torres García, José David Sepulveda Sánchez, Sergio Huerta Ochoa, Gerardo Díaz Godínez, Maura Téllez Téllez, Rubén Díaz Godínez, Carmen Sánchez. Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala
60.	**RAS-1 function during growth and differentiation of *Neurospora crassa*: effect of cysteine substitutions.** *Itzel A. Vargas-Pérez*, Sammy I. Gutiérrez Terrazas, Wilhelm Hansberg Torres. Instituto de Fisiología Celular, UNAM
61.	**Characterization of the Δ*aps-2* mutant in *Neurospora crassa*.** *Fausto Martín Villavicencio Aguilar*, Olga Alicia Callejas Negrete and Rosa Reyna Mouriño Perez. Centro de Investigación Científica y Educación Superior de Ensenada

62.	**Simultaneous expression of mating type information a and alpha in *Candida glabrata*, confers susceptibility to oxidative stress.** *Karina Asyade Robledo Márquez*, Irene Castaño Navarro. División de Biología Molecular, IPICyT
63.	**Germplasm of strain fungus "COBIOCH-UAEM" of the Center of Biological Investigations, (biological and genetic resources of México).** *Isaac Tello Salgado*, Elizur Montiel Arcos y Daniel Martínez Carrera. Centro de Investigaciones Biológicas, Laboratorio de Micología, Universidad Autónoma del Estado de Morelos.
64.	**In search of putative glutathione transporter in *Candida glabrata.*** *Alvarado Rodríguez Gloria Guadalupe*, Gutiérrez Escobedo María Guadalupe y De Las Peñas Nava Alejandro. Instituto Potosino de Investigación Científica y Tecnológica
65.	**Role of the Atf1 transcription factor to different kinds of stress in *Trichoderma atroviride.*** *Víctor Alejandro Correa Pérez* y Edgardo Ulises Esquivel Naranjo. FCN, UAQRO
66.	**Functional Characterization of the MAPKK Pbs2 in *Trichoderma atroviride*.** *Jorge Luis Parra Arriaga*, Fidel Landeros Jaime, José Antonio Cervantes Chávez y Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro
67.	**Physiological analysis of an acatalasemic *Saccharomyces cerevisiae* complemented with the catalases from the euryhaline yeast *Debaryomyces hansenii*.** *Ángel García-Campos*, Román Castillo-Díaz, Diego Noriega, Mónica Ramírez-Hernández, Viviana Escobar, Víctor Valdés-López, Luisa Alba-Lois, Claudia Segal-Kischinevzky. Facultad de Ciencias, UNAM
68.	**Fluconazole resistance in *gsh1Δpro2-4* mutant *of Candida glabrata.*** *Ma. Guadalupe Gutiérrez Escobedo*, Alejandro De las Peñas Nava, Irene Beatriz Castaño Navarro. Instituto Potosino de Investigación Científica y Tecnológica
69.	**Functional heterologous complementation of *Δtps2 Ustilago maydis* mutants impaired in the synthesis of trehalose.** *Edda Hurtado Santiago*, Edgardo Ulises Esquivel Naranjo, Fidel Landeros Jaime, José Antonio Cervantes Chávez and José Ruiz-Herrera. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro
70.	**Elucidating the role of the hydrolysis of intracellular trehalose in *ustilago maydis´* physiology.** *Alonso López-Cabrera***,** Laura Valdés-Santiago, Claudia Geraldine León-Ramírez, Ulises Esquivel Naranjo, José Ruiz-Herrera and José Antonio Cervantes-Chávez. Facultad de Química, Universidad Autónoma de Querétaro
71.	**ROS accumulation in idiophase and its relationship with lovastatin biosynthesis in *Aspergillus terreus*.** *Roxana U. Miranda*, Francisco Fierro, Luis Enrique Gómez-Quiroz and Javier Barrios-González. Universidad Autónoma Metropolitana
72.	**Study of colonization of *Arabidopsis thaliana* plants by *Trichoderma atroviride* expressing a *Coriolopsis gallica* laccase.** *Denise Lizeth Aceves Zamudio*, Edgar Balcázar López Marcela Ayala Aceves y Jorge Luis Folch Mallol. Centro de Investigación en Biotecnología, UAEM
73.	**Over expresion of the *swo1* gene in *Trichoderma atroviride* and evaluation of the micoparasitic activity of the transformants strains.** *Karina Atriztán-Hernández*, Edgar Balcázar-López, Alfredo Herrera-Estrella, Jorge Luis Folch-Mallol. Facultad de Ciencias Biológicas, Universidad Autónoma del Estado de Morelos
74.	**Phylogenetic analyses of endoxylanases from *Colletotrichum* sp.** *Ulises Conejo-Saucedo*, Horacio Cano-Camacho, Everardo López-Romero, Alicia Lara-Márquez, Maria Guadalupe Villa Rivera, María G. Zavala-Páramo. Centro Multidisciplinario de Estudios en Biotecnología, Universidad Michoacana de San Nicolás de Hidalgo

75.	**In depth transcriptome analysis of *Trichoderma spp.-Arabidopsis thaliana* –interaction** *Magnolia Estrada-Rivera,* Miguel A. Hernández-Oñate, José de Jesús Gallardo-Negrete, Alfredo Herrera-Estrella and Sergio Casas-Flores. División de Biología Molecular, IPICYT
76.	**Study of Cultivable Fungi Associated with Rihzospher of *Pinus Chiapensis*.** *Silvia María del Carmen García García*, Cristina Domínguez Castillo, Marcos Flores Encarnación, Dolores Castañeda Antonio, Francisco Bersain Moreno Luna, Ricardo Carreño López, Ricardo Munguía Pérez. Centro de Investigaciones en Ciencias Microbiológicas, Benemérita Universidad Autónoma de Puebla
77.	**MAPKs mediating the capacity of biocontrol in *Trichoderma atroviride* MAPKs: mediadoras de la capacidad biocontroladora de *Trichoderma atroviride*.** *Rosa Laura Hernández-Fuerte*, Emma Beatriz Hernández-Beltrán and Alfredo Herrrera-Estrella. CINVESTAV-Irapuato, IPN
78.	***Fusarium* species infecting maize in Sinaloa, Mexico.** *Karla Y. Leyva-Madrigal*, Claudia P. Larralde Corona, Ignacio E. Maldonado-Mendoza. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, IPN
79.	**Aggressiveness of *Gibberella fujikuroi* isolates on maize seedlings.** *Karla Y. Leyva-Madrigal*, Claudia P. Larralde Corona, Ignacio E. Maldonado-Mendoza. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, IPN
80.	**Search of the gene coding for the histidine kinase (*HK*) protein of the nematophagous fungus *Pochonia chlamydosporia* var. *mexicana*.** *María Gabriela Medina-Canales*, Gerardo Zúñiga, Rosa Helena Manzanilla-López, Alejandro Tovar-Soto, and Aída Verónica Rodríguez-Tovar. Escuela Nacional de Ciencias Biológicas, IPN
81.	**Mutation of gene *Swo1* of fungus *Trichoderma atroviride* and evaluation of its mycoparasitic activity against pathogenic fungi.** *Richa Mehta*, Edgar Balcázar Lopés, Karina Atriztán Hernández y Jorge Luis Folch Mallol. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos
82.	**Potentially pathogenic yeasts in the nostril of volunteers and outdoors at the Universidad Autónoma Metropolitana Unidad Xochimilco.** *Arturo Miranda Calixto*, Raúl Venancio Díaz Godoy, Judith Castellanos Moguel. UAM - Unidad-Xochimilco
83.	**Methyl jasmonate restores the effect of root silencing of PvLOX2 on mycorrhiza induced resistance in common bean leaves.** *Arlene Mora-Romero*, Alejandra González-Ortiz, Sergio Medina-Godoy, Lauro Cervantes-Chávez, Quiroz-Figueroa, Francisco, María del Carmen Martínez-Valenzuela, Melina López Meyer. CIIDIR, IPN/ Instituto de Investigación en Ambiente y Salud, Universidad de Occidente
84.	**Identification of entomopathogenic fungi from Queretaro State to control maize pests.** *Joel Acis Mosqueda Anaya*, Fidel Landeros Jaime, Santiago Vergara Pineda, José Antonio Cervantes Chávez, Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro
85.	**Biochemical mechanism involved in the onion growth promotion and control of *Alternaria porri* and *Sclerotium rolfsii* by *Trichoderma*.** *Gilberto Ortega García*, Valeria Camacho Luna, Roberto Montes Belmont, Gabriela Sepúlveda Jiménez. Centro de Desarrollo de Productos Bióticos, IPN
86.	**Characterization of the cell wall and its role in the innate immune response against *Candida parapsilosis*.** *Luis Antonio Pérez-García*, Attila Gácser, Eine Estrada-Mata, Mercedes Guadalupe López-Pérez, Arturo Flores-Carreón, Héctor Manuel Mora-Montes. División de Ciencias Naturales y Exactas, Universidad de Guanajuato

87.	**Comparison of abundance and infectivity of Arbuscular Micorrhizal Fungi isolated of two different zones of Tlaxcala, Mexico.** *Carolina Pérez Morales*, Martha Bibbins Martínez, Miguel Ángel Villalobos López. CIBA, IPN
88.	**Identification of fungi associated with avocado scab.** *Juan Enrique Pérez Reséndiz*, Fidel Landeros Jaime, José Antonio Cervantes Chávez y Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales**,** Universidad Autónoma de Querétaro
89.	**Structural and molecular analysis of biofilm formation *in vitro* by *Aspergillus fumigatus*-*Staphylococcus aureus*.** *Adrián Ramírez Granillo*, María Gabriela Medina Canales, María de los Ángeles Martínez Rivera, Victor Manuel Bautista de Lucio, Aída Verónica Rodríguez Tovar. Escuela Nacional de Ciencias Biológicas-IPN
90.	**The relevance of the cell wall in the immune sensing of *Candida parapsilosis, C. orthopsilosis and C. metapsilosis* by human mononuclear cells.** *Vianney Ramírez-Vargas*, María de Jesús Navarro-Arias and Héctor Manuel Mora-Montes. Departamento de Biología, Universidad de Guanajuato
91.	**Phenotypic characterization of the phytopathogenic fungi *Fusarium temperatum* and construction of a chimeric vector for the interruption of the histidine kinase gen.** *Karen F. Robles-Barrios*, Aída V. Rodríguez-Tovar y Néstor O. Pérez-Ramírez. ENCB, IPN
92.	**Antifungal and phytotoxic activity of endophytic fungi from *Gliricidia sepium* (Fabaceae).** *Rosa Elvira Sánchez Fernández*, Mónica Flores Reséndiz, Sergio Sánchez Esquivel, Martha Lydia Macías-Rubalcava. Instituto de Química, UNAM
93.	**Gene expression of *glx-I* in coleoptiles from maize "resistant to aflatoxin contamination".** *Sánchez-Medina M.A.*, Coconi-Linares, L.N., Pina-Canseco M.S., Pérez-Santiago A.D., Guzmán-Ortíz D.A. Instituto Tecnológico de Oaxaca
94.	**Biological evaluation of endophytic fungus PB3f isolated from *Haematoxylon brasiletto* (fabaceae).** *Brenda Lorena Sánchez Ortiz*, Martha Lydia Macías-Rubalcava. Instituto de Química, UNAM
95.	**Effect of *Trichoderma* and *Bacillus* on the content of phenolic compounds and flavonoids, and antioxidant activity of onion.** *Gabriela Sepúlveda-Jiménez*, Jorge Humberto Betanzos-Rodríguez, Alma Rosa López-Laredo. Centro de Desarrollo de Productos Bióticos, IPN
96.	**Genetic characterization of ant associated Ophiocordyceps.** *Vallejo Camarena Mariana*, Hernández Miguel, Cibrian Jaramillo Angélica, Herrera Estrella Alfredo, Janda Milan. CINVESTAV-Irapuato, IPN
97.	**Expression analyses of an endo-β-(1-6)-D-galactanase gene in two races of *Colletotrichum lindemuthianum* with different life style.** *María Guadalupe Villa Rivera*, María Guadalupe Zavala Páramo, Ulises Conejo Saucedo, Everardo López Romero, Alicia Lara Márquez, Horacio Cano Camacho. Centro Multidisciplinario de Estudios en Biotecnología, Universidad Michoacana de San Nicolás de Hidalgo
98.	**Multilocus analysis of *Histoplasma capsulatum* associated with six molecular markers.** *Tania Vite-Garín*, Daniel Estrada-Bárcenas, Gabriela Rodríguez-Arellanes, Antonio Ramírez-Bárcenas, María Lucia Taylor**.** Facultad de Medicina, UNAM
99.	**Participation of Effector-Like Proteins in the Establishment of the Biological Relationship of *Trichoderma – Arabidopsis*.** *Vianey Olmedo-Monfil*, Alfredo Herrera-Estrella, Mario Iván Alemán-Duarte, Paulina Guzmán-Guzmán, Claudia Ramírez-Valdespino, Ma. Daniela Porras-Troncoso. Departamento de Biología, DCNE, Universidad de Guanajuato

100.	**Construction of a Bacterial Artificial Chromosome (BAC) Genomic Library for Physical Mapping and Genome Sequence Validation of a Brewing Yeast.** *Gómez-Muñoz Cintia*, Riego-Ruiz Lina, Damas-Buenrostro Luis. IPICyT
101.	**Functional characterization of *Candida tropicalis MNN4* and *OCH1*.** *Hernández-Chávez Marco J*, Mora-Montes Hector M. Departamento de Biología, Universidad de Guanajuato
102.	**Analysis of nitrogen catabolite repression in *Lachancea kluyveri*.** *Jiménez Benítez José Ángel* y Riego Ruiz Lina Raquel. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica
103.	**Isolation and functional characterization of *Sporothrix schenckii OCH1*.** *Nancy E. Lozoya-Pérez* and Héctor M. Mora-Montes. Departamento de Biología, Universidad de Guanajuato
104.	**Cellular localization and gene transcriptional regulation have a role on the functional divergence of *Saccharomyces cerevisiae* Bat1 and Bat2 isozymes.** *Javier Montalvo-Arredondo*, José Ángel Jiménez-Benítez, Maritrini Colón-González, Alicia González-Manjarrez, Lina Raquel Riego-Ruiz. Instituto Potosino de Investigación Científica y Tecnológica
105.	**The GATA type factor Gln3 is involved in nitrogen uptake in the yeast *Candida glabrata*** *Francisco Javier Pérez de los Santos* and Lina Raquel Riego Ruiz. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica.
106.	**Functional characterisation *Candida tropicalis MNN4*.** *Martha M. Rangel Sosa*, Marco J. Hernández-Chávez and Héctor M. Mora-Montes. Universidad Autónoma de Aguascalientes
107.	**The Pep4Um vacuolar proteinase is involved in morphogenesis and pathogenesis of *Ustilago maydis*.** *Cinthia V. Soberanes-Gutiérrez.*, Melissa Vázquez-Carrada., Claudia León-Ramírez., José Ruiz-Herrera and Lourdes Villa-Tanaca. Escuela Nacional de Ciencias Biológicas, IPN
108.	**Functional characterization of three mannosyltransferases of *Saccharomyces cerevisiae*.** *Nahúm Valente Hernández*, Héctor Manuel Mora Montes. División de Ciencias Naturales y Exactas, Universidad de Guanajuato
109.	***In silico analysis and expression profile of Candida albicans yeast* glucanases.** *César Isaac Bazán Méndez* and Juan Pedro Luna-Arias. CINVESTAV, IPN
110.	**Transcriptional regulation of *GDH3* in glucose grown-cultures of *Saccharomyces cerevisiae*.** *Brisa Aranzazú Campos-Oliver*, Maritrini Colón-González, José Carlos Campero-Basaldúa, Cristina Aranda-Fraustro and Alicia González-Manjarrez. Instituto de Fisiología Celular. Universidad Nacional Autónoma de México
111.	**The *Trichoderma atroviride* photolyase-encoding gene is transcriptionally regulated by non-canonical light response elements.** *Mayte Guadalupe Cervantes-Badillo*, Tania Muñoz-Centeno, Edith Elena Uresti-Rivera, Gerardo Rafael Argüello-Astorga, Sergio Casas-Flores. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica
112.	**Expression divergence of *BAT1* and *BAT2 Saccharomyces cerevisiae* paralogous genes determines their functional diversification**. *James E. González Flores*, Juan C. Martínez Morales and Alicia González Manjarrez. Instituto de Fisiología Celular, UNAM
113.	***GDH1-GDH3* Paralogus Genes Show Divergent Transcriptional Regulation in *Saccharomyces cerevisiae*.** *Alan Anuart González Rangel*, Cristina Aranda and Alicia González. Instituto de Fisiología Celular, UNAM

114.	**Dosage matters: Revealing the fitness effects of experimental gene duplication.** *Diana I. Ascencio* & Alexander de Luna. LANGEBIO CINVESTAV-IPN. Unidad Irapuato
115.	**Regulatory divergence in paralogous genes *ALT1* and *ALT2* in *Saccharomyces cerevisiae.*** *Dariel Márquez Gutiérrez* and M. Alicia González Manjarrez. Instituto de Fisiología Celular, UNAM
116.	**Inhibition of local silencing increases the resistance of *Candida glabrata* to fluconazole and oxidative stress.** *Emmanuel Orta-Zavalza*, Gehenna Guerrero-Serrano, Ma Guadalupe Gutiérrez-Escobedo, Israel Cañas-Villamar, Jacqueline Juárez-Cepeda, Irene Castaño and Alejandro De Las Peñas. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica
117.	***Aspergillus terreus* higher lovastatin production in solid-state Fermentation correlates with higher expression of genes *laeA* and *lovE.*** *Teresa Pérez-Aguirre*, Armando Ordaz-Hernández y Javier Barrios-González. Universidad Autónoma Metropolitana
118.	**Lovastatin production, ROS accumulation and sporulation in *Aspergillus terreus* with a silenced gene *Atyap1.*** *Ailed Pérez S*, Esmeralda Bibian L, Roxana Miranda U, Armando Mejía A, Javier Barrios-González. Universidad Autónoma Metropolitana Unidad Iztapalapa
119.	**Regulation of the expression of the acidic ribosomal proteins in *Saccharomyces cerevisiae.*** *Juan Ismael Rea Hernández* y Samuel Zinker Ruzal. CINVESTAV, IPN
120.	**Analysis of *xyl3* gene expression in *Fusarium oxysporum* f. sp. *lycopersici* strains.** *María Alejandra Reyes Medina*, Karla Lizbeth Macías Sánchez. Unidad Profesional Interdisciplinaria de Ingeniería, IPN
121.	**Quantitative expression analysis in *Sporothrix schenckii*: identifying constitutively expressed genes for data normalization.** *José Elías Trujillo Esquivel*, José Asunción Martínez Álvarez, Patricia Ponce Noyola and Héctor Manuel Mora Montes. Departamento de Biología, Universidad de Guanajuato
122.	**Expression analysis of information contained in the *Candida glabrata MTL* loci: Possible heterodimeric molecules a1/alpha2, a1/alpha3 and a1/alpha4.** *Patricia Yáñez Carrillo* e Irene Castaño Navarro. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica
123.	**Recombinant Expression of an immunodominant antigen from *Mycobacterium tuberculosis* in *Pichi*a *pastoris*.** *Daniel Juárez López*, Mauricio Alberto Trujillo Roldán, Norma Adriana Valdez Cruz. Instituto de Investigaciones Biomédicas, UNAM
124.	**Caracterización de los genes de *Kluyveromycesmarxianus* involucrados en la producción del 2-feniletanol mediante la Vía de Ehrlich.** *Ortega-Domínguez CI*, Rutiaga-Quiñones OM, Aréchiga-Carvajal ET, Rojas-Contreras A, Soto-Cruz NO. Instituto Tecnológico de Durango

Plenary Session

Keeping the shape. Endocytosis and hyphal morphogenesis

Rosa R. Mouriño Pérez

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Morphogenesis in filamentous fungi depends mainly on the establishment and maintenance of polarized growth. This is accomplished by the orderly migration and discharge of exocytic vesicles carrying cell wall components. We have been searching for evidence that endocytosis, an opposite process, could also play a role in morphogenesis. We found that coronin deletion (*Neurospora crassa* mutant, Δ*crn-1*) causes a decrease in endocytosis (measured by the rate of uptake of FM4-64) together with marked alterations in normal hyphal growth and morphogenesis accompanied by irregularities in cell wall thickness. The absence of coronin destabilizes the cytoskeleton and leads to interspersed periods of polarized and isotropic growth of the hyphae. We used CRIB fused to GFP as an exocytic reporter of activated Cdc-42 and Rac-1. We found that CRIB-GFP was present in wild-type hyphae as a thin hemispherical cap under the apical dome, i. e. when growing in a polarized fashion and with regular hyphoid morphology. In the Δ*crn-1* mutant, the location of CRIB-GFP shifted between the periods of polarized and isotropic growth, it migrated to the subapical region and appeared as localized patches. Significantly, cell growth occurred in the places where the CRIB-GFP reporter accumulated, thus the erratic location of the reporter in the Δ*crn-1* mutant correlated with the morphological irregularity of the hyphae. We found that the Δ*crn-1* mutant had a higher proportion of chitin than the WT strain (14.1% and 9.1% respectively). We also compared the relative cell wall area (TEM images) and we found a different ratio wall/cytoplasm between the Δ*crn-1* mutant and the WT strain. In conclusion, we have found that the mutant affected in endocytosis has an altered pattern of exocytosis as evidenced by its distorted morphology and displaced exocytic markers. A direct cause-effect relationship between endocytosis and exocytosis remains to be established.

Cell Differentiation and Resilience in Fungi

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Psychological resiliencecan be defined as the human capacity to cope with stress and adversity and to"bounce back" to a previous state of normal functioning. However, coping with extreme situations might require becoming different either transiently or permanently. We proposed that confronted with oxidative stress, microorganisms evolved mechanisms not only to cope with stress but to actually use this ancestral form of stress to regulate cell differentiation.Our research is aimed at establishing the role of reactive oxygen species (ROS) as growth and cell differentiation signals and to understand the mechanisms that modulate ROS production, perception, detoxification and action in filamentous fungi.We have shown that the ROS-producing enzymes NADPH oxidases (NOX) are essential for sexual differentiation in *Aspergillusnidulans* and *Neurosporacrassa* and for polar growth and cell-fusion in *N. crassa*. Currently we are trying to determine NOX regulation, cell localization and possible action mechanisms. Regarding ROS perception and detoxification, wefoundthat response regulator (RR) SskAtransmitsoxidative stress signalstothe stress MAPKSakA, which in turnsphysicallyinteractswith ATF/CREB transcription factor AtfA in thenucleus. This defines a general stress-signallingpathway, whichplaysdifferential roles in oxidative stress responses duringgrowth and development. WehaveshownthatSakAphosphorylationis a conservedmechanismtoregulatetransitionsbetween non-growing (spore) and growing (mycelia) states. The SrkA protein kinase ispart of the SakA pathway and mediates its repressing functions during sexual development.In additiontoSakA, the RR SrrA andNapAtrancriptionfactors are differentiallyinvolved in ROS signalling and celldifferentiation.

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How does the opportunistic pathogen *Candida glabrata* avoid sexual reproduction?

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Candida glabrata is an opportunistic fungal pathogen frequently isolated in hospital-acquired infections.The frequency of isolation of this organism from blood-stream infections has risen significantly in North America in the last two decades in part due to its inherentlydecreased susceptibility to the antifungal drug fluconazoleand resistance to oxidative and osmoticstress,compared to other *Candida* species.

C. glabrata is an haploid yeast more closely related to *Saccharomycescerevisiae*than to other *Candida* species. In *C. glabrata*, like in *S. cerevisiae*, theregionsclosetothetelomeres are subjecttotranscriptionalrepression by a chromatin-based silencing that depends on Sir2-4, Rap1, Ku70/80 and Rif1 proteins. Subtelomeric silencing in *C. glabrata* plays a role in virulence by regulating the expression of a large family of genes encoding putative cell wall proteins (the *EPA* genes) the majority of which are located close to several telomeres. *C. glabrata* adheres tightly to cultured mammalian epithelial cells mainly through the expression of the adhesin Epa1. *EPA1* gene is located close to the right telomere of chromosome E and is subject tosubtelomeric silencing. Subtelomeric silencing however, varies between subtelomeric regions, and this probably depends on specific sequence elements present at these regions that can confer different requirements for the silencing proteins Sir2-4, Ku70/80 and Rif1.

C. glabrata has no known sexual cycle and does not maintain a sexual cell-type identity, even thoughit contains the vast majority of the genes required for sexual reproduction, including three mating type-like loci (*MTL*) encoding orthologues to the genes that control sexual reproduction in *S. cerevisiae* and other fungi. The *MTL1* locus can contain **a**-type information (**a1** gene), or alpha-type information (alpha1 and □lpha2 genes),*MTL2* almost always contains **a**-type and *MTL3*alpha-type information. *MTL1* and *MTL2* are both transcriptionally active while *MTL3* is subject to an incompletesubtelomeric silencing nucleated at the left telomere of chromosome E.

Simultaneous expression of **a**-type and □lpha-type information in *Candida albicans*and *S. cerevisiae*, results in the formation of a heterodimer between the proteins **a1** and □lpha2 that represses several genes, some of them involved in the response to osmotic and oxidative stress.It is not known whether a similar heterodimer is formed in *C. glabrata*.We have used Bimolecular Fluorescence Complementation (BiFC) assays to determine whether a heterodimer is formed in *C. glabrata*. We have also constructed strains that simultaneously express **a** andalpha information from *MTL1*, and characterized the phenotype under several stress conditions. We found thatstrains simultaneously expressing **a** and □lpha information are more sensitive to oxidative stress than the wild-type, suggesting that the genes encoded in the *MTL* loci may be involved in the response to some types of stress in *C. glabrata*.

Intracellular trafficking along microtubules in fungi

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Long-distance trafficking of membranous structures along the cytoskeleton is crucial for secretion and endocytosis in eukaryotes. Molecular motors are transporting both secretory and endocytic vesicles along polarized microtubules. In the model microorganism Ustilago maydis the transport mechanism and biological function of a distinct subset of large vesicles marked by the G-protein Rab5a are well-studied. These Rab5a-positive endosomes shuttle bi-directionally along microtubules mediated by the Unc104/KIF1A-related motor Kin3 and dynein Dyn1/2. Rab5a-positive endosomes exhibit diverse functions during the life cycle of U. maydis. In haploid budding cells they are involved in cytokinesis and pheromone signaling. During filamentous growth endosomes are used for long-distance transport of mRNA, a prerequisite to maintain polarity most likely via local translation of specific proteins at both the apical and distal ends of filaments. Endosomal co-transport of mRNA constitutes a novel function of these membrane compartments supporting the view that endosomes function as multipurpose platforms.

Transcriptional Regulation of the Yeast Stress Response through a Dynamic Interplay of Signaling Networks

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All cells perceive and respond to environmental stresses and do so through elaborate stress-sensing networks that allows cells to evaluate rapidly their ever changing environment. In yeast cells, these sensing pathways converge on stress sensitive transcription factors, including Msn2 and Msn4. Regulation of Msn2 occurs through modulation of its nuclear localization. By performing time lapse studies of Msn2-GFP localization in living cells subjected to media fluctuations in microfluidics chambers, we find that the Msn2 responds to most stresses by an initial entry into the nucleus followed by random and rapid cycles into and out of the nucleus. We find that dynamic interplay among several different signaling pathways, including Ras/Protein kinase A, AMP activated kinase, the HOG map kinase pathway and protein phosphatase 1, regulate the activation of Msn2 in different ways in response to different stresses such as nutrient limitations and changes in osmolarity. In addition, our studies have demonstrated the critical role of noise in this regulation, which imparts quite diverse behaviors to genetically identical cells, allowing the individual cells in a population to hedge their bets against an uncertain future. Using a combination of ODE and stochastic modeling of the relevant signaling networks, we have been able to reproduce in silico the responses of Msn2 to different stresses and thus define the molecular bases of both the initial nuclear influx and the subsequent bursts of activation. These studies highlight the means by which cells balance growth versus survival in an uncertain environment and how information acquisition through signaling pathways inform that balancing act.

Oral

Dissecting the epigenetic mechanisms of blue light perception in the filamentous fungus *Trichoderma atroviride*

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Sunlight regulates circadian rhythms, phototropism, synthesis of β-carotenes and sexual and asexual development in fungi. In the filamentous fungus *Neurospora crassa*, the White Collar -1 and 2 proteins (WC-1 and WC-2) regulate all known responses to blue light. The WC-1 protein is the photoreceptor and together with WC-2 function as transcription factors of blue light inducible genes. The histone acetyltransferase NGF-1, the orthologue of Gcn5 from *Saccharomyces cerevisiae*, is the co-activator of blue light induced genes in *N. crassa* and physically interacts with WC-1 to regulate blue light response. *Trichoderma atroviride* is a filamentous fungus that responds to light by forming a ring of asexual structures called conidia on the colony perimeter where the stimulus was given. In this fungus, the WC-1 and WC-2 orthologues, BLR-1 and BLR-2, regulate as well blue light responses. BLR-1 has been proposed as the blue light receptor. In eukaryotes, DNA interacts with histone proteins, forming an structure named chromatin. The amino termini of histones are subject to posttranslational modifications, including methylation, acetylation and deacetylation, which are conducted by histone methyltransferases (HMTs), histone acetyltransferases (HATs) and histone deacetyltransferases (HDACs) respectively. These modifications or their combination lead to fine tuning on gene expression. In this work we generated mutants in *tgf-1, tahos2* and *taset5* genes whose products putatively encode for a HAT, an HDAC class I and a HMT respectively. Here we show that blue light induced a global acetylation of histone H3. Deletion of *tgf-1,* which encodes a putative acetyl transference, the orthologue of NGF-1, led to a pleiotropic phenotype, mainly affecting growth and development. Transcription and Chromatin immunoprecipitation (ChIP) analyses showed that TGF-1 is the co-activator of blue light regulated genes, probably by acetylating the K9 and K14 lysine residues of histone H3 on the promoters in *T. atroviride*. Furthermore, we demonstrate that TGF-1 is necessary to maintain the basal level of expression of *bld*-2 in the dark. TGF-1 was necessary for histone H3 acetylation of the promoter of *phr*-1 (blue light upregulated) after a blue light pulse, since the Δ*tgf-1* mutant showed no histone H3 acetylation on the *phr*-1 promoter. The Δ*tahos2* strain was impaired in conidiophore development and photoconidiation. Furthermore the Δ*taset5* showed more sensitiveness to light than the wild type strain. In addition, the Δ*taset5* and Δ*tahos2* strains are altered in expression of light regulated genes. Δ*tahos2* is unable to conidiates in response to mechanical injury, whereas Δ*taset5* showed hyperconidiation phenotype when injured.

The NADPH oxidases and tetraspanin PLS-1 in fusión and cell differentiation in the fungus *Neurospora crassa*

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Hansberg and Aguirre proposed that reactive oxygen species (ROS) play essential roles in cell differentiation in microorganisms. ROS are generated mainly during mitochondrial electron transport and by certain enzymes. The NADPH oxidases (NOX) are enzymes that catalyze the production of superoxide by transferring electrons from NADPH to O_2. In the fungus Neurospora crassa, we identified two NADPH oxidases: NOX-1 and NOX-2. Δ*nox*-1 mutant grew lesser than the wild type strain (are affected in cell fusion by CAT, shows a decrease in radial growth, a reduction on the formation of aerial micelium and conidiation) and was defective in asexual and sexual differentiation: Δ*nox*-1 mutant was female sterile, as it was unable to develop primordial sexual fruty bodies (protoperithecia), and also show a decreased production of aerial mycelia and conidiospores. The lack of NOX-2 did not affect any of these processes but led instead to the production of sexual spores that failed to germinate.

Tetraspanins are small integral membrane proteins that were first identified in mammals as cell-specific antigens. These proteins act as organizers of membrane signalling complexes, additionally this proteins regulate cell morphology, motility and fusión. So far only three tetraspanin families have been identified in fungi (PLS-1, TSP-2 and TSP-3).

In *P. anserina* mutants with mutation of *nox*-2 (which encodes the NADPH oxidase of the NOX-2 family), display the same ascospore-specific germination defect as the Δ*pls*-1 mutant, while in *B. cinerea* the mutants Δ*nox*-B and Δ*pls*-1 substantiate a link between BcNOX-B and BcPLS-1 because both deletion mutants have overlapping phenotypes (especially a defect in penetration).

We generate the mutant Δ*pls*-1 in *Neurospora crassa*. This strain are affected in cell fusion by CAT, shows a decrease in radial growth, a reduction on the formation of aerial micelium and conidiation. Also PLS-1 is essential for protoperithecia development ; this phenotype is similar to the mutant Δ*nox*-1.

THE ROLE OF CHITIN SYNTHASES IN DEVELOPMENT AND MORPHOGENESIS OF *Neurospora crassa*

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Chitin is one of the most important carbohydrates in the cell wall of filamentous fungi. It is a linear homopolymer of β-(1-4)-linked *N*-acetyl glucosamine. Chitin synthesis is catalyzed by chitin synthases (CHS), which mediate the transfer of N-acetyl-glucosamine to a growing chitin chain. The filamentous fungus *Neurospora crassa* has one representative for each of the seven CHS classes described. All CHS were found at the core of the Spitzenkörper (Spk) and in forming septa in growing hyphae of *N. crassa*. As the septum ring develops, CHS-2-GFP moves centripetally until it localizes around the septal pore. A partial colocalization of CHS-1-mCherry and CHS-5-GFP occurs in the Spk. Both, CHS-5 and CHS-7 have a myosin motor-like domain at their amino termini, suggesting that they interact with the actin cytoskeleton. By total internal reflection fluorescence microscopy analysis putative chitosomes containing CHS-5-GFP were revealed moving along wavy tracks, presumably actin cables. The actin inhibitor Latrunculin A prevented the accumulation of CHS-4, CHS-5 and CHS-7 in the Spk, further supporting the participation of actin cables in the transport of CHSs. In addition, to investigate whether myosin motor proteins are involved in the traffic of CHS, we co-expressed CHS-1-mChFP and MYO-2Δdil-GFP (Class V). Arrival of both proteins to the Spk suggests that CHS-1 is transported independently of MYO-2.
CoIP experiments using GFP-TRAP and mass spectrometry for strains expressing GFP tagged CHS-1, CHS-4 and CHS-5 were carried out; several putative CHS interacting proteins were identified; these include CSR-4 (homologue at Chs4p in *Saccharomyces cerevisiae*), a serine protease, a homoserine kinase, α-tubulin and also proteins involved in traffic such as SEC-10 and SEC-14. Future analyses will determine the role of these candidate proteins in CHS secretion.
Knock out mutants for each *chs* were analyzed to study the role of each CHS during different developmental stages. Both, *chs-6* and *chs-7* exhibited an important role in vegetative growth; *chs-5* and *chs-7* were important during asexual reproduction; and *chs-7* and *chs-3* had a significant role during formation of sexual structures. Analysis of double mutants allowed elucidating epistatic interactions between pairs of *chs* genes. The double mutant *chs-1Δ;chs-3Δ* displayed a stronger aberrant phenotype than the corresponding single mutants, suggesting that these genes are involved in compensatory pathways. By contrast the double mutant *chs-5Δ;chs-7Δ* showed an intermediate phenotype, suggesting genetic suppression, indicative that these genes are on the same pathway.
Collectively our results suggest that there are different populations of chitosomes, each containing a class of CHS with specific roles during different developmental stages

Role of the photoreceptors BLR1 and BLR2 in the response to mechanical injury in *Trichoderma atroviride*

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Trichoderma atroviride has been widely used as biological control agent due to its mycoparasitic ability, which as a soilborne filamentous fungus is continuously exposed to changes in environmental conditions. *T. atroviride* responds to exposure to blue light, possibly as a cue to the potentially damaging effects of light, by forming conidia. Recently, it has been reported that in *T. atroviride* mechanical damage triggers a series of cellular processes, which culminate in the formation of conidia at the injured area. These cellular processes are very similar in plants and animals suggesting that the three kingdoms share a conserved defense-response mechanism.

The light and injury responses appear to be independent at the molecular level, however it is thought that there is a convergence point between both pathways. Thus, we wondered if there was any difference in the response of the fungus to injury in darkness and when injury was followed followed by a light pulse (double stimulus). Interestingly the production of conidia in response to injury with light is 10 times greater than without it, which suggests that both pathways converge increasing the production of conidia.

In *T. atroviride* injury triggers NADPH oxidase (Nox)–dependent ROS production and Nox1 and NoxR are essential for asexual development in response to damage. Here, we show that when the *Δnox1* and *ΔnoxR* strains are injured and exposed to light, there is production of conidia in the damaged area. However, there is no significant increase in ROS production in these mutants neither after injury or double stimulus. Experiments performed with the WT, Δ*nox1*, Δ*nox2* and Δ*noxR* strains and the antioxidant N-acetyl cystein (NAC), which eliminates all superoxide produced, showed a complete inhibition of conidiation in response to light and mechanical damage, when applied independently, however when subjected to double stimulus all of them formed conidia exclusively in the area of damage.

The response to blue light in *Trichoderma atroviride* is mediated by the transcription factors BLR1 and BLR2. We observed that the Δ*blr1* and Δ*blr2* mutant strains do not show the cumulative effect in conidia production when subjected to the double stimulus and there is no conidiation in response to injury in the presence of the antioxidant NAC even in the light. Interestingly, when we quantified the number of conidia generated in response to injury in the Δ*blr1* and Δ*blr2* strains, we found a decrease of 90.7% and 77.1% compared to the WT, respectively. These data suggest that the BLR proteins regulate genes that are responsive to mechanical damage even in the absence of light. To corroborate this, we analyzed the BLR1 and BLR2 overexpressing strains when subjected to injury, and showed a 2.5 and 2 fold increase in conidiation compared with WT, respectively.

Based on these data we suggest that Nox–dependent ROS are essential to respond to damage in the dark, which could activate BLR's through the BLR1 LOV domain and the

coupled FAD, which is also used to perceive blue light. When the damaged hyphae are stimulated by light, the effect is cumulative and increases the response to injury because light can directly activate the BLR´s making the reactive oxygen species dispensable for this response.

Analysis of the effect of *hda1* disruption on the telomere length maintenance in *Ustilagomaydis*

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Telomerase is aribonucleoprotein complex that synthesizes telomeres in most eukaryotic cells.The enzyme has a central core of two subunits: catalytic subunit, with*te*lomerase *r*everse *t*ranscriptase activity(TERT); and the *te*lomerase *R*NA subunit (TER), which posses the template sequence for telomere synthesis.Expression of the enzyme is regulated at different levels by a variety of transcriptional and post-transcriptional mechanisms during the cell cycle and cellular development programs, and by epigenetic mechanisms as DNA and histone methylation ofhuman TERTpromoter; acetylation and deacetylation of hTERT-promoter histones, and phosphorylation events on regulatory proteins. *hda1* gene from the dimorphic fungus *Ustilago*maydisencodes an RPD3-related histone deacetylase involved in pathogenic development. Deletion of *hda1*results in up-regulation of genes involved in the filamentous life style with slight phenotypic changes. In several other fungi, inhibition of RPD3-class histone deacetylases with trichostatincausedderepression of subtelomeric-harbored genes. In human, global chromatin decondensationinducedby histone deacetylase inhibitors produce erroneous epigenetic regulation of TERTby means not completely elucidated.In our previous studies, telomerase activity from *U.maydis*was sought in tumor galls using TRAP-PFGE and TRAP-ELISA techniques without consistent results. Similar outcomes were obtained when*utert1* transcript measurement was tried by qRT-PCR, or when terminal restriction fragment (TRF) length was sought by southern blot. In an attempt to avoid the use of tumor galls to investigate thetelomere maintenance in mycelia of *U. maydis*, here we analyze the contribution of *hda1* to telomere/telomerase metabolism in the fungus. We initiate with one-step disruption of the *hda1*gene, which lies in the um02065 locus of*U. maydis* genome sequences (http://mips.helmholtz-muenchen.de). The cassette was composed by thecarboxin resistance gene, flanked by approximately 1 kb of sequences located to each side of the chimeric gene.Transformation was achieved by protoplast fusion as reported, and disrupted strains where characterized by PCR. Total DNA from mutants was extracted and digested to completion with *Pst*I. DNA was resolved by electrophoresis, nylon transferred and hybridized to a $(TTAGGG)_n$ probe. Autoradiogram was used to measure telomere length. Southern blot resultssuggest no changes intelomere length, which could be interpreted as lack of epigenetic control of *hda1* on the telomerase reverse transcriptase (*utert1*) gene in filamentous phaseof the fungus. However as an increase of ALT recombination events occurs in human histone-deacetylasemutants to maintain telomere length, we are committed to measure transcript level of *utert1,* and from subtelomeric*UTASa*element.

GRB and APS1 are components of the Microtubule Organizing Centers of *Neurospora crassa*

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Gamma-tubulin is the main Microtubule (Mt) nucleator in the Microtubule Organizing Centers (MTOCs) found in most eukaryotic cells. The pericentrin proteins are responsible of the recruitment of the gamma-tubulin complex to the inner plate of the Spindle Pole Body (SPB), and are important to ensure the correct spindle assembles. The recruitment of gamma-tubulin complex to the outer plat of the SPB is carried out for other proteins. In order to find other proteins involved in the MTOCs of *N. crassa*, we identified by bioinformatics analysis the proteins GRB with 20% identity to the pericentrin Pcp-1 of *S. pombe* and APS-1 with 23% identity to ApsB of *A. nidulans*. These proteins contain 1,492 aa and 1,749 aa respectively. GRB, APS1 and gamma-tubulin were tagged with GFP and/or mCherry. Gamma-tubulin was present in the SPB as a single bright spot in interphase nuclei and as two opposite spots in mitotic nuclei, there was no gamma-tubulin in any other location. GRB was also observed in the SPBs of the nuclei co-localizing with gamma-tubulin. Gamma-tubulin and GRB have the same dynamics. GRB co-localizes with centromeric histone 3 (CenH-3) in interphase nuclei and it is embedded in the nuclear envelop. It seems that the positioning of the gamma-tubulin complex in the inner side of the SPB is GRB dependent but it was not possible to be corroborated because the deletion of *grb* gene is lethal. APS-1 is present in the SPB co-localizing with gamma-tubulin and also is present as gamma-tubulin free accumulations in the cytoplasm but not in septa. Gamma-tubulin in *N. crassa*, as in other organisms, is essential but not APS-1. To conclude, Gamma-tubulin and GRB are present exclusively in the SPBs and both are essential. APS-1 is a possible Gamma-tubulin free MTOC, but this needs to be confirmed.

Ustilago maydis adaptation to alkaline pH, trough Rim101 regulation

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As a general characteristic Fungal cells have an outstanding metabolic plasticity, so they are capable to tolerate, adapt and even grow in extreme ambient conditions. We have been studying the metabolic strategies of *Ustilago maydis* as a model for alkaline pH adaptation by microarray data analysis. Alkalinity triggers in fungi an adaptation mechanism, mediated by Rim101, a zinc finger type transcription factor. Trough gene expression comparison between *U. maydis* FB2 (*wt)* cells against BMA2, (*rim101*⁻*)* we found that it modifies the expression of approximately 20% of it´s Genes. Adaptation to pH 9 in growth media involves very high control of at least 33 ionic membrane transporters, modification of the expression of genes involved in nutrient and vesicle transport and a reorganization of polysaccharide, lipids and the protein composition of the cell wall mainly. Phenotypic characteristics observed in the *rim101*⁻ mutant of *U. maydis* cells growing at pH 9 can be explained via the analysis of changes in gene expression on genes associated with the establishment of cell polarity, the re - organization of the actin cytoskeleton organization and budding patterns. In this analysis we saw that Rim101 can regulate gene expression in a direct or indirect way, through the expression or repression of at least 63 transcriptional factors. Further studies must analyze if these mechanisms are completely conserved among other Basidiomycetes or if they present variation depending on the ecological fungal niche.

Suboptimal response to stress underlies adaptation by gene inactivation

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Beneficial mutations are the fuel of adaptive evolution; such changes are required to improve the fitness of populations. However, why some genetic variants are beneficial under certain environments remains mostly elusive (1). Network theory suggests a possible mechanism for the emergence of beneficial effects of mutations: A metabolic network that has been perturbed by the loss of a gene can be rescued by removing additional genes that alleviate or even cancel-out the suboptimal metabolic state caused by the first mutation (2). Given that cells may respond suboptimally to changes in the environment (3-4), we screened a collection of metabolic mutants for beneficial mutations in phosphate limitation. We found 22% from 490 knockouts mutants with fitness higher than wild type (5) four functional classes are overrepresented among such 110 beneficial mutations: Oxidative phosphorilation, protein glycosilation, sterol metabolism, and phospholipid synthesis. To test the relevance of loss-of-function within such processes in actual evolution, we evolved yeast strains in the laboratory for 200 generations under phosphate limitation and will show results from genetic and biochemical analyses on adapted strains. We will also use metabolic modeling enhanced by transcriptiomics in order to simulate suboptimality in the response to phosphate limitation to explain the systemic grounds rendering the beneficial effect of loss-of-function adaptive mutations.

Our study suggests that certain types of beneficial mutations are a consequence of systemic properties and responses to genetic and environmental perturbations, rather than a simple result of their specific molecular functions.

1. Tenaillon O, Rodriguez-Verdugo A, Gaut RL, McDonald P, Bennett AF, et al. 2012. Science 335: 457-61
2. Motter AE, Gulbahce N, Almaas E, Barabasi AL. 2008. Mol Syst Biol 4: 168
3. Schuetz R, Zamboni N, Zampieri M, Heinemann M, Sauer U. 2012. *Science* 336: 601-4
4. Fong SS, Palsson BO. 2004. *Nat Genet* 36: 1056-8
5. Díaz-García LA. 2012. *CINVESTAV Irapuato*

Functional genomics of *Bjerkandera* sp. UAMH8525: Bio-prospecting of a new glycosyl hydrolase

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Functional genomics enables the study of new sequences coding for enzymes and structural proteins that contribute to better understand microbial physiology. Moreover, the description of new biocatalysts with functionality in industrial conditions is a novelty for modern biotechnology. Limitations as *in vitro* microbial cultures, the expression of functional proteins in specific culture conditions, and methods for the detection of enzymatic activities, are disadvantages to study cultivable microorganisms. Enhancing genomic studies are a powerful tool for bio-prospecting of new metabolites. One disadvantage of this type of study is the methods of scrutiny. The massive sequencing stands out among new search methods for coding sequences and allows the annotation and characterization of new transcriptional units. *Bjerkandera* is a fungal genus that groups several Bacidiomycetes causing white rot of wood, and their enzymes have great potential for biotechnology applications. Due to this reason, this fungus is a model system for the study of new lignocellulolyticenzymesin our laboratory. A genomic library was constructed from *Bjerkandera adusta* and screening through PCR using degenerate primers for xyanases allowed us to detect a clone with a partial sequence that showed homology to glycosyl hydrolases family. The characterization demonstrated that the gene sequence was incomplete at the 5' and 3' ends. Methodologies were used as Genome Walker and 5'RACE to complete the sequence towards the 3' and 5' ends respectively. These methodologies allow progress in sequence from genomic DNA (Genome Walker) and cDNA (5 'RACE). The sequence of the gene from genomic DNA is comprised of 1500 bp. Blastx with a sequence showed the existence of domains of glycosyl hydrolases and homology with fungal glycosyl hydrolases. The best hits of the Blast analysis suggests this gene codes for a xylanase. Furthermore it was demonstrated that the gene was complete as we detected initiation and stops codons for translation. Currently, we are working on the cloning and expression of the gene in *Pichia pastoris*, for the subsequent functional characterization of the enzyme. This study demonstrates that the application of genomic tools for annotation, description and characterization of new genes is a novel and useful approach to study the physiology of poorly characterized organisms.

Horizontal gene transfer of a caleosin gene from host to pathogen in pathosystem *Phaseolus vulgaris/Colletotrichum lindemuthianum*

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Horizontal gene transfer (HGT) means transference of genetic material between different lineages leading to new biological diversity. HGT events are less abundant in eukaryotes than in prokaryotes. Their occurrence, in eukaryotes, comes often by viral infection, phagocytosis, symbiosis, or parasitism[1, 2]. The genetic transfer frequently is unidirectional, from host to the parasite or pathogen, but it is known that it also occurs in both directions. Reports of HGT between plants and fungi are really scarce. In phytopathology, HGT offers new insights of host-pathogen coevolution [2]. There are evidences that suggest HGT from plants to an ancestor of the phytopathogen fungal *Colletotrichum*, particularly the subtilisins genes a serine protease group [3]. Here we describe evidences of a genetic transference from the host plant *Phaseolus vulgaris* to the anthracnose causal fungal agent, *Colletotrichum lindemuthianum*. The genetic sequence transferred belongs to a protein family widely distributed in plants and fungi, named caleosin (involving in lipidic bodies biogenesis; pfm05042). This caleosin, previously described as calmodulin (CaM), was obtained in an incompatible interaction between *P. vulgaris* and an isolate of this same pathogen [4]. To test the hypothesis that the caleosin is derived from plants by HGT, firstly, a molecular phylogeny reconstruction of protein family (228 members) was done. Phylogenetic tree of the family is, in general, congruent with the species tree, showing a dichotomic distribution, with plant protein sequences forming a cluster (group I), and those of fungus in another (group II). However this tree shows that the caleosin from *C. lindemuthianum* share a common lineage with caleosins from plants, while the genus *Colletotrichum* caleosins (six members) share a common lineage with other fungal caleosins, which are congruent whit previously reported [4]. Afterwards we performed a genomic PCR amplification of caleosin gene in some pathogen fungi (*C. lindemuthianum, Rhizocctonia solani*, *Fusarium solani* and *Fusarium oxysporum*). We only found caleosin gene amplification in *C. lindemuthianum* isolates. TAIL-PCR reactions to accomplish the knowledge of the entire gene sequence in the fungal pathogen genome are underway.

1. D. Song, W. K. Cho, S. H. Park, Y. Jo and K. H. Kim, PLoS One **8** (5), e64270 (2013).
2. B. K. Wijayawardena, D. J. Minchella and J. A. Dewoody, Trends Parasitol **29** (7), 329-338 (2013).
3. V. D. Armijos Jaramillo, W. A. Vargas, S. A. Sukno and M. R. Thon, PLoS One **8** (3), e59078 (2013).
4. A. Alvarado-Gutiérrez, M. Del Real-Monroy, R. Rodríguez-Guerra, L. Almanza-Sánchez, E. Lozoya-Gloria and S. Fraire-Velázquez, Physiological and Molecular Plant Pathology **72** (4–6), 111-121 (2008).

Analysis of the oligomeric organization between paralogous proteins in *Saccharomyces cerevisiae*

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Introduction

A trascendental aspect related to the quaternary organization of paralogous proteins is the study of the transition from homomeric to heteromeric structures and the importance of this transition in the appearance of new functions. Such functional versatility may imply a mayor stability of the complexes or the acquisition of different kinetic properties. To determine the simultaneous existence and the biochemical properties of different isoforms of paralogous proteins is important to understand their impact in the physiology and biochemistry of *Saccharomyces cerevisiae*.

Goal

The project goal is to determine the existence *in vivo* of monomers and heteromers between paralogous proteins in *S. cerevisiae*, to establish for a specific pair its biochemical and physiological impact.

Methodology

Saccharomyces cerevisiae strains Y8205 MATα y BY4741 MATa were used in this study. The analysis of the different interactions between paralogous proteins was assessed by BiFC. This technique consists in the phusion of complementary half's of GFP to the paralogous proteins. If there is an interaction between these proteins, the GFP is reconstituted producing a fluorescence signal which can be analyzed by confocal microscopy.

Results

The pertinent constructions to determine the formation of oligomers of the following products of paralogous genes: *ENO1, ENO2, GDH1, GDH3, HXK1, HXK2, LEU4, LEU9, LYS20, LYS21, TDH1, TDH2, TDH3* were prepared. The formation of different isoforms (homo and hetero-oligomers) of these proteins was evaluated *in vivo* using confocal microscopy. We are currently studying the enzymatic and physiological role of homodimers Leu4 and Leu9 and a third isoform composed of the heterodimer Leu4/Leu9.

Aims

Determine the dynamics of associations between paralogous proteins in *S. cerevisiae* was assessed by BiFC with confocal microscopy which allows us to:

We have found that that the hetero-oligomerization between products of duplicated genes analyzed in this study occurs frequently when both paralogues are localized in the same compartment.

Compare the physiological and biochemical role of different isoforms of alpha-*isopropyl* malate synthases homodimeric-Leu4 and homodimeric-Leu9 heterodimeric-Leu4-Leu9.

Systematic identification of subtelomeric silencing pathways in *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae* and other eukaryotes, many of the genes encoded close to telomeres are transcriptionally repressed in a process called subtelomeric silencing or telomere position effect (TPE). This epigenetic process underlies diverse biological phenomena including aging, virulence of unicellular pathogens, and processes of industrial relevance in yeasts. The Sir complex plays a central role in TPE, through the action of the histone deacetylase Sir2. To date, more than 100 genes affecting Sir mediated TPE have been reported in *S. cerevisiae*. The precise function and interactions for all these genes in silencing remains largely unknown.

In order to study the TPE in a systematic and quantitative way, we developed an experimental approach using a dual reporter *URA3-GFP*. The reporter was integrated in different subtelomeric loci and assayed for loss of transcriptional repression in Sir complex mutants. As a result of this study, we identified the *COS8* locus as an alternative to evaluate TPE in a genome wide scale. To characterize the effect of the *trans* factors involved in TPE and describe the genetic interactions which determine it, we crossed the yeast nonessential knock-out collection to a strain bearing the *URA3-GFP* reporter at the *COS8* locus using the SGA method. We will present results of this genetic screen, in which TPE trans-acting factors will be screened at the genome-wide level for the first time.

Strains from the generated collection can be further analyzed by flow cytometry to quantitatively measure the reporter expression level and to identify particular effects on distribution of silencing level within cells of the same mutant population. From the results obtained from the single-deletion mutant screen, we also plan to construct paired double mutants of the silencing factors identified and use the genetic interaction network to reveal the genetic architecture of principal pathways affecting silencing.

This work represent the first high-throughput genetic approach intended to screen for silencing factors in a genome-wide scale and to describe the functional associations that determine TPE silencing in yeast.

Secondary metabolites in the fungal pathogen *Candida glabrata*

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Candida glabrata is an opportunistic fungal pathogen that in recent decades its prevalence has increased due to intensive use of azoles. *C. glabrata* is exposed to oxidative stress inside macrophages and *C. glabrata* must neutralized the reactive oxygen species in order to assure its survival. Furthermore, *C. glabrata* can multiply inside the macrophages and has been shown to inhibit the maturation of the phagolysosome.. Interestingly, the oxidative stress response of *C. glabrata in vitro* is mediated by the single catalase (Cta1), however, Cta1 is dispensable in a model of systemic infection in mice.
We have shown that stationary phase cells of *C. glabrata* are more resistant to oxidative stress than log phase cells. However, log phase cells exposed to conditioned medium (a filter sterilized supernatant from a *C. glabrata* stationary phase culture) were able to survive to oxidative stress. We identified phenyl ethanol (PE), an aromatic alcohol, as major compound in the conditioned medium of stationary phase cells. We determined that the concentration of PE in the medium increases at day 7 and depends on the nitrogen source of the culture medium. In the presence of conditioned medium or only PE, log phase cells become resistant and the *CTA1* gene is induced and the activity of the Cta1 is increased. The transcription factors, Msn2 and Msn4, control this conditioned-medium response. Furthermore, Aro8 and Aro9 are required for the production of PE. The conditioned medium of the double mutant *aro9*Δ *aro8*Δ, cannot protect log phase cells. We have identified other volatile organic compounds produced by *C. glabrata* such as isoamyl alcohol, nerodiol, and farnesol. We are currently characterizing whether these compounds could be quorum sensing molecules in *C. glabrata*.

"Functional analysis of the NADP- Dependent glutamate dehydrogenase (NADPH-*Kl*Gdh1) of *Kluyveromyces lactis*"

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Gene redundancy is a common characteristic of living organisms, which may occur for a single gene, chomosomic segment or the whole genome. Gene duplication may be a source for genetic material useful to develop new or specialized functions.The genome of the yeast *Saccharomyces cerevisiae (S. cerevisiae),* arose from a whole genome duplication event (WGD). It has been proposed, that a selective loss and retention of a particular set of genes, allowed the development of a facultative metabolism.

The paralogous genes *GDH1* y *GHD3* codify for NADP-glutamate dehydrogenases. These enzymes are organized in homo and hetero-hexameric isozymes, implicated in glutamate biosynthesis. Gdh1 and Gdh3 show 87% of identity in amino acid sequence. Previous studies in our laboratory have shown that Gdh1 and Gdh3 display different kinetic properties. Gdh1 has a higher capacity to utilize alpha-ketoglutarate than that displayed by Gdh3. *GDH1* is expressed in the presence of glucose as sole carbon source while *GDH3* expression is repressed under this condition, derepression is attained when glucose is exhausted or when ethanol is provided as sole carbon source. Additionally, we have shown that glucose repression of *GDH3* is promoter dependent, accordingly, when the endogenous promoter was changed by the constitutive TetO7 promoter, *GDH3* was expressed regardless the carbon source. Thus, expression divergence forms part of the functional diversification observed between GDH1 and GDH3.

Kluyveromyces lactis (*K. lactis*), diverged from the *Saccharomyces* lineage before the WGD event and has a unique orthologous NADP-dependent glutamate dehydrogenase (NADP-*Kl*Gdh1), this enzyme is 74% identical in amino acid sequence to the paralogous Gdh1 and Gdh3 of *S. cerevisiae.* The aim of this project is to characterize the *Kl*Gdh1 enzyme following three different approaches: i) Phenotypic analysis of the single mutant *Klgdh1* grown under different physiological conditions ii) kinetic characterization of the enzyme and, iii) analysis of the expression profile.

So far, we have shown that the klgdh1 mutant is a glutamate braditroph and lacks NADP-Gdh activity. Enzyme purification is under way.

Proteomic analysis of signaling pathways mediated by the heterotrimeric Gα protein Pga1 of *Penicillium chrysogenum*

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Introduction. Heterotrimeric Gα protein Pga1 of *Penicillium chrysogenum* controls conidiation, conidia germination and secondary metabolite production [1]. Mass spectrometry-based proteomics is a new methodology for the study of cell signaling networks [2]. The aim of this study is to determine the effect of the Heterotrimeric Gα protein Pga1 in protein expression levels.

Methods. *P. chrysogenum* Wis54-1255 and *Δpga1* (deletion of *pga1*), were grown for 6 days on Power solid medium [1]. Intracellular protein sample preparation was performed as described [3]. The first dimension (Iso Electric Focusing) for two-dimensional (2D) gel electrophoresis, was performed on a PROTEAN® IEFCell (Bio Rad) with IPG strips. The second dimension was run by SDS-PAGE. 2D images were captured by scanning stained gels using the ChemiDoc™(Bio Rad) and digitalized with Quantity One® software (Bio Rad). The analysis of protein expression were performed by PDQuest 2-D Analysis Software (Bio Rad), the protein spots of interest were manually excised from 2D gels, The proteins were digested with trypsin, and the resulting peptides were analyzed by mass spectrometry using a MALDI-TOF equipment (Waters).

Results. In Figures 1 and 2 proteins that are differentially expressed between the strains can be observed. Six proteins were analyzed and identified by mass spectrometry (table 1). Some proteins related to secondary metabolism and aminoacid metabolism are overrepresented in the Wis 54-1255 strain. The comparison of proteomes show the effect of the heterotrimeric Gα protein Pga1 in cellular metabolism.

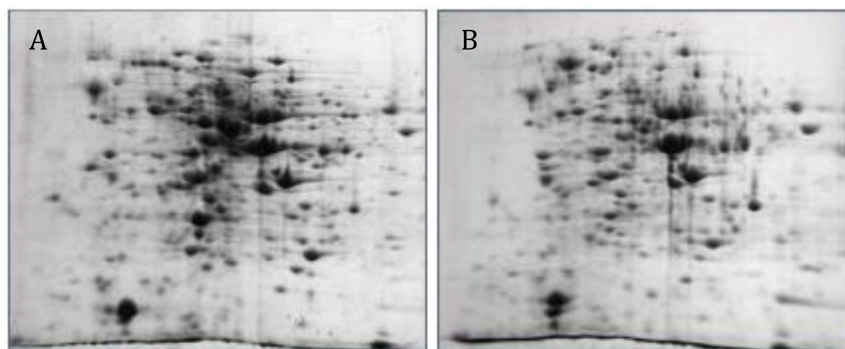

Figure 1. Comparison of the intracellular proteomes of Wis 54-1255 (A) and the *Δpga1* (B) strains.

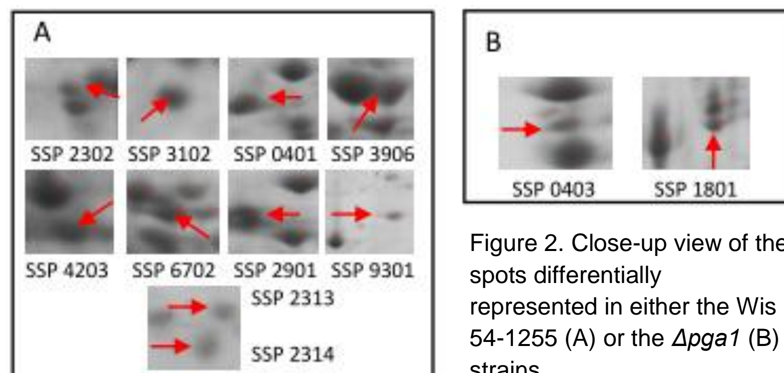

Figure 2. Close-up view of the spots differentially represented in either the Wis - 54-1255 (A) or the *Δpga1* (B) strains

Table 1. Protein identification (spots overrepresented in the Wis-54-1255 strain)

SSP number	Protein name	Function
6202	uroporphyrinogen-III synthase	Biosynthesis of secondary metabolites, porphyrin metabolism
4203	proteasome subunit α	Protein Degradation
2901	phenol 2-monooxygenase	Histidine metabolism
2314	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase	Cysteine and methionine metabolism
	N- acetyltransferase	Tyrosine metabolism
9301	NADH-dependent FMN oxydoreductase	Oxidation-reduction process

References. **[1]** García-Rico R, Fierro F, Mauriz E, Gómez M, Fernández-Bodega, Martín J. The heterotrimeric G α protein Pga1 regulates biosynthesis of penicillin, chrysogenin and roquefortine in *Penicillium chrysogenum*. 2008. Microbiology. 154: 3567-3578. **[2]** Choudhary C, Mann M. Decoding signalling networks by mass spectrometry-based proteomics. 2010. Nature Reviews/Molecular Cell Biology. 11: 427-439. **[3]** Jami M-S, Barreiros C, García-Estrada C, Martín J. 2010a. Proteome Analysis of the Penicillin Producer *Penicillium chrysogenum*. Molecular & Cellular Proteomics. 9: 1182-1198.

Morphological changes in the *Candida albicans* ultrastructure caused by the transglutaminase competitive inhibitor cystamine

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Candida albicans is an opportunistic fungal pathogen. The cell wall of this fungus is the main structure that has contact with the host and constitutes one of the major virulence factors. There are proteins that have a key role in the biology of this fungus. One of them are transglutaminases (TGases), which are involved in the formation of covalent bonds between proteins through the Nє-(γ-glutamyl)-lysine from one protein to glutamine from the other. This activity has been demonstrated in subcellular fractions of both *Saccharomyces cerevisiae* and *C. albicans*. Most of the activity has been found in cell walls, which was blocked by cystamine, a specific inhibitor of these enzymes, reducing the growth rate of several strains of *S. cerevisiae.* It is in our interest to identify proteins with transglutaminase activity in *C. albicans*. Our results corroborate that TGase activity is in greater proportion in the cell wall (74%). However, we found an activity in the cytosolic fraction (14%) that was not inhibited by cystamine, but it was increased by 36%, contrary to findings in *S. cerevisiae* and *C. albicans* by other authors. By transmission electron microscopy, we could observe remarkable changesin the ultrastructure of *C. albicans*, mainly with electron dense material accumulationin thevacuole. Theses damages were more evident at 100 mM and 200 mM of cystamine. In addition, we could observe an alteration of the normal cell division pattern of *C. albicans* yeast cells. Our results suggest that cystamine induces extensive autophagic degradation of cytosolic components in the vacuoles of *C.albicans***.**

Search S6 ribosomal protein not associated with ribosomes in nuclei of Saccharomyces cerevisiae

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The ribosomal Biogenesis is an event which begins in the nucleolus with ribosomal gene transcription generating the 35S rRNA. Subsequently forming the 90S pre-ribosomal particle composed by the 35S rRNA, snoRNA, some ribosomal proteins and non ribosomal. The pre-ribosomal subunits continue their processing and maturation along nucleoplasma to finally exported to the cytoplasm and end maturation. The mature rRNA are associated with ribosomal proteins forming 40S and 60S subunits of the ribosome. One of the proteins that form part of the 40S subunit is rpS6. rpS6 is an evolutionarily conserved protein in all eukaryotes and is found in the 40S subunit. In Saccharomyces cerevisiae, the rpS6 has two serine residues that are susceptible to phosphorylation in the C-terminal end. RpS6 phosphorylation has been associated with messengers translation increased with oligopirimidinas domain in the 5 'end. Recently reported in HEK293T cells a population of rpS6 in the cytoplasm that are not associated with ribosomes and that interacts with Hsp90 chaperone. In another work group using rat liver cells, found rpS6 associated with chromatin in a fraction of the nucleoplasm. Saccharomyces cerevisiae has been used as a study model to better understand the mechanisms of translation, ribosomal biogenesis and functions of ribosomal proteins. In our working group we are interested in identifying rpS6 in nuclear fraction of Saccharomyces cerevisiae to know if rpS6 is only associated with pre-ribosomal subunits or there rpS6 free populations.

Mycoviral presence on *Isaria fumosorosea* isolates from Mexico

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Isaria fumosorosea is an entomopathogenic fungi widely used in Mexico for whitefly biological control. This fungus produces conidia and in submerged culture, yeast-like cells or blastospores with pathogenic activity, studied to obtain a great amount of infectivity units. One of the most valuable features of an entomopatogenic strain is virulence. Several factors can affect fungal virulence, for example, mycovirus, also known as double stranded RNA elements (dsRNA). Presence of dsARN in a fungus is an unequivocal sign of micoviral infection. Many authors classify micovirus as asymptomatic infections, but in several cases can cause phenotypic alterations such as low virulence, irregular conidiation, and low enzymatic activities. Another sign of mycoviral infection is killer phenotype that has been observed in yeasts infected with mycovirus: the fungi secrete a toxic glycoprotein which inhibits killer sensitive cells of the same species or species related or not. We studied killer phenotype and dsRNA presence on fifteen isolates of *Isaria fumosorosea* used for biological control of whiteflies in Mexico. Eight of the isolates (EH-503, EH-504, EH-506, EH-510, EH-511, EH-519 and EH-520) are from the Centro Nacional de Referencia en Control Biologico (Tecomán, Colima) and six (CC1, CC2, CC3, CC4, CC5 and CC6) are commercially used for biological control. One reference isolate (EH-453) from ARSEF was used too. All isolates were maintained in H media. We tested killer phenotype of isolates on YEPG-metilene blue media; extracted total RNA and tested resilience to high concentrations of salt. All isolates tested had killer phenotype and all showed dsRNA bands. This results suggest that mycovirus are common in Isaria fumosoroseus isolates used for biological control of whiteflies in Mexico. Killer phenotype shows that mycoviral infection is not asymptomatic in the isolates tested. To our knowledge, this is the first time that mycoviral presence on Isaria fumosorosea is reported in Mexican isolates.

"*Saccharomyces cerevisiae* ALT2: A bioinformatics approach to uncover its function"

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In *Saccharomyces cerevisiae* evidence of whole genome duplication (WGD) 100-120 million years ago is present, which is coincident with the emergence of angiosperms. Thereafter a massive genome reduction happened which left around 10% of the original WGD event. Syntenic blocks scattered across the genome are the only traces left by this genomic "erosion".

After a DNA duplication event, the new sequences start to diverge and might follow three main different paths. 1) One of the copies can accumulate mutations and lost its function. 2) Each copy retains some aspects of the ancestral function, which is called subfunctionalisation. 3) One of the sequences can gain a new function, which is referred as neofunctionalisation. Therefore, functional annotation in duplicated genome is a daunting task because we can imagine all sorts of combination of the previous scenarios. Which can be misleading if we use only sequence similarity as the sole criterion for finding the function of a duplicated gene.

Alanine aminotransferase 1 and 2 (ALT1& ALT2) are paralogous genes which origin is consistent with the WGD event. Sequence analysis shows a 65% identity at the amino acid level between these genes. Furthermore, multiple-alignment reveals that 11 residues involved in the transaminase activity are conserved. Previous work in the lab has shown that ALT1 shows pyruvate transaminase activity while ALT2 lacks this property. Alanine pool measurements do not show any appreciable difference between the WT and *alt2* mutant, which contrasts with the *alt1*. Something remarkable is that both ALT1 and ALT2 are regulated by alanine. While ALT1 expression is induced in the presence of alanine. ALT2 expression is repressed in the presence of this amino acid. Therefore, the retention of ALT2 in the *Saccharomyces cerevisiae* genome remains as an open question.

Because sequenceanalysis has not yielded any cue of ALT2 function, we decided to use a systems biology approach. With this in mind we used the Synthetic Genetic Array (SGA) data from Boone lab, which contain around 5 million gene-gene interactions. We think that genes that present a similar epistatic pattern might be involved in similar process. With this in mind, Pearson correlation coefficient was used as a measured of similarity between ALT2 and other genes across the metabolism. Then genes that are are correlated or anticorrelated with ALT2 were analysed. Only those with consistent epistatic signature where kept for further analysis. Consistent means that if anticorrelated, then its genetic interaction should be positive, and if correlated then negative. Among this set of genes, ALT1 was present and showed to be anticorralted with ALT2 and consistent. When looking the interacting genes shared by ALT1 and ALT2, genes from NAD biosynthesis pathways popped up immediately. After mapping the genetic interactions onto the biochemical network and analysing our results, we want to suggest that a phenotype might be uncovered in anaerobic condition in the presence of nicotinic acid. Verification of our hypothesis is on going and the outcome might lead up to the functional annotation of this elusive gene.

Analysis of *Trichoderma atroviride* strains that express a laccase from*Pycnoporus sanguineus*

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Laccase belongs toa class of redox enzymes called multicopper oxidases. These enzymes cancatalyze the oxidation of a variety of organic and inorganic substrates and use oxygen as electron acceptor. Laccases have been isolated from higher plants, prokaryotes, insects and fungi. This enzyme has various functions, for example,in fungiit carries out a variety of physiological functionsincluding morphogenesis, fungal-plant interaction, defensemechanisms and lignin degradation.Applications of laccases in biotechnology are increasing mainly because they have a wide substrate range, for example, diphenols, monophenolsmetoxy-substituted aromatic compound and aliphatic amines. Moreover, some substratesthat cannot be oxidized directly because of steric hindrance or by having a high redox potential,can be oxidized by small laccase-radicalized mediators (for example, ABTS o veratryl alcohol). The production of laccase from native sources cannot meet the increasing market demand due to low yields orthe incompatibility of the industrial processes of fermentation standards with the conditions required for the growth of microorganism. In this work we were able to successfully express a laccase from *Pycnoporus sanguineus* in the filamentous fungus *Trichoderma atroviride*. For this purpose, a transformation vector was designed to integrate the gene of interest in an intergenic*locus* near the *blu*17 terminator region. Although monosporic selection was still necessary, stable integration at the desired locus was achieved. The native signal peptide from *P. sanguineus* laccase was successful to secrete the recombinant protein into the culture medium. The heterologously expressed laccase maintained similar properties to those observed in the native enzyme (thermostability, substrate range, pH optimum, etc). Zymograms of the native and recombinant proteins showed a slightly different mobility in the gel, probably due to differences in post-translational modification. The laccase-overexpressing *Trichoderma* strain was able of degrading bisphenol A (an endocrine disruptor) more efficiently than the wild type strain and the heterologously expressed laccase was capable of discoloration of dyes *in vitro*, showing its potential for xenobiotic compound degradation.

Endophytic fungi associated with *Taxus globosa* Schltdl. with potential anticancer taxol production. Claudia López Sánchez[1], Felipe de Jesús Palma Cruz[2] and Lucia Martínez Martínez[3]. [1]- Technologic Institute of Oaxaca Valley, [2]- Technologic Institute of Oaxaca, [3]- Faculty of Medicine and Surgery, Autonomus University Benito Juárez of Oaxaca. ExHacienda de Nazareno, Xoxocotlán, Oaxaca. Tel. 951-1684417. claudina1963@gmail.com .

103 endophytic fungi strains were isolated from *Taxus globosa* Schltdl. collected in Ixtlan, Oaxaca, which belong to 24 different genera. Of these strains, only 98 fungi were identified belonging to the following taxa generic: *Botrytis, Penicillium, Aspergillus, Alternaria, Varicospirium, Cladosporium, Fusarium, Cephalosporium, Diplosporium, Chaetomium, Cylindrocarpon, Dactylaria, Trinacrium, Brachysporium, Cercosporella, Verticilium, Nigrospora , Thielaviopsis, Humicola, Monilia, Gyrothrix, Sporonema, Gonatobotrix* and *Rhizoctonia*. For the determination of the production of taxanes (Taxol or paclitaxel precursors) prospective analyzes were performed on thin layer chromatography (TLC) using as eluent methylene chloride: methanol 9:1 v / v., which were compared with standard paclitaxel. The reference factor (Rf) indicating the presence of taxanes (Rf = 0.733), reveals the probable content of the compound (taxol) in extracts of the mycelium of eight endophytes isolated and extract medium in which cultured three strains, meaning that 44% of genera isolates are potential producing the compound in question.

Analysis of the fungal community associated with litter from two species of oak

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Fungi are the main microorganisms that degrade organic matter in forest systems, playing an important role in ecological and biogeochemical processes. Numerous biotic and abiotic factors can cause variations in fungal communities of forest ecosystems, including physical and chemical characteristics of the litter. Although fungi are key mediators in the decomposition of litter, little is known about the distribution, abundance and community structure of fungi in it. The objective of this study was to compare the litter fungal community from two different species of oak. We collected litter samples associated with *Quercus deserticola* (Qd) and with *Quercus castanea* (Qc) in monoculture conditions in a forest fragment in Cuitzeo Lake Basin, Michoacan. We obtained 158 clones of the nuclear ribosomal internal transcribed spacer (ITS) region of fungi. 84 clones were associated with Qc litter and 74 clones were associated with Qd litter. We did a BLAST search in Genebank database for the sequence analysis of the ITS region from the 158 clones. The analysis identified 84 of them (53%) in the phylum Ascomycota, 57 of them (36%) in the phylum Basidiomycota and 17 (11%) as fungi uncultivated non identified. The main order found were Agaricales (25%), Capnodiales (19%) and Pleosporales (12%) in Qc, and Corticiales (19%), Capnodiales (16%) and Thelephorales (15%) in Qd. Using the DOTUR software we determined that the number of Operational Taxonomic Units (OTU's) was 18 for Qd and 24 for Qc, with a Shannon-Weaver diversity index of 2.48 for Qd and 2.88 for Qc. The UniFrac test showed that fungal community structure is significantly different between the two studied litter (P= 0.05). This is supported by the clustering environments, that placed Qc and Qd on different branches. Our results indicate that the different types of litter studied have an effect on fungal community structure, possibly by differences in chemical quality in combination with the microenvironment.

Specific and fast molecular detection of several common species of *Candida* in human infections: *Candida albicans, Candida tropicalis* and *Candida parapsilosis*

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Introduction: Candidiasis is an infectious disease caused by different yeasts of the genus *Candida*. *Candida* is considered the main cause of opportunistic fungal infections in humans worldwide. Some species of clinical interest are: *Candida albicans,* the predomintant species; *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis* and *Candida krusei*. The frequency with which each of these species is isolated depends on the geographical area where these yeasts were detected, the particular organ in the host from which the organisms were found and the age of the infected patients. Given the high incidence and mortality of candidiasis and the antifungal resistance of some species, the specific identification of the particular species involved, is crucial. There are many methods available for clinical diagnosis; however, conventional methods are sometimes nonspecific, or have low sensitivity, and/or are time consuming. Therefore it is important to design a method that can identify the *Candida* species present in clinical samples in a specific and fast manner. This would result in an adequate and timely treatment to infected patients and thus reduce the rate of mortality of this infection.

Objective: To develop a molecular technique that allows specific and fast detection of several common species of *Candida* in human infections: *Candida albicans, Candida tropicalis* and *Candida parapsilosis.*

Results: We designed 19 pairs of primers that align to specific sequences of 3 *Candida* species: *C. albicans* (6 pairs)*, C. tropicalis* (11 pairs) and *C. parapsilosis* (2 pairs). The designed primers were tested in PCR reactions using as template genomic DNA from standard strains from each species. We selected pairs of primers that were specific for each of the three species. For each selected pair of primers we determined the optimal PCR conditions: temperature alignment, primer and deoxynucleotide triphosphate concentration. Specific primers and optimal conditions were used to diagnose clinical isolates obtained from various hospitals.

Heterologous expression in *Pichia pastoris* of transcription factors PcYap1, RsmA and AtfB of *Penicillium chrysogenum*

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INTRODUCTION. AP-1 transcription factors are bZIP (leucine zipper) DNA binding proteins that activate transcription in response to different stimuli. In *Saccharomyces cerevisiae* the AP-1-type family of transcription factors named Yap has been described [1], which bind the DNA sequence TTACTAA. AP-1-type transcription factors have also been described in filamentous fungi, such as AfYap1 from *Aspergillus fumigatus* [2] and RsmA from *Aspergillus nidulans* [3], which regulate oxidative stress response and secondary metabolism respectively. The non AP-1-type transcription factor AtfB, regulating secondary metabolism, has been described in *Aspergillus parasiticus* [4]. The aim of this work was the expression of the genes encoding the transcription factors PcYap1, AtfB and RsmA of *Penicillium chrysogenum* in *Pichia pastoris* as His-tag fusion proteins, for subsequent use in binding assays to regulatory TTACTAA-like sequences present in the penicillin biosynthetic *pcbAB* gene promoter.

METHODS. The *PcYap1, RsmA* and *AtfB* genes were amplified by PCR From genomic DNA of *P. chrysogenum*. The coding DNA sequences (CDS) of the genes were obtained by different methods: recombinant PCR for *RsmA*, RT-PCR for *PcYap1*, and conventional PCR for *AtfB*. The CDS of each gene was cloned into the expression vector pPICZ (Fig 1. A) yielding plasmids pPICZ-A/*PcYap1*, pPICZ-B/*RsmA* and pPICZ-B/*AtfB* (Fig 1. B), which were transformed into *Pichia pastoris* by electroporation. For heterologous recombinant protein production, *P. pastoris* transformants carrying each of the plasmids were cultured and selected by phleomycin, using methanol as inductor. Extraction of intracellular heterologous His-tag fusion proteins was performed after 72 hours of incubation, and purification was performed by affinity chromatography using an imidazole gradient. The eluted fractions were analyzed on polyacrylamide gels under denaturing conditions (SDS-PAGE).

RESULTS. In the case of the PcYap1 protein fused to the *c-myc* epitope and 6-histidine tail, a molecular weight of 66 kDa was expected; a band of this size was observed partially purified in the fraction corresponding to the elution with 500 mM imidazole (Fig 2). For RsmA, a fusion protein of 35 kDa was expected; a protein of this molecular weight was detected in the gels from the fraction eluted with 200 mM imidazole, and purified in the fraction eluted with 500 mM (Fig 3). In case of AtfB, a fusion protein of 38 kDa was expected, which was detected in the gel from the fraction eluted with 200 mM imidazole, and purified with 500 mM imidazole elution (Fig 4). These purified proteins will be used for EMSA (Electrophoretic Mobility Shift Assay) with regulatory sequences present in the *pcbAB* gene promoter.

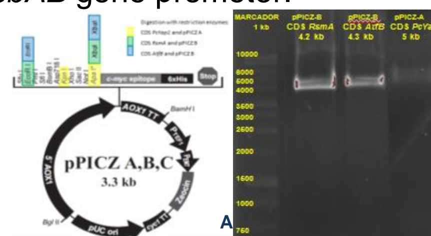

Figure 1. (A) Vector pPICZ. (B) Expression plasmids: pPICZ-A/*PcYap1*, pPICZ-B/*RsmA* and pPICZ-B/*AtfB*

Figure 2. SDS-PAGE of the imidazole gradient fractions for protein purification of PcYap1.

Figure 3. SDS-PAGE of the imidazole gradient fractions for protein purification of RsmA.

Figure 4. SDS-PAGE of the imidazole gradient fractions for protein purification of AtfB

REFERENCES. **[1]** Fernandes L, Rodrigues-Pousada C y Struhl K. 1997. Mol. Cell. Biol. 17: 6982-6993. **[2]** Lessing et al. 2007 **[3]** Yin W-B, Amaike S, Wohlbach DJ, Gasch AP, Chiang Y-M, Wang CCC, Bok JW, Rohlfs M y Keller NP. 2012. Mol. Microbiol. 83: 1024-1034. **[4]** Roze, L.V., Chanda, A., Wee, J., Awad, D., and Linz, J.E. 2011. J. Biol. Chem. 286: 35137-35148.

Analysis and characterization of the *YLR177W* gene overexpression in *Saccharomyces cerevisiae* during Agave juice fermentation.

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Tequila is defined as: "Regional alcoholic beverage distilled from must". Tequila is prepared from the heads of the *Agave Tequila Weber* Blue Variety, previously or subsequently hydrolyzed or cooked, and subjected to alcoholic fermentation with yeast, cultivated or not, and then the must is capable of being enhanced and blended together to formulate with other sugars to a ratio not higher than 49% of total reducing sugars expressed in units of mass." (NOM-006-SCFI-2005)

The Tequila elaboration process involves 1) *Agave* cultivation, 2) The Jima, 3) Baking and mashing of the agave heads, 4) Malt Fermentation by *S. cerevisiae*, 5) Distillation and finally 6) Maturation and bottling.

Although *S. cerevisiae* is traditionally ethanol-producing, it is also sensitive to higher concentrations of ethanol. Accumulation of ethanol in a medium inhibits cell growth and viability, affects different transport systems, and reduces ethanol titer in the culture. Ethanol stress affects the integrity of cell membrane and perturbs protein conformation causing protein denaturalization and dysfunction.

Nowadays, the demand for improved yeast strains has increased. One of characteristics desired in these strains is that it possesses a high ethanol tolerance, a feature that can be achieved by genetic engineering of the genes involved in ethanol tolerance in *S. cerevisiae* tequileras strains.

Madrigal Pulido (2005) analyzed by microarrays the response of *S. cerevisiae* AR5 Tequilera strain, at 7% ethanol shock during 30 min and compared with a laboratory strain (S288c). He found that 16% overexpressed genes encode for proteins not classified. One of these genes is the open reading frame, *YLR177W*.

The goals of this project were:

1) Overexpress the *YLR177W* gene in *S. cerevisiae* AR5, Tequilera satrain, characterization of the strain generated by growth kinetics at different ethanol concentrations, assess ethanol tolerance and during Agave juice fermentation.
2) Subcellular localization of gene product *YLR177W,* by GFP-tagging.

We found that *YLR177W* gene overexpression in *S. cerevisiae* increases ethanol tolerance. However, the *YLR177W* gene overexpression does not confer advantages during the normal yeast growth. The result of subcellular localization showed that the YLR177W protein has cytoplasmic localization.

PvLOX2 silencing on mycorrhiza induced resistance in common bean

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Arbuscularmycorrhizal symbiosis is an association between roots of most plants and fungi of the Glomeromycota phylum. During this symbiosis, exchange of nutrients between the plant and the fungus occurs. In addition to the nutrimental benefits, mycorrhiza colonization induces resistance (or MIR, mycorrhizal induced resistance) against necrotrophic pathogens. We have previously shown that mycorrhiza colonization of common bean with *Rhizophagusintraradices* induces MIR against the foliar pathogen *Sclerotiniasclerotiorum.*Thereare evidences suggesting that MIR requires an intact jasmonic acid signaling pathway and that mycorrhiza colonization is able to promote a priming state,however, little is known about the molecular mechanism that controls it. To gain insight on the molecular basis of systemic priming, common bean LOX2 gene (PvLOX2), which encodes for a lipoxygenase (LOX), was selected to be silenced in composite common bean plants. Six common bean lipoxygenases have been described so far, and phylogenetic analysis group PvLOX2 with 13-LOXs (subgroup 1), in contrast to PvLOX6, which encodes for a 13-LOX (subgroup 2) and is involved in the JA biosynthesis. Composite plants of common bean (Negro Jamapa variety) were obtained using a pTDTLOX2RNAi construct, or empty vector as control. Plants were then colonized with *R. intraradices,* and leaves were challenged with *S. sclerotiorum* using a detached leaf assay. Before pathogen challenging, RNA was extracted from roots and leaves of mycorrhiza colonized plants for gene expression analysis by qPCR. PvLOX2 silencing did not alter the percentage of mycorrhiza colonization nor the morphology of infection units or arbuscules; however, the onset of MIR failed. PvLOX2 silencing in roots caused down regulation of PvLOX6 in leaves, as well as its own expression. No significant changes on expression of allene oxide synthase (AOS) were observed on roots and leaves. This suggests that PvLOX2 expression in roots is required for MIR, possibly by interfering with the onset of systemic priming. Since PvLOX2 is not directly associated to JA biosynthesis, but to other oxylipins, it is possible that the root signal required for systemic priming is not JA. The dependency of PvLOX6 expression in leaves of the expression of PvLOX2 in roots suggests that a non-chloroplastic 13-C derived oxylipin can be the signal for priming to be transport from the root to the leaves. Interestingly, PvLOX2 silencing caused up regulation of COI1 gen in roots, which suggests that systemic priming induced by mycorrhiza colonization is COI1 dependent, but possibly JA-independent. (This work was supported by: CONACYT grant 102237 and scholarship for AMR; SIP-IPN grants20113493, 20120496, PIFI-IPN)

"Role of the small RNAs synthesis machinery on the antagonistic capacity of Trichoderma atroviride"

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RNA silencing is a process based on the recognition sequences specific for small RNA molecules to regulate gene expression and cellular processes highly conserved in plants, animals and fungi. In the latter our knowledge about this mechanism of regulation is limited. Therefore, in order to improve our understanding of the role of small RNAs in processes such as development and conidiation, we generated mutants in all components of the small RNAs synthesis machinery in the fungus *Trichoderma atroviride*.

Trichoderma atroviride is a soil saprophytic fungus that has numerous applications in biotechnology. One of these applications makes use of its antagonistic power for the biological control of plant pathogens. *Trichoderma spp*. display different antagonistic strategies, it is recognized as a mycoparasite, but it is also good competitor for space, using antibiotics as a barrier against other microorganisms. The antibiotics produced by *Trichoderma* are volatile and non-volatile compounds. Most *Trichoderma* antagonism studies have focused on non-volatiles compounds. Although volatile compounds have been shown to be a source of communication in plants, in fungi little is known about their function. Therefore we wonder if a highly conserved gene regulatory machinery as that mediated by RNAi (RNA interference) modulates antagonism by *Trichoderma*.

For this aim we evaluated the antagonistic capacity of a battery of mutants affected in the small RNAs synthesis machinery (*ago1, ago2, ago3, rdr1, rdr2, rdr3, dcr1, dcr2, dcr1dcr2*). The antagonistic effect of the mutants and the parental strain *T. atroviride* (IMI206040) as a control was evaluated in direct and indirect confrontations against a wide range of phytopathogenic fungi: *Rhizoctonia solani, Phytophtora capsici, Alternaria solani, Botrytis cinerea, Fusarium oxysporum* and *Sclerotium rolfsii*. We have found antagonistic differences in direct confrontation, antibiosis assays and in control by volatiles, in all mutants. To clarify these differences, we decided to study the metabolic profiles of volatile and non-volatile compounds in the mutants, independently and during the *T. atroviride*-pathogen interaction by gas chromatography coupled to mass spectrometry. Briefly, the *Δdcr2* and *Δdcr1dcr2* mutants produce high levels of important antibiosis compounds including 6 pentyl-α-pyrone, while the *Δago1, 2*, and *3*, as well as the *Δrdr1, 2, 3* mutants poorly produced these metabolites or did not produce them at all, affecting the inhibitory capacity of *T. atroviride* towards certain pathogens. Additionally, we have evaluated the ability of the mutant strains to communicate with plants in dual cultures, using marker lines carrying a reporter gene under the control of promoters (*pLox2:uidA* and *PPR1:uidA*) that are induced during the plant defense response in Arabidopsis thaliana. In these experiments, the *Δago1, 2,* and *3*, as well as the *Δrdr1, 2,* and *3* mutants failed to induce plant defense responses in *A. thaliana*, correlating, apparently, with their deficiency in the production of metabolites, indicating the importance of this regulation process in direct and indirect *Trichoderma* biocontrol capacity.

Immune sensing of *Sporothrix schenckii* and *S. brasiliensis* by human mononuclear cells

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Sporothrix schenckii and *S. brasiliensis* have been recently described as members of the *S. schenckii* complex, which includes dimorphic fungi with ability to cause sporotrichosis. The infection is generally fixed and may become disseminated in immunocompromised patients. Since the host's immune status is relevant for the disease control, the study of the fungus-immune system interaction is quite relevant to understand basic aspects of this disease. Studies using mice with chronic granulomatous disease showed that the reactive oxygen species-based killing mechanism is essential for killing of phagocytosed *S. schenckii* cells, and that TLR4 and TLR2 are immune receptors relevant for its sensing. Despite this significant progress in the *S. schenckii* immune sensing, little is known about the interaction between the fungus with human immune cells, the identity of the main pathogen-associated molecular patterns, and the relevance of the dimorphism in such interaction. Here, using peripheral blood mononuclear cells and different *S. schenckii* and *S. brasiliensis* morphotypes we show that cytokine stimulation is dependent on the presence of certain cell wall components and cell morphology. TNFα and IL-1β stimulation was significantly higher in conidia and hyphae from *S. schenckii*, and this was dependent on the presence of *O*-linked glycans on conidia surface and dectin-1-independent; whereas yeast cells from two organisms stimulated similar levels of these cytokines, but in *S. schenckii* this required recognition of both *O*-linked glycans and β1,3-glucans, while in *S. brasiliensis* TNFα production was *O*-linked glycan-independent. Hyphae stimulated poor TNFα and IL-1β levels that were *O*-linked glycan-dependent, but *O*-linked glycan independent, in *S. brasiliensis* and *S. schenckii*, respectively. Cell morphology had no impact in IL-6 production upon stimulation with *S. brasiliensis*, but in *S. schenckii*, yeast cells stimulated significantly higher IL-6 production when β1,3-glucan was unmasked. Finally, we did not found significant differences in stimulation of IL-10 by conidia or yeast cells, but hyphae from *S. brasiliensis* stimulated higher IL-10 levels than *S. schenckii* cells. Overall, these results suggest that morphology and cell wall components play significant roles during *S. schenckii* and *S. brasiliensis* sensing by innate immune cells.

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Arsenic transport and speciation in *Glomus intraradices* by molecular and synchrotron radiation spectroscopic analysis

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The protective mechanisms employed by arbuscular mycorrhizal (AM) fungi to reduce the toxic effects of arsenic on host plants remain partially unknown. AMF potentially enhance arsenate uptake while also increasing arsenate tolerance for the plant host. Mycorrhizal fungi employ the same mechanisms found in other microorganisms to reduce the toxic effects of As by relying on As exclusion. Our research group identified a gene with high similarity to a putative As efflux pump (*GiArsA*) expressed in arbuscules and the extra-radical hyphae of *Glomus intraradices*. Based upon this data and previously published results we have proposed the following As detoxification mechanism in fungi. First As(V) absorbed by the fungal hyphae symbiont is probably transformed via an As(V) reductase to produce As(III). Next, the As(III) is translocated to the extra-radical hypha and effluxed outside the plant's rhizosphere by a membrane-bound As(III) pump designated *GiArsA/B*. The reduction, translocation, and elimination of As(III) has a detoxification effect on the plant host thereby increasing the fitness of both symbionts and enhancing plant As tolerance.
The goal of the present research was to acquire more information on the possible mechanisms in AM fungi evolved in arsenate tolerance through the in situ localization and speciation of As in the AM fungus *Glomus intraradices* and mycorrhizal roots exposed to As(V). Under our proposed molecular model for *G. intraradices* about As, the present research gives additional *in situ* data on As localization and speciation supporting the molecular evidence provided by the work on *GiArsA* As efflux pump and validate our hypotheses: *G. intraradices* is able to reduce As(V) and pump it out from the extra-radical mycelium.
By means of the usage of the two-compartment *in vitro* fungal cultures of *Glomus intraradices*-transformed carrot roots and micro-XANES and XRF techniques, we observed that arsenic is absorbed in the hyphae of AM fungi and is associated mainly to Fe and Mn accumulation. Bulk XANES data showed that gradual As(V) reduction occurred in the AM hyphae between 1-3 h after arsenic exposure and was completed after 6 h. Additionally, we also corroborated that arsenic is pumped out from the extra-radical hyphae; which validated our hypotheses that *G. intraradices* directly participate on the arsenic detoxification. The possible arsenic changes in the external mycelium will be discussed.

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The 4-phosphopantetheinyl transferase of *Trichoderma virens* plays a role in activation of plant immunity through volatile organic compound emission

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Abstract

Trichoderma virens is a free-living fungus that in association with roots can induce plant growth and defense responses. This fungus is well-known for its ability to produce a wide variety of metabolites and parasitize other fungi. Here, we examined the effects of volatile organic compounds from *T. virens* in growth promotion and induction of defense responses in *Arabidopsis thaliana* seedlings using a co-cultivation system *in vitro*. Plants exposed to volatiles from *T. virens* showed 2-fold increase in fresh weight when compared to axenically-grown seedlings that correlated with increased root branching. Fungal VOCs elicited defense responses as revealed by an enhanced expression of the jasmonic acid-responsive marker *pLox2:uidA*, and accumulation of jasmonic acid and hydrogen peroxide. Gas chromatography-mass spectrometry analyses of the volatiles released by *T. virens* revealed a series of hydrocarbon terpenes, including the sesquiterpenes β-Caryophyllene, (-)-β-Elemene, Germacrene D, τ-Cadinene, α-Amorphene, and τ-Selinene and the monoterpenes β-Myrcene, *trans*-β-Ocimene, and *cis*-β-Ocimene that were absent in the 4-*phosphopantetheinyl transferase 1* (TvPPT1) mutant strain. The contribution of VOCs to plant defense and immunity was assessed by comparing the effectiveness of WT strains of *T. virens* and *ppt1* mutant in the formation of lateral roots and protection conferred against *Botrytis cinerea*. Our results indicate a specific role of fungal-produced terpenes in plant immunity through the *PPT1* gene.

Keywords: Volatile organic compounds, *Trichoderma*, *Arabidopsis*, plant defense, growth and development.

Cytokines profile during the innate and the inter-phase innate-adaptive immune response in murine pulmonary histoplasmosis induced with fungal mycelial phase propagules

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Histoplasma capsulatum (*Hc*) is a dimorphic fungal pathogen. The saprobe-geophilic multicellular morphotype (mycelial phase) of *Hc* develops at 25-28°C. Aerosolized infective propagules of the mycelial phase, mainly microconidia and small hyphal fragments, can produce a respiratory infection when inhaled by humans and other mammals. The virulent unicellular morphotype (yeast phase) of this pathogen is related to intracellular parasitism in susceptible hosts, and it can grow at 37°C in special culture media. The most common clinical manifestation of acute *Hc* infection is the pulmonary disease. Pulmonary symptoms include cough, dyspnea, and chest pain. The initial infection effectors' mechanisms (innate response) can destroy fungal pathogens and limit their infection, blocking disease development. Neutrophils have been studied due to their ability to inhibit *Hc* yeast phase growth. However, scarce data are available concerning the innate response from to mycelial phase. To address this problem, our laboratory has developed a model of pulmonary infection in BALB/c mice that mimics closely the course of self-limited infection in humans with primary pulmonary histoplasmosis. Using Luminex-Multiplex Analyst Platform, we report the detection and quantification of cytokines in lung homogenates from BALB/c mice infected with the mycelial phase of two virulent *Hc* strains, G217-B from the USA or EH-46 from Mexico. For both strains, two innate cytokines, IL-1β and TNF-α, developed the highest concentration at 6 h and 7th day post-infection, respectively, whereas the innate-chemokine MIP-2 had an early expression at 3 h post-infection, particularly in animals inoculated with G-217B strain. Regarding innate-adaptive inter-phase, peptides of IL-12 (IL-12p40 and IL-12p70) and the IL-10 cytokines were not detected after mice inoculation with G-217B strain. IL-12p40, IL-12p70, and IL-10 varied their concentrations in mice inoculated with the EH-46 strain. The innate response of the host against *Hc* depends on different PAMPs exposed on the surface of the infective morphotype (mycelial phase) that could be presented distinctly to the host recognition mechanisms. This fact could promote differences in the establishment and clinical course of the infection in susceptible hosts, supporting the high variability and plasticity of this dimorphic fungal pathogen. The experimental model used seems to be very useful to extrapolate the events in immunocompetent humans that inhale infective propagules of *Hc*.

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References. Gómez BL. Curr Fungal Infect Rep. 2011; 5:199–205. Brummer E. et al. J Infect Dis. 1991; 164:158-162. Kurita N. et al. Mycopathologia. 1991; 115:207-213. Newman SL. et al. J Clin Invest. 1993; 92:624-631. Zhou P. et al. J Immunol. 1998; 160:1359-1368.

Fungal oxidoreductases applied to bioremediation: opportunities and challenges

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Amongst the oxidoreductases studied for bioremediation applications, fungal peroxidases and laccases stand out. These enzymes are naturally efficient biocatalysts for environmental applications due to their catalytic versatility, broad substrate specificity and certain stability. Because these enzymes are able to catalyze the oxidation of compounds that are very different in terms of their chemical identity, it is possible to use them to transform complex mixtures of pollutant compounds into less toxic and less recalcitrant, more biodegradable compounds. For example, crude oil asphaltenes are a complex mixture of thousands of molecules that are of recalcitrant nature. Nevertheless, a single enzyme, chloroperoxidase from *Caldariomyces fumago*, is able to catalyze the oxidation of the asphaltenic fraction of several heavy oils. According to thermogravimetric studies, this enzyme-catalyzed oxidation translates into a higher reactivity of treated asphaltenes, thus reducing its recalcitrant character. There are challenges for the use of fungal oxidoreductases in the environmental or other industrial fields: reducing the inactivation due to the catalytic cycle (i.e. instability due to oxidative intermediates and/or free radicals generated during the reaction); improving activity and stability in non conventional reaction media (i.e. organic solvents); and developing and efficient production system (i.e. heterologous expression). Some of the strategies and experimental and theoretical tools utilized to meet some of these challenges will be analyzed.

An acidic MES buffered media prevents alkaline hydrolysis of cephalosporin C in *Acremonium chrysogenum pacC^{C}* fermentation cultures

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Introduction: *Acremonium chrysogenum* is known as the only producer of cephalosporin C (CPC), a broad spectrum β-lactamic antibiotic highly effective against gram-positive and gram-negative microorganisms (1). CPC is mainly obtained by liquid or solid-state fermentation using complex media. However, it has been observed in many cases that when the pH in these cultures reaches values close to 8.0, CPC production decreases significantly, affecting directly the productivity despite that under normal conditions the β-lactamic production is positively regulated by neutral to alkaline pH conditions (2). Recently, we have developed a *pacC^{C}* type *A. chrysogenum* strain, which shows an alkaline mimicking phenotype capable of producing β-lactamic antibiotics even under acidic conditions (3).

Objective: In this work, we tested different buffering systems, pH values and concentrations, so that growth would not be seriously affected while the pH remained constant, leading to β-lactamics production in this system by *A. chrysogenum pacC^{C}*.

Methods: We used triple-baffled 500-mL flasks containing 100 mL of complex or synthetic media buffered with either acetate or citrate or MES (2-(N-morpholino) ethanesulfonic acid) buffer at pH 4.0, 5.0, and 6.0 for acetate and citrate buffers and 5.0 for MES; inoculated with *A. chrysogenum* wild type. Fermentation was carried out during 144 h at 25 ºC and 250 rpm. Biomass, pH, and β-lactamics production was measured every 24 h. Then, 0 – 0.5 M concentrations of the selected buffer were tested on complex media fermentation and, finally, a last fermentation with *A. chrysogenum pacC^{C}*, including WT strain, was performed using the concentration and selected buffer.

Results: Acetate buffers at pH 4.0 and 5.0 and citrate buffer at 4.0 were not effective for growth. Although citrate buffer at pH 5.0 and 6.0 and acetate buffer at pH 6.0 allowed good growth, they did not keep the pH value constant along the fermentation time. The most effective system was MES buffer at pH 5.0. Hence, 0.3 to 0.5 M MES concentrations had a positive effect on growth as well as on pH maintenance along the whole fermentation process. MES at 0.3 M was selected as buffer system because it allowed good growth, besides it kept the pH constant and the production time was increased during fermentation. Finally, when *A. chrysogenum pacC^{C}* was tested in 0.3 M MES buffered complex media under the same fermentation conditions, we were able to distinguish the main effects of our *pacC^{C}* transformants, among them we found at least three strains with until 10-fold higher β-lactamics production than the wild type strain, even when using the routine complex media previously reported (4).

References: **1.** Schmitt EK, Hoff B, Kück U. (2004). *Adv. Biochem. Engin/Biotechnol.* (88):1-43. **2.** Velasco J, Gutiérrez S, Casqueiro J, Fierro F, Campoy S., Martín JF. (2001). *Appl. Microbiol Biotechnol.* (57):360-356.
3. López-Calleja. (2010). Master Thesis in Biotechnology. Universidad Autónoma Metropolitana – Iztapalapa. México. **4.** Caltrider PG, Niss HF. (1966). *Appl Microbiol.* (14):746–753.

High-Level Expression of Manganese Peroxidase, Lignin Peroxidase, and Versatile Peroxidase in Ligninolytic Fungus *Phanerochaete chrysosporium*

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Lignin is an insoluble and heterogeneous aromatic polymer, very abundant in nature and highly recalcitrant to degradation. Only white rot fungi are able to mineralize this compound by employing a complex enzymatic machinery. Four types of extracellular ligninolytic enzymes have been identified and characterized in different species of fungi: manganese peroxidase (MnP), lignin peroxidase (LiP), versatile peroxidase (VP), and laccase.
In recent years there has been an increasing interest in these enzymes due to several reasons: they can be utilized as a pretreatment strategy for lignin elimination in the production of biofuels, are capable of oxidizing a wide range of environmental pollutants and are effective in biobleaching of pulp paper.
The white rot fungus *Phanerochaete chrysosporium* is a valuable species in lignin degradation since it produces several LiP and MnP isoenzymes, although no VP, that is generally thought important for ligninolysis. VP has been described in several species of the genera *Pleurotus* and *Bjerkandera*. This enzyme oxidizes both phenolic and non-phenolic aromatic compounds, which cannot be oxidized directly by peroxidases from *P. chrysosporium* due to enzyme inactivation or lack of mediators (such as Mn^{2+}) necessary to complete the process. Availability of these peroxidases has been limited due to the fungal slow growth and low productivity of the enzymes. Also, lack of an efficient genetic transformation method for expression of multiple enzymes into a single fungal strain has been an obstacle.
Expression of recombinant peroxidases in *P. chrysosporium* is highly desirable, because this system may provide adequate posttranslational modifications and the recombinant enzymes may act in synergy with endogenous enzymes.
In this work we have inserted the genes encoding endogenous MnP and LiP, and the heterologous gene VP under the control of the *gpd* constitutive promoter in *P. chrysosporium*. For this purpose we employed a new and highly efficient transformation method based in the use of shock waves. This novel method of transformation has recently been developed by our group. The expression of recombinant proteins was verified by PCR, Southern blot and RT-PCR. The extracellular secretion and overexpression of peroxidases were improved up to four-fold as compared with wild type species. Assays of enzymatic activity showed substrate degradation several times higher in the early stages of liquid culture than in non-transformed strains. The specific activity of VP was comparable to that produced by *Pleurotus eryngii*. A *P. chrysosporium* strain expressing these peroxidases may have several uses in various industrial processes.

GENE CLONING AND EXPRESSION OF A FUNGUS CARBOHYDRATE ESTERASE OF *Bjerkandera adusta* in *Pichia pastoris*, AND EVALUATION OF ITS EFFECT ON LIGNOCELLULOSIC MATERIAL DEGRADATION

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The use of lignocellulosic material as raw material in various industrial processes, has received much interest for several decades, because it is considered the most abundant agro-industrial by-product, is a renewable raw material source, and its three main components (cellulose, hemicellulose and lignin) are important practical applications. Among the enzymes that degrade these polymers, which are produced by different organisms, including basidiomycetes, the carbohydrate esterase (CE) are required for complete degradation of plant material. Hemicellulose in particular shows modifications such as the presence of acetyl groups that may be eliminated by the carbohydrate esterases. In particular, las CE 4-family catalyze the N or O-deacetylation of acetylated substrates as xylan, chitin and peptidoglycan. Performing an analysis of sequences from a cDNA library of *B. adusta* grown in the presence of Maya crude oil, a sequence of interest, with similarity to EC was found. Knowing the roles of these proteins, it will be interesting to express the carbohydrate esterase gene of *B. adusta* in a heterologous system such as *Pichia pastoris*, allowing us to characterize biochemically, and evaluate their efficiency in the treatment of lignocellulosic material. Justification: The use of fungal proteins such as biological pretreatment methods of lignocellulosic material, represent a potential tool for use in the production of biofuels and other chemicals of industrial interest from agricultural residues. Objective: Express a carbohydrate esterase of *B. adusta* fungus in *P. pastoris*, and evaluating its effect on the degradation of lignocellulosic material. Methodology: To predict the full size gene Northern blot was performed, and genomic and cDNA sequences were obtained using the kit from Clontech and GenomeWalkerTM FirstChoice ® RLM-RACE, respectively. The cDNA was cloned into the vector pPICZ-αA, for expression in the X-33 strain of *P. pastoris* and the activity of transformant strains was analyzed using culture supernatant and 2-naphthyl acetate as substrate. Results: The complete gene for carbohydrate esterase has an ORF of 470 aa and high identity (> 60%) with carbohydrate esterase (CE) family 4. The protein was partially characterized from supernatant with a temperature and a pH optimum of 28°C and 6.0 respectively, and the substrate specificity was better for the 2-naphthyl acetate and 4-ethyl-PN as compared to the other substrates tested. Acetic acid release was measured from natural substrates as bagasse and pastures, confirming the activity of deacetylase, results correlate with the possible function of the protein predicted in silico analysis.

Esterase prospecting for pesticide degradation through the screening of a DNA library from activated sludge

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The microbial esterases (EC3.1.1.1) show considerable potential due to their regiospecifity and enantioselectivity. They have shown to be effective in the degradation of organophosphorus pesticides by attacking the ester linkage that binds functional groups to phosphoric acid structure, making its structural conformation less stable. In fact several genes encoding esterases have been identified in metagenomic libraries prepared from several environmental samples. Esterases could be detected from activated sludge metagenomes and expressed in heterologous systems; genes encoding enzymes potentially active against organophosphate pesticides could make possible the obtaining of efficient biocatalysts that allow a viable use for bioremediation processes. The aim of this study was the analysis and screening of a metagenomic DNA library to search for enzymes with esterase activity. To explore the genetic resources activated sludge samples were collected in the sludge digestor of a mexican wastewater treatment plant. DNA was extracted and then nebulized in nitrogen flow. *Escherichia coli* DH5α was transformed using fragments of 3 to 10 kb ligated in pJet vector. Screening library for esterase positive clones was conducted by identifying clear zone formation around bacterial colonies on the tributyrin agar plates. 1.4 mg of high molecular weight DNA was isolated from a sample of 10 g of sludge. The library size was 18,000 clones. The total length of the library was estimated at 87.7 Mb. Restriction analysis showed that clones had different *Xho* I restriction patterns indicating that the library consists of random DNA clones. One positive clone was selected and showed an 2.2 kb insert. Sequence analysis revealed a putative gene showing a hot-dog type conserved domain, described in the 4-hydroxibenzoil-CoA thioesterase from *Pseudomonas* related to hydrolysis of thioesters, phenylacetic acid degradation and 4-chlorobenzoate. The open reading frame of interest showed 64 percent homology with a *Mycobacterium* thioesterase, indicating it may be a new role in protein degradation of organic contaminants. Phylogenies analysis and structural modeling of protein sequence infer evolutionary relationships with thioesterases family 11. Our perspectives include the gene expression in yeast heterologous system like *Pichia pastoris*. This project is supported by the National Council for Science and Technology (CONACyT) through grant 153789-Q.
Key Words: Esterase, activated sludge, metagenomic.

Antifungal activity of silver nanoparticles against *Candida albicans*

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Infectious diseases represent one of the major health problems worldwide. There are several difficulties associated with the treatment of these diseases, such as unwanted side effects associated with antibiotics, slow development of new substances and treatments as well as the evolution of resistant strains among others. Therefore, it is necessary to find new ways to combat infectious diseases applying the latest technologies. Silver has long been recognized for its antimicrobial effects and recently, silver nanoparticles (AgNPs) have been reported to possess antifungal properties. In this work we evaluate the effect of AgNPs against *Candida albicans*, the main agent causing candidiasis. To understand the mechanism how AgNPs interact with the living cell and how to improve their use in the clinic, we determined the minimum inhibitory concentration (MIC) and the ultrastructural distribution of AgNPs in *C. albicans* cells. Results show high antifungal activity at very low concentrations (MIC= 150 µg/mL; LD_{50}= 60 µg/mL), lower than those of common antifungal agents. It was found that AgNPs applied to inhibit *Candida*, accumulate outside the cell but also smaller NPs localize throughout the cytoplasm. Energy dispersive spectroscopy (EDS) analysis confirms the presence of silver inside the cells. Due to the obtained results it is assumed that the AgNPs used in this study do not penetrate the cell, but possibly free silver ions infiltrate into the cell and AgNPs are formed through reduction by soluble proteins present in the cytoplasm as no relationship was found to the cell membrane or any membranous organelle.

Posters

Establishment of *Fusarium solani* biofilm and differential protein expression analysis in two differentials states of growth

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Introduction. Opportunistic fungal infections became common in the 1990s. One of the most common fungus that causes infections is *Fusarium* spp. (Dignani and Anaissie, 2004). This mould, is a genus hyaline, saprophytic and considered the second cause of human hyalohyphomycosis (Alastruey-Izquierdo *et al*., 2008; Loussert *et al*., 2009; Daher *et al*., 2011). Biofilm formation is one adaptation that allowed fungus pathogenicity in humans. Fungal biofilm is an exopolymeric matrix and highly organized structure that protects fungus for environmental conditions (Branda *et al*., 2005). Virulence factors have been described in *Aspergillus* spp. For example, transcriptional regulators of proteins synthesis, cell membrane proteins, and adhesins efflux pumps. Those are overexpressed in *Aspergillus* spp. biofilm (Villena *et al*., 2010; Fanning and Mitchell, 2012). Filamentous fungi developed adaptive strategies to colonize different environments. Which require differential genes and proteins expression involved in biofilm formation compared to agitation growth. Proteins expressed are probably related to virulence factors. In this paper we established the *Fusarium solani* biofilm and analyzed fungus proteomic expression in biofilm condition and agitation growth.
Methods. Genomic DNA was obtained from two isolates. The ITS region and elongation factor α (EFα) were amplified by PCR. The sequences obtained were analyzed by Blast (NCBI). Biofilm formation standardization was carried in 96-well plates at 4, 8, 12, 24 and 48h at concentrations of $1x10^4$, $1x10^5$ and $1x10^6$ conidia/mL at 28°C and 37°C. Optical microscopy was made also at the same times and concentrations described. Fluorescence microscopy was performed in both isolated used propidium iodide and calcofluor white at 4, 8, 12, 24 and 48h at a concentration of $1x10^5$ conidia/mL. Proteomic analysis was made on two isolates, grown in agitation and biofilm conditions. This analysis consisted in total proteins extraction, quantitation proteins, isoelectrofocusing and 2D gels.
Results. Both isolates were identified as *F. solani*. Isolates showed 100% percentage of similarity with the specie. The optimum conditions for biofilm formation for both isolates grown in 96-well plates was observed at a concentration of $1x10^5$ conidia/ml, 48h at 28°C. Micrographs were obtained from optical and fluorescence microscopy showed biofilm formation in both culture. Proteomic profile was made from the two isolates grown in biofilm condition and in agitation. Analysis showed differences in expression of certain proteins or their isoforms.
Conclusions. Isolates were identified as *F. solani*. Biofilm formation was standardized. Optic and fluorescence microscopy showed biofilm growth of both isolates. Proteomic analysis showed significant differences between both isolates in the two different growths.

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Proteolitic activity comparision among entomopathogenic fungi *Isaria fumosoreosea* isolates from Mexico

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The population regulation potential of entomopathogenic fungi depends on virulence. During fungal penetration, cuticular components must be hydrolyzed in order to allow the fungal infection. Extracellular proteases are one of the most important virulence factors due protein is the main component of insect cuticle. We studied extracellular protease production of fifteen isolates of *Isaria fumosorosea* used for biological control of whiteflies in Mexico. Eight of the isolates (EH-503, EH-504, EH-506, EH-510, EH-511, EH-519 and EH-520) are from the Centro Nacional de Referencia en Control Biologico (Tecomán, Colima) and six (CC1, CC2, CC3, CC4, CC5 and CC6) are commercially used for biological control. One reference isolate (EH-453) from ARSEF was used too. All isolates were maintained in H media. For enzymatic assays skim milk with phosphate buffer media was used. For enzymatic production in solid media, enzymatic halo diameter was measured. Submerged culture protease activity was measured by azocasein method. One unit of protease activity (PU) was defined as the amount of enzyme that produced a change of 0.010 in optical density at 440 nm.

Protease halos at 24 h were more evident on solid media. EH-504, EH-511 and EH-509 had the largest halos (33.1 mm, 28.3 mm and 24.5 mm respectively); the isolates EH-520 and CC5 showed a very low enzyme production halos (18.6 mm and 17.9 mm respectively) when compared with the rest of isolates.

Submerged culture allowed measure qualitatively extracellular activity of unspecific proteases. Activity had a peak at 72 hours of incubation; according to their activity, isolates were classified in four groups: high, medium, low and minimal protease production. Isolates with high activity were EH-509 (49.87 PU/mL), CC6 (43.36 PU/mL) and CC2 (31.25 PU/mL). Medium activity isolates were CC3 (28.17 PU/mL), EH-511 (25.95 PU/mL) and EH-504 (23.20 PU/mL). Low activity isolates were CC1 (16.46 PU/mL), EH-519 (12.43 PU/mL), EH-506 (10.96 PU/mL), EH-510 (10.95 PU/mL), CC5 (10.62 PU/mL) and EH-520 (10.22 PU/mL). Minimal activity isolates were CC6 (6.16 PU/mL), EH-453 (6.03 PU/mL) and EH-503 (3.19 PU/mL). Results shows that skim milk media is an effective media to produce extracellular proteases and to evaluate isolates according to their enzyme production, which makes them suitable to an adequate biological control of whiteflies in Mexico.

Analysis of *GDH3* glucose-repression in *Saccharomyces cerevisiae*

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Gene duplication is a key evolutionary mechanism providing material for the generation of genes with new or modified functions. The fate of duplicated gene copies has been discussed and several models have been put forward to account for duplicate conservation. It is accepted that *Saccharomyces cerevisiae* genome arose from complete duplication of 8 ancestral chromosomes; functionally normal ploidy was recovered because of the massive loss of 90% of duplicated genes. It has been suggested that the selective retention of some paralogous genes promoted the acquisition of the facultative metabolism.

Previous studies from our laboratory have shown that the NADP-dependent glutamate dehydrogenases encoded by the paralogous genes *GDH1* and *GDH3*, have been maintained because of the differential expression profile of the genes and the biochemical specialization of the enzymes. *GDH3* expression is repressed under fermentative conditions (glucose-grown cultures), in contrast to *GDH1*, and derepressed under respiratory conditions (ethanol- or carbon limited-grown cultures). Additionally, it was demonstrated that *GDH3* de-repression is accompanied by chromatin remodeling. However a detailed analysis of the transcription factors required for glucose-repression of *GDH3* has not been addressed yet.

On the other hand, biochemical characterization of Gdh1 and Gdh3 revealed that these isoenzymes differ in their allosteric properties and rates of use of α-ketoglutarate, being Gdh1 the enzyme with the highest affinity for this substrate. It has been suggested that this specialization (different kinetic properties of the enzymes and expression profile of the genes) has resulted in an improved glutamate biosynthesis and α-ketoglutarate utilization rate without compromising the energy yielding role of the tricarboxylic acid cycle under respiratory conditions. Conversely, under fermentative growth conditions, Gdh1 could be able to use α-ketoglutarate at a faster rate in order to achieved a higher glutamate biosynthesis and promote a higher growth rate.

The aim of this project is to i) evaluate the NADP-dependent glutamate dehydrogenase activity in mutants of a set of transcription factors that could mediate *GDH3* glucose repression, and ii) to analyze the fitness impact of the over-expression of *GDH3* under fermentative conditions.

Cloning of *cat-1* and expression in *E. coli*

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H_2O_2 is toxic to cells because it can give rise to singlet oxygen and the hydroxyl radical. In addition, H_2O_2 is involved in signal transduction, cell growth (proliferation), cell differentiation and apoptosis. Catalase dismutates H_2O_2 into dioxygen and two water molecules. *Neurospora crassa* has three monofunctional heme catalases, two large-size subunit catalases (CAT-1 and CAT-3) and one small-size subunit catalase (CAT-4). CAT-1 is associated with non-growing cells and is accumulated in asexual spores (conidia) [1].

A long channel leads to the active site of the enzyme [2]. In the *Saccharomyces cerevisiae* catalase A, different substitutions of substrate channel residues affect enzyme activity [3]. Residues of the main channel are critical for H_2O_2 selection in water [4].

We have cloned the *cat-1* gene to study the relationship between the main channel amino acid residues and enzyme activity. mRNA was extracted from conidia, cDNA synthesized, and the isolated *cat-1* gene was cloned in the pQE70 vector to tag the C-terminal domain with 6x-His. After heterologous expression in *E. coli* (M15/pREP4) strain, CAT-1 was purified by Ni-affinity chromatography. Purified CAT-1 was separated by PAGE-SDS under native conditions and CAT-1 in-gel activity was detected. We are currently improving expression of the gene in *E. coli*. Then we will do site-directed mutagenesis of critical amino acid residues in the substrate channel to determine their roll in selecting H_2O_2 in water [4].

1) Michán, Lledías, Baldwin, Natvig, Hansberg, (2002) Regulation and oxidation of two large monofunctional catalases, Free Radic, Biol. Med 33:521–532
2) Díaz, Loewen, Fita, Carpena, (2012) Thirty years of heme catalases structural biology. Archives of Biochemistry and Biophysics 525: 102-110
3) Zámocky and Koller (1999) Understanding the structure and function of catalases: clues from molecular evolution and in vitro mutagenesis. Progress in Biophysics and Molecular Biology 72:19-66
4) Domínguez, Sosa-Peinado and Hansberg. How catalase recognizes H_2O_2 in a sea of water. Proteins: Structure, Function and Bioinformatics (in press).

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Study Molecular and Biochemical characterisation of gene ICL from S. *schenkii*

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Glyoxylate route is widely distributed among prokaryotes, lower eukaryotes and plants. Apparently is absent in vertebrate. This route along with the oxidation is essential for maximum energy production during the growth cell in carbon sources such as fatty acids.

In pathogenic organisms such as Mycobacterium tuberculosis and Candida albicans has been shown that when infected macrophages activates the genes of this way and the gene coding for isocitrate lyase (ICL) is required for virulence. Therefore, it is proposed that agents that inhibit the expression of this gene or its product could function as new therapeutic alternatives.

The objective of this work is determine the role of ICL gene in the physiology of Sporothrix schenkii using biochemical and molecular tools. For that reason, will be isolate and sequence the ICL gene coding region, and determine the number of copies present in the genome of the fungus.

The cloned sequence will be overexpressed in a system of heterologous to biochemically characterize the enzyme isocitrate lyase. On the other hand, will be determined gene expression during growth on carbon sources and during infection of macrophages, in order to obtain information about the conditions which favor the expression and repression, as well as, if this gene is activated during the stay of the fungus within phagolysosomes. Finally, by gene disruption, will be generated mutants to eval the contribution of glyoxylate path to retention within macrophages and will be determined in a model of murine sporotrichosis, if the mutant strains have a modification in its virulence. This will be a significant advance in the search for new virulence factors from S. schenkii, as well as identifying new therapeutic targets.

Functional Divergence of Genes Implicated in the Leucine Biosynthesys in *Sacharomyces cerevisiae* and the Ancestor Type Yeast *Kluyveromyces lactis*

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Gene duplication is an important force of evolutionary innovation, allowing the acquisition of a new function or the improvement of ancient ones. The paralogous pair of genes *LEU4* and *LEU9* present in *Saccharomyces cerevisiae* (*S. cerevisiae*) encode two alpha isopropyl malate synthases which catalyze the formation of α-isopropilmalate from α-ketoisovalerate which is the first step in leucine biosynthesis, both genes were originated from the event of whole genome duplication (WGD) of the ancestor of *S. cerevisiae* this event had place approximately 100 million years ago. *K. lactis* is a yeast related with S. cerevisiae which derived his lineage before the event of WGD, nevertheless *K. lactis* also has two genes *KlLEU4* and *KlLEU4bis* which codify two α-isopropyl malate synthase isozymes. Synteny analysis of *K. lactis* and *S. cerevisiae* genome suggests that the duplication of the ancestor type *KlLEU4bis* originated *LEU4* and *LEU9* in *S. cerevisiae*. The presence of the gene *KlLEU4* on *K. lactis* that doesn´t have such a direct relationship with the genes in *S. cerevisiae* offers a new perspective to analyze the physiological role of paralogous pairs in *S. cerevisiae* and in the ancestral type yeast *K. lactis*, which has not been previously analyzed.

This work has the objective of determinate the reciprocal complementation between the genes of *K. lactis* and *S. cerevisiae*, this study will provide a new point of view of the fate of paralogous genes.

The analysis of the mutants *Klleu4Δ* and *Klleu4bisΔ* shows a decrease of the α-isopropyl malate activity in crude extracts; This phenotype is similar to that found for both *leu4Δ* and *leu9Δ*. The *Klleu4Δ* mutant shows a reduced growth rate on ethanol as compared with glucose.. The mutant Leu4Δ shows a minor growth rate in ethanol VS glucoseas the only source of carbon, nevertheless the mutant Leu4Δ also shows a diminution when grow in glucose as the only source of carbon. The mutants Klleu4bisΔ and Leu9Δ have similar growth rate on glucose or ethanol. The double mutant S. cerevisiae Leu4ΔLeuΔ is leucine auxotroph.

"Studies on functional divergence between *Saccharomyces cerevisiae ALT1* and *ALT2* using *Kluyveromyces lactis KlALT1* and *Lachancea Kluyveri* SkALT1 as ancestral type"

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Gene duplication has a relevant role in evolution, since diversification of paralogous genes allowd the emergence of new or specialized functions from the preexisting ones. There are different models for the possible destiny of duplicated genes: 1) lost of function, 2) neofunctionalization, 3) subfunctionalization and 4) gene dosage.

Saccharomyces cerevisiae experimented a whole genome duplication (WGD) about 100 millon years ago, but only 10% of the paralogues have survived untill today, thus, these yeast is considered to be a degenerate tetraploid. Comparisons between *Saccharomyces* and *Kluyveromyces* linages suggest that the last linage diverged after whole genome duplication of *Saccharomyces*. It is thus possible to consider that Kluyveroyces physiology its more similar to the ancestor which did not underwent duplication and gave rise to the lactis lineage, The ancestor probably was an obligate aerobe, crabtree negative and incapable of generating petit mitochondrial mutants. For these reasons *Kluyveromyces lactis* and *Lachancea kluyveri* are considered as ancestral type yeasts.

ALT1 and *ALT2* are two paralogous genes present in *S. cerevisiae* genome, which are localized in one of the duplicated blocks which were generated as a result of the above described WGD The phenotypic characterization of knock out *alt1Δ* and *alt2Δ* mutants and biochemical analysis of the encoded gene products has established that *Alt1* is an alanine aminotransferase, responsible for the biosynthesis and catabolism of alanine. Previous results from our laboratory indicate that Alt2 plays no role in alanine catabolism and could have a marginal role in its biosynthesis. Since a double *alt1Δ alt2Δ* mutant is not an alanine auxotroph it can be concluded that there exists a yet unidentified alternative pathway for the alanine biosynthesis.

K. lactis and *L. kluyveri* genome shows there is only one *ALT1-ALT2* ortholog, identified by sequence homology and sinteny: *KlALT1* and *LkALT1* respectively. Through the characterization of the null mutant of the orthologous genes, we discovered that *KlALT1* and *SkALT1* are the principal pathways of alanine biosynthesis and catabolism, in each one of these yeasts, although in both cases an alternative alanine biosynthetic and catabolic pathway is present, although it has not been identified yet.

The specific activity of SkAlt1 its similar to that of Alt1 and they both show a 5 and 7-fold increased activity respectively when extracts are prepared from alanine-grown cultures. In the case of *Alt1*, increased activity corresponds to increased transcription. We have not been able to measure KlAlt1 specific activity.

The characterization of *KlALT1* and *SkALT1* and their encoded products will allow the proposition of a model for the evolutionary origin of *ALT1* and *ALT2*.

Ustilago maydis as model of Ustilaginales to evaluate antifungal effects with chitosan and derivatives

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INTRODUCTION.
Ustilaginales family is known and classified as smunt fungi. They are serious plant pathogen with the dikaryotic stage as obligated parasite, which need the plant for development. *Ustilago maydis* is a dimorphic fungus that belongs to this family, with yeast like non pathogenic and other filamentous pathogenic form that induces tumors formation in corn. Its complete genome has been elucidated, and is easy to handle at lab. Because the importance of phytopathogenic infection for this family, new alternatives to prevent fungal development are needed. The use of chitosan, a natural polymer derived from chitin, which contain glucosamine units, as been tested as antifungal for some filamentous fungi. Two derivatives, oligochitosan and glycolchitosan have been suggested as better replacements of the original molecule. However, evidences of the proper mechanism of action by these molecules have not been fully elucidated. In the present investigation we compare the effects and mode of action of these molecules on *Ustilago maydis* as model of ustilaginal.

MATERIAL AND METHODS.
U. maydis commercial strain FB2 ATCC201384 was grown in minimal medium in the presence of chitosan (CH), oligochitosan (OCH) and glycolchitosan (GCH), at a concentration of 1mgmL^{-1}. Cell growth was evaluated at different times by optical density. To determine changes in cell morphology TEM and SEM was used. Assessment of cellular respiration and potassium efflux was by a specific electrode for O_2 and K^+ respectively. H^{+-}ATPase activity was measured by spectrophotometric methods. Membrane potential was determinate by fluorimetric methods. SDS-PAGE was used to evaluate changes in the membrane fraction proteins.

RESULTS.
Cells grown in the presence of chitosan showed a marked inhibition compared to the other two molecules. SEM and TEM indicated effect on cellular membrane and wall. Chitosan affected principally wall and membrane. Oligochitosan caused cytoplasm disorder and glycolchitosan accumulated glycogen and caused increase in fungal size.
The K^+ efflux was increased by the presence of chitosan, as compared to the other two molecules. H^+-ATPase activity was reduced in 50% for chitosan and oligochitosan. Changes in external pH during cultivation with the polymers indicated that Oligochitosan acidified the external media more than chitosan and glycolchitosan. Membrane potential was affected by chitosan but not by oligochitosan and glycolchitosan. Oxygen consumption was increased three-fold over the control cells by Oligochitosan and reduced by glycolchitosan. Membrane proteins pattern analyzed by SDS-PAGE, was different for each treatment in terms of expression as response to the antifungal activity.

CONCLUSIONS.
U maydis response to antifungal treatments indicates that the impact on the plasma membrane is different. Greater affectation on the membrane was caused by chitosan. Oligochitosan and glycolchitosan might be internalized and produce the different response observed**.**

PURIFICATION OF HETERO-OLIGOMERS OF THE ISOZYMES GDH1, GDH3 OF *S. CEREVISIAE* AND GDH1 OF *S. KLUYVERI*

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Organization of hetero-oligomeric isoenzymes (HISO) may function as a strategy which promotes adaptation to facultative metabolism this is only possible if the different monomers of the isozymes have similar interaction surfaces. In this work, we induced the formation of hetero-oligomers "*in vitro*" between glutamate dehydrogenases of *S. cervisiae* **(**Gdh1p, Gdh3p) and that of *S. kluyveri* (KGdh1p) based in a novel serial steps of denaturalization and renaturalization process.
The HISO are enzymes composed of functional units (monomers, dimers, etc.) encoded by paralogous genes. Glutamate dehydrogenases (GDHs) are enzymes that catalyze the irreversible NAD(P) dependent oxidative deamination of glutamate to 2-oxoglutarate and ammonium, generating NAD(P)H simultaneously. *S. cerevisiae* is the first microorganism described in which the NADP-GDH activity is encoded by two different genes generating two hexameric isozymes Gdh1p, Gdh3p. When *S. cervisiae* is grown on glucose and ammonium, Gdh1p is expressed, and when it is grown in ethanol ammonium, both, Gdh1p and Gdh3p are synthesized. Gdh1p and Gdh3p have 87% amino acid identity indicating that they could have similar interface that support the formation of HISO displaying different kinetic properties and biological roles.
The evolution of the interfaces could be the key to understand the transition from an homo-oligomeric interface to an hetero-oligomeric interface, in order to analyze the possibility of hetero-oligomeric organization, we studied the formation of HISO between the ancestral type monomers encoded by KGdh1p from *S. kluyveri*, with *S. cervisiae* encoded monomers.
KGdh1p monomers were induced to organize HISO with Gdh1p and Gdh3p respectively. The first data suggest that the stability of Gdh1p, Gdh3p, KGdh1p, is different when a chaotropic agent is present, and re-naturalization is efficient when we used low concentration of this chaotropic agent. The formation of HISO is currenctly being analyzed. The formation of HISO between different GDHs was achieved through this denaturation-renaturation, we are currently determiningbkinetic properties of the hetero-oligomeric enzymes.

Ustilago maydis has two plasma membrane H^+-ATPases related to fungi and plants

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The plasma membrane H^+-ATPases play critical roles in the physiology of yeast, plant and protozoa cells. In the genome of the basidiomycete *Ustilago maydis* a Blast was performed using the sequence of *Saccharomyces cerevisiae* Pma1, and two genes encoding plasma membrane H^+-ATPase were identified, *um01205* and *um02581*. Expression of mRNAs was confirmed by real time qRT-PCR on three conditions of growth (YPD, minimal medium plus glucose or plus ethanol). The relative abundance levels of mRNA for *um01205* gene was higher than for *um02581* gene at all conditions. Biosynthesis of proteins Um-Pma1 and Um-Pma2 were confirmed by mass spectrometry analysis with a sequence coverage of 52 to 38%, respectively. Percent identity between the pair of sequences from *U. maydis* in the alignment was 32%, which is low compared with the 89% identity for Pma1 and Pma2 from *S. cerevisiae*. Phylogenetic analysis of orthologous plasma membrane H^+-ATPase proteins from fungi and plants revealed a complex relationship between fungi and plants-type ATPases. The results indicated that some basidiomycetes have both a fungal and a plant-type ATPases. In the case of *U. maydis*, Um-Pma1 has close phylogenetic correlation with the fungal-type, whereas the Um-Pma2 has close relationship with the plant-type.The possibility that the observed relationships are due to horizontal gene transfers from plant to fungi can be supported on the basis of other groups of fungi that contain also the two types of ATPases. When cells were grown in minimal medium plus ethanol, proton pumping activity was 5-fold higher compared to cells cultured in rich YPD medium, but total vanadate-sensitive ATPase activity was the same in both conditions. In contrast, this activity in cells cultured in minimal medium plus glucose was 2-fold higher than in YPD or ethanol, underlying unknown mechanisms for the regulation of the plasma membrane ATPase activity in *U. maydis*. Unlike baker´s yeast and plant H^+-ATPases, where the activity is increased by a short incubation with glucose or sucrose, respectively, *U. maydis* H^+-ATPase activity did not change in response to these sugars. Sequence analysis of the two *U. maydis* H^+-ATPases revealed the lack of canonical threonine and serine residues which are targets of protein kinases in *S. cerevisiae* and *Arabidopsis* plasma membrane H^+-ATPases, suggesting a different post-translational regulatory mechanism.

Purification and characterization of Alt1 and Alt2 of *Saccharomyces cerevisiae*: Study of functional divergence in metabolism of alanine

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Saccharomyces cerevisiae genomes arose from complete duplication of eight ancestral chromosomes, this **W**hole **G**enome **D**uplication occurred approximately 100 millions years ago at the same period in which angiosperms appeared. Most of these new genes (90%) were massively lost. 10% of duplicated genes that were retained had different fates, some of them they conserved the function of the ancestral gene, while other ones went through functional divergence resulting in subfunctionalization. In 1% of the cases, one of the duplicate copies acquired a new function while the paralogue conserved the ancestral function.
Did WGD gave *Saccharomyces* cerevisiae an advantage? Some hypothesis suggest that the selective retention of some duplicated genes favoured that *Saccharomyces cerevisiae* could develop facultative metabolism, this fact was determinant to conquer the new ecologic niche offered by the angiosperms.
Many of the duplicated genes that were retained are related to carbon and nitrogen metabolism. One example of duplicate genes associated with nitrogen and carbon metabolism are *GDH1* and *GDH3*. This pair of paralogous are glutamate deshidrogenase that catalyze the synthesis of glutamate from ammonium and -ketoglutarate, this genes show different expression patterns and the encoded enzymes Gdh1 and Gdh3 have different kinetic properties. These differences have resulted in a regulatory mechanism that supports facultative metabolism, through the control of the -ketoglutarate flux.
ALT1 and *ALT2* are paralogous that originated from the WDG. These genes show striking differences, in the expression patterns and subcellular localization, Alt1 has a catabolic and a biosynthetic role, and it is localized in the mitochondria. ALT2 displays a biosynthetic regulatory pattern and the protein is in cytosol. Previous works from the laboratory demonstrate that Alt1 has alanine aminotransferase activity, and it constitutes the sole catabolic pathway for alanine utilization, and the major pathway for alanine biosynthesid. Contrastingly, Alt2 function has not been understood, and it is devoid of alanine aminotransferase enzymatic activity.

The objective of this project is the purification of Alt1 and Alt2. With these proteins we will perform an structural comparison in order to determine their structural differences with the aim to identify those that affect Alt2 enzymatic activity.

Immunogenicity of the cell wall of *Sporothrix brasiliensis* and *S. globosa*

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Sporotrichosis is a subcutaneous mycosis of worldwide distribution and is especially endemic in tropical and subtropical areas of Latin America. It is caused by several species of the dimorphic fungi *Sporothrix* spp. These fungi have become emerging pathogens, primarily in immunocompromised patients, affecting both humans and animals. Recent molecular and phenotypic studies have shown that *S. schenckii* is not a single species but rather a complex of several cryptic species of which some are of medical importance such as *S. schenckii sensu stricto, S. brasiliensis* and *S. globosa*.

The definitive method for diagnosis of lymphocutaneous and fixed cutaneous forms of sporotrichosis is based on the isolation of the fungus from biological samples. The disadvantage of culture methods is that they are difficult to apply to systemic and disseminated sporotrichosis. Alternative methods based on antibody response in patients have been developed. The cell wall of members of the *S. schenckii* complex contains highly immunogenic molecules, which are potentially useful for diagnosis and treatment of sporotrichosis. The aim of this study was therefore to investigate the presence of immunodominant antigens in CW extracts obtained from the yeast morphotypes of S*. brasiliensis* and *S. globosa* using two-dimensional gel electrophoresis and 2D-immunoblotting with anti-*S. schenckii* antibodies and to determine the potential role of these antigens as biomarkers for the diagnosis of sporotrichosis. The analysis of proteins in 2D PAGE gels revealed 100 spots in *S. brasiliensis*, 120 spots in *S. globosa*, and 115 spots in the control strain of *S. schenckii*, in a weight range of 30 to 80 kDa. 2D-immunoblotting analysis with anti-*S. schenckii* antibodies revealed that both *S. brasiliensis* and *S. globosa* showed the same two immunoreactive antigens of 70 (pI's 4.0-5.0) and 60 (pI's 4.5-5.5) kDa observed in *S. schenckii*, with several isoforms. The Gp70 and Gp60 antigens may therefore considered as potential protein biomarkers for the diagnosis of sporotrichosis.

Functional characterization of proteins encoded by *LkLEU4* and *LkLEU4BIS* of *Lachancea kluyveri* as a model of duplicated genes in ancestral type yeast that were selectively retained

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Gene duplication is one of the most important sources for genetic evolution. Duplicated genes are present in most of organisms. The yeast *Saccharomyces cerevisiae* is an excellent model to study duplicated genes. The genome of this yeast arose from an ancient whole-genome duplication (WGD) followed by massive gene loss. Some duplicated genes were selectively retained, and there is the possibility that retention of a selected group of genes conferred advantages to *S. cerevisiae* for the establishment of facultative metabolism. Among duplicated genes of *S. cerevisiae* those involved in the metabolism of nitrogen compounds are particularly abundant. We can mention as examples: *GDH1/GDH3*, *LYS20/LYS21*, *BAT1/BAT2* and *LEU4/LEU9*, concerned in glutamate, lysine and leucine metabolism, respectively.

Lachancea kluyveri, whose lineage diverged before the WGD event, in contrast with *S. cerevisiae*, is a strict aerobic organism and has some paralogous genes that originated from isolated duplication events. Interesting examples of this, are *LkLEU4 (SAKL0E10472g)* and *LkLEU4BIS (SAKL0F05170g)* genes; which encode for two isopropyl malate synthase isoenzymes. The presence of these two genes suggests metabolic advantages which allowed it to adapt to different environments.

The present study is a preliminary physiological characterization of *Lkleu4*Δ and *Lkleu4BIS*Δ mutants of *L.kluyveri*. Our results show that the *Lkleu4*Δ strain exhibits a lower growth rate than the wild type on YPD and glucose-ammonium media. Interestingly, this growth defect is not restored even in presence of leucine, suggesting that *LkLEU4* would play another role in yeast metabolism. *Lkleu4BIS*Δhas the same growth rate than wild type strain in all media except on ethanol-ammonium, where it displays a decreased growth rate. Loss of either gene causes a decrease in isopropyl malate synthase activity, and as expected the sum of the activities of both mutants completely restores the activity of the wild type strain.

Further studies on these strains should be performed for to complete their physiological characterization. Furthermore, we will to purify the *Lk*Leu4 and *Lk*Leu4BIS isoenzymes in order to determine the kinetic parameters of these proteins and compare them with those reported for their orthologs in *S. cerevisiae*.

Biochemical characterization of the recombinant catalase-peroxidase from *Neurospora crassa* and four monofunctional mutants

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Catalase peroxidases (CPs) are the sole oxidoreductases within the superfamily of non-animal heme peroxidases that are capable of both the reduction and efficient oxidation of H_2O_2. CPs are encoded only within eubacterial, some archaebacterial, and fungal (Ascomycota and Basidiomycota) genomes. The original gene probably derived from a proteobacterial peroxidase gene that was duplicated. The two genes were fused and fungi later acquired the gene, possibly by lateral transfer. Most filamentous fungi have a single gene for an intracellular CP; some phytopathogenic fungi have two genes, one for an intracellular and another for an extracellular CP [1]. In contrast to prokaryotic CPs, the corresponding eukaryotic proteins have hardly been described and only few enzymes have been characterized. The CP function has not been elucidated; it is believed to contribute to hydrogen peroxide degradation deriving from fungal metabolism.

Point mutations in several conserved amino acid residues in the heme cavity of bacterial CPs cause the loss of the catalase but not the peroxidase activity. We have generated several of these variants in *cat-2*: D120A, Y238A, M264A and R426A. Wt and variant enzymes were expresses in *Escherichia coli* and then purified. Determining the in gel catalase and peroxidase we observed that all these mutants exhibited catalase but not peroxidase activity. This study is focused on the biochemical characterization of Wt and the different point mutants of the enzyme. Catalase activity was determined polarographically and peroxidase activity by a spectrophotometrical assay. CAT-2 catalase and peroxidase activity was dependent on the pH. We are determining the kinetics of the Wt CAT-2 and the D120A, Y238A, R426A and M264A mutants. Thereafter we will complement a *Neurospora* Δ*cat-2* strain with a monofunctional mutant gene to analyze which of the two activities of CAT-2 is more relevant for the fungus.

References:

1) Zamocky, M., Furtmüller, P.G. and Obinger, C. (2009) Biochem Soc Trans **37**: 772-777.

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Role of PARP/PARG [(Poly-ADP-ribosyl) polymerase/glycohydrolase] in the pathogenic fungus *Fusarium oxysporum f.sp. lycopersici*

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Fusarium oxysporum f. sp. lycopersici is a fungal plant pathogen for tomato, present in the soil, causing the disease called "vascular wilt". In response to damage, the plants are able to producing substances that may be genotoxic, fungi have evolved mechanisms to reverse this damage. In yeast and humans these pathways of DNA damage response have been characterized, all together make a network that, in addition to controlling cell cycle responses to genotoxic injury, preventing the spread of damaged genomes when the injury is irreversible.

In higher eukaryotes has been reported that poly (ADP-ribosyl) polymerase (PARP) is activated in response to DNA breaks generated by genotoxic agents such as reactive oxygen species, ionizing radiation, alkylating agents and/or free radicals. This modification of nuclear proteins with the poly-(ADP)-ribose (PAR) is a type of posttranslational modification required for activation of cellular processes, and is degraded by the enzyme poly (ADP-ribosyl) glycohydrolase (PARG). PARG has endo and exo glycohydrolase activity giving ADP-ribose free as product. The genome of *Fusarium oxysporum f. sp. lycopersici* has a gene encoding a protein orthologous to the human-PARG. We are interested in demonstrating the presence and function of this protein in this fungus and its possible role in physiology and/or pathogenesis. Previously, we amplified and cloned ORF from DNA and mRNA FOXG_05947. The fragment was expressed as His6-PARG. The recombinant soluble enzyme was purified, and its activity evaluated by assaying enzyme activity revealing that the purified enzyme exhibits activity of poly ADP-ribosyl glycohydrolase, and is inhibited by DEA (6,9-Diamino-2-ethoxyacridine lactate monohydrate) and tannic acid both reported as inhibitors of PARG.

In order to evaluate its function in the fungus, we generated a Δ*parg* mutant by using Double-joint PCR technique. The pathogenicity of mutant was assayed in two models; tomato plants and *Galleria melonella* larvae. The pathogenicity of this mutant was no affected since the wild type and the mutant had the same pattern of infection. To determine if the complex PARP-PARG is involved in the virulence or pathogenicity, we generated a deleting mutant in both genes, using the Split-Marker technique. The pathogenicity of Δ*parg*-Δ*parp* mutant in the models above mentioned showed a delay in the induction of the infections signs. However the pathogenicity was no affected, possibly both proteins are no essentials to establish the infection

Is the histidine kinase important for *Candida glabrata*?

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Candida glabrata is an opportunistic human fungal pathogen that is closely related to *Saccharomyces cerevisiae*. The two-component signal transduction pathway have been identified and characterized in several pathogenic fungi, such as *C. albicans,* and has been observed that the protein kinases are indispensable for virulence and pathogenicity expression. The *SLN*1 gene encodes for a histidine kinase part of the two component signal transduction pathway. Our group has identified in *Candida glabrata SLN*1 gene that is regulated negatively (Guzmán-González *et al*., 2013). The expression of the Cg*SLN*1 gene is down regulated under different environmental stress as pH, temperature, osmotic and oxidative conditions. We also observed a change in the expression during the interaction of the yeast with murine fibroblasts. This data suggest that the Cg*SLN*1 is related with the adaptation and virulence. The aim of this work is to obtain a mutant of Cg*SLN*1 gene to clarify its role.

The wild type Candida glabrata CBS138 was characterized using the micro-dilution method on plates with different stress conditions including pH, osmolarity, and oxidative stress. The MIC for Zeocin was also determined. Additionally, we will use the vector pTEF1Zeo to obtain a mutant of *SLN*1 gene of *C. glabrata*, we will obtain a constructions for replacement of gene *SLN*1 by homologous recombination with the Zeocin cassette or a by the double PCR fusion method.

The next step in our research is to obtain the mutant and characterized them using the micro-dilution method to evaluate its response to different stress conditions, and evaluated its pathogenicity *in vitro* and *in vivo*.

ROS AND cAMP SIGNALLING IN *Neurospora crassa* CONIDATION

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Cyclic adenosine monophosphate (cAMP) plays key roles in regulation of cellular functions such as gene expression, growth, cell division, cell differentiation, chemotaxis, pathogenicity, and cell death. The intracellular cAMP level is regulated by the activity of adenylate cyclase (AC) and phosphodiesterases (PDE). Protein kinase A is the principal cAMP target, however other targets have been reported.
Cell differentiation in *Neurospora crassa* is a response to a hyperoxidant state (HO), which is a brief unstable state. Conidiation involves three morphogenetic transitions: growing hyphae to adhered hyphae, adhered hyphae to aerial hyphae and aerial hyphae to conidia. A HO state develops at the start of each transition [1]. In *N. crassa*, cAMP is involved in the morphology of mycelia, growth of aerial hyphae and conidia development. Mutant strains without AC activity (*crisp*) do not form aerial hyphae but conidiate profusely, indicating cAMP requirement for aerial hyphae growth but not conidia formation [2]. The high affinity phosphodiesterase null mutant strain (*acon-2*) does not conidiate [3].
We measured cAMP levels in Wt, $ras\text{-}1^{bd}$, Δpde_L and Δpde_H strains. The redox state of the cell and the small GTPase RAS-1 partially determined cAMP levels during conidiation. During the first two morphogenetic transitions, the $ras\text{-}1^{bd}$ mutant strain had higher cAMP levels than the wild type. In both strains, cAMP levels decreased sharply during the first two HO states and also were restored after 30 min to initial levels. The low affinity phosphodiesterase (PDE_L) was mainly responsible for the decrease during the first HO because in the Δpde_L strain there was a sharp transient cAMP increment instead of a decrease and also a delay in the adhesion of hyphae. The high affinity phosphodiesterase (PDE_H) was required to restore cAMP levels after the first HO state. In the Δpde_H strain, a similar decrease as the Wt but to a lesser degree was observed; adhesion of the hyphae was also similar to Wt. Both phosphodiesterases were required for the cAMP decrease during the second HO state; both mutant strains Δpde_L and Δpde_H had an increment (instead of a decrease) in cAMP levels during the second HO state.
To find out how the cAMP signal is generated during the HO state, we measured phosphodiesterases activity in cell extracts and partially purified the enzymes. H_2O_2 increased PDE_L and inhibited PDE_H activity and, at least, the PDE_L was activated by phosphorylation.

References:
[1] Hansberg, W., H. de Groot, and H. Sies (1993). Reactive oxygen species associated with cell differentiation in *Neurospora crassa. Free Radic Biol Med*, 14: 287-293.
2) Terenzi, H.F., Flawia, M.M., Tellez-Inon, M.T. and Torres, H.N. (1976) Control of Neurospora crassa morphology by cyclic adenosine 3', 5'-monophosphate and dibutyryl cyclic adenosine 3', 5'-monophosphate. Journal of Bacteriology 126: 91-99.

3) Matsuyama, S.S., Nelson, R.E., Siegel, R.W. (1974) Mutations specifically blocking differentiation of macroconidia *Neurospora crassa.* Dev. Biol. 41: 278-287.

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Role of G-protein heterotrimericα subunit in glucose sensing and cell differentiationin *Y. lipolytica*

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Introduction: cells absorb and assimilate nutrients that determine the cellular response (growth and differentiation) of organisms by activating signaling cascades. In fungi are two major pathways of signal transduction, which have been relate to dimorphism: MAPK kinase and PKA (Protein-kinasecAMP-dependent); in the latter via the G-protein heterotrimericalpha-subunit (GPa) participates in signaling enabling cAMP synthesis. It is suggested that in fungi, inresponse to nutritional conditions, the PKA pathway controls the morphological transitionfrom yeasts to hyphae.

Objective: to determine the role of Gpa1 in glucose sensing in the dimorphic fungi *Yarrowialipolytica*by analysis of mutants generated by GPA1 gene deletion.

Methodology: by*in silico* analysis of the genome of*Yarrowialipolytica,*2*Yl*GPAgenes were identified; using molecular techniques, mutants were generated by deletion of GPA1 gene. Their ability to grow in culture medium with different glucose concentrations, were analyzed.

Results: the genome of *Y. lipolytica* has tentatively two GPA genes encoding the G-protein heterotrimericalpha subunit. Mutants generated by deletion of GPA1 showed no difference in growth relative to the parent strain in YPD medium added with different glucose concentrations.

Discussion and conclusions: *Yl*-ΔGPA1 mutants did not show growth differences relative to the parental strain, it is possible that the presence of GPA2 gene in the genome of *Y. lipolytica* is playing a compensatory role in the mutant ΔGPA1 activating the pathway PKA, to probe it, we are generating mutants *Yl*GPA2and double mutants ΔGPA1/ΔGPA2.

Keywords: glucose, nutrient sensing, GPA genes, *Yarrowialipolytica.*

The contribution of transcription factors NapA, SrrA and AtfA to the antioxidant response and cell differentiation in *Aspergillus nidulans*

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Reactive oxygen species (ROS), primarily generated during respiration, are partially reduced oxygen derivatives that are more reactive than oxygen and cause cell damage. However, ROS also participate in diverse biological processes, including development. Aerobic organisms have to regulate ROS levels to allow signaling without reaching toxic concentrations.

Aspergillus nidulans has three transcription factors (TFs), which are required for survive oxidative stress: SrrA, NapA and AtfA. SrrA contains a heat-shock-like DNA-binding domain and is closely related to Skn7 and Prr1 from *S. cerevisiae* and *S. pombe,* respectively. NapA is a peroxide sensor homologous to *S. pombe* Pap1, which is responsible for induction of multiple antioxidant genes. AtfA is a b-ZIP protein homologous to mammalian ATF-2, which has been show to associate with the stress MAPK SakA under different stress conditions.

To determine the contribution of these TFs to the antioxidant response, were have used GFP functional fusions to study its cellular localization and generated double (*ΔsrrA ΔnapA* and *ΔsrrA ΔatfA*) and triple mutants (*ΔsrrA ΔnapA ΔatfA*) to test them under different stress conditions. SrrA::GFP and AtfA:.GFP show constitutive nuclear localization, while NapA::GFP accumulates in the nucleus in the presenece of oxidative stress. Regarding stress sensitivity, we found that spores and mycelia displayed different patterns of oxidative stress sensitivity: *ΔnapA* mutation conferred the highest sensitivity to H_2O_2 to the spores, while the inactivation of *srrA* made mycelia more sensitive to this stress. *ΔatfA* mutants were in general less sensitive to H_2O_2 and only the lack of NapA made both, spores and mycelia, sensitive to menadione. Our results indicate that although AtfA, NapA and SrrA are all required for a proper antioxidant response, each TF plays dfferential roles in this response, also showing different roles in the regulation of *Aspergillus* development.

Roles of the MAPK cascade components NRC-1 and STE50 in *Neurospora crassa*

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Reactive oxygen species (ROS) have been considered as signaling molecules mediating several cellular processes. In *Neurospora crassa*, asexual and sexual cell differentiation results from an increase of ROS. The mechanism through which the ROS signal mediates these morphogenetic transitions is still unclear.

The strain *ras-1*^{bd} could enable us to study this mechanism since it alternates growth and conidiation in a cyclic manner. Raising or lowering ROS levels affects cyclic conidiation, suggesting that RAS-1 regulates the cellular fate depending on the redox state of the cell [1, 2]. To elucidate the pathways downstream of RAS-1, we made a genomic screening for putative effectors, genes having a RAS association domain. Only three genes were detected: adenylate cyclase, NRC-1 (a MAPKKK) and STE50 (an adapter protein). The aim of the present work is to determine the phenotype of the absence of NRC-1 or STE50 under physiological and oxidative stress conditions.

Both strains were female sterile: Δ*nrc-1* formed protoperithecia-like structures that could not be fertilized and Δ*ste50* was unable to form protoperithecia at all. Δ*nrc-1* strain grew as Wt but Δ*ste50* grew less well. With regard to the asexual cell cycle Δ*nrc-1* strain produced normal amounts of aerial hyphae and conidia while Δ*ste50* formed less. Germination and viability of conidia were affected into both strains. To analyze if NRC-1 and STE50 participate in the cellular response to ROS, the strains Δ*nrc-1* and Δ*ste50* were analyzed under oxidative stress. Δ*nrc-1* strain showed increased sensitivity to H_2O_2 during germination, but an increased resistance during growth compared to Wt. Δ*ste50* showed increased sensitivity to H_2O_2 during germination and growth of hypha.

Results indicate that NRC-1 and STE50 are mainly required for sexual cell differentiation and for germination of conidia. In general, Δ*ste50* was more severely affected than Δ*nrc-1* indicating an additional role of STE50 besides acting together with NRC-1.

References: 1) Belden, W. J., *et al.* 2007. The band mutation in *Neurospora crassa* is a dominant allele of *ras-1* implicating RAS signaling in circadian output. Genes Dev. 21:1494-1505. 2) Chávez-Canales, María d J. (2010) Modulación de la conidiación cíclica de *Neurospora crassa* con oxidantes y antioxidantes. Tesis de Licenciatura, Facultad de Biología, Universidad Nacional Autónoma de México, México.

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Site-directed mutagenesis of the *Acremonium chrysogenum aga1* gene to obtain constitutively active and inactive Aga1 Gα subunits

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Introduction. In filamentous fungi, the Gα subunits of G proteins have been classified in three subgroups I, II and III [1]. The subgroup I has been implicated in regulation of several process, including growth, conidiation, sexual development, pathogenicity and secondary metabolism [2].
We are interesting in the study of the Gα Aga1 subunit of *Acremonium chrysogenum*, to understand its role in fragmentation of hyphae (Fig. 1A), process closely related to cephalosporin C production.

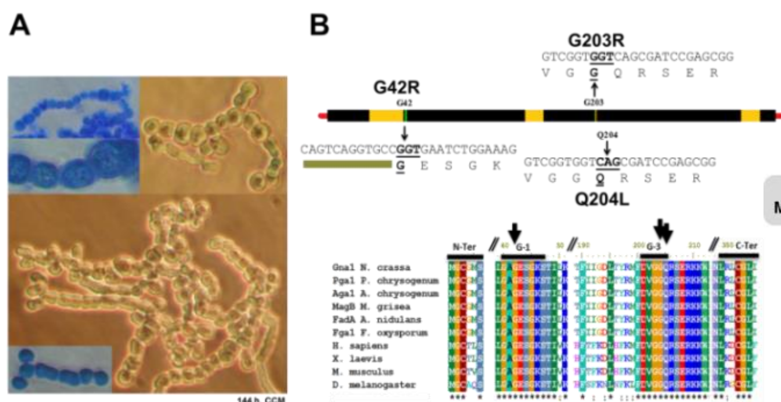

Fig. 1. (A) Hyphae and arthrospore chains of *A. chrysogenum*. (B) Site-directed mutagenesis of the *aga1* gene.

Methods. The Gα subunit encoding gene *aga1* was isolated from *A. chrysogenum* and cloned in the pJET2.1 vector. The *aga1* gene was submitted to *in vitro* site-directed mutagenesis (Fig. 1B) using Pfu Ultra II Fusion HS DNA Polymerase (Agilent) with three sets of primers (F-G42R, R-G42R; F-G203R, R-G203R; F-Q204L, R-Q204L). The mutant alleles were inserted in the integrative plasmid pC43 [3]. Transformation of *A. chrysogenum* protoplasts was carried out as described previously [4]. Transformants were selected in tryptic soy agar TSA with sucrose (10.3%) supplemented with 30 µg/ml phleomycin.

Results. The constructed plasmids (Fig. 2) were analyzed by digestion with restriction endonucleases in agarose gels, and mutations were confirmed by sequencing. The mutant alleles generated were the dominant activating *aga1*^{G42R} and *aga1*^{Q204L} alleles (expressing a constitutively active Aga1 subunit), and the dominant inactivating *aga1*^{G203R} allele (expressing a constitutively inactive Aga1 subunit). Transformants strains with these alleles were obtained for analysis of the Gα Aga1 subunit functions.

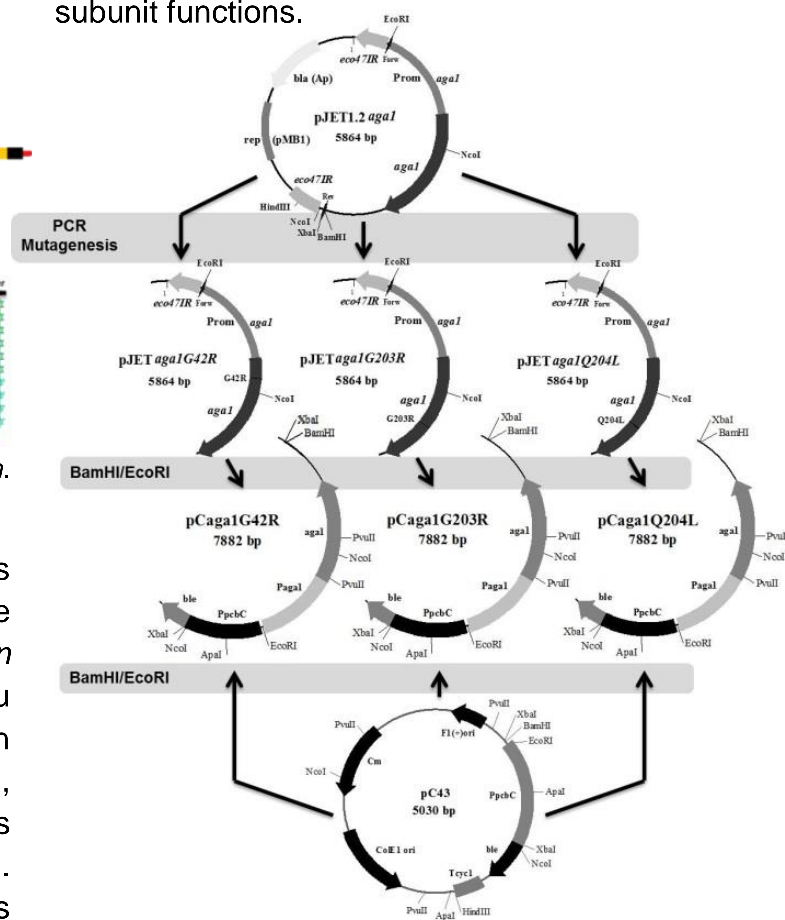

Fig. 2. Diagram of plasmid constructions.

References.

1. Bö ker M. (1998). *Fungal. Genet. Biol.* 25:143-156.
2. Li L, Wright SJ, Krystofova S, Park G, Borkovich KA. (2007). *Microbiol. Annu. Rev.* 61:423–452.
3. Gutiérrez S, Velasco J, Marcos AT, Fernández FJ, Fierro F, Barredo JL, Díez B, Martín JF. (1997). *Appl. Microbiol. Biotechnol.* 48:606–614.
4. Gutiérrez S, Díez B, Alvarez E, Barredo JL, Martín JF. (1991). *Mol. Gen. Genet.* 225:56–64.

X Congreso Nacional de Biología Molecular y Celular de Hongos
Sociedad Mexicana de Bioquímica, A.C.
Oaxaca, Oaxaca. 27 al 31 de Octubre de 2013. http://www.smb.org.mx/

GROWTH OF FILAMENTOUS FUNGI ON DIBUTYL PHTHALATE AND TOXICITY OF ITS BREAKDOWN PRODUCTS SHOWN ON THE BASIS BACTERIAL GROWTH

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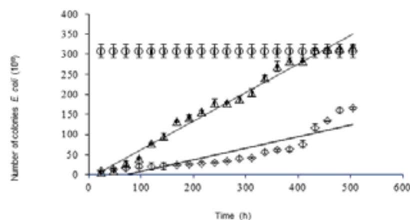
Key words: mycelial growth, dibutyl phthalate, filamentous fungi

Introduction. Phthalate are plasticizers widely used in the manufacture of plastics and are often discharged by the paper and plastics industries during the manufacturing processes into de ecosystem, contributing to the environmental pollution. Dibutyl phthalate (DBP) is one of the most widely used phthalates and it is mutagenic, carcinogenic and teratogenic[1]. In this work was to evaluate the growth of filamentous fungi on DBP and to determinate the toxicity of their degradation compounds.

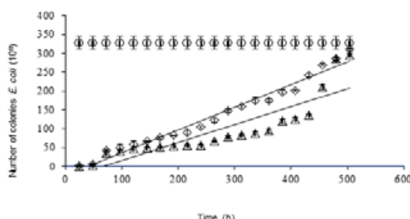
Methods. Radial (μ_r)[2], specific growth (μ) rates[3] and biomass[3] of *Neurospora* sp, *Thichoderma harzianum* and *Aspergillus niger* were evaluated in media containing mineral salts (SM) and different concentration of DBP (mg/l): 1) medium containing SM (without DBP), 2) 500 of DBP+SM and 3)1000 of DBP+SM. The toxicity of breakdown products of DBP was evaluated by counting viable colonies of *Escherichia coli* grown on R2A agar containing the supernatant of each fermentation.

Results *Neuropora* sp, *T. harzianum* and *A. niger* were able to grow on DBP-containing media. *Neurospora* sp had higher μ_r compared to the other two fungi. *A. niger* had higher biomass and *T. harzianum* had higher μ. The pH of the culture media was decreased as time increases fermentation. IC_{50} of DBP was 473 mg/l. The breakdown products of DBP were not toxic to *E. coli.*

a)



b)



c)

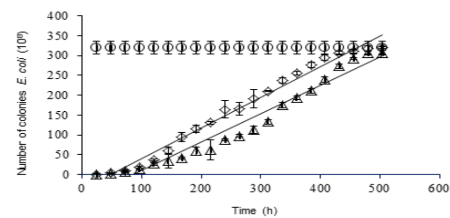


Fig. 1 Number of colonies of *E. coli* grown on the supernatant of cultures of *Neurospora* sp (a), *T. harzianum* (b) and *A. niger* (c) in 1000 mg/l of DBP (♦), 500 mg/l of DBP (▲) and without phthalate (●) under submerged fermentation conditions

Table 1. μ_r and biomass of *Neurospora* sp, *T. harzianum* and *A. niger* grown in 1000 mg/l of DBP, 500 mg/l of DBP and without phthalate

Strain	Culture media					
	Without phthalate		DBP (mg/l)			
			500		1000	
	μ_r (mm/h)	Biomass (mg/ml)	μ_r (mm/h)	Biomass (mg/ml)	μ_r (mm/h)	Biomass (mg/ml)
Neurospora sp	$1.07^{a}\pm0.02$	$0.06^{c}\pm0.03$	$1.31^{b}\pm0.02$	$0.12^{d}\pm0.05$	$1.31^{b}\pm0.00$	$0.12^{d}\pm0.02$
Trichoderma harzanium	$0.74^{a}\pm0.02$	$0.16^{c}\pm0.02$	$0.56^{b}\pm0.01$	$0.08^{d}\pm0.00$	$0.57^{b}\pm0.00$	$0.06^{c}\pm0.03$
Aspergillus niger	$0.33^{a}\pm0.00$	$0.12^{d}\pm0.04$	$0.72^{b}\pm0.02$	$0.12^{d}\pm0.12$	$0.69^{c}\pm0.01$	$0.13^{c}\pm0.12$

Means with the same letter within a row are not significantly different. Data were evaluated ANOVA and Tukey test. ($P<0.01$). Numbers in parenthesis correspond to SD of three separate experiments.

Conclusions. *Neurospora* sp had higher μ_r and biomass than the other strains in media containing DBP. This strain degraded DBP to compound less toxic since the amount of colonies were positively correlated to the fermentation time

References.

1. Lee SM, Lee JW, Koo B, Kim MK, Choi DH and Choi IG. (2007). Dibutyl phthalate biodegradation by the White rot fungus *Polyporus brumalis. Biotechnol Bioeng.* 97 (6):1516-1522**.**
2. Téllez-Téllez M, Díaz-Godínez G and Sánchez C. (2003). Physiology of a colony of Pleurotus pulmonarius grown on medium overlaid with a Cellophane membrane. Appl. Microbiol. Biotechnol. 63(2):212-216.
3. Díaz R, Sánchez C, Bibbins-Martínez M and Díaz-Godínez G. (2011). Effect of medium pH on laccase zymogram patterns produced by *Pleurotus ostreatus* in submerged fermentation. Afr J Microbiol Res. 5(18):2720-2723.

qPCA: a scalableassaytomeasuretheperturbation of protein–proteininteractions in living cells

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One of the most important challenges in systems biology is to understand how cells respond to genetic and environmental perturbations. Here we show that the yeast DHFR-PCA, coupled with high-resolution growth profiling (DHFR-qPCA), is a straightforward assay to study the modulation of protein–protein interactions (PPIs) in vivo as a response to genetic, metabolic and drug perturbations. Using the canonical Protein Kinase A (PKA) pathway as a test system, we show that changes in PKA activity can be measured in living cells as a modulation of the interaction between its regulatory (Bcy1) and catalytic (Tpk1 and Tpk2) subunits in response to changes in carbon metabolism, caffeine and methyl methanesulfonate (MMS) treatments and to modifications in the dosage of its enzymatic regulators, the phosphodiesterases. Our results show that the DHFR-qPCA is easily implementable and amenable to high-throughput. The DHFR-qPCA will pave the way to the study of the effects of drug, genetic and environmental perturbations on in vivo PPI networks, thus allowing the exploration of new spaces of the eukaryotic interactome.

A role for Hof1p and the Dbf2p/Mob1p Mitotic Exit Network kinase as a sensor for the mitochondrial inheritance checkpiont

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Organelle inheritance is the process whereby organelles are segregated between mother and daughter cells during cell division. The inheritance of organelles like mitochondria, which cannot be formed de novo, is essential for daughter cell viability. In the yeast *Saccharomyces cerevisiae*, cell division is an asymmetric process, which relies on an activeprocess of organelle segregation from mother to daughter cells.As for most organelles, segregation of mitochondria in *S. cerevisiae*depends on the actin cytoskeleton. This process is coordinated with the progression of the cell division cycle. In addition, emerging evidence has shown that failure to transfer mitochondria into daughter cells blocks cell cycle progression. In *S. cerevisiae*, defects in mitochondrial segregation caused by defective association of mitochondria with the actin cytoskeleton orby deficient retention of mitochondria in developing daughter cells inhibit cytokinesis. Analysis of the cytokinesis apparatus hasrevealed that these defects are due to failure in contractile ring closure.Furthermore, evidence has also implicated a conserved checkpoint signalingpathwaythat regulates nuclear inheritance, the mitotic exit network (MEN), in the regulation of this mitochondrial inheritance checkpoint. Here we show that the terminal kinase complex of MEN signaling pathway, Dbf2p/Mob1p, as well as the F-BAR protein Hof1p, a target of Dbf2p/Mob1p that regulates actomyosin ring contraction, is recruited to mitochondria when there are severe defects in mitochondrial inheritance. We present data showing that recruitment to mitochondria of these proteins modulates their activity. Our data suggest that these proteins participate in a system,which allows cells to sense and regulate the distribution of their mitochondria.

Isolation and identification of fungi able to degrade polyurethane

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Polyurethane (PU) is a synthetic polymer developed by Otto Bayer in 1937. It is produced by condensation of polyisocyanate and polyol. PU is very versatile, and used in the manufacturing of many products due to its durability and resistance. Therefore, PU production has increased, and its utilization has widely contributed to the global pollution crisis. PU degradation by different kinds of microorganisms (mo), fungi and bacteria, has been proven feasible. The diversity of micromycetes and the large amount of enzymes and metabolites they secrete allow them to survive in limited environments, making them excellent candidates for PU degradation. Based on this, we decided to isolate fungi able to degrade PU.

For this work, various strains from different environmental samples, with the ability to use a PU varnish (Impranil DLN) as their only carbon source, were isolated. Once obtained, the strains were analyzed in solid MM-PUi (minimal medium with 0.3% Impranil), and those that formed a degradation halo around fungal growth (Impranil clarifies when it is degraded), were selected. Tests were then conducted in liquid MM-PUi to spectrophotometrically quantify Impranil degradation by turbidity, using different MM-PUi dilutions as standard curve. Growth in differential solid media was conducted in order to determine extracellular enzyme activities (urease, protease, and esterase). Liquid MM-PUi inoculated with selected fungi strains was analyzed by infrared spectroscopy (FTIR), in order to know which functional groups fungal enzymes attacked. These strains were also tested for degradation of PU-foam. In this casefungi was supplied with 50% of potato-dextrose-broth medium. Finally, the best PU-degraders fungi were identified by macro- and microscopic analysis in accordance to Saccardo's classification.

A total of 42 filamentous fungi strains were isolated, 32 of which produced a degradation halo in solid MM-PUi. From them, eight fungi strains were able to degrade liquid Impranil more than 70%, in 15 days. Using differential media, it was observed that most of the fungi had extracellularesterase activity, two of them showed urease activity and five showed protease activity. FTIR analysis of PU, after being incubated with fungi by 21 days, showed changes in several bands that denote attack to the urethane bond. When these strains were tested for its capacity to degrade PU-foam, there were reductions of more than 40%in form, size, and mass, after being incubated with specific fungi for 21 days. Among the fungi able to attack PU were: *Trichosporum sp., Aspergillus sp., Cladosporium sp., Penicillium sp.,* and *Trichoderma sp.*

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In silico analysis of two new thermostable fungalxylanases (GH11) domains

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Introduction

The study of stable and active at high temperature xylanases are very important, since their industrial applications. This enzymes cleave internal linkage on the β-1,4-xylanopiranose backbone of xylan [1]. The filamentous fungus is an option to produce thermostable xylanases. In this work we present two new alternatives of thermostable fungal xylanases from *Talaromyces spectabilis* and *Neurosporasitophila,* Tsp Xyn11A and Nsi Xyn11A, respectively. Both of them are classified in Family 11 glicosil hydrolases.

Results

According to *in silico* analysis, there are some aspects to indicate that Nsi Xyn11A and Tsp Xyn11A are thermostable xylanases, the sticky patches compound for Tyr and Trp, which generate a stable framework, another is a high percentage of Arg and an elevated ratio of Thr/Ser in the catalytic domain[2,3].

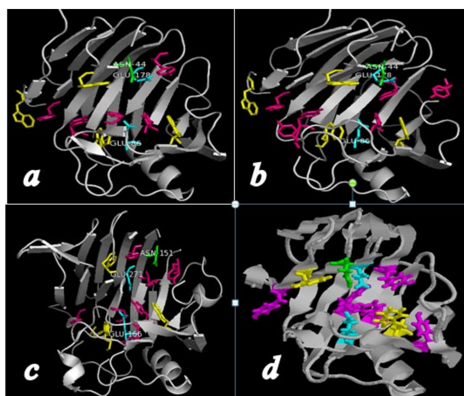

Figure 1.Thermostabilityissuesin the structures of (a) *P.variotii*, (b) *T. lanuginosus*, (c) *N. sitophila*and (d) *T. spectabilis*. Inall casescyan Eofcatalyticdyad, green pH related residue, W and Y making up thearomatic channel yellow and pink respectively.

In this work, we made a comparison between predicted structural xylanases domains, two previously reported from *Paecilomyces variotii and Thermomyces lanuginosus* (Protein Data Bank), this proteins are know it for their thermostability. The sequence of the otherstwo xylanases were obtain by our work group from *Neurospora sitophila* and *Talaromyces spectabilis*, their residual activity is comparable with thermostable xylanases, 50% after 1 h at 85°C, 40% after 4h at 70°C (enzymatic extract). The figure 1, shows the sticky patches in the four xylanases. The ratio of Thr/Ser and the high presence of Argis consistent in the four xylanases too (0.9-1.3 and 6-14, respectively).

Conclusions

According to *in silico* analysis, the new fungal xylanases, Nsi Xyn11A and Tsp Xyn11A,contain the principal aspects to be identified like thermostables.

References

1. **Collins, T., Gerday, C. and Feller, G. (2005).** "Xylanases, xylanase families and extremophilic xylanases". FEMS MicrobiolRev 29:3-23.

2. **Paës Gabriel, Berrin Jean-Guy, Beaugrand Johnny (2012).** "GH11 xylanases: Structure/function/properties relationships and applications". Biotechnology Advances, 30:564-592.
3. **Sapag A., Wouters J., Lambert C., Ioannes P., Eyzaguirre J. y Depiereux E. (2002).** "The endoxylanases from family 11: computer analysis of protein sequences reveals important structural and phylogenetic relationships". Journal of Biotechnology. 95:109-131.

Title: Heterologous expression of the enzyme 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGR) from *Candida glabrata*

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Introduction: The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is a glycoprotein involved in the onset of the cholesterol biosynthesis pathway in humans and ergosterol in fungi (Friesen and Rodwell, 2004). This enzyme has been proposed as a target of antifungal drugs, such as statins Development of new antifungals is relevant as many fungi are now resistant to drugs like azoles.This is the case of the yeast *Candida glabrata*.**Background:** Our research group has synthesized a number of fibrates and statins analogs that inhibit ergosterol synthesis and affects the viability of *C. glabrata* (Sanchez-Sandoval, 2010) and *Schizosaccharomyces pombe* (Argüelles et al., 2010). Moreover, these compounds were able to inhibit *C. glabrata* HMGR (HMGRCg) enzyme activity in enriched extract. Human recombinant HMGR is commercially available; nevertheless, the commercialization of this recombinant protein is restricted because it is the product of a human gene (Mayer et al., 1988). **Objective:** To clone gene *HMGRCg* in heterologous expression vectors pPICZαB pPICZB for overexpression of this recombinant protein in a *Pichia pastoris* system.**Methodology:**Primers were designed to amplify the sequence encoding the soluble fraction of the protein,*HMGRCg*gene was amplified and cloned in pPICZαB and pPICZB expression vectors.*P. pastoris*was transformed and rec-HMGR-Cg protein wasoverexpressed and detected.**Conclusions and results:** We have expressed *HMGRCg*gene in*P. pastoris*. The rec-HMGR-Cghas been detectedand is working on the purification as well as in activity tests. (*Grant: CONACyT 133695*).

References:

1.-Argüelles Nancy; Sánchez-Sandoval Eugenia; Mendieta Aarón; Tanaca-Lourdes Villa; Garduño-Siciliano- Leticia; Jiménez Fabiola; Cruz María del Carmen; Medina-Franco José L.; Chamorro-Cevallos Germán; Tamariz Joaquín.2010. Design, synthesis, and docking of highly hypolipidemic agents: Schizosaccharomyces pombe as a new model for evaluating α-asarone-based HMG-CoA reductase inhibitors. Bioorganic and Medicinal Chemistry. 18(12):4238-4248.

2.-Friesen JA, Rodwell V.W. 2004. The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductases. Bio. Central Med. 5: 248-254.

3. -Mayer J.R., Debouck I.C., Metcalf B.W.1988. Purification and properties of the catalytic domain of human 3-hydroxy-3-methylglutaryl-CoA reductase in *Escherichia coli*. Arch Biochem Biophys. 267 (1): 110-118.

4. -Sánchez-Sandoval M. 2010. Effect of inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) against *Candida albicans* and *Candida glabrata*. Master's thesis. ENCB, IPN.

Effect of Chitosan Films and Thyme Essential Oil on Mycobiota and Biological Quality in Stored Corn

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The 90% of crops for food and feed requiere the use of grains and sedes, hence these are the main input for the production of plant foods. Moreover, in Mexico it is estimated the lost from 25 to 30% of grains due to insects, rodents and fungi. So there is interest in producing safe food, high quality with longer life and packed or protected materials compatible with the environment, in this context the study of films and coatings has become an alternative for the convervation of these. The aim of this study was to evaluate whether different chitosan-based coating, thyme essential oil and chitosan with thyme essential oil have effects on natural mycobiota, germination, and vigor of maize seed.
We performed in determination mycobiota in malt salt agar plates, from seed maize H-48 previously coated with different treatments, 1% chitosan (Q 1%), chitosan supplemented with two different concentrations of thyme essential oil (QAT 0.05 and QAT 0.1 %), and two concentrations of thyme oil (AT 0.1 and AT 0.2 %), and control (T), stored in 85% HR chambers, by sampling every 20 days with tree replicates per treatment. We also determined moisture content, germination, and vigor, according to ISTA. The mainly fungi finding belong Fusarium, Colletotrichum, Paecilomyces, Papulaspora, Cladosporium, Asperguillus, Eurotium, and Penicillium. There was an increased incidence of fungi in the T with a presence of 53.6%, and AT 0.2% treatment had the greatest fungistatic effect with an incidence minimum of 0.99%. Regarding the percentage of germination all treatments were superior to T being the best the QAT 0.05, QAT 0.1%, and AT 0.2%. The most vigorous seedlings were chitosan treatments with added thyme for both concentrations and Q 1%. Most antifungal activity on the development of fungi mainly introduced the AT. However, the chitosan supplemented with thyme essential oil remained high biological quality of maize.

Bioleaching of Cr (VI) contaminated effluent and soil by fungi

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Chromium is a naturally occurring element that is present in nature, can exist in different valence states, but the most common and stable are trivalent Cr (III) and hexavalent Cr (VI). (Cheung and Gu, 2007). The Cr (VI) has been established as toxic and carcinogenic in humans because it is very soluble in water, their permeability of biological membranes and their subsequent interaction with intracellular proteins and nucleic acids.

In the remediation of soils contaminated with chromium several techniques are used as stabilization / solidification, physico-chemical extraction, washing soil and phytoremediation, which offer temporary solutions or simply immobilize the contaminant.

Metals are not mineralized by microorganisms but may be oxidized, reduced or complexed by organic metabolites. The bioleaching offers an attractive approach for the extraction of metals from solid materials, such as low cost and low power requirements, environmental safety and operational flexibility. (Ren et al., 2009).

Some species of microorganisms, such as *Aspergillus* and *Penicillium*, have shown potential for metal bioleaching due to the microbial production of amino acids, organic acids and other metabolites, which dissolved metals from ores by displacement of metal ion soil matrix by complex formation between the soluble metal chelators. (Burgstaller and Schinner, 1993).

The aim of this work is to determine the ability of different environmental strains of fungi for the removal of Cr (VI) effluent and contaminated soils.

The fungi Ed8, SN, 14 and 1, were grown in culture medium supplemented with Lee sodium salicylate and pH adjusted to 5.3. After growing for different time, we investigated the ability of the growth media to reduce Cr (VI) contaminated effluents, it was observed that they have the ability to reduce to 200, 225, 75 and 225 ppm Cr (VI), respectively.

Preliminary results are shown for the removal of Cr (VI) from soil contaminated with chromates.

Burgstaller, W., Schinner, F. J. (1993). J. Biotechnol. 27:91-116
Cheung, K.H. and Gu, J. D., (2007). Biodeter Int. Biodegr., 59:8-15.
Ren, W., Li, Q., Geng, Y., and Li X., (2009). J. Hazar. Mat. 167:164-169.

MOLECULAR IDENTIFICATION OF LEAD-TOLERANT RHIZOSPHERE FUNGI ISOLATED FROM A POLLUTED SITE.

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Key words: phylogeny, rhizosphere fungi, lead tolerance.

Introduction. It has been isolated, identified and classified a wide variety of fungi which tolerate high concentration of heavy metals. The morphology-based taxonomy will remain imperative to complete the information about fungi. However to obtain a correct identification and classification is necessary to supplement the information with molecular techniques. About 17 fungal strains were isolated from rhizosphere in a polluted site in Avalos, Chihuahua. Some of the isolates show high tolerance to Pb and could not be correctly identified by conventional microbiologic methods. We have specially two fungal strains that remain unidentified which are able to tolerate 3000 mg $Pb \cdot kg^{-1}$. The aim of this project was identify two strains of rhizosphere fungi (ANBM-01 and ANBM-05) by molecular methods, analyzing fragments of ribosomal genes (LSU y SSU) and intergenic regions (ITS-1).

Methods. Both fungal strains were grown in potato dextrose agar plates. DNA extraction from fungal mycelia was carried out by the modified protocol of Kieser *et al.* (1). Fragments of the SSU rDNA gen (2), LSU rDNA gen (3) and ITS-1 (4) were amplified. The PCR products were sequenced by Macrogen and then analyzed using ClustalX2 and MEGA5.1 Software.

Results. Based on phylogenetic analysis the strain ANBM-01 was found to be related to *Neosartorya hiratsukae* (97% similarity). Also, the characteristics of the fungus isolated were examined and were found similarities with previous reports. Besides this work and its background, there is only one paper available that refers to the relation between Pb and this fungus (5).The strain ANBM-05 was subjected to the same analysis, finding that it is closely related with *Microascus cirrosus* (99% similarity). To our knowledge, this fungus had never been reported as Pb tolerant.

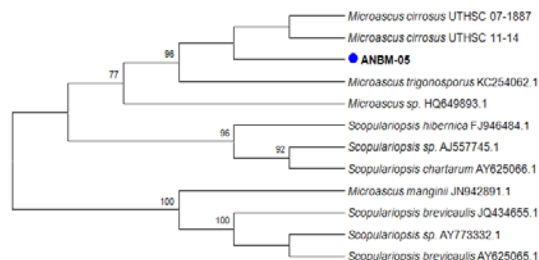

Fig.1 Phylogenetic reconstruction based on distance methods obtained from ITS-1 sequencing data from ANBM-05.

Conclusions. Phenotypic and phylogenetic analysis indicated that strain ANBM-01 is related to *N. hiratsukae* and ANBM-05 is associated to *M.cirrosus.* Neither of these fungi have been investigated enough for its Pb tolerance. To our knowledge, this is the first report of a heavy metal tolerant strain of *M.cirrosus*.

References.

1. Kieser *et al.* (2000). Practical Streptomyces Genetics. Norwich: The John Innes Foundation.
2. González-García, E. (2005). Tesis para obtener el grado de Maestro en Ciencias. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional.

3. Kurtzman, C. P., & Robnett, C. J. (1998). *Antonie van Leeuwenhoek*, *73*(4), 331-371.
4. White *et al*. (1990). PCR protocols. A guide to methods and applications. Academic Press, Inc., San Diego, Calif.
5. Hongxiang, Ou, *et al.* (2010). *Information Science and Engineering (ICISE), 2nd International Conference on*.

Development of a methodology for the molecular detection of Sclerotium cepivorum in soils, based on the amplification of internal transcribed spacer sequences (ITS).

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Abstract

In Mexico, the garlic and onion cultivation are among the first 10 most important, in terms of production and exportation. Therefore any factors that may cause the loss of crops to be detected and treated early. White rot, caused by *Sclerotium cepivorum* Berck., is the main disease affecting the *allium* genus causing even total losses.

Thus a simple, fast and sensitive detection method, becomes a priority. The methods used are mainly based on selective cultures do (especially for soil-borne microorganisms) but the recovery that is associated with the method of choice, the pollutants, the best competitors, often overcome the target pathogen, more than one species share the same morphological characteristics as the bias that the researcher used in this method is a key factor in the microbial detection.

The gene cluster ribosomal DNA (rDNA) is a target used widely for the detection of pathogenic fungal, using PCR for the detection of certain sequences. The rDNA gene is composed of three subunits: a large (LSU) of the 28S and small (SSU) 18S which are separated by a much smaller area of 5.8S gene. The three subunits are connected together with two internal transcribed spacer (ITS1 and ITS2).

The presence of variable sequences as ITS regions between the subunits permits discrimination of closely related species of a particular genus of fungal.

This study aims to standardize a method for detection of *Sclerotium cepivorum* based on the amplification of ITS sequences with specific primers directly from DNA extraction made from the soil sample, optimizing conditions for subsequent accreditation in Mexican Accreditation Entity.

References

Pérez-Moreno, L., Rodríguez-Aguilera, A. y Sánchez-Pale, J. R. Efecto de Coniothyrium minitans Campbell en esclerocios de Sclerotium cepivorum Berk. Revista Mexicana de Fitopatología, 2004; 22: 429-434.

Paplomatas, E.J. Molecular Diagnostics of Fungal Pathogens. Arab J. Pl. Prot. 2006; 24: 147-158.

Popcorn irradiation with UV-C light to control the nature mycobiota

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The presence of various kinds of fungi on popcorn kernels have been reported and the most frequent genera found were: *Fusarium*, *Penicillium,* and *Aspergillus*, some species of these genera are involved in seed germ discoloration and are the mayor mycotoxin producers, which represent a potential risk for industry and human health. The use of chemical treatments cause ecological problems as well as being potentially harmful to humans, so it was considered important to develop an alternative physical and secure control method, as the use of radiation with UV-C, which confers several advantages, including that it is a nondestructive method and is environmentally friendly. The aim of this study was to determine whether non-ionizing UV light has effects on natural occurring mycobiota popcorn kernels *(Zea mays everta*). It was performed to determine the internal and external mycobiota in 15 samples of bulk, packaging and industrial popcorn, all different brands sold in Mexico. Finding 25 species of fungi belonging to the following genera *Fusarium*, *Cladosporium*, *Alternaria, Helminthosporium, Paecilomyces*, *Aspergillus*, *Eurotium*, *Penicillium*, *Chaetomium*, *Rhizopus,* and *Mucor*, storage and advanced deterioration fungi were found with the highest percentage of incidence. All samples were treated in a UV-C radiator prototype at a wave length 254nm an output current of 65.2 μw/cm^2 and different time exposure (0, 5, 10, and 20 minutes). Twenty minutes being the most effective treatment with a reduction ratio of 67.5% for $\alpha = 0.05$. Popcorn irradiated were popped in a conventional home popping-machine conditions at 185 °C for 50 seconds per rep (3), there was a significant increase of 2.4% in burst of popcorn kernels treated for 20 minutes with UV-C with respect untreated popcorn kernel and also found that moisture content determined by dry weight significantly was reduced to 20.5% which might explain the increase in burst.

MYCELIAL GROWTH AND HYPHAL STRUCTURE OF EDIBLE MUSHROOMS GROWN ON DI (2-ETHYL-HEXYL) PHTHALATE

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Key words: Edible mushroom, phthalate, DEHP

Introduction. Edible mushrooms belong to the phyla Basidiomicetes are responsible for the biodegradation of natural polymer complex there, lignin and aromatic compounds structurally relacionados[1]. These fungi produce ligninolytic enzymes capable of oxiding lignin and some xenobiotics such as phthalates[2]. Phthalates are aromatic esters used as plasticizers[3]. Di (2-ethyl-hexyl) phthalate (DEHP) provide flexibility and malleability to polyvinyl chloride (PVC)[4]. The objective of this work was to evaluate the growth and la ultraestructura de la hifa de hongos comestibles desarrollados sobre DEHP.

Methods. Radial growth (u_r) and biomass (Bm) of *Pleurotus ostreatus* ATCC26, *P. ostreatus* ATCC37 and *Lentinula edodes* were evaluated in media containing mineral salts (MS) and different concentrations of DEHP (mg/l): 1) medium containing MS (without DEHP), 2) 750 of DEHP+MS, 3) 1200 of DEHP+MS and 4) 1500 of DEHP+MS. Diameter of hyphae (DH) and thickness from the cell wall (TCW) from the young zone (YZ) and mature zone (MZ) were evaluated using image analysis on (Image Pro-Plus program).

Results. *P. ostreatus* 26 had higher u_r and DH on the medium containing 1200 of DEHP+MS compared with the other two fungi. *P. ostreatus* 37 had higher Bm on the medium containing 1200 of DEHP+MS compared with the other strains (Table 1). *L. edodes* had higher TCW on the medium containing 1200 of DEHP +SM compared with other fungi. *L. edodes* presented the highest DH in 1500 mg/l of DEHP (Tables 1,2).

Table 1. Radial growth rate and biomass of different strains of edible mushrooms grown on different concentrations of DEHP.

Strain	Culture media							
	u_r (mm/h)				Bm (g/cm^2)			
	MS	MS+750 mg/l DEHP	MS+1200 mg/l DEHP	MS+1500 mg/l DEHP	MS	MS+750 mg/l DEHP	MS+1200 mg/l DEHP	MS+1500 mg/l DEHP
P. ostreatus 37	0.09^{a} (0.004)	0.07^{b} (0.008)	0.09^{a} (0.017)	0.02^{c} (0.010)	0.004^{c} (0)	0.008^{a} (0.001)	0.008^{a} (0.001)	0.005^{b} (0.0005)
L. edodes	0.14^{c} (0.002)	0.15^{b} (0.005)	0.16^{b} (0.003)	0.20^{a} (0.009)	0.002^{d} (0.0005)	0.005^{b} (0.001)	0.004^{c} (0.001)	0.007^{a} (0.001)
P. ostreatus 26	0.14^{c} (0.008)	0.25^{a} (0.006)	0.24^{a} (0.013)	0.20^{b} (0.036)	0.005^{b} (0.0005)	0.007^{a} (0.001)	0.003^{c} (0.003)	0.007^{a} (0.001)

Means with the same letter w thin a row are not significantly different. Data were evaluated using ANOVA and Tukey test. (P<0.01). Numbers in parenthesis correspond to SD of three separate experiments.

Table 2. Thickness from the cell wall of different strains of edible mushrooms grown on different concentrations of DEHP.

Strain	TCW (µm)							
	Culture media							
	MS		MS+750 mg/l DEHP		MS+1200 mg/l DEHP		MS+1500 mg/l DEHP	
	YZ	MZ	YZ	MZ	YZ	MZ	YZ	MZ
P. ostreatus 37	0.49^{d} (0.030)	0.47^{d} (0.032)	0.61^{b} (0.016)	0.61^{b} (0.034)	0.57^{c} (0.026)	0.72^{a} (0.041)	0.63^{b} (0.044)	0.50^{d} (0.034)
L. edodes	0.59^{c} (0.028)	0.66^{b} (0.097)	0.68^{b} (0.016)	0.66^{b} (0.012)	0.60^{c} (0.031)	0.73^{a} (0.078)	0.64^{b} (0.009)	0.65^{b} (0.029)
P. ostreatus 26	0.65^{a} (0.029)	0.65^{a} (0.012)	0.64^{a} (0.036)	0.66^{a} (0.017)	0.60^{b} (0.014)	0.64^{a} (0.024)	0.60^{b} (0.030)	0.62^{a} (0.018)

Means with the same letter w thin a row are not significantly different. Data were evaluated using ANOVA and Tukey test. (P<0.01). Numbers in parenthesis correspond to SD of three separate experiments.

Table 3. Diameter of hyphae of different strains of edible mushrooms grown on different concentrations of DEHP.

Strain	DH (µm)							
	Culture media							
	MS		MS+750 mg/l DEHP		MS+1200 mg/l DEHP		MS+1500 mg/l DEHP	
	YZ	MZ	YZ	MZ	YZ	MZ	YZ	MZ
P. ostreatus 37	2.12^{f} (0.064)	2.00^{g} (0.265)	2.29^{c} (0.158)	2.24^{d} (0.180)	2.29^{c} (0.053)	2.59^{a} (0.217)	2.41^{b} (0.275)	2.19^{e} (0.223)
L. edodes	3.08^{c} (0.111)	2.97^{d} (0.275)	2.80^{e} (0.286)	2.95^{d} (0.120)	3.16^{b} (0.267)	2.81^{e} (0.605)	2.84^{e} (0.298)	3.31^{a} (0.112)
P. ostreatus 26	2.48^{f} (0.154)	2.86^{c} (0.081)	3.33^{a} (0.329)	3.11^{b} (0.184)	2.76^{d} (0.148)	2.84^{c} (0.140)	2.36^{g} (0.070)	2.54^{e} (0.159)

Means with the same letter w thin a row are not significantly different. Data were evaluated using ANOVA and Tukey test. (P<0.01). Numbers in parenthesis correspond to SD of three separate experiments.

Conclusions. In general, *L. edodes* presented the highest u_r, Bm, TCW and DH in 1500 mg/l of DEHP, which suggest that this organism has a less specific enzymatic system in relation to *P. ostreatus* that allows it to grow in high concentrations of this compound.

References.

1. Rodríguez Sánchez Enrique. "Carcterización molecular de lacasas de *P. eryngii*: Expresión heteróloga de estas enzimas y aplicaciones en la degradación de contaminantes aromáticos". *Universidad Complutense de Madrid*, Facultad de Ciencias Biológicas, Madrid, pp. 11- 12. 2006.
2. Sánchez C. (2009). *Biotechnol advans*, vol (27.): 185-194.
3. Liang D, Zhang T, Herbert H y He J. (2008). *Appl microbiol biot.* Vol. (80): 183-198.
4. Mersiowsky I., Weller M. and Ejlertsson J. (2001). *Water Res*, vol. (35): 3063-3070.

Production of extracellular xylanase produced by *Wickerhamia* sp.

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INTRODUCTION

Xylan is a major component of hemicellulose in cell walls of monocots and hard woods, and a potentially valuable resource as a supply of xylose for a variety of microbial fermentation. It consist of a linear backbone of β-1,4-linked D-xylopyranoside residues, which are commonly substituted with arabinose, glucoronic acid, or methylglucoronic acid. The insoluble substrate xylan, is degraded by numerous bacteria and filamentous fungi using multiple enzymatic activities. Extracellular endo-1,4-β xylanases hydolyze the internal β-1,4-xylosidic linkages in the xylan backbone. β-xylosidases and several debranching enzymes complete the hydrolysis. Endoxylanases have received considerable attention because of their practic value[1].

Xylanases produced by yeasts have been scarcely studied. *Wickerhamia* sp. was found to produce extracellular endoxylanase activity using different substrates as carbon and energy sources, although the activity is not very high, is one of the few reports of a xylanase producing yeast.

MATERIAL AND METHODS

Batch cultures were carried out using 1L Erlenmeyer flasks containing 200 mL of Castañeda medium suplmented with different substrates, such as cellobiose, xylose, lactose, xylan, corn cob ans sorghum. Enzymatic activity was measured by the dinitro-salycilic acid method using xylan as substrate at pH 7.0 and 50°C.

RESULTS AND DISCUSSION

From the assayed substrates, under the tested conditions, the highest activity was obtained using cellobiose (64 U/mL), followed by xylan (11 U/mL), corn cob (2.8 U/mL) and sorghum (1.6 U/mL), with P_{max} expressed as U/L·h of 1777, 916, 116 and 66 respectively. The greatest xylanase activity occurred prior to late exponential production of yeast biomass using cellobiose as carbon source.

From the obtained results, we infer that the presence of the β-1,4- linkage in the cellobiose molecule is the trigger of the induction of the extracellular endoxylanase synthesis produced by *Wickerhamia* sp.

Enzyme characterization and purification are necessary to complete the basic profile of this novel extracellular endoxylanse produced by a yeast.

Ohta, K., *et. al.,* 2001, Purification and Characterization of an acidophylic xylanase from *Aureobasidium pullulans* var. *melanigenum* and sequence analysisof the encoding gene. J Biosci Bioeng. **92(3):262-270**

Evaluation of *Lentinula edodes* and *Pleurotus ostreatus* extracts as bioactive compounds antibacterial

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Parallel to the overproduction of agricultural wastes has increased world production of edible mushrooms over 7 million tons, of which 70% are Basidiomycetes, such as *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. Basidiomycetes produce several bioactive compounds such as polysaccharides, peptides, nucleosidesand phenols, with hypoglycemic, immunomodulators, anti-inflammatory, antitumor, antiviral, antibacterial or antiparasitic action. The purpose of this research was obtained aqueous extracts residues of *Pleurotus ostreatus* (Jacq.) P. Kumm. (1871) UAEH-004 strain of 23 d cultivation with barley straw (*Hordeumvulgare*) and *Lentinul aedodes* residues (Berk.) Pegler (1976), UAEH_015 strain 90 d cultivation, with 80% of oak (*Quercussp.*) and 20% sorghum (*Sorghum sp.*), to evaluate activity antimicrobial against *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella tiphymorium*, determining the minimum inhibitory concentration (MIC) at 24 h *in vitro*. The results showedhigher antibacterial activity (P <0.01) against *Salmonella typhimurium*(UFC 1.11 x 10^7/200 μL)from 12 mg *Pleurotus ostreatus* extracts, with 30.11 mm $\pm$ 0.97 of inhibition halo, 6 mg *Lentinula edodes* extracts (P <0.01), with 28.11 mm $\pm$ 0.435 of inhibition halo. Antibacterial activity against *Staphylococcus aureus* (1.0 × 10^7UFC / 200 μL)and *Micrococcus luteus* (1.04 × 10^7UFC / 200μL) was 50 mg *Pleurotus ostreatus* extract, with 8.8 mm $\pm$ 1.18inhibition halo and 50 mg *Lentinula edodes* extracts; the most activity was obtained in 1.04 × 10^7UFC /200 μL of *Micrococcus Luteus* and 1.0 × 10^7UFC /200 μL of *Staphylococcus aureus,* contrary to *Escherichia coli* (7.7 x 10^6UFC /200 μL) where not present antibacterial activity (P> 0.01), only with 6 mg *Pleurotus ostreatus* extracts, with 9.55 mm $\pm$ 0.39 inhibition halo and6 mg *Lentinula edodes* extracts, with 7.95 mm $\pm$ 1.34Inhibition halo. In conclusion *Pleurotus ostreatus* and *Lentinula edodes* extracts, have greater antibacterial activity against *Salmonella typhimurium*, and lower activity against *Staphylococcus aureus, Micrococcus luteus* and *Escherichia coli*. *Pleurotus ostreatus* and *Lentinula edodes* extracts offers a viable alternative as antibacterial activity.

DEGRADATION OF LIGNOCELLULOSE BY ACELLULOLYTIC STRAINS (C-) OF *Pleurotus ostreatus*

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keywords: lingocellulose degradation, *Pleurotus,* acellulolytic strains

Lignin is the main obstacle for bioutilization of lingocellulosic residues as fodder for ruminants. White-rot fungi like the edible mushroom *Pleurotus ostreatus*, have the ability to attack lignin but degradation of cellulose and hemicellulose is produced at the same time. This fungus is cultivated on agricultural and forestry wastes and cultivated wild strains produce a simultaneous degradation of polysaccharides and lignin.

In order to increase lignin degradation and preserve cellulose, acellulolytic mutants of *Pleurotus ostreatus* were used to bred acellulolytic dicaryotic strains (C-) to promote aselective degradation of lignin, Three of these acellulolytic strains and four *Pleurotus* strains used for commercial cultivation, were tested under commercial production conditions. Production of mushrooms and changes in substrate composition during the whole production cycle was followed on a commercial substrate prepared with fermented straw. Lignin, glucan, xylan and ash content were determined at spawning (t=0), at the end of the incubation period (t=1) and after the first flush of mushrooms (t=2)

Wild strain IAP was the most productive with 82 % biological efficiency (BE) (g fresh mushrooms /100 g dry substrate. Acellulolytic strain 71x512 with 39 % BE was in a second group together with the two commercial *Pleurotus* strains K8501 y K1508, normally used by the company where this test was carried out.

Ash content in substrate increased with all strains when substrate was fully invaded and after the first crop of mushrooms indicating extensive substrate degradation. Highly significant differences in lignin, glucan and xylan degradation were observed. Although acellulolytic strains (C-) did not produced selective lignin degradation, they caused larger degradation of substrate than commercial strains. Notably, the most productive acellulolytic strain 71x512(C-) showed identical lignin and xylan degradation as commercial strain K8501 and glucan degradation was also identical to that of another commercial strain, K1508.

Correlations of mushroom production and substrate degradation were evaluated for all strains. Remarkably, mushroom production was directly correlated with xylan degradation and total substrate consumption in spent substrate. Moreover, degradation of all substrate components was directly correlated with total substrate consumption after spawn running and at the end of cropping; however, lignin degradation in spent substrate was not correlated with total substrate consumption.

Production of lipolytic activity by the thermophilic fungus *Chaetomium sp.* using different carbon sources.

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Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are ubiquitous hydrolytic enzymes that catalyze the breakdown of triacylglycerols to free fatty acids and glycerol and operate at the interface of emulsified lipid substrates. Lipases constitute one of the most important groups of enzymes because they are used widely in industrial applications, e.g., as food additives, industrial reagents, cleaners, cosmetics, pharmaceutical and chemistry synthesis.

The thermophilic fungus *Chaetomium sp*. strain CO5-B4 was isolated from sugarcane bagasse compost and selected for its ability to produce ligninolytic activity; however, there are no reports on the lipolytic activity produce by this fungus. The aim of this work was to study the effect of different carbon source on the production of lipolytic activity by *Chaetomium sp*.

For identification of *Chaetomium sp*. as a lipolytic strain, the fungus was grown on tributyrin agar at 45°C for 48 h. For lipase production, *Chaetomium sp*. was grown in liquid basic basal medium with different carbon sources at 1%: glucose, soybean oil, olive oil and glycerol. Cultures were incubated at 45 °C, for 240 h and 8000 rpm. All assays were done by duplicate. Every 24 h, a sample culture was filtered, and the cell-free filtrate was used as a source of extracellular lipase activity; mycelia were dried to constant weight. Lipase activity was assayed using *p*-nitrophenyl palmitate (*p*-NPP) as substrate at 45 °C for 10 min. One unit (U) was defined as the amount of enzyme needed to liberate 1 µmol of p-nitrophenol per minute under the assay conditions.

Extracellular lipolytic activity was detected in the culture supernatant from *Chaetomium sp*. grown in liquid basic basal medium supplemented with olive and soybean oil as inducers. The highest lipase activity was observed after 96 h (46.1 U/ml) and 144 h (64.1 U/ml) using olive oil and soybean oil, respectively. For glycerol and glucose as inducers, the highest lipase activity was observed after 120 h (18 U) and 96 h (48.6 U), respectively. When the fungus was grown on olive oil, the lipolytic activity was produce over 10 days; while in the case of glucose, the lipolytic activity was detected only during the first 6 days of incubation.

In this study, we report the production of extracellular lipolytic activity by *Chaetomium sp*. using olive oil, soybean oil, glycerol and glucose as carbon sources. Findings here suggest olive oil was the best inducer of lipolytic activity by *Chaetomium sp*.

"Isolation, identification and phenotypic characterization of yeast obtained from mine tailings of Zacatecas, Mexico."

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In Mexico there are large accumulations of mine tailings with high levels of heavy metals. These residues are left over the ground and generate an extreme man-made environment with low concentrations of assimilable organic carbon and nitrogen. These ecosystems can be colonized by yeast that promotes the growth and development of other microorganisms and pioneer plants, contributing to the genesis of a proper soil and mitigating the toxicity of the environment. In this research endophytic yeast strains isolated from the pioneer plants *Prosopis loevigata*, and tailings of mines from Zacatecas State, with low nitrogen requirements, were identified and characterized. Four oligothrophic yeast strains were isolated in nitrogen-free culture media. They exhibited a low nitrogen requirement and an extensive capsule. The strain exhibited a characteristic morphology, with mucoid, translucent and large colonies. DNA was extracted, and ITS region amplified and phylogenetically analyzed. The strains were identified as *Cryptococcus flavescens* and *Cryptococcus albidus*. Finally, the strains were able to tolerate concentrations ≥20% of tailings added to Winogradsky medium.

Heterologous expression of a lipase gene from the basidiomicete fungus *Bjerkandera adusta* UAMH 8525 in *Pichia pastoris*

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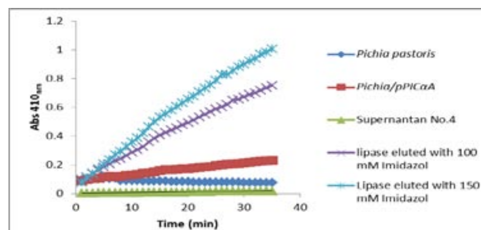
Key words: Lipase, *Bjerkandera adusta and Pichia pastoris.*

Introduction The interest in lipases arises due to their ability to catalyze the hydrolysis of triacylglycerols and the synthesis of esters from glycerol and long-chain fatty acids under certain conditions (1). We report the cloning and sequencing of the genomic DNA of a lipase gene from *Bjerkandera adusta* UAMH 8258, its cDNA expression and characterization in *P. pastoris* X-33.

Results and Discussion The lipase cDNA product was cloned and its sequence confirmed. It also shows identity with proteins annotated as alpha/beta hydrolases of *Trametes versicolor*, *Aspergillus niger*, *Aspergillus oryzae* with maximum identity of 43%, 30% y 27%. The lipase cDNA was cloned in pPICZαA expression vector and a fusion protein with a His tag was generated. The heterologous protein was purified and lipase activity was determined with ρ-nitrophenil laurate as the substrate. One unit of lipase activity was defined as the amount of enzyme that liberated 1 mmol of fatty acid per minute per litre. We obtained a maximum activity of 8.95 U/L.



Sample	[Proteín]	volumetric activity U/L
Pichia pastoris	0.649	0 259459459
Pichia pastoris /pPICαA	0.653	1.32972973
Supernatant No. 4	1.094	0.162162162
Elution with 100 mM imidazole	0.063	6 291891892
Elution with 150 mM imidazole	0.015	8 951351351

Graph and table of the hydrolisis of ρ-nitrophenil laurate for the lipase of *Bjerkandera adusta*. Supernant of transformant No. 4, lipase eluted with 100 mM of imidazole, lipase eluted with 150 mM of imidazole.Controls *Pichia pastoris* wild-type, *Pichia pastoris*-pPICZαA.

Conclusion Lipase of *Bjerkandera adusta* was functionally expressed in *Pichia pastoris.* Its biochemical characteristics will be discussed.

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References (1) Treichel H, De Oliveira D, Mazutti M, Di Luccio M, and Olivieira J. 2010. *Food Bioprocess Technol.* 3:182–19.

Functional analysis of *arg2* gene encoding the small subunit of carbamoyl-phophate synthetase and its usefulness as a selection marker in *Trichoderma atroviride*

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*Trichoderma atroviride*is a filamentous fungus of great economic and biotechnological interest due they are mycoparasites of fungi that affect agricultural important plants and they show high production of metabolites. There are genome analysis of several species of the genus *Trichoderma* but mostly hypothetical functions have been about 40% of the genes. Hence the importance of a thorough analysis of functional genomics to understand the role of genes in the different life stages of the fungus.

Strategies for functional genomics, are scarce and existing are using auxotrophics election markers that were generated by mutagenesis (physical or chemical), this not being specific causes changes in the genetic background of *Thichoderma*. Therefore the implementation of new techniques for the generation of selection marker and consequently the study of genes, is necessary tools that generate selection markers without he noise created in the gene by mutagenesis. A first PCR amplifies the 5 'and 3' of gene *arg*2, with specific oligonucleotides and excludes the full open reading frame (ORF). A second PCR binds the fragments obtaining DNA strands lacking of *arg*2 ORF (Δarg2). A final PCR amplify the mutagenic cassette using nested primers, continuing transformation through protoplasts of *T. atroviride*. The strains were inserted with pCB1004 plasmid and selected on minimal medium (MM) supplemented with arginine, its use as a tool for investigating the genetic background is being evaluated.

Production of INVA and INVB invertases from *Zymomonas mobilis* in *Pichia pastoris*, under the control of AOX1 and GAP1 promoters

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Key words: AOX1, GAP1 promoters, invertases, Pichia pastoris

Introduction. *Zymomonas mobilis* produce two invertases: an intracellular (INVA) and an extracellular (INVB). These enzymes have attractive features for the food industry. For this reason, is attractive to produce these enzymes in greater amounts, compared to the low yield obtained natively. *Pichia pastoris* has been widely used for obtaining high amount of recombinant enzyme using the AOX1 (inducible expression) and GAP1 (constitutive expression) strong promoters. The aim of this study was to express the INVA and INVB from *Z. mobilis* in *P. pastoris* under the control of the AOX1 and GAP1 promoters and compare the production of both enzymes in *P. pastoris* to that obtained from *Z. mobilis*.

Methods. *AOX1* promoter: pPICZαB-*invA* and pPICZαB-*invB*. *GAP* promoter. pGAPZαA-*invA* and pGAPZαA-*invB*. Fermentations were carried out in 1 L baffled flasks (working volume of 100 mL) at 28ºC, 180 rpm during 120 h. For AOX1 promoter, 1% methanol was added every 12 h. For GAP1 promoter a batch fermentation was performed, using glucose as carbon source. Samples were taken every 12 h and the response variables were: invertase activity (DNS method), quantification of biomass (dry weight) and determination of protein (Lowry method).

Results. Summary of all fermentations are described in table 1.

Table 1. Production of recombinant and native INVA and INVB invertases.

	Specific activity (U/mg)	Total biomass (g/L)	Total protein (g/L)	Specific production (INV/g BM)
Native INVA	221±1	2±0.2	1.49±0.13	37
GAP INVA	4433±69	17±0.8	1.51± 0.26	**208**
AOX1 INVA	2280±87	12±1	1.18 ± 0.11	170
Native INVB	317±10	2±0.3	1.17±0.13	79
GAP INVB	4684±139	12±1.4	5,2± 0.41	284
AOX1 INVB	5637±117	10±1.4	2.9 ± 0.12	**490**

Conclusions.

Production of INVA and INVB in *P. pastoris* was higher under the control of GAP (1.2 fold) and AOX1 (1.7 fold) promoters, respectively. Production of the enzymes in *P. pastoris* was greater compared to that observed in *Z. mobilis* by 5.6 and 6.2 fold for INVA and INVB, respectively.

References.

Lowry N J, Rosenbrought F & Randall A L (1951). *J Biol Chem.* **193**:265-275; **Miller G J** (1959) *Analytical Chem.* 31: 426-428;**Arjmand S,** Lotfi A S, Shamsara M & Mowla S J (2013). *Electronic Journal of Biotecnology*. 16: 1-14

Sanitary Quality of Nixtamalizated Masses with Nejayote

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Nejayote is by-product of nixtmalization that contain high amounts of organic compounds. Ramírez-Romero et al., 2011, proposed added nejayote solids in mass to improve its consistency. Consequently, aim of research was to perform microbiologic analysis at nixtamalizated masses with nejayote, in which sanitary quality will be determinate. Microbiologic analysis in corn kernel variety Sinaloa, were performed in two culture media; potato dextrose agar (PDA) and malt extract agar (MEA). Method used was agar plate. Total aflatoxins determination were achieved by monoclonal antibodies method. Nixtamalization process conditions were 90°C and 12 h of steeping time. Masses obtained were: test mass (MT), nejayote mass 1:1 (nejayote/water) (N1) and nejayote 1:2 (nejayote/water) (N2). Microbiologic analysis of the masses were performed by serial dilutions method in day 1 and 4 post-process to identify molds, yeasts, aerobic mesophiles and total coliforms. In corn kernel was observed *Fusarium verticillioides, Cladosporium sp., Alternaria sp.*, *Penicillium aethiopicum, P. chrysogenum, Aspergillus niger, A. parasiticus*, and *Rhizopus sp.,* which some species are potentially mycotoxins producers. Results show 7ppb of aflatoxins in corn kernel, this value is lower than the Official Mexican Standard. In microbiologic analysis of the masses were observed in mass N1, day 1 at dilution 10^{-2}, slower growth of molds, yeasts (*F. verticillioides*, *Cladosporium sp.* y *Rhodotorula sp.*) and aerobic mesophylls, compared to MC. While N2 the growth of these microorganisms were significantly lower. In the microbiological analysis of 4 day was found increased growth of molds and yeast, and aerobic mesophylls in both masses N1 and MC, however in N2 the growth of molds, yeasts, and aerobic mesophylls were also lower. In none of the masses was found the presence of coliforms. Nixtamalization eliminates fungus and bacteria from corn kernel, but in the mass N2, there was less growth of microorganisms and this can help extend the life of the products. In another aspect reuse a ratio of nejayote for nixtamalized not affect the sanitary quality of the masses and reduces the drainage disposal.

Transformants of *A. rouxii*, an option for increase tyrosinase activity

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Pentachlorophenol (PCP) is a toxic compound and is the most common xenobiotic that produces during the process of papermaking, and is widely used as fungicide. *Amylomyces rouxii* is a zygomiceto that tolerate and remove PCF in submerged and solid cultivation and it has showed that tyrosinase is involved in PCP removal by this fungus. *A. rouxii* produces extracellular protease; this enzyme has a negative effect on tyrosinase activity [1-3]. In this study a gene of aspartate protease, the rhizopuspepsin-2, was silenced using a strategy of RNAi [4].

Four transformants strains showed lower protease activity and higher tyrosinase activity than the parental strain, these results confirming that rhizopuspepsin-2 was silenced. The four transformant showed low stability, in 4 or 6 months they lost the informatios nad the enzymatic activities became similar as the parental strain.

References

1. Marcial, J., Pérez de los Santos, A.I., Fernández, F.J., Díaz, G. G., Montiel-Gonzales, A.M. and Tomasini, A. Caracterización de una aspartato proteasa producida por *Amylomyces rouxii. Revista Mexicana de Ingeniería Química.* 10:9-16. (2005)
2. Montiel, A. M., F. J. Fernández, J. Marcial, J. Soriano, J. Barrios-González, and A. Tomasini. A fungal phenoloxidase (tyrosinase) involved in pentachlorophenol degradation. *Biotechnol. Lett.* 26:1353-1357. (2004)
3. Tomasini, A., Flores, V., Cortés, D. and Barrios-González, J. An isolate of *Rhizopus nigricans* cazable of tolerating and removing pentachlorophenol. *World J. Microbiol.* 17:201-205. (2001)
4. Liu H., Cottrell T.R., Pierini L.M., Goldman W.E., Doering T.L. (2002). RNA interference in the pathogenic fungus *Cryptococcus neoformans.* Genetics 160: 463-470.

BIODEGRADATION OF LIGNOCELLULOSIC MATERIALS BY SOLID STATE CULTURES OF TWO STRAINS OF THE FUNGUS *Penicillium*

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Lignocellulosic materials are composed primarily of three polymers: cellulose (50%), hemicellulose (25-30%), and lignin (15-25%). The structure of this type of materials is highly complex, exhibiting recalcitrance and heterogeneity as its main features. Cellulose, the most abundant polysaccharide on earth, is a linear polymer of D-glucose units linked by beta-1-4 glucosidic bonds. Hemicelluloses, commonly known as xylans, are heterogeneous polymers of pentoses (D-xylose, L-arabinose), hexoxes (D-glucose, D-galactose, D- mannose), and sugar acids (glucuronic, galaturonic). The third component, lignin, is not a polysaccharide but a polyaromatic molecule constituted by phenylpropanoid subunits, whose functions consist in conferring the plant with rigidity and preventing microbial attacks. In addition to the main components, pectin, a galacturonic acid polymer is also present in plant-cell walls.

The biodegradation of these compounds is basically accomplished by microbial consortia, which in turn, possess enzymatic systems, known as cellulases, xylanases, ligninases, and pectinases, depending on their action target. During the past decades, this process has received much attention due to the fact that 1) it represents a key aspect in carbon recycling and 2) the products derived from it such as fermentable sugars, constitute the starting point for the synthesis of alternative fuels. An excellent alternative for the degradation of these molecules at laboratory scale, is represented by solid-state fermentation (SSF), defined as a process in which microorganisms grow on solid materials in the absence of free liquid. Selecting a suitable substrate for SSF depends on several factors, mainly related to cost and availability, so lignocellulosics in the form of agricultural, industrial, and forest residues may account for a partial solution.

In our research group, two different residues (dried pine needles and brewed coffee wastes) were employed as substrates for SSF. Their potential for the microbial synthesis of enzymes with the capacity to break down plant-cell walls, was evaluated, using as biological model the genus *Penicillium* (*P. chrysogenum* as a known control and a recently isolated microorganism belonging to this genus). This fungus was obtained from forest soil of *La Marquesa* (*Insurgente Miguel Hidalgo y Costilla*) National Park, where pine needles were also collected. In our opinion, due to its natural habitat, it is believed that *Penicillium* sp. will display higher enzymatic activities in comparison to the selected fungal model, which represents a strong advantage for biotechnological applications.

Functional analysis of *pyr*4 gene encoding orotidine-5'-monophosphate decarboxylase and its usefulness as a selectable marker in *Trichoderma atroviride*.

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Trichoderma atroviride is a filamentous fungus of great biotechnological interest due to their production of enzymes and secondary metabolites, also is a mycoparasite of plant parasitic fungus of commercial interest. So a comprehensive study of functional genomic at different stages of its life cycle is very important for understanding the role of different genes in its biology. Molecular strategies for the functional study of its genome are scarce, mainly the selectable markers. In addition to a high cost, the selection markers which provide resistance to antibiotics have different effectiveness. For a long time auxotroph strains have been obtained to use their genes that complement the mutation as selectable marker, however, they have been generated by saturating mutagenesis (chemical or physical) causing accumulative changes into its genome.

Nowadays, the whole genome sequences for a number of filamentous fungi including the model organisms are currently available. With the goal to improve methods for functional genomic, in this work we are generating novel tools which will be an impact in gene study. Specific oligonucleotides were designed to amplify the 5 'and 3' flanks of *pyr*4 gene, excluding the complete open reading frame (ORF) in a first PCR reaction. In a second PCR, the fragments were joining together to obtain DNA molecules which lacking of *pyr*4 ORF (∆pyr4). Finally, a PCR is performed to amplify the mutagenic cassette using nested primers and then, it was used to transform protoplasts of *T. atroviride*. The transformants resistant to 5-fluororotic acid were selected on minimal medium (MM) supplemented with uracil and its use as genetic background in investigation is being evaluated.

Study of phenoloxidases, proteases and peroxidases in *Rhizopus oryzae* ENHE

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The objective of this project was study the extracellular and intracellular enzymes such as proteases (acid, neutral and basic), phenoloxidases (laccase and tysosinase) and peroxidases (manganese and lignin) in *Rhizopus oryzae* ENHE which is a filamentous fungi that is able to grow in contaminated environments with xenobiotics as pentachlorophenol (PCF), so the study on their enzymatic system is useful for biotechnology developments.

R. oryzae ENHE was cultivated on Lee medium with citrate buffer 0.1M pH 5.8, at 30°C and 150 rpm. In these conditions *R. oryzae* ENHE reached its maximum biomass production from 24 hours. Samples were collected from 24 up to 96 hours of cultivation. With these conditions we could quantify acid, neutral and alkalyne proteases in the intracellular extract, and acid, neutral and alkalyne proteases, along with lignin peroxidase (LiP), in the extracellular extract.

The LiP could be quantified only until 48 hours of cultivation, although when 0.1 g L^{-1} was added to the medium at time zero the activity could be quantified during the whole kinetics. Involvement of LiP in PCF removal is probable given the known metabolic activity of this enzyme.

Protease enzymes participate in the turnover of cellular protein, so finding the proteases which regulate other enzymes of interest with specific inhibitors might contribute to enhance PCF removal. In this work we managed to identify some aspartatic, serine and cysteine proteases.

The intracellular extracts were subjected to zymographic studies with L-DOPA as substrate, where a dark band of aproximately 203 kDa could be detected which corresponded to phenoloxidase activity. However, we could not confirm that this activity was tyrosinase, as results turned out negative using spectrophotometric and zymographic techniques with MBTH and tyrosine as substrates.

Extracellular β-glucosidase activity from the thermophilic fungus *Corynascus sepedonium*

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β-glucoside glucohydrolases (EC 3.2.1.21), commonly called β-glucosidases, catalyze the hydrolysis of β-glucosidic bonds in polysaccharides, releasing glucose from the non-reducing end. These enzymes are widespread in nature, occurring in microorganisms, plants and animals, and have attracted interest due to their participation in the conversion of cellulose to glucose. The efficient enzymatic hydrolysis of cellulose is still considered as the major bottleneck for the economical production of biofuels. One way to overcome deficient production of biofuels is to raise the reaction temperature, thus thermostability is a desirable enzymatic property.

The aim of the present study was to determine the extracellular β-glucosidase activity from the thermofilic fungus *Corynascus sepedonium*. For enzyme production, *C. sepedonium* was cultivated in basal medium of Mandel and Weber supplemented with 1% wheat bran at 45°C, in a rotary shaker at 140 rpm for 15 days. Samples were collected and analyzed every 24 h during cultivation. Crude extracellular enzyme was obtained by centrifugation at 8000 rpm for 10 min at 4°C. The supernatant was used as the source of crude extracellular enzyme. The β-glucosidase activity was assayed at 50°C in 50 mM citrate buffer, pH 5 using 5 mM p-nitrophenyl-β-D-glucopyranoside as substrate. After 5-15 min of incubation with crude extracellular enzyme, the reaction was stopped by addition of sodium carbonate 200 mM and the Absorbance of the product (p-nitrophenol) was read at 405 nm. One enzyme unit (U) was defined as the amount of enzyme that produces 1μmol of p-nitrophenol per min.

C. sepedonium produce β-glucosidase activity (42 U/ml) after 5 days of incubation at 45°C in liquid basal medium supplanted with 1% wheat bran as carbon source. The β-glucosidase activity in the culture supernatant from *C. sepedonium* showed 2-fold higher activity at 60°C than 50°C. The production of β-glucosidase activity by *C. sepedonium* was dependent of grown conditions and decreased to 4 U/ml after 5 days of culture when the fungus was grown in a medium with lower concentration of mineral salts. Currently, we are analyzing the biochemical properties (optimal temperature, pH, thermostability, etc.) of the extracellular β-glucosidase activity from *C. sepedonium*.

Heterologous expression of a protein with amorphogenetic activity on cellulose from B*jerkandera adusta* in A*rabidopsis thaliana* plants.

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Key words: Loosenin, Heterologous expression, Plants.

Introduction. The saccharification process during second- generation ethanol production from lignocellulosic material (plant biomass) is a major challenge given the highly recalcitrant structure of cell wall of plants, the main source of fermentable sugar storage in the form of polymers (cellulose and hemicellulose). To make the process more efficient, the use pretreatments that allow disruption of the lignocellulose structure, improving cellulose and hemicellulose accessibility has been proposed (1). The most common pretreatments are the physical and chemical, however biological pretreatments have additional advantages over the first two, such as low cost, safety and environmentally friendly (1). Cell wall remodeling proteins, such as expansins, swollenins and loosenin have recently been considered as a potential biological pretreatment tool. Loosenin is a protein from basidiomycete fungus *Bjerkandera adusta*. It showed to have the ability to modify the structure of cellulose, tested on cotton fibers and make them more susceptible to saccharification (2).
The aim of this work is to generate plants whose cell walls are more susceptible to enzimatic degradation through the heterologous expression of the protein LOOS1 of *B. adusta* in *A. thaliana* plants.
Methods. We cloned the *loos1* gene in a plant expression vector under an inducible promoter, pBINRD29A. For insertion of the construct pBINRD29::*loos1* in the Arabidopsis genome *Agrobacterium tumefaciens* 4404 will be used. Plant transformation will be accomplished by the floral dip method. Gene transcription and protein production will be corroborated by RT-PCR and Western blot analysis respectively. To evaluate the effect of the expression of LOOS1 on the susceptibility of the cell wall, plant material of transgenic plants will be treated with cellulolytic cocktails and reducing sugars assays will be performed.
Results. The gene was cloned into the intermediate vector pJET 1.2/Blunt, and thereby transformed cells *E. coli* DH5α. A clone with the correct sequence was selected to carry out subcloning into the expression vector pBINRD29-A. This clone was then transformed into *A. tumefaciens*. The *Agrobacterium* clone with the pBINRD29 :: *loos1* construction was selected for transforming plants of *A. thaliana* strain Columbia by the floral dip method on one month old plants. The first selection of transgenic plants by germination and growth of seeds on plate MS medium supplemented with kanamycin (resistance conferred by the vector) was obtained. This plants was transferred to pot. After three weeks growing under same conditions, transformed plants presented different phenotype compared to wild type plants.
Conclusions. The difference in the phenotype of the plants could have given by the early expression of the *loos1.*
References. 1.Salvachúa, Prieto A, López-Abelarias M, Lu-Chau T, Martínez A, Martínez M. (2011). *Bioresource Thechnology.* vol.(102.):7500-7506.
2. Quiroz-Castañeda R, Martínez-Anaya R, Cuervo-Soto L, Segovia L, Folch-Mallol J. (2011). *Microbiol Cell Factories* Vol (**10)**:8
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RADIAL GROWTH RATE, BIOMASS AND CELLULAR ULTRASTRUCTURE OF THE VEGETATIVE PHASE OF FUNGI FILAMENTOUS GROWN ON DI OCTIL PHTHALATE ISOLATED FROM A PAPER INDUSTRY

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Key words: filamentous fungi, di octil phthalate, vegetative phase

Introduction. Filamentous fungi are able to degrade lignocellulosic substrates. These fungi had two phases of growth; vegetative and reproductive. It has been reported that the wall of hyphae from the central zone of the colony that corresponds to mature hyphae (MH) was around twice the thickness of the wall from the periphery of the colony or young zone (YH)[1]. It has been reported that these fungi are able to grown on phthalates[2]. These are plasticizer that contributing to the environmental pollution[3]. The objective of this work was to evaluate the diameter of hyphae (DH), thickness from the cell wall (TCW) and growth of filamentous fungi grown on di octil phthalate (DOP).

Methods. Radial growth rate (u_r)[4] and biomass (Bm)[5] of filamentous fungi were evaluated in media containing mineral salts (MS) and different concentrations of DOP (mg/l): 1) medium containing MS (without DOP), 2) 500 of DOP+MS and 3) 1000 of DOP+MS. DH and TCW were evaluated using image analysis (Image Pro-Plus)[1].

Results. *Neurospora sitophila* had higher u_r than the other fungi. Strain P7 (in process of identification) had highest biomass (Table 1), DH and TCW in media containing 1000 mg/l of DEHP (Tables 2, 3).

Table 1. Radial growth rate and biomass of filamentous fungi grown on different concentrations of DOP.

Strain	Culture media					
	u_r (mm/h)			Bm (g/cm^2)		
	SM	SM+500 mg/l DOP	SM+1000 mg/l DOP	SM	SM+500 mg/l DOP	SM+1000 mg/l DOP
Neurospora sitophila	1.06^{a} (0.525)	1.19^{a} (0.039)	1.05^{a} (0.017)	0.004^{a} (0.0006)	0.008^{a} (0.001)	0.012^{b} (0)
Fusarium colmorum	0.75^{a} (0.005)	0.89^{b} (0.023)	0.55^{c} (0.009)	0.004^{a} (0.001)	0.010^{a} (0.002)	0.015^{b} (0.004)
Trichoderma atroviride	0.65^{a} (0.016)	0.50^{b} (0.048)	0.64^{a} (0.004)	0.002^{a} (0.0006)	0.004^{b} (0.0006)	0.005^{b} (0.001)
Hipocrea lixii	0.60^{a} (0.006)	0.66^{a} (0.001)	0.78^{a} (0.016)	0.003^{a} (0.0006)	0.003^{a} (0.001)	0.006^{b} (0.006)
Trichoderma harzianum	0.74^{a} (0.019)	0.44^{b} (0.072)	0.97^{c} (0.002)	0.008^{a} (0.006)	0.004^{b} (0.0004)	0.008^{a} (0.003)

Means with the same letter within a row are not significantly different. Data were evaluated using ANOVA and Tukey test. (P<0.01). Numbers in parenthesis correspond to SD of three separate experiments.

Table 2. Thickness from the cell wall of filamentous fungi grown on different concentrations of DOP.

Strain	TCW (µm)					
	Culture media					
	SM		SM+500 mg/l DOP		SM+1000 mg/l DOP	
	YZ	MZ	YZ	MZ	YZ	MZ
Neurospora sitophila	0.233^{e} (0.002)	0.234^{e} (0.003)	0.274^{d} (0.002)	0.304^{b} (0.004)	0.284^{c} (0.003)	0.329^{a} (0.001)
Fusarium culmorium	0.269^{d} (0.003)	0.264^{e} (0.003)	0.260^{e} (0.003)	0.307^{b} (0.005)	0.289^{c} (0.003)	0.346^{a} (0.002)
Trichoderma atroviride	0.232^{e} (0.002)	0.234^{e} (0.002)	0.252^{d} (0.003)	0.273^{b} (0.001)	0.258^{c} (0.002)	0.284^{a} (0.003)
Hypocrea lixii	0.233^{e} (0.002)	0.235^{e} (0.002)	0.256^{d} (0.002)	0.284^{b} (0.003)	0.268^{c} (0.001)	0.285^{a} (0.002)
Trichoderma harzianum	0.237^{e} (0.003)	0.235^{e} (0.003)	0.257^{d} (0.002)	0.272^{c} (0.003)	0.278^{b} (0.001)	0.280^{a} (0.001)

Means with the same letter w thin a row are not significantly different. Data were evaluated using ANOVA and Tukey test. (P<0.01). Numbers in parenthesis correspond to SD of three separate experiments.

Table 3. Diameter of hyphae of filamentous fungi grown on different concentrations of DOP.

Strain	DH (µm)					
	Culture media					
	SM		SM+500 mg/l DOP		SM+1000 mg/l DOP	
	YZ	MZ	YZ	MZ	YZ	MZ
Neurospora sitophila	1.59^{d} (0.086)	1.94^{b} (0.038)	1.76^{b} (0.021)	1.88^{c} (0.026)	1.96^{b} (0.01)	2.11^{a} (0.015)
Fusarium culmorium	1.59^{e} (0.015)	1.97^{c} (0.040)	1.85^{d} (0.053)	2.08^{b} (0.072)	1.99^{c} (0.026)	2.29^{a} (0.040)
Trichoderma atroviride	1.51^{f} (0.055)	1.71^{d} (0.036)	1.67^{e} (0.055)	1.89^{b} (0.079)	1.77^{c} (0.04)	1.97^{a} (0.040)
Hypocrea lixii	1.51^{e} (0.055)	1.71^{c} (0.036)	1.67^{d} (0.02)	1.89^{b} (0.015)	1.75^{c} (0.012)	1.97^{a} (0.035)
Trichoderma harzianum	1.51^{f} (0.055)	1.71^{c} (0.036)	1.62^{e} (0.02)	1.79^{b} (0.015)	1.65^{d} (0.012)	1.90^{a} (0.035)

Means with the same letter w thin a row are not significantly different. Data were evaluated using ANOVA and Tukey test. (P<0.01). Numbers in parenthesis correspond to SD of three separate experiments.

Conclusions. In general, the u_r, biomass, DH and TCW of the YZ and MZ were higher in media containing 1000 mg/l of DOP than in the rest of the media for all fungi. These results suggest that these fungi are able to use DOP as a sole source of carbon and energy.

References.

1. Sánchez C, Téllez M, Díaz G and Moore D. (2004). *Lett Appl Microbiol.* Vol. (38.) (6): 483-487.
2. Hwang, S. S., Kim, H. Y., Ja, K. O., Song, H. G. (2012). *J. Microbiol and Biot.* Vol (22) (2): 239-243.
3. Liang D, Zhang T, Herbert H y He J. (2008). *Appl microbiol biot.* (80.): 183-198.
4. Téllez-Téllez M, Díaz-Godínez G and Sánchez C. (2003). *Appl. Microbiol. Biotechno*l. Vol. (63)(2):212-216.
5. Díaz R, Sánchez C, Bibbins-Martínez M and Díaz-Godínez G. (2011). *Afr J Microbiol Res.* (5)(18):2720-2723.

Analysis of the overexpression of the *TRX1* gene and the deletion of *ATH1* gene in the production of dry active yeast

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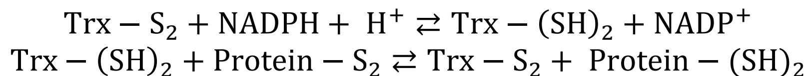
The yeast *Saccharomyces cerevisiae* is the most important microorganism in the alcohol production and alimentary industry. This feature makes this yeast the microorganism with the greatest commercial demand. For this reason it has been designed industrial process to generate yeast ready to be used. One of these processes is the elaboration of dry active yeast; this process is based in the aerobic production of yeast followed by a dried process. Nevertheless, this process is very aggressive, due to high tension of oxygen and the high osmolarity during the culture process, and the high temperature during the dried process, these parameters in the global process attempt against the viability of the cells.

We are analyzing the possibility to generate stronger yeast strains against the adverse effects during the elaboration of dry active yeast, by genetic modification of industrial strains.

Thioredoxin (Trx) is a small protein that can react with other oxidized proteins to reduce the damage caused by oxidative stress:



$$\mathrm{Trx} - \mathrm{S}_2 + \mathrm{NADPH} + \mathrm{H}^+ \rightleftarrows \mathrm{Trx} - (\mathrm{SH})_2 + \mathrm{NADP}^+$$
$$\mathrm{Trx} - (\mathrm{SH})_2 + \mathrm{Protein} - \mathrm{S}_2 \rightleftarrows \mathrm{Trx} - \mathrm{S}_2 + \mathrm{Protein} - (\mathrm{SH})_2$$

Thioredoxins can also facilitate the refolding of disulfide-containing proteins and modulate the DNA binding activity of some transcription factors. Furthermore, Trx is an efficient antioxidant able to reduce hydrogen peroxide, to scavenge free radicals, and to protect cells against oxidative stress. Additionally, It has been demonstrated that industrial yeast strains contains around of 20% of their dry weight of trehalose, this compound has been related with the resistance against heat shock. The levels of trehalose are regulated by synthesis and degradation, the first responsible of the degradation of trehalose is the acid trehalase encoded by gene *ATH1*.

In this work we analyzed the effect of the overexpression of the thioredoxin gen, *TRX1.* The ORF of the *TRX1* gene was fused to the promoter of the *PGK* gene and transformed industrial *S. cerevisiae* strains. We are analyzing the effect of the overexpression in the resistance against the oxidative stress. Furthermore we analyzed the effect of the deletion of the gene *ATH1,* in the *S. cerevisiae* strain Y07145, against the damage caused by high osmolarity and high temperature.

With the strains created we will analyze the biomass generated and cell viability during the production of active dry yeast in a pilot scale.

Hyphomycetes endophytes of *Taxus globosa* Schltdl. antagonists of *Fusarium* sp. and *Alternaria* sp. phytopathogenics of *Capsicum annuum* L.

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Introduction. The pepper (*Capsicum annuum*) is a vegetable that during production is affected by diseases caused by fungi of the genus *Fusarium* and *Alternaria*. And although there are several strategies to combat this study is to contribute to reducing the impact of agriculture on the environment, using *Taxus globosa* endophytes with antagonistic capabilities to control fungal pathogens in question.
Materials and Methods. Were isolated and identified from *Capsicum annum* phytopathogenic fungi of the genus *Alternaria* and *Fusarium*. Microcultures were made to identify and simultaneously reactivated and replated 22 strains of endophytes Hyphomycetes of *Taxus globosa*. For the in vitro bioassay double cultures were established (1, 2) and measured growth rate of both antagonists and plant pathogens as to determine antagonism.
Results and Discussion. 22 endophyte strains showed antagonistic activity against two plant pathogens, however only 4 strains showed the highest percentage of inhibition again *Alternaria*, and invaded to 75% of the surface of the petri dish with culture medium, showing the highest antagonistic activity after confronted by two weeks. Whereas with *Fusarium* only three endophytes had the best percentage of inhibition where antagonists colonized only half the surface of the medium contained in the petri dish so antagonistic activity are considered average.
Conclusion. Fungal endophytes of *Taxus globosa* have the potential to compete and inhibit the mycelial growth of *Fusarium* and *Alternaria* plant pathogens, so they can be considered as potential agents for biological control fungi that affect *Capsicum annuum* crops.
Literature Cited.
1. Bell, D. K.; Wells, Markham H.D. and C.R. 1982. In vitro Antagonism of *Trichoderma* species Against Six Plant Fungal Pathogens. Ecology and Epidemiology. Phytopathology 72:379-382.
2. Ting, A.S.Y., Mah, S. W. and T, C. S. 2009. Prevalence of endophytes Antagonistic Towards *Fusarium oxysporum* F. spp. *cubense* Race 4 in Various Plants. Am.-Eurasian J. Sustain. Agric. 3 (3): 399-406.

Regulation and function of NADPH oxidases in polarized growth and cell fusion in *Neurosporacrassa*

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Hansberg and Aguirre (1990)proposed that reactive oxygen species (ROS) play essential roles in cell differentiation in microorganisms. ROS are generated mainly during mitochondrial electron transport and by certain enzymes. The NADPH oxidases (NOX) are enzymes that catalyze the production of superoxide by transferring electrons from NADPH to O_2.*Neurosporacrassa* has two NADPH oxidases; NOX-1 and NOX-2 and the regulatory subunit NOR-1.The inactivation of *nox-2* only affects ascospore germination while deletion of *nox-1* results in mutants unable to differentiate sexual fruiting bodiesand defective asexual development and polar growth. The mutant phenotype of Δ*nor-1*issimilar to Δ*nox-1* and Δ*nox-2*phenotypes, indicatingthat NOR-1 regulates NOX-1 and NOX-2 activity.Δ*nox-1* and Δ*nor-1* strains are also impaired in cell-fusion, which might explain the growth defects in these mutants. Germinating conidia fuse through conidial anastomosis tubes or CAT.In the presence of the antioxidant N- acetyl cystein (NAC)the production CAT is delayed without of affecting the germination process. In contrast, a mutant strain lacking CuZn superoxide dismutase (Δ*sod-1)*shows increased CAT formation.Preliminary data show that a NOX-1::GFPfusion is located to the perinuclear ER and vacuoles during CAT fusion.In mature hyphae, NOX-1::GFP is locatedalong the cell membrane and also accumulatesat the hyphal tips, while NOR-1::GFP localizes at the hyphal apex and also at the cytoplasm in a particulate pattern.Partial co-localization of NOX-1 and NOR-1 duringhyphal elongation suggests that NOR-1 activates NOX-1 only atspecifics sites.Results obtained support that NOX-1 and NOR-1 act duringCAT formation and vegetative fusion, and both processes depend of the redox state of the cells. NOX-1 and NOR-1 also contribute to the polar growth in *N. crassa.*

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"In vivo" analysis of the transcriptional expression of the telomerase reverse-transcriptase gene in *Ustilago maydis*

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Telomerase is a specialized reverse transcriptase that synthesizes telomeres in eukaryotic cells. The enzyme has two central limiting components required for telomere synthesis. The protein subunit known as TERT (*te*lomerase *r*everse *t*ranscriptase) and an RNA subunit named TR o TER (*te*lomerase *R*NA). Expression of telomerase is regulated in metazoans by transcriptional, post-transcriptional and by epigenetic mechanisms during the cell- and life cycles. In those organisms telomerase become gradually down regulated as cellular development programs progress, until it is undetectable in differentiated cells. In human, TERT promoter is controlled through a plethora of transcriptional factors that recognize motifs scattered on approximately 2kb upstream the start ATG of the gene (Cairney and Keith 2007). *Ustilago maydis*, the dimorphic fungus causative of the corn smut disease, experiences developmental transition from saprophytic yeast to pathogenic mycelium. Transcriptional expression of genes controlling that developmental process requires of sequence elements distributed on tracts of up to 2 kb at 5' from its start ATG (Basse et al. 2006, FEMS Microbiol Lett.). *U. maydis*, is interesting for telomerase studies; the gene encoding the telomerase reverse transcriptase gene (*utert1*) is located in the locus um11198 (MIPS *Ustilago* database; http://mips.helmholtz-muenchen.de). Regulation of *utert1* through the two life styles of this fungus has been difficult because of the limiting quantities of transcripts and protein synthesized, mainly when tumors are used for analysis. Here we initiated the analysis of transcriptional regulation of *utert1* in wild type sporidia of *U. maydis.* To achieve this purpose, transcriptional fusions of the weak promoter of *utert1* with the reporter gene green-fluorescent protein (GFP) were assembled on shuttle vectors. Fusions were made on pUC18 backbone, using the UARS1 and the cassette harbored in the pMF3-c plasmid (Brachmann, 2004, Mol Gen Genomics), which contains the GFP encoding gene lacking promoter and with t_{nos} termination signals, and by the chimeric gene conferring carboxin resistance (Cbx^r). *utert1* promoter sequences were cloned 5' collinear to the reporter gene. After transformation using protoplasts, faint fluorescent signals were obtained in 8 - 10% of sporidia cultures grown in YEPS plus carboxin 4.2 µM, 24hs at 30°C with aeration. Stronger signals were obtained after 48h of incubation under the same conditions. Recently transformed cells show a higher percent of sporidia exhibiting gene reporter expression. Standardization of GFP detection by fluorometry is under way to settle conditions to measure GFP expression from constructs with progressively truncated versions of promoter regions.

GROWTH OF THE COLONY OF *PLEUROTUS OSTREATUS* GROWN ON DI (2-ETHYLHEXYL) PHTHALATE: FRACTAL DIMENSIÓN AND BIOCHEMICAL ANALYSIS

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Key words: Pleurotus ostreatus, Dimensión fractal, Di (2-etilhexil) ftalato

Introduction. *Pleurotus ostreatus* is a fungus able to degrade lignocellulosic substrates[1], It has been reported that these fungi are able to grown on phthalates[2]. Di (2-ethylhexyl) phthalate (DEHP) is a xenobiotic compound, provides flexibility and malleability to plastics[3]. The filamentous fungi that develop on toxic substrates, have colonies with irregular borders; This irregularity has been measured using tools such as the fractal dimension (Fd)[4]. In this work enzymatic activities, content of glucans and glycogen, and Fd were evaluated using image analysis (software Image J 1.43u).

Methods. The enzymatic activity and content of glycogen and glucans[5] of *Pleurotus ostreatus* ATCC26 were evaluated in media containing mineral salts (SM) but glucose and SM and different concentration of DEHP (mg/l): 1) medium containing SM (without DEHP), 2) SM+gluc, 3) 500 of DEHP+SM and 4) 1000 of DEHP+SM. Fd were evaluated using image analysis on (software Image J 1.43u).

Results. *P. ostreatus* had higher enzymatic activity of proteases, glucanases and chitinases in media containing 1000 mg/l of DEHP. Higher laccases production in media containing 500 mg/l of DEHP. The highest content of glycogen and glucans in media containing SM+gluc. **Tabla 1**. *P. ostreatus* had lower Fd in media containing 1000 mg/l of DEHP at 72h of growth**. Fig. 1 and 2.**

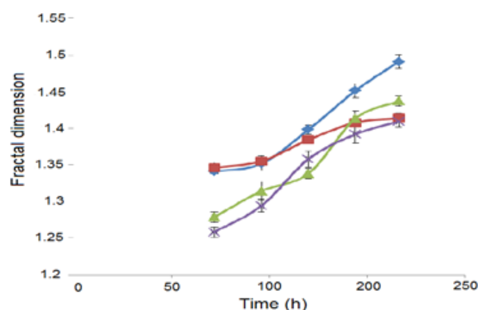

Fig. 1. Fractal dimensión in media containing SM (◆), SM+Gluc (■), SM+500 mg/L DEHP (▲) y SM+1000 mg/L DEHP (×).

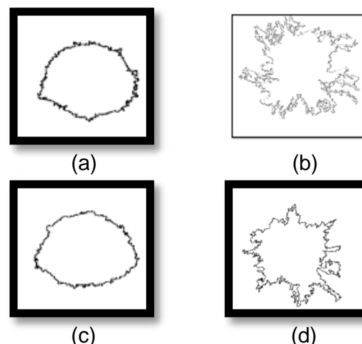

Fig. 2 Fractal dimension colony periphery *P. ostreatus* in media 500 mg/l at 24 h (a), 72 h (b) and 1000 mg/l of DEHP at 24 h (c), 72 h (d).

Tabla 1. Biochemical analysis of *P. ostreatus* in different media.

Biochemical parameters	Culture media			
	MS	MS+Glucose	MS+500 mg/L DEHP	MS+1000 mg/L DEHP
Laccases (U/gX)	39.11	27.65	150.52	60.95
Proteases (U/gX)	6532.10	11951.11	11048.15	9995.24
Chitinases (U/gX)	0.39	0	0.09	0.70
Glucanases (UI/gX)	7.02	5.61	21.31	21.53
Glycogen (mg/gX)	0.14	0.15	0.09	0.14
S-Glucans (mg/gX)	23.84	56.99	23.29	17.02
R-Glucans (mg/gX)	198.86	358.45	163.88	167.03

Conclusiones. *P. ostreatus* 26 had higher activity of proteases in media SM+gluc and SM+500 mg/l of DEHP. Had lower Fd in media containing SM+gluc and 1000 mg/l of DEHP at 72h of growth. Proteases involved in the formation of new hyphae, which allow minor colony irregularity present in the periphery.

References.

1. Sánchez C. (2009). *Biotechnol Advans*, Vol. (27.): 185–194.
2. Hwang SS, Hyoung TC y Song HG. (2008). *J Microbiol Biotechnol.* Vol. (18.): 767-772.
3. Liang D, Zhang T, Herbert H y He J. (2008). *Appl microbiol biot.* (80.): 183-198.
4. Lundy SD, Payne RJ, Giles KR y Garrill A. (2001). *FEMS Microbiol Lett*, Vol. (201.):259-263.
5. Díaz R, Sánchez C, B bbins-Martínez M and Díaz-Godínez G. (2011*). Afr J Microbiol Res.* (5) (18):2720-2723.

DIAGNOSIS AND MOLECULAR CHARACTERIZATION POISONING USING GENDER *AMANITA* ITS3 E ITS4 SEQUENCE OF *CANDIDA SP*

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INTRODUCTION. The poisoning by ingestion of fungal pathogens is performed by specific clinical picture of the patient, particularly if there have a history of mushroom ingestion. There isn't a specific and sensitive study that could confirm the poisoning by fungi as genus *Amanita*. The *Amanita muscaria* poisoning is one of the most common problems in countries that ingest natural edible mushrooms. ITS regions (Internal Transcribed Spacer regions) have been used in molecular biology for detecting and identifying pathogens in humans. Is difficult to identify poisoning by Amanita sp., so in this paper we proposes the use of endpoint PCR amplification using the primers panfungal ITS3 and ITS4 from *Candida sp* as a diagnostic for determining the presence in blood human, *Amanita muscaria* DNA. **METHODOLOGY**. DNA extraction was performed using the technique of chloroform isoamyl alcohol. PCR amplification was performed by the Quiagen kit and Taq PCR Master Mix. We were used the primers ITS3 and ITS4 from *Candida sp*. then with 1.4% gel agarose by electrophoresis were observed bands. Was used as positive control *Aspergillus* with a molecular weight of 400bp. **RESULTS** *Amanita* DNA was showed with two different molecular weight bands (300bp and 450bp), patients who ingest Amanita sp. showed a 300bp band. **CONCLUSION.** The primers from ITS3 and ITS4 regions of *Candida sp.,* could be used as a approach in the diagnostic of poisoning *Amanita poisoning*.

Neurospora crassa catalase-3 catalytic and C-terminal domains: an *in vitro* assay

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Catalase dismutates hydrogen peroxide (H_2O_2) and is important for fungal antioxidant response. *Neurospora crassa* has three catalases: two large size-subunit catalases (CAT-1, CAT-3), a small subunit catalase (CAT-4) and a catalase peroxidase (CAT-2). Large-subunit catalases have a carboxyl-terminal domain whose structure is similar to DJ-1 protein [1], a chaperone that inhibits aggregation of α-synuclein in the human brain. Mutation in DJ-1 causes congenic Parkinson disease. When the carboxyl-terminal of the CAT-1 is eliminated with subtilisin, the protein looses stability when it is incubated at high temperature and its capability to be activated with detergents. The complete CAT-1 avoids denaturation of others proteins such as citrate synthase and beta-galactosidase. In contrast, CAT-1 without the C-terminus domain is unable to stabilize other proteins [2]

We have expressed the C-terminal domain of CAT-3 in *E. coli* to determine if it also can stabilize other proteins. We amplified the last 465 pb of *cat-3* corresponding to the C-terminal domain, the complete gene and the gen without the C-terminal domain. These fragments were inserted in the plasmids pTYB1 and pTYB11 to fuse them to an intein then and the E. coli ER2566 strain was transformed with them. Expression was induced with IPTG at 15° C for 24 h and the proteins were then purified with a chitin affinity column. Intein is cleavage in the presence of DTT. However the expressed C-terminal domain remained bound to the column unless SDS was added. With the eluted C-terminal domain we will do experiments to determine stabilization of other proteins.

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1) Hansberg, W., Salas-Lizana, R:, Dominguez, L. (2013), Fungal catalases: Function, phylogenetic origin and structure, Archives of Biochemistry and Biophysics, 525, 170-180

2) Rangel-Silva P, Hansberg W, unpublished results.

The chromatin modifying enzymes HOS-2 and SET-5 are involved in development and response to light in *Trichoderma atroviride*

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Abstract

In *Trichoderma atroviride*, blue light regulates several processes such as development, conidiation and gene transcription. These responses are regulated by the BLR complex, which is formed by the BLR-1 and BLR-2 proteins, where the first has been proposed as the light sensor, and both function as transcription factors. Gene expression is strictly regulated at different levels, e.g. epigenetic regulation. This kind of regulation includes chemical modifications at the histone N-terminal tails, which affects chromatin structure and dynamics. These modifications on chromatin structure are performed by histone methyltransferases (HMT), histone acetyl transferases (HAT), histone deacetylase (HDAC), to allow the activation or repression of gene transcription. This on-off switch depends on the modified amino acid residue at the histone tails, the number and combination of these modifications. This work is aimed to dissect the role of some genes that encode for chromatin-modifying proteins that are expressed in response to light, such as the HDAC, *hos*-2 and the HMT *set*-5 encoding genes. The *hos*-2 deletion mutant show slow growth rate and is unable to conidiates in response to light stimulation or mechanical damage. On the other hand, *set*-5 deletion mutant conidiates applying low blue light intensities with an enhanced conidia production. In this sense, the *hos-2 and set-5* mutants showed altered levels expression of early and late conidiation after a blue light pulse. In addition, the *hos*-2 mutant was severely growth affected when exposed to different sources of oxidant stress at different concentrations in the media, like hydrogen peroxide (H_2O_2) and menadione ($C_{11}H_8O_2$). The *set*-5 mutants had similar growth rates when compared to wild type at all tested conditions. Thus, our data suggest that *hos*-2 y *set*-5 proteins could be regulating genes that are important for development, light perception and oxidative stress in *T. atroviride*.

Key words: *Trichoderma*, *hos-2* and *set-5* mutants, oxidative stress, development, conidiation genes

The adenylate-forming enzyme AfeA regulates asexual development in *Aspergillus nidulans*

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In the fungus *Aspergillus nidulans*, asexual sporulation (conidiation) is triggered by environmental signals such as exposure to air, starvation for nutrients and the presence of self-generated chemical signals. Conidiation depends on activation of the *brlA* gene, which encodes a Zn-finger transcription factor (TF).

The inactivation of genes *flbA* to *-E*, *fluG* and *tmpA* results in similar *fluffy* phenotypes, characterized by a delay in conidiophore and asexual spore formation. FlbA is a regulator of a heterotrimeric G protein signaling pathway that stimulates vegetative growth and inhibits conidiation. *flbB* and *flbC* encode transcription factors needed for proper *brlA* expression. *flbD* encodes a Myb TF that regulates both asexual and sexual differentiation. In contrast, *fluG* is responsible for production of an extracellular signal required for activation of *flbD-E*. *tmpA* encodes a flavoenzyme required for the production of a sporulation chemical signal independent of the *fluG* pathway.

Here we show that *afeA* as new gene whose inactivation causes a *fluffy* phenotype and decreased *brlA* expression. AfeA is a member the adenylate-forming enzyme superfamily. AfeA is most similar to unknown function coumarate ligase-like (4CL-Lk) and acetyl-coA ligase enzymes. A functional AfeA::mRFP protein shows cytosolic localization and consistent with its acyl-AMP function, active site replacement K540N eliminated most of AfeA function. We propose that AfeA participates in the biosynthesis of an acylated compound, either a fatty acid or a p-cuomaryl type compound, which could be oxidized by TmpA to produce the precursor of a PKS or NRPS-derived chemical signal that regulates cell communication and reproduction.

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Oaxaca, Oaxaca. 27 al 31 de Octubre de 2013. http://www.smb.org.mx/

GROWTH OF THE MYCELIAL AND REPRODUCTIVE PHASES OF *PLEUROTUS OSTREATUS:* BIOCHEMICAL ANALYSIS AND CELLULAR ULTRASTRUCTURE

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Key words: Pleurotus ostreatus, cellular ultrastructure, lignocellulosic residues

Introduction. *Pleurotus ostreatus* is a fungus able to degrade lignocellulosic substrates[1]**.** This organism had two phases of growth; vegetative and reproductive. It has been reported that the wall of hyphae from the central zone of the colony that corresponds to mature zone (MZ) was around twice the thickness of the wall from the periphery of the colony or young zone (YZ). It was also observed that the content of intracellular protein and glycogen from YZ was higher than that observed in MZ[2]. In this work enzymatic activities, content of glucans and glycogen, diameter of the hyphae and thickness of the cell wall were evaluated in the two phases of growth of *P. ostreatus.*

Methodology. *P. ostreatus* was grown on petri dishes containing dextrose potato agar (DPA) and MH was separated from YZ using a scalpel[2]. Fruit bodies of *P. ostreatus* of 0.5, 1.0, 2.0 and 4.0 mm tall were obtained in crystal trays containing APD. Activities of laccase, protease and glucanase, and content of glycogen and glucans were determined. In the vegetative phase and in the fruit bodies of the different stages of growth the diameter of the hyphae and thickness of the cell wall were evaluated[3].

Results. MZ and YZ showed the highest activity of laccases, proteases and cellulases. The fruit bodies showed higher content of *R*-glucans and *S*-glucans than the vegetative zone. YZ and the fruit body of 0.5 mm had the highest content of glycogen (Table 1). The thickness of the cell wall and the diameter of the hyphae were different in mature and peripheral zone of the colony (Fig. 1).

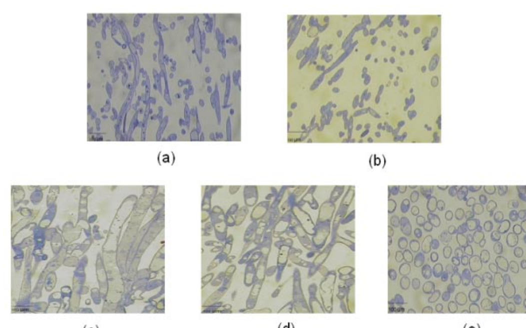

Fig1. Transmission electron micrograph of YH (a) and MH (b) of a colony of P. ostreatus, and from the base (c), stem (d) and pileus (e) of a fruit body 4 mm tall of *P. ostreatus*. Scale = 100 µm.

Table 1. Enzymatic activities and content of glucans and glycogen of the vegetative phase (YZ and MZ) and of fruit bodies 0.5, 1, 2 and 4 mm tall of *P. ostreatus*.

Biochemical parameters	Phases of growth					
	Vegetative phase		Reproductive phase			
	YZ	MZ	0.5 mm	1 mm	2 mm	4 mm
Laccases (U/gX)	17.645 ±0.334	16.108 ±0.670	0.830 ±0.017	5.577 ±0.358	4.754 ±0.200	4.619 ±0.085
Proteases (U/gX)	4.056 ±0.274	7.018 ±0.727	0	1.135 ±0.629	0	1.911 ±0.147
Glucanases (U/gX)	0.366 ±0.020	0.489 ±0.016	0.830 ±0.017	0	0.013 ±0.002	0.133 ±0.079
Endocellulases (U/gX)	0.126 ±0.040	0.443 ±0.080	0	0	0	0
Chitinases (U/gX)	0.008 ±0.002	0.006 ±0.001	0.054 ±0.003	0.039 ±0.004	0.031 ±0.006	0.132 ±0.032
Glycogen (U/gX)	0.981 ±0.067	0.297 ±0.040	0.843 ±0.068	0.838 ±0.060	0.995 ±0.070	1.297 ±0.112
S-Glucans (U/gX)	0.988 ±0.062	1.077 ±0.421	1.146 ±0.384	0.542 ±0.012	9.004 ±0.478	14.856 ±2.325
R-Glucans (U/gX)	2.406 ±0.177	9.593 ±0.508	0.415 ±0.045	0.451 ±0.017	7.120 ±1.336	31.528 ±4.547

Conclusions. Laccases and proteases activity play and important role in the invasion of the substrate and in the reproductive phase of the fungi, respectively. Fruit body of 4 mm tall had the highest content of glucans since it had the highest amount of mature hypha.

References.

1. Sánchez C. (2009). *Biotechnol Adv.* Vol. (27.): 185–194.
2. Sánchez C, Téllez M, Díaz G and Moore D. (2004). *Lett Appl Microbiol.* Vol. (38.) (6): 483-487.
3. Sánchez, C. (2004). *Appl Microbiol Biotechnol.* Vol. (64.) (5):691-4.

RAS-1 function during growth and differentiation of *Neurospora crassa*: effect of cysteine substitutions

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Ras is a small G protein (GTPase), which is related to various signaling pathway that regulates cell growth, proliferation and differentiation in eukaryotic cells. To function properly, Ras is post-translationally modified (farnesylated) and recruited to the plasma membrane where it interacts with its effector molecules. Ras cycles between the GDP bound (active) and GDP bound (inactive) forms. Alternation between the active and inactive form enables Ras to serve as a molecular switch, coupling membranes receptors to downstream signaling molecules. RAS activation increases with oxidative stress and reactive oxygen species activated Ras [1]. Ras interacts with proteins that have a Ras Association Domain.

In the fungus *Neurospora crassa*, the *ras-1^{bd}* mutant alternates between growth and conidiation in a cyclic manner. ROS levels affect cyclic conidiation, suggesting that RAS-1 regulates the cellular fate depending on the redox state of the cell [2]. Only three genes of *N. crassa* were detected to have a Ras Association Domain: the genes for adenylate cyclase, NCR-1 (a MAPKK) and Ste50 (an adapter protein).

RAS-1 has three cysteine residues in the C-terminal: C210 which is required for the location of RAS-1 to the cell membrane and C206 and C207 that could be related to its targeting to the membrane or to redox regulation of RAS activity. In the N-terminal, C23 could also be related to the regulation of RAS-1 by the redox state of the cell. The aim of this work is analyze the role of RAS-1 cysteine residues in the growth and cell differentiation of *N. crassa*. We tagged RAS-1 with the Human influenza hemagglutinin (HA) and thereafter C23S, C206S, C207S and C210S substitutions were introduced HA-RAS-1. With these mutant strains we are determining the effect of these substitutions on the growth and development of the fungus.

References:

1) Heo J, Campbell SL. 2006. Ras regulation by reactive oxygen and nitrogen species. Biochemistry. 45(7):2200-10

2) Belden WJ, Larrondo LF, Froehlich AC, Shi M, Chen CH, Loros JJ, Dunlap JC. 2007. The band mutation in Neurospora crassa is a dominant allele of ras-1 implicating RAS signaling in circadian output. Genes Dev. 21(12):1494-505.

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CHARCTERIZATION OF THE Δ*aps-2* MUTANT IN *Neurospora crassa*

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Microtubule organization depends on several associated proteins (MAPs),these proteins are related to the plus or minus end of cytoplasmic or mitotic microtubules (MTs). In filamentous fungi, the behavior of astral MTs and the movement of the mitotic spindle seem to be regulated by a plus end protein APSA(anucleate primary sterigmata), first described in *Aspergillusnidulans*.We found the homolog of ApsA in *Neurospora crassa* and we named it APS-2. The *N. crassaaps-2*genhas a high identity with ApsA and AMI1 of *Podosporaanserina*. In this study we analyzed the effect of the lack of APS-2 in a deletion mutant and observed the nuclear dynamics and the kinetic features of the null mutant during vegetative growth, asexual and sexual reproduction in comparison with a wild type strain (WT). We are showing preliminary data about the cellular features of the Δ*aps-2*deletion mutant compared with the WT. The Δ*aps-2* mutant showed a decrease of the elongation rate of 25%.Although hyphal growth is polarized, hyphae in the mutant grow in a meandering fashion. The mutant displays a higher number of branches and ishyperseptated. The cell wall distribution in the Δ*aps-2*mutant is abnormal; there is a higher concentration close to the hyphal tips. So far, we can conclude that the *aps-2*gene of*N. crassa*is not essential but affectscell growth probably due the effect of the alterations innuclear distribution.

Simultaneous expression of mating type information a and alpha in *Candida glabrata*, confers susceptibility to oxidative stress.

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Candida glabrata is an opportunistic fungal pathogen frequently isolated in nosocomial infections. *C. glabrata* is an asexual haploid yeast that has a closer phylogenetic relationship with *Saccharomyces cerevisiae* than with other *Candida* species such as *Candida albicans*. Even though *C. glabrata* is an asexual organism, it has conserved orthologues of the genes that control mating in *S. cerevisiae*. In *S. cerevisiae*, and *C. albicans* the control of cell-type identity, which is essential for mating, is achieved through a regulatory circuit between the **a1**, alpha1 and alpha2 proteins encoded at the mating type loci (*MAT* or *MTL*). The **a1** and alpha2 proteins in *C. albicans* and in *S. cerevisiae* form a heterodimer that is crucial for cell-type identity control and for other processes like the morphological switch (in *C. albicans*) and the response to some types of stress (in *S. cerevisiae*). *C. glabrata* contains the genes encoding these proteins, however, it does not maintain cell-type identity. It is possible that a heterodimer is also formed in *C. glabrata* and that it is involved in the response to stress.

We have asked first whether *C. glabrata* can form a heterodimer (**a1**/alpha2) using bimolecular fluorescence complementation (BiFC). This technique also allows us to determine the intracellular localization of the proteins **a1** and alpha2. We have constructed a positive control strain that expresses the yKu70 and yKu80 proteins with amino and carboxyl terminus of the fluorescence protein YFP respectively and in the opposite configuration. The results show a fluorescent signal in these strains, therefore validating the technique to detect protein-protein interactions in *C. glabrata*.

In addition, in order to determine whether the information at the *MTL1* locus (**a** or alpha), participates in the response to stress we have characterized phenotypically strains of *C. glabrata* expressing different mating information from *MTL1* under various stress conditions. Our results show that the strains expressing simultaneously both types of information are slightly more sensitive to different concentrations of H_2O_2 than the parental strain, suggesting that these genes may participate in the response to oxidative stress in *C. glabrata*.

Germplasm of strain fungus "COBIOCH-UAEM" of the Center of Biological Investigations, (biological and genetic resources of México)

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The confine and use of material fungus native Mexican in the investigation, are of great importance, since, our nation to the being one of the main countries more mega diverse, has a great quantity of biological resources that arrive to be considered as genetic resources by their great importance carry out in the development of the nation through investigations. The Laboratory of Mycology of the Center of Biological Investigations, has a maintenance program, conservation, characterization and improvement of the native Germplasm coming from different regions of the country. The strains, they are maintained active biologically through serial field, also, of the spores collection and in some cases genetic molecular elements. The Germplasm it has a total of 280 specimens including the Phylum Ascomycotas and Basidiomycota, being the Basidiomycotas those most represented ones, including orders like: Agaricales, Auriculariales, Boletales, Geastrales, Hymenochaetales and Polyporales. Inside the order Agaricales is the families Agaricaceae, PleurotaceaeSchizophyllaceae, Mycenaceae and Tricolomataceae, families very represented in modern genetic, molecular, cellular, biotechnology and social studies, for its great potential as genetic and biological resources, also, of its important rolethey play as essential elements in strategies for the social development to the around of the world. In the same way, inside the order Polyporales is the family Ganodermataceae which occupies the first place in studies with biotechnical and molecular focus for its great potential as medicines bio manufacture against diverse diseases, also, of the use of the enzymes in the biotechnical industry. The Germplasm maintains special collections of organisms used in the alimentary industry as *Pleurotus*spp, *Agaricus*spp, and *Ganoderma* spp., at the moment. This material is deposited in the Center of Genetic Resources of Edible Mushrooms (CREGENHC) of postgraduates' School (Mexico). At the moment, the Germplasm has been used in important studies for the bio-drugs production, and enzymes involved in the regeneration of places contaminated by compound xenobiotic.

In search of putative glutathione transporter in *Candida glabrata*

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Candida glabrata is an opportunistic human fungal pathogen, able to counteract oxidative stress both intrinsic and extrinsic generated by macrophages. Some of defense systems of *Candida glabrata* toward oxidative stress are catalases, superoxide dismutases and peroxidases. Also, glutathione is one of the most important non-enzymatic defense systems for this response. This metabolite is a non-protein thiol essential in almost all eukaryotic organisms, which participates in the detoxification of drugs and metals, and is important for the storage of sulfur and nitrogen, and the protection of proteins from oxidation by a process called glutathionylation. The biosynthesis of glutathione requires two enzymes, the γ-Glutamylcysteine Synthase and Glutathione Synthase, encoded by the *GSH1* and *GSH2* genes, respectively. We know that *GSH1* is an essential gene, and growth of the *gsh2*Δ mutant strain is improved when grown in the presence of glutathione. These results suggest that *Candida glabrata*, despite not having the orthologues Opt1 and Opt2 which are glutathione transporters in *Saccharomyces cerevisiae* (closely phylogenetically related yeast), could have a glutathione transporter which helps to improve its growth. In this study, we constructed a genomic library of *C. glabrata* and developed a genetic screening to identify the glutathione transporter in a *gsh1*Δ strain complemented with the *GSH1* gene in a plasmid. We screened for genomic library plasmid that would allow the *gsh1*Δ p*GSH1* strain loose the covering plasmid. We identified 2 plasmids, one containing the *GSH1* and the second which contains 4 different contiguouos genes. Further characterization of this plasmid suggests that one of the 4 genes is responsible for suppressing the lose of *GSH1*.

Role of the Atf1 transcription factor to different kinds of stress in *Trichoderma atroviride*

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In fungi, the Atf1 transcription factor, homologous to Atf2 of humans, is a target of stress-activated protein kinase (SAPK) which together acts regulating gene expression. Atf1 plays an important role due to it regulates stress response genes, and this is carried out once this protein is activated by phosphorylation through SAPK (Sty1/ SakA/p38) in the nucleus. In *Trichoderma atroviride*, light regulates expression of MAPK Tmk3 homologous to Sty1/SakA/p38. This MAPK protects the cell of different kinds of stress related to sunlight, in the same way it regulates some light responses in *T. atroviride*. To better understand this pathway and its relationship with light, in this work we obtained *T. atroviride* mutants lacking *atf1* gene. This gene encodes a basic-leucine zipper (bZIP) transcription factor which has all domains described in fungal homologous such as osmotic stress activation, homologous recombination activation and homologous recombination repression. Also, Atf1 has a bipartite nuclear localization signal suggesting its specific location into the nucleus. The strains lacking *atf1* (Δ*atf1*) were subjected to different kinds of stress, such as osmotic, oxidative, heavy metals (Cadmium) and DNA damaged by UV irradiation. Also we analyzed integrity of cell wall, growth and sporulation, always comparing with WT and Δtmk3 strains. The Δ*atf1* strains are sensitive to osmotic stress similar to the sensitivity observed in Δtmk3 strains, suggesting that Atf1 is an element of SAPK pathway such as has been described in other model organisms like *Schizosaccharomyces pombe* and *Aspergillus nidulan*. However, all Δ*atf1* strains grown slower that Δtmk3 and WT, indicating additional roles of this transcription factor probably independent of MAPK Tmk3. Additionally, we are analyzing gene expression by RT-PCR analysis to elucidate if Atf1 regulates genes in response to light or to other different kinds of stresses.

Functional Characterization of the MAPKK Pbs2 in *Trichoderma atroviride*

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Eukaryotic cells adapt their physiology to respond to changing extracellular conditions for enhanced fitness and survival. There are three MAPK (Mitogen-Activated Protein kinase) signaling pathways highly conserved in fungi, which they use to respond appropriately to the environmental stimuli. In *T. atroviride*, light induces expression of the stress-induced MAPK pathway, specifically the genes encoding MAPK Tmk3 (Hog1/Sty1/SakA/p38) and MAPKK homolog to Pbs2 of *Saccharomyces cerevisiae*. The MAPK TMK3 protects the cell of different kinds of stress related to sunlight and it regulates some light responses in *T. atroviride*. To better understand this pathway and its relationship with light, in this work we generated mutants lacking of gen *pbs*2 (IdTa 157499) in *T. atroviride*. The strains lacking of *pbs*2 gene (Δ*atf1*) were subjected to different kinds of stress, such as osmotic, oxidative, heavy metals (Cadmium) and DNA damaged by UV irradiation. Also we analyzed integrity of cell wall, growth and sporulation, comparing with Wild Type (WT) and Δtmk3 strains. The Δpbs2 strains show phenotypes practically identical to those detected in Δtmk3 mutants. All Δpbs2 strains are more sensitive to osmotic and oxidative stress, heavy metals and to UV irradiation than WT strain. The Δpbs2 and Δtmk3 strains grown slower than WT and their conidia production are less abundant both in constant illumination as well as with a light pulse. Also, the conidia pigment is pale (light yellow) in Δpbs2 strains as compared to WT.

Physiological analysis of an acatalasemic *Saccharomyces cerevisiae* complemented with the catalases from the euryhaline yeast *Debaryomyces hansenii*

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Debaryomyces hansenii has two catalases genes (*Dh*CTAand *Dh*CTT), both exhibit higheractivity than those of *S. cerevisiae*. In previous experiments with *D. hansenii* we observedcatalase T activity only in cells on late stationary phase growing on rich media with glucose, whereas cells growing on non-fermentable carbon sources as ethanol showed high catalaseA activity. However, the corresponding transcripts were always present (Segal-Kischinevzky,et al, 2011). Two plasmids with either catalase T or A coding gene were constructed, including1000 bp upstream and 400 bp downstream to keep the 5´-UTR, the promoter and the 3´-UTR. The genes were amplified by PCR and cloned into pRS316. Both plasmids weretransformed separately into anacatalasemic double mutant of *S. cerevisiae*. Our resultsshowed that both genes successfully complement catalase activity, measured byspectrophotometry and zymograms. The expression of *DhCTA*gene improves the doublingtime of the complemented mutant when it is grown on YP ethanol medium, whereas *DhCTT*improves the maximum biomass reached when it is grown on rich medium (YPD). The present work shows the differences seen between wild type *S. cerevisiae* strain and our complementedstrains, growing in rich media with a fermentable (glucose) or a non-fermentable (ethanol) source of carbon, and in absence or presence of NaCl at average sea water molarity (0.6M); throwing light into possible hypothesis and implications of the expression of these genes and their resulting physiology. As an instance of these differences, the expression of both *DhCTA*and *DhCTT*genes improvedthe resistance to a peroxide shock in exponential growth phase in rich media with glucose, being *DhCTT*the gene that bestowed a better resistance.

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Fluconazole resistance in *gsh1Δpro2-4* mutant *of Candida glabrata*

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Candida spp. cause serious infections in immunocompromised patients. Their prevalence has increased in the last decade. The common treatment for fungal infections is based in azolic agents. *Candida glabrata* has innate resistance to fluconazole, and this is dependant of the Pleiotropic Drugs Resistance (PDR) system, which is regulated by the transcriptional controller Pdr1 and two main transporters, Cdr1 and Cdr2. When fluconazole is present, Pdr1 conformation changes and promotes transcription of the efflux fluconazole pumps. Fluconazole inhibits the lanosterol 14 α-demethylase (encoded by *ERG11*), a key enzyme in the biosynthesis of ergosterol. As a consequence of accumulation of toxic 14-methyl sterols, cell membranes destabilize. Moreover, fluconazole induces oxidative and nitrosative stress. Glutathione (GSH) is a pivotal molecule to detoxify xenobiotics such as fluconazole and to remove reactive species. GSH is synthesized by sequential action of Gsh1 and Gsh2, encoded by *GSH1* and *GSH2*, respectively. Therefore, we wanted to evaluate the fluconazole resistance of a *gsh1Δ* null mutant. Suprisingly we found that a suppressor mutation in *PRO2* (which encodes a key enzyme of the proline biosynthesis pathway) is necessary for the construction of a *gshΔ1* null mutant, thus generating a *gsh1Δpro2-4* mutant. In this work, we evaluated the fluconazole resistance of a *gsh1Δpro2-4* mutant. We found *gsh1Δpro2-4* mutant to be more resistant to fluconazole than the wild type strain. In order to know if the resistance depends of the PDR system, we deleted the *PDR1*, *CDR1* and *CDR2* genes in the *gsh1Δpro2-4* mutant background. We found that the double mutants are more resistant to fluconazole than single mutants. When we reconstituted the *GSH1* gene to the *gsh1Δpro2-4* mutant in its chromosomic context, we also found it to be more fluconazole resistant. However, a *pro2-4* mutant maintains wild type fluconazole resistance levels. We suggest a link between the glutathione and proline biosynthesis pathways. This connection could help *C. glabrata* to export the fluconazole through unknown transporters, not Cdr1 or Cdr2.

FUNCTIONAL HETEROLOGOUS COMPLEMENTATION OF *Δtps2 Ustilago maydis* MUTANTS IMPAIRED IN THE SYNTHESIS OF TREHALOSE

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Trehalose is a disaccharide formed by two molecules of glucose, we can find it in bacteria, fungi, insects, plants but not in vertebrates. In fungus, trehalose is synthesized by the TPS/TPP (Trehalose Phosphate Synthase/Trehalosa Phosphate Phosphatase) pathway, initially described in *Saccharomyces cerevisiae*. The trehalose-phosphate phosphatase Tps2, dephosphorylates trehalose 6-phosphate generating trehalose (T) and inorganic phosphorous (Pi). In some organisms, trehalose metabolism is involved in cell´s response after challenged with different kind of stresses, such as temperature shifts, osmotic or oxidative stress, ions and even the presence of heavy metals. In some fungi, the concentration of intracellular trehalose is increased when the organism is under stress. There is evidence, that mutants unable to synthesize trehalose are sensitive to different stress conditions, and the virulence is diminished, like in *Magnaporthe grisea, Cryptococcus neoformans* or *Candida albicans*.

Ustilago maydis is a basidiomycete fungus pathogen of maize, which causes the disease named “huitlacoche”. Keeping in mind the importance of trehalose in some fungi, we pursued to study its role in *U. maydis*, then we deleted the encoding trehalose phosphate phosphatase gene (*UmTPS2*). The phenotype shown by these mutants, revealed its importance during the response to cell wall, oxidative and osmotic stresses, and also to ultraviolet radiations. Besides, *Δtps2* mutants are less virulent against corn plants, revealing the importance of trehalose in the general physiology of *U. maydis*. In order to determine if the phenotypic traits displayed by *Δtps2* are due to *UmTPS2* deletion, we proceeded to the genetic complementation either heterologous or homologous. Here, we presented our results obtained using a quimeric version of *TPS2* gene. We used *S. cerevisiae´s TPS2* (ORF), whose protein has the two conserved glycosyltransferase and the trehalose phosphatase domains. We cloned this ORF under the control of the regulatory sequences of the native *UmTPS2* gene. With this construction we transformed *U. maydis Δtps2* mutants. Currently we are analyzing the phenotype of some complemented strains under different stress conditions.

ELUCIDATING THE ROLE OF THE HYDROLYSIS OF INTRACELLULAR TREHALOSE IN *Ustilago maydis´* PHYSIOLOGY

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Trehalose is a non-reducing disaccharide that is commonly found as a storage carbohydrate in eukaryotic cells, but also implicated in the response to various environmental stresses. The exact mechanisms of trehalose protection are not fully understood, but during stress or dehydration, the sugar can stabilize proteins by replacing water molecules, being considered as a molecular chaperone. Research conducted to elucidate the biological role of trehalose in eukaryotes has been limited predominantly to the study of free-living species such as the yeast model *Saccharomyces cerevisiae*. Regarding to fungal plant pathogens, little is known about the trehalose metabolism, in the case of *Magnaporthe grisea*, degradation of trehalose is important before cuticle penetration. About plant pathogens, it is also important to consider that in order to have success during plant colonization /infection, fungi must quickly respond to several challenges, some of them considered as stress.

We wonder if there is any relevance of trehalose metabolism during this whole process in *Ustilago maydis*. We would try to understand the role of trehalose catabolism in this basidiomycete plant pathogen. This fungus is the causal agent of the smut disease in corn, commonly called in Mexico “Huitlacoche”, considered as a delicacy in Mexico and in some countries, but in the rest of the world. Worth to mention is the fact that after an organism increases its intracellular trehalose content due to a stress condition, this carbohydrate should rapidly be reduced to normal values, being hydrolyzed to glucose by the action of neutral trehalose enzyme. In yeast, there are two encoding neutral trehalases genes: *NTH1* and *NTH2*. We conducted an *in silico* search in *U. maydi*s´ genome, and we identified the putative and unique *U. maydis* neutral trehalase gene named Um11661.1 (*NTH1*). A DelsGate strategy to interrupt this gene was adapted, putative transformants are currently being analyzed to determine the proper gene deletion. The corresponding mutants will be studied regarding its general response to abiotic stress, its virulence and the quantification of the amount of intracellular trehalose accumulated after cells are under stressed conditions.

ROS ACCUMULATION IN IDIOPHASE AND ITS RELATIONSHIP WITH LOVASTATIN BIOSYNTHESIS IN *Aspergillus terreus*

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Lovastatin (LOV) is a secondary metabolite and it has anticholesterolemic properties[1]. This molecule is produced by *Aspergillus terreus* by submerged fermentation (SmF) and also by solid-state fermentation (SSF). *sod1* expression, ROS content, and redox balance kinetics were measured during SmF and SSF using an industrial type culture medium and *A. terreus* TUB F-514, a high producing strain. Results showed that *A. terreus sod1* gene (oxidative stress defense enzyme) was intensely expressed during rapid growth phase (trophophase) of LOV fermentations. This high expression decreased abruptly, just before the onset of production (idiophase). However, ROS measurements detected high concentrations only in idiophase, suggesting a link between ROS and LOV biosynthesis. Apparently *sod1* down regulation promotes the rise of ROS during idiophase. This oxidative state in idiophase was further supported by a high redox balance observed in trophophase that changed to a low value in idiophase (around six-fold lower). The patterns of ROS accumulation, *sod1* expression, and redox balance behavior were similar in SmF and SSF. However, *sod1* expression and ROS concentration (ten-fold) were higher in SmF. Our results indicate a link between ROS and LOV biosynthesis. In a subsequent work we showed that ROS regulated LOV biosynthesis (Miranda et al., in press).
Moreover, analysis *in silico* of *lovE* promoter (transcription factor of LOV biosynthesis) was performed using The MEME Suite[4] and JASPAR database software[5]. The analysis of *lovE* promoter revealed the existence of one putative binding site for Yap1p. This protein is a transcription factor that responds to changes in redox cellular status[2]. To study the mechanism by which ROS may regulate LOV biosynthesis, the expression of *yap1* during LOV SSF and SmF, was analyzed by Northern-blot. The transcription analysis indicated that *sod1* and *yap1* show similar patterns of expression during LOV fermentations: strong expression during trophophase and down regulation in idiophase, generating an increase in ROS concentration during production phase. These results suggest that, in *A. terreus*, Yap1 regulates *sod1* gene, as has been shown in yeast and other fungi. Furthermore, the down regulation of these genes in idiophase appears to contribute to generate the ROS accumulation in idiophase, which in turn induces and sustains LOV biosynthesis (Miranda et al, in press). Results also suggest that AtYap1 acts as a negative regulatory protein, since its production is down regulated in idiophase.

References:

1. Manzoni M and Rollini M (2002) *Appl Microbiol Biotechnol*. 58:555-564.
2. Moye-Rowley W (2003) *Eukaryotic Cell*. 2(3): 381-389.
   Web resources:
3. http://meme.nbcr.net/meme/
4. http://jaspar.binf.ku.dk/

Study of colonization of *Arabidopsis thaliana* plants by *Trichoderma atroviride* expressing a *Coriolopsis gallica* laccase.

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Trichoderma is a genus of filamentous fungi that display a remarkable range of lifestyles and interactions with other fungi, animals and plants. Because of their ability to antagonize plant-pathogenic fungi and to stimulate plant growth and defense responses, some *Trichoderma* strains are used for biological control of plant diseases (1).

Besides the different molecular mechanisms involved in the *Trichoderma*-plant interaction which trigger the defense response of the plant, there are physical barriers such as the plant cell wall that confers protection against biotic and abiotic stresses. Therefore, one of the challenges facing *Trichoderma atroviride* to efficiently colonize the plant is to modify the plant cell wall. This structure is made up of lignocellulose that consists of three biopolymers: cellulose, hemicellulose and lignin, this polymer is insoluble, chemically heterogeneous, amorphous and highly branched. It provides structural support, rigidity and protects the structural polysaccharides (cellulose and hemicellulose) from degradation.

Laccases are polyphenoloxidases having four copper ions in the active site and catalyze the oxidation of various phenolic compounds in the presence of mediators, and coupling the reduction of molecular oxygen to water. These enzymes oxidize lignin and generate highly volatile aromatic radicals that favor depolymerization (2).

This work aims to evaluate the colonization of *Arabidopsis* plants by *Trichoderma atroviride* strains expressing a *Coriolopsis gallica* laccase. Our hypothesis is that degradation of lignin in the root mediated by laccase could favor the colonization of *Arabidopsis* plants, causing increased plant defense mechanisms against pathogens and promoting its growth. This transformant strain could represent an option for use as a bio-fertilizer for plants.

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1) Hermosa R. Viterbo A., Chet I. y Monte E. (2012) Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology*. 158:17–25.
2) Giardina P., Faraco V., Pezzella C., Piscitelli A., Vanhulle S. y Sannia G. (2010) Review: Laccases: a never-ending story. *Cell. Mol. Life Sci.* 67:369–385.

OVER EXPRESION OF THE *swo1* GENE IN *Trichoderma atroviride* and EVALUATION OF THE MICOPARASITIC ACTIVITY OF THE TRANSFORMANTS STRAINS.

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Introduction:

Chemical fungicides are hazardous compounds affecting human healthand the environment, application of biological control agents like *Trichoderma spp*. is a promising alternative for plant protection. Members of the genus *Trichoderma* (*Teleomorph Hypocrea*) are potent mycoparasitic fungi that not only compete for nutrients but also secrete cell wall-degrading enzymes (CWDEs) such as chitinases, glucanases, and proteases.Expansins are plant proteins, which are known to be involved in the remodeling of cell wall structures during growth and other processes (1). Other proteins with expansin-like activity have been identified in fungi, which haven been named Swollenins (Swo), the first *swo1*, was identified in *Trichoderma reesei* (2). Another *Trichoderma* species, *T. atroviride*, has been used as a model in phytopathogenic fungi control studies (3). A gene that encodes for a protein with similarity to s*wo1* was found to be highly expressed in the first stages in the process of mycoparasitism of *Trichoderma atroviride* with several phytopathogenic fungi (4).

Is of our interest to study the function of the *swo1* gene of *T. atroviride* during mycoparasitism.

Results.

The *swo1* gene was cloned via RT-PCR from whole mycelium RNA of *T. atroviride* collected during direct confrontations with phytopathogens.The gene was subcloned in the expression vector *pUE10*. Transformation of *T. atroviride* was made through a protoplasts technique. After three monosporic passes, several transformant strains of *T. atroviride* that overexpress the *swo1* gene were obtained and confronted to different pathogenic fungi.

The *swo1* overexpresant strains show a more aggressive mycoparasitic behavior as judged by large necrosis zone evident in the confluent zone of the interacting fungi. This is probably due to the loss of structure in the cell wall of the phytopathogen caused by swollenin activity.

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References. 1. Qiang Y., Ting-Ting S., Wei-Feng L., Guan-Jun C. (2008). *Biosci. Biotechnol. Biochem.* 72(11):2799-2805. 2. Saloheimo M., Paloheimo M., Hakola S., Pere J., Swanson B., Nyyssönen E., Bhatia A., Ward M., Penttilä M. (2002). *Eur. J. Biochem.* 269: 4202-4211. 3. Argumedo-Delira R., Alarcon A., Ferrera-Cerrato R., Peña-Cabriales J.J. (2009). *Rev. Int. Contam. Ambient.* 25(4):257-269. 4. Reithner B., Ibarra-Laclette E., L. Mach R., Herrera-Estrella A. (2011). *Appl. Environ. Mocrobiol.* 77(13):4361-4370.

Phylogenetic analyses of endoxylanases from *Colletotrichum sp.*

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The genus *Colletotrichum* includes a number of plant pathogens of major importance, causing diseases of a wide variety of mono and dicotiledons plants. *C. lindemuthianum* is an intracellular hemibiotrophic pathogen of bean. After penetration of a host epidermal cell, an infection vesicle is formed and the fungus extends into adjacent cells by large primary hyphae which invaginate without penetrating the host cell membrane, thus persisting as a biotrophic interaction. Once a large area of the plant tissue has been colonized, necrotrophic hyphae develop and this event closely correlates with the production of a battery of host cell wall-degrading. Among these, xylanases are thought to play a major role in pathogenesis.
Deduced amino acid sequences of xilanases reported for eight Colletotrichum species, xilanases Clxil1 from a pathogenic and a saprophitic races of *C. lindemuthianum* isolated in this study, and a xylanase sequence from *Phaedon cochleariae* as an external group, were used to generate distance trees. Phylogenetic analysis showed that the xylanases are grouped into three clades, where Clxil1 xylanase from *C. lindemuthianum* Race 1472 and R0 were grouped with xilanase from *C. gloeosporioides Nara_gc5* and in another clade with *C. orbiculare* (goup I). The results show an basal separation of xylanases from *C. graminicola M1.001* and C. *higginsianum* (Group III), both pathogens of monocotyledoneus. Apparently there is a different source for these enzymes, those from pathogens of monocotyledonous on one side and on the other side enzymes from dicotyledonous pathogens hosts (group I) followed by a diversification of these enzymes in more recent evolutionary process (group II). Interestingly, for fungi that have more of a xylanase in their repertoire, these enzymes are not in the same group, as in the case of *Colletotrichum gloeosporioides* and *C. graminicola*.

We believe that these xylanases of *Colletotrichum sp.* evolved independently according to its host (mono or dicotiledons).

In depth transcriptome analysis of *Trichoderma* spp.-*Arabidopsis thaliana* – interaction

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Trichoderma spp. are haploid, filamentous hyphomycetes. Several *Trichoderma* species have been used as model systems to investigate mycoparasitism, antibiosis, induction of tolerance to stress to plants, plant growth promotion, and induction of systemic resistance to pathogens (jgi, 2013). It is well known that *Trichoderma* spp. prime the plant systemic resistance to pathogens through jasmonic acid/ethylene-induced systemic resistance (ISR) and salicylic acid-systemic acquired resistance (SAR) signaling pathways (Tucci *et al*., 2011; Shoresh *et al*., 2010). Studies on *Trichoderma*-plant interaction had shown that the plant proteome and transcriptome changes as result of coculture with *Trichoderma spp.* (Marra *et al*., 2006). These results have been associated with priming and enhanced resistance to pathogens. Until now, the *Trichoderma* research community has focused mainly on the response of the plant to *Trichoderma*, the response of the fungus to the presence of the plant has been poorly studied, so the question about the involved mechanisms that mediate this interaction remains to be elucidated. *Arabidopsis* plants were grown in MS solid medium and covered with a layer of cellophane, then the plants were inoculated at the root tips with *T. virens* or *T. virens* wild type strains. Samples were taken at 48, 72 and 96 h post-inoculation and total RNA was extracted to generate cDNA libraries. Validation of the transcriptome was performed by means of the evaluation of *sm1* gene induction. We used SOLID sequencing and identified 518, 236 and 53 genes differentially expressed at 48, 72 and 96 h respectively. Our results showed a transcriptional reprogramming in *Trichoderma* spp. associated with the presence of the plant. To validate the RNA-Seq results, we will use real-time RT-PCR to analyse the expression levels of differentially expressed genes.

Study of Cultivable Fungi Associated with Rihzospher of *Pinus Chiapensis*

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Key words: Pinus chiapensis, rizosfera, hongos.

INTRODUCTION. The forests and jungles around the world are really important because of their carbon storage capacity and for harboring a large number of organisms that contributes to the ecological balance of earth. Mexico occupies the 12th place in terms of forests and jungles existence. About 70 species of pines of 120 existing species in the world are in Mexico and approximately half are endemic from the national territory. Unfortunately a dozen of they are in danger of extinction for different reasons.

Pinus Chiapensis is found in states of Puebla, Veracruz, Chiapas and Guerrero as part of their cloud forests. Due their exploitation and another causes no well know, this pine is cataloged as a threatened species and with special protection.

Preliminary studies have shown that the seed germination of *P.Chiapensis* can be influenced by soil microorganisms. Is well know how the microorganisms can decisively influence in the establishment of a large number of plants, with activities of bio control of pathogens, moving populations, biofertilizers, phytostimulants, and biosolubilizing.

In this study was identified the cultivable fungal microflore associated with *P.Chiapensis* in their natural environment.

METHODS. Two stands of *P.Chapensis* were sampled in northern highlands zone of Puebla state, extracting small amounts of rhizosphere soil that formed a composite sample. The samples was sieved, homogenized, and were realized dilutions that were seeded in different media, for obtaining cultures in different conditions of temperature and wet. The morphologic identification was made according to the traditional criteria. For the microscopic identification of fungal, axenic cultures were obtained, and from these, micro cultures technique were made, which were stained with blue cotton and identified through a microscopic test (40x), following taxonomic keys described in specialized texts.

Results. Species richness from basal cultivable mycobiota of soil asociated to *Pinus chiapensis* from locations of Hueyapan and Cuautempan, ubicated at the Northern highlands of state of Puebla, was composed by 66 species of microorganisms incluiding actinomycetes, yeasts and filamentary fungal, cover in 15 genera and 19 species (environmental, pathogen, and opportunistic). The dominant genera found were *Paecilomyces* sp (Tichocomaceae Family) and *Trichoderma* sp (Hypocreaceae Family).

Conclusions. Were found genera and species of fungal with beneficial and pathogen potential for *P.Chiapensis* establishment.

References. Del Castillo R.F. Trujillo Argueta S. and Sáenz Romero C. 2009. *Pinus chiapensis*, a keystone: Genetics, ecology and conservation. Forest Ecol Manag 257: 2201-2208.

Farjon, A., J. A. Pérez de la Rosa & B.T. Styles. 1997. Guía de los Pinos de México y América Central. Royal Botanic Garden & Universidad de Oxford. pp. 3-5

Villaseñor, J.L & O, Téllez-Váldez.2004.Distribucion potencial de las especies del género *Jefea* (Asteraceae) en México. *Anales del Instituto de Biología*. Serie Botánica 75(002):205-220.

MAPKs mediating the capacity of biocontrol in *Trichoderma atroviride*

MAPKs: mediadoras de la capacidad biocontroladora de *Trichoderma atroviride*

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Abstract.

Trichoderma atroviride is a potent biocontrol agent, which attacks and parasitizes a number of plant pathogenic fungi using different mechanisms such as formation of infection structures, secretion of cell wall degrading enzymes, antifungal metabolites or antibiotics. Furthermore, it induces plant defense responses. We analyzed the activity of three genes encoding mitogen-activated protein kinases (MAPK's): *tmk1, tmk2* and *tmk3* to evaluate the participation of the corresponding signal transduction pathways. Mutants were examined during fungal growth, conidiation, mycoparasitic interaction and antibiosis, as well as in assays to determine their capacity to induce plant defense responses mediated by the jasmonic and salicylic acid pathways. All mutants exhibited a reduction in radial growth (Δ*tmk1* 14%, Δ*tmk2* 50%, Δ*tmk3* 25%) when exposed to light, as compared to the parental strain, but there were no significant differences in darkness. In confrontation assays, Δ*tmk1* and Δ*tmk3* mutants showed reduced mycoparasitism effect against *Botrytis cinerea and Rizoctonia solani* AG-5. In contrast, the Δ*tmk2* mutant produced a clear zone of inhibition of growth on *Fusarium oxysporum, Botrytis cinerea, F.* sp, *Sclerotium rolfsii* and *Alternaria alternata* that is not observed when confronted with the wild type. In experiments to evaluate the antibiotic effect of Trichoderma, all strains were capable of completely inhibiting the growth of the phytopathogens, except *F.* sp and *F. oxysporum.* These data indicated that MAPK's signal transduction pathways are involved in the expression of mycoparasitism-related genes but no in antibiotic production. We are currently conducting experiments towards determining the role of MAPKs in the induction of plant defense responses.

Key words: *Trichoderma atroviride*, mycoparasitism, MAPK's, antibiosis.

Fusarium species infecting maize in Sinaloa, Mexico

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Stalk, ear and root rot caused by *Fusarium* species is a recent problem in Northern Sinaloa, México. In 2006, the Municipal Agency for Plant Health in Northern Sinaloa, detected a high percentage (70-84%) of maize plots showing wilting, foliage death, stalk rot, lower leaves yellowing, stunting, proliferation of aerial roots, among other symptoms (unpublished data). This symptomatology was first attributed to *F. oxysporum* and later, using molecular techniques the pathogen was identified as *F. verticillioides*. To date, there is still controversy about the identity of the fungus or fungi causing fusariosis. In the present work we characterized 93 isolates of *Fusarium* isolated from maize seeds, stalks and roots in Sinaloa, Mexico during the years 2007-2009 and 2011. Total genomic DNA was extracted from frozen mycelium by grinding in liquid nitrogen and using the DNeasy Blood and Tissue kit (QIAGEN). A portion of the calmodulin gene was amplified with the PCR primer pair Ver1 5´-cttcctgcgatgtttctcc-3´ and Ver2 5´-aattggccattggtattatatatcta-3´. Following PCR, amplicons were purified with the QIAquick PCR Purification Kit and sequencing made in Langebio (Cinvestav, Irapuato). Sequence alignment and phylogenetic analysis were performed in Mega 5 beta using MUSCLE aligner and the neighbor-joining method.

Three *Fusarium* species were detected; *Fusarium verticillioides* (*Fv*), *F. nygamai* (*Fnyg*) and *F. thapsinum* (*Fthap*). This is the first report of *Fnyg* and *Fthap* in Mexico. 64 isolates were identified as *Fv*, 27 as *Fnyg* and 2 as *Fthap*. The distribution of species was different in each plant part. *Fv* was the most abundant species found in maize seeds (47 isolates) followed by *Fnyg* (3 isolates) and *Fthap* (2 isolates), while in roots *Fnyg* was the most abundant species (24 isolates) followed by *Fv* (16 isolates) and *Fthap* was not detected. The isolate obtained from stalk was identified as *Fnyg.* These differences in the proportion of species, suggests a spatial distribution or tissue preference for each species on maize plants. Mixed infection (*Fv* and *Fnyg*) was detected in several individual plants.

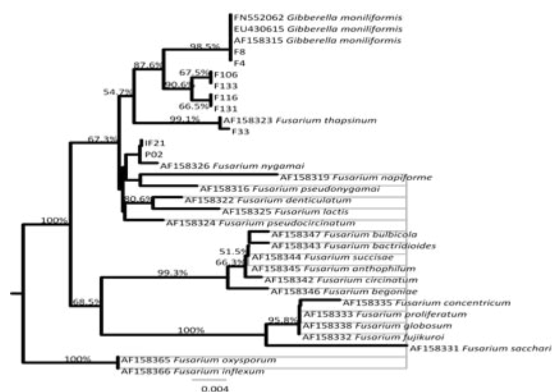

Fig. 1 Distance tree (Neighbour-Joining) derived from partial sequences of the calmodulin gene of 9 Fusarium isolates and 23 reference sequences (O´Donnell et al., 2000). Tree was constructed using Mega 5 Beta (bootstrap = 1000) using Kimura 2 parameters substitution model with gamma distribution. Bootstrap values are indicated as percentage

Aggressiveness of *Gibberella fujikuroi* isolates on maize seedlings

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The genus *Fusarium* comprises some of the most important pathogens in agriculture. *Fusarium verticillioides* (*Fv*) is the most common pathogen of maize in the world and the cause of stalk, root and ear rot. Recently, other species such as *F. nygamai* and *F. thapsinum* have been associated with the same symptoms in Sinaloa, México. Mixed infections were detected in several cases, which arose the question of whether all species of *Fusarium* isolated from maize are pathogenic. Aggressiveness of sixty one isolates of *Fv*, fifty isolates of *Fnyg* and two isolates of *Fthap* was determined. Disinfected maize seeds were soaked for 5 min in a 1 x 10^6 mL^{-1} conidial suspension and control seeds were soaked in sterile distilled water. The rolled paper towel technique was used for pathogenicity assessment. All treatments were done by triplicate using fifty seeds per treatment. The bioassay was maintained at 25 ± 2°C with a photoperiod of 14:10 h (L:D) for 14 days. Severity damage in root and stalk was measure with the scale of García-Espinoza (2009). Height, germination percentage, root and stalk biomass were determined at the end of the bioassay. All isolates were able to cause fusariosis symptoms. Differences in aggressiveness were observed between *Fusarium* species and isolates of the same species. The isolate F52.1 was the most aggressive of all isolates causing a 93.3% of root and stalk damage. Isolates were grouped into three aggressiveness groups (Figure 1); slightly aggressive (SA; 20 isolates), moderately aggressive (MA; 24 isolates) and highly aggressive (HA; 34 isolates).

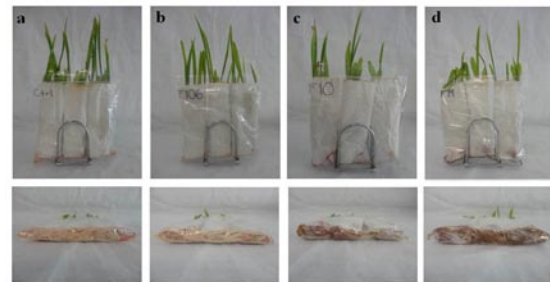

Fig. 1. Aggressiveness groups. a) Control plants; b) SA group; c) MA group; d) HA group.

Fv isolates were evenly distributed into the three groups, while *Fnyg* isolates were mainly HA. *Fthap* isolates registered low damage percentage (24% and 45%) and grouped in SA and MA groups (Figure 2). This could be explained by differences in host preference by *Fthap*, since this is a well known pathogen of sorghum.

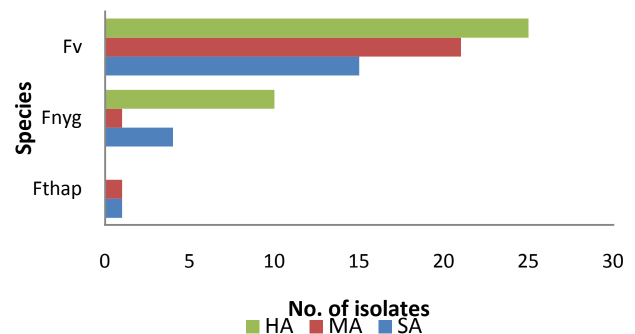

Fig. 2. Aggressiveness groups in each species

Search of the gene coding for the histidine kinase (*HK*) protein of the nematophagous fungus *Pochonia chlamydosporia* var. *mexicana*

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The aim of this work was to find the gene encoding the *HK* protein in the genome of the nematophagous fungus *Pochonia chlamydosporia*. We did an *in silico* search for nucleotide or amino acid sequences homologous to the *HK* protein. We selected the sequence XM_003004935.1 of *Verticillium albo-atrum*. We designed primers, taking part of the region encoding the histidine kinase domain, and the first and second HAMP domain. The native isolate Pcp21 (*P. chlamydosporia* var. *mexicana*) was grown on corn meal agar and the genomic DNA was extracted. PCR primers were designed from *HK* of *V. albo-atrum*. The amplification reaction of the *P. chlamydosporia HK* gene had shown two amplicons (400 and 900 bp). The PCR products were purified and then sequenced. The nucleotide sequences obtained were subjected to a Blast analysis in GenBank (http://www.ncbi.nlm.nih.gov) and we observed an identity >70% with genes encoding *HK* protein of *V. albo-atrum*, *Sporothrix schencki* and *Nectria haematococca*. The nucleotide sequences were translated into protein and they were were subjected to a Blastp analysis, additional we did other analysis on the prosite Expasy platform. Such analysis shown: phosphorylation sites of protein kinase C and protein Casein kinase II, N-glycosylation sites and the N-myristoylation. These sites are characteristic of *HK* protein which indicates that the protein is found in the *P. chlamydosporia* genome. From the 400 bp fragment primers were designed to perform PCR-RACE and thus find the complete gene coding for the *P. chlamydosporia HK* protein. So far we had a 500 bp fragment which includes the 3 'end gene. This is a preliminary work that reveals the presence of histidine kinase gene in the genome of *P. chlamydosporia* var. *mexicana* and therefore the two component system which could be involve in the saprotrophic to parasitic transition of *P. chlamydosporia*.

Mutation of gene *Swo1* of fungus *Trichoderma atroviride* and evalution of its mycoparasitic activity against pathogenic fungi

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Plants are constantly faced with the risk of being attacked by fungal pathogens. Plant pathogens are estimated to cause yield reductions of almost 20% in the principal food and cash crops worldwide. Although these losses can be minimized by the use of disease-tolerant crops, crop rotation etc. In modern agriculture has progressively increased the use of chemicals as pesticides to protect plants. But it can also cause bioecological and human health problems. The control of plant pests by biocontrol agents is a better alternative and friendly to the environment.
The genus *Trichoderma* is used as a biological control agent. Filamentous fungi of the genus *Trichoderma* are common inhabitants of the rhizosphere. Moreover, many years ago *Trichoderma* species known to improve plant growth and productivity of axenic systems and soil are directly protected from the pathogens.

Reithner et al., (2011) made a comparative analysis of the transcriptome of *Trichoderma atroviride* IMI206040 during interaction with phytopathogenic fungi *Rhizoctonia solani*. In this study, transcript fragments of 7,797 genes of *Trichoderma* were sequenced. The differential transcription of 13 genes was confirmed by RT-PCR (including swo1, encoding an expansin-like protein) in the presence of *Rhizoctonia solani*. The swollenin (expansins type protein) in fungi have the ability to deconstruct different cell wall polysaccharides.
Preliminary data in the laboratory show that overexpression of the gene *Swo1* in *Trichoderma atroviride* increased pathogenicity against phytopathogenic fungi. To corroborate the role of swollenina in mycoparasitism, in this work we want to analyse mutant strain *Swo1* of *Trichoderma atroviride* and evaluate mycoparasitic activity against phytopathogenic fungus.

Potentially pathogenic yeasts in the nostril of volunteers and outdoors at the Universidad Autónoma Metropolitana Unidad Xochimilco

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The bioaerosols are microscopical particles of biological origin or organism fragments that can be found in the air and could cause different diseases such as allergies, toxicities or infections in human beings. As a part of the bioaerosols, we could find the yeasts that use the air as a media of dispersion by currents or by adhesion to another particle. When they find an adequate substrate and an ideal temperature that can facilitate the growth, the yeasts easily develop. Therefore, they have high impact in human health. Approximately exist 30 species that cause human diseases, within them we could find *Candida albicans, C. tropicalis, C. guillermondii, C. lusitaniae, Rhodotorula glutinis, Cryptococcus neoformans*. However, these yeasts are found as commensals in the human body, although if any immune alteration in the common human microbiota, diseases can be triggered. The aim of the present study was identify the yeasts that can be founded in the nostril from 13 volunteers, and those obtained from environmental samples at the Universidad Autónoma Metropolitana Unidad Xochimilco. Nasal washes were performed to the volunteers to obtain the samples that were cultured in Rose Bengal Agar supplemented with chloramphenicol, and Petri dishes were placed in 5 areas of the University with the same medium to obtain environmental samples by impaction. Identification of yeasts was performed by using the API® 20 C AUX system. The assay allowed the identification of four genera and eleven species, including *C. guillermondii, C. lusitaniae, C. tropicalis, Cr. laurentii, Cr. neoformans, R. mucilaginosa, Trichosporon mucoides*. Results suggest that yeast could be airborne organisms that can maintain viability and germinate in the nostril. Many of these yeasts are potentially pathogenic to immunocompromised hosts, but on air can be found in low amounts. To our knowledge this is the first report of airborne yeasts of Universidad Autónoma Metropolitana Unidad Xochimilco.

Key words Yeasts, nasal washes, environment samples.

Methyl jasmonate restores the effect of root silencing of PvLOX2 on mycorrhiza induced resistance in common bean leaves

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Additionally to the nutritional benefits that the mycorrhiza association brings to colonized plants, arbuscular mycorrhiza confers resistance against necrotrophic pathogens. Similarly to rhizobacteria, mycorrhiza colonization is able to induce a priming state, which developed previously to the pathogen attack, and allow the plant to activate defense responses faster and stronger when challenged to the pathogen. Although there are evidences indicating that mycorrhiza induced resistance (MIR) require an intact jasmonic acid (JA) signaling pathway, the mechanism that regulates priming by mycorrhiza colonization is still unknown. In previous studies in our lab, we have shown that RNAi silencing of lipoxygenase 2 gene of common bean (PvLOX2) in roots of composite plants, blocked the onset of MIR on common bean leaves against *Sclerotinia sclerotiorum*, a necrotroph foliar pathogen. PvLOX2 silencing, however, did not alter the establishment and development of mycorrhiza colonization. It was also shown that silencing of PvLOX2 in roots, down-regulated PvLOX6 in leaves. Since PvLOX2 is a 13-LOX (subgroup 1), and putatively cytoplasmic, which is suggested by the lack of a chloroplastic transit peptide, the role of this gene in JA biosynthesis is ruled out. PvLOX6, on the other hand, is a 13-LOX (subgroup 2), localized in chloroplasts and involved in the JA biosynthetic pathway. Based on the fact that silencing of PvLOX2 in roots caused a significant reduction of PvLOX6 in leaves, and possibly a decreasing on JA production, which correlates with the blockage of MIR, it was hypothesized that exogenous methyl jasmonate (MeJa) to leaves of composite PvLOX2RNAi plants could restore MIR. In order to test this hypothesis, leaves of colonized PvLOX2 RNAi plants were excised and treated with 50 μM MeJa and challenged to *S. sclerotiorum* in a detached leaf assay. Necrotic lesion measurements showed that MeJa-treated PvLOX2RNAi leaves from mycorrhiza colonized plants developed fewer lesions than non-MeJa colonized PvLOX2RNAi controls, restoring the resistance observed in non-transformed mycorrhiza colonized plants. Up regulation of PvLOX2, PvLOX6, and AOS genes in MeJa-treated leaves of PvLOX2RNAi mycorrhiza colonized plants suggests that this phytohormone might be responsible for restoring induction of resistance by affecting the expression of some oxylipin pathway genes.

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Identification of entomopathogenic fungi from Queretaro State to control maize pests

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The chemical control of pest in crops with the goal of increase agricultural yield and supply enough food for the population has a negative impact on the environment and human health. The pesticides are highly toxic, persistent, and unspecific causing pollution of water natural resources and soil, besides beneficial organisms are killed as well. This situation has been generated concern, then changing the agricultural practices and also looking for friendlier strategies leading to get a healthy environment are important. In this sense, biological control has been successfully applied and when it is appropriately established, it will work for long time. The entomopathogenic fungi are commonly used to control plant- pest, in fact there are several commercial products based mainly on *Metarhizium* and *Beauveria* conidia. Given that the fungus establishment can be affected by the particular whether conditions at some specific location, is important to isolate and identify native fungal strains, then after its physiological characterization use them to try to implemented an biological control strategy at a specific area. Nowadays, in collaboration with CESAVEQ (Comité Estatal de Sanidad Vegetal de Querétaro, A.C.) we are collecting dead insects (apparently killed by fungi) mainly grasshoppers (*Melanoplus* spp., *Sphenarium purpurascens* y *Brachystola* spp.), fall armyworm (*Spodoptera frugiperda*), white grubs (*Phyllophaga* spp. y *Anomala* spp.), earworm (*Helicoverpa zea*). At the lab, insects are incubated in a humid chamber until fungal growth is evident and then, it fungus is propagated on PDA plates with antibacterial agents. In our collection, we identified at least three different fungal species which are being identified by taxonomic dichotomous keys, considering mainly growth, colony morphology, pigments, fruiting body and conidia among others. Also, these dataset obtained will be corroborated by sequencing of the genomic ribosomal DNA.

Biochemical mechanism involved in the onion growth promotion and control of *Alternaria porri* and *Sclerotium rolfsii* by *Trichoderma*

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The *Trichoderma* fungi are used in the biological control of plant diseases. Principally, *Trichoderma* are antagonistic fungi of soil pathogen but could be also used in the control of foliar pathogens. The control mechanisms of *Trichoderma* involve parasitism, competition and antibiosis. Furthermore, *Trichoderma* promotes the plant growth and resistance. Onion production is affected by diseases caused for fungi such as *Sclerotium rolfsii* and *Alternaria porri* in the Morelos state, Mexico. *S. rolfsii* is a soil fungus that generates the disease "southern blight". *A. porri* is a foliar pathogen and causes the disease "purple stain". The two pathogens could be controlled by *Trichoderma* through of different cellular and biochemical mechanisms and a specific mechanism can vary within different pathosystems. In this study were evaluated some of the mechanisms of *Trichoderma* strains to promote growth of two onion varieties and to control *S. rolfsii* and *A. porri*.
The TrC1-TrC4 strains were isolated of onion crops and the TrJ strain of tomato crop from state of Morelos, México. T337, T479 and T359 strains were obtained of the fungi collection from “Colegio Superior Agropecuario del estado de Guerrero”, Mexico. The commercial products used were Tricovel and PHC T22. The strains of *S. rolfsii* and *A. porri* (AP1 and AP2) were isolated of onion crops from the Morelos state, Mexico. The HA039 strain of *A. porri* (AP3) was obtained of the fungi collection from “Colegio de Postgraduados”, Mexico state, Mexico.
The T337, TrC1, TrJ strains inhibited the mycelia growth reach up 40% and presented antagonism against both pathogens. TrC2-4 strains also inhibited the mycelia growth of *A. porri* by antibiosis and/or parasitism. The Ticovel product not inhibited the mycelia growth and did not show antagonism against the two pathogens. The T22 product inhibited the mycelia growth reach up 40% of the three *A. porri* strains, but not inhibited the mycelia growth of *S. rolfsii*. Only, the TrC1 and TrJ strains promoted the bulbs growth of the two onion varieties. This effect was related with their ability to produce indoleacetic acid (phytohormone) and by liberating phosphorous from organic compounds such as phytates. The morphological characteristics of TrC1 and TrJ indicate that correspond to the species *T. atroviride*. These two *Trichoderma* strains have potential to promote the onion growth and to control the diseases caused by *S. rolfsii* and A*. porri*.

Characterization of the cell wall and its role in the innate immune response against *Candida parapsilosis*

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Candida parapsilosis is an opportunistic fungal pathogen commonly associated with nosocomial infections in newborns and immunocompromised patients. The fungal cell wall is the first point of contact with the host cells, thus, this structure influences in a very strong manner the fungus-host interaction and plays an important role in the recognition of the pathogen and the triggering of the host immune response. In this work we aimed to characterize the cell wall of *C. parapsilosis* and how it influences the recognition by peripheral blood mononuclear cells (PBMCs). We found that *C. parapsilosis* cell wall is mainly composed by polysaccharides of glucose, mannose and glucosamine, as well as proteins, distributed in two layers. An inner layer mainly composed by chitin and β-glucans, which become exposed when the cells are heat inactivated, and an outer layer rich in mannoproteins. Although this results are similar to those reported in *C. albicans,* the cell wall porosity is higher in *C. parapsilosis*, suggesting that the β-glucan layer is more exposed at the wall surface, probably due to a small size of the mannans. In agreement, *C. parapsilosis* showed lower levels of cell wall phosphomannan. These results are significant for the immune recognition, since *N*- and *O*-mannans linked to glycoproteins, along with β-glucans, represent the major pathogen-associated molecular patterns recognized by the immune receptors MR, TLR4 and dectin1, respectively. In order to investigate the importance of the *N*-linked mannans during the *C. parapsilosis* immune recognition, we generated an *och1*Δ null mutant, which has truncated *N*-linked mannans at the cell wall. The characterization of the mutant showed an increment in the chitin and glucan amounts, together with higher relative porosity of the cell wall. An increment in the phagocytosis by monocyte-derived macrophages compared with the wild type was also evident, along with a different profile of secreted cytokines when PBMCs were stimulated with the null mutant. Taking together, these results provide new information about the immune response elicited against *Candida* non-*albicans* species, showing remarkable differences in the pro-inflammatory cytokine profile compared to those reported in *C. albicans* despite the similarity in the cell wall composition of these two species.

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Comparison of abundance and infectivity of Arbuscular Micorrhizal Fungi isolated of two different zones of Tlaxcala, Mexico

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Key words: Arbuscular Micorrhizal Fungi, organic soil, stress conditions.

The Arbuscular Micorrhizal Fungi (AMF) are among the most common organisms of soil rhizosphere and one of the most important symbiotic associations in nature that can colonize extensively the roots of many plants. They can assists its plant host in phosphorus (P) and nitrogen (N) uptake, also benefit plants by increasing water uptake, plant resistance and biocontrol of phytopathogens, adaptation to a variety of environmental stresses such as drought, heat, and salinity, and that helps to maintaining soil fertility. We know that survive in diverse extreme conditions and environments, therefore, they are potentially useful to apply in sustainable agriculture. The objective of this work was isolate, identify and evaluate infective propagules of AMF from two zones of Tlaxcala, Mexico, one of them located in Xalostoc, Tlaxcala. The soil was an organic soil cultivated with common bean *(Phaseolus vulgaris)*. The second one was located in El Carmen Tequexquitla, Tlaxcala, a zone with salt and drought conditions where the flora was grass. We made a physicochemical analysis of the two soils. We isolated and counted the spores of 15 soil sub-samples from each one, spores were extracted by wet sieving and decanting, we identify the spores by taxonomic characters, the infectivity and most probable number (MPN) of AMF propagules were quantified through this bioassay. We found 3 predominant genera: *Acaulospora, Glomus* and *Gigaspora*. The abundance and infectiveness were significantly different. We conclude that the zone that is not used for agriculture did not shown elimination of propagules, and the spore abundance is higher in contrast with the organic soil used for agriculture. Probably as a consequence of minor agricultural practices is the natural regeneration in soil that promotes the maintenance of the natural micro-rhizosphere which includes the AMF.

Identification of fungi associated with avocado scab

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Avocado scab is a disease affecting avocado cortex producing a corky aspect, such damage apparently has not impact on organoleptic characteristics, been still consumed by farmers. However, commercialization is seriously affected and considering that Mexico is the main producer on the world, then it is necessary to identify which is or are the causal agents of this disease, how this agent gets into the plant tissue and penetrates, damaging in this way the fruit. Nowadays, this avocado´s disease has been attributed mainly to the fungus *Sphaceloma perseae* although research about it is really scarce.

This study was performed on samples collected from orchards located at San Joaquin municipality in the Queretaro State. These samples were taken from tissues of flowers, leaves and fruits. Different fungal strains were isolated from these samples including the genera: *Colletotrichum*, *Sphaceloma*, *Fusarium*, *Rhizoctonia* and *Rhizopus* by using dichotomous taxonomic keys. Showing in this way that *Sphaceloma perseae* is not the only fungus associated with avocado scab. In order to determine the molecular identity of these fungi associated with avocado scab, we amplified the ribosomal DNA using primers ITS1 and ITS4.

These fungi associated with avocado scab will be used to infect fruit and avocado leaves under controlled laboratory conditions in order to determine which of those are directly related to the typical symptoms observed during avocado scab disease.

Structural and molecular analysis of biofilm formation *in vitro* by *Aspergillus fumigatus-Staphylococcus aureus*

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Background. The mixed biofilms are events present in nature that bring us closer to the real conditions of survival. They can be found in causing negative effects such as keratitis (Peters et al., 2012), which has been characterized as coinfections caused by bacteria and fungi, being *A. fumigatus* second in frequency of isolation (Hernández-Camarena et al., 2012). Therefore, it is important, to conduct studies on microbial interactions.

Objective. Analyze and determine the structure of the biofilm formed *in vitro* for *A. fumigatus*, *S. aureus* and fungus-bacteria interactions, clinical isolates from patients with infectious keratitis.

Methodology. Identification of the etiological agents was confirmed. Optimum conditions molecular were established for biofilm formation *in vitro* of *A. fumigatus*, *S. aureus* and their interaction in polystyrene plates of 96 wells. Biofilm formed was quantified by the method already described (Peeters et al., 2008), and observations were made by scanning electron microscopy (SEM).

Results. The identity of *A. fumigatus* (Af) and *S. aureus* (Sa) was confirmed. Standardization of biofilm formation for all models (Af, Sa, Af-Sa) was accomplished. The mixed biofilm (Af-Sa), compared with single fungal biofilm (Af), is limited. This effect is evident in the establishment and formation *in vitro* of the biofilm, which was verified by SEM.

Conclusion. Our results suggest an antagonistic phenomenon between *A. fumigatus* and *S. aureus* in the formation *in vitro* biofilm mixed, when compared with each of the microorganism biofilms.

References.

- Peters B. *et al.,* (2012). Clin. Microbiol. Rev.; 25(1):193-213.
- Hernández-Camarena *et al.*, (2012). Rev. Mex. Oftalmol. ; 86(4):213-222.
- Peeters E. *et al.*, (2008). J. Microbiol. Methods; 72: 157–165.

The relevance of the cell wall in the immune sensing of *Candida parapsilosis, C. orthopsilosis and C. metapsilosis* by human mononuclear cells

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Candida parapsilosis is an opportunistic fungal pathogen usually associated with infections in hospitalised patients, especially in neonatal wards and immunocompromised patients. It has the second place among the causative agents of candidemia, and a natural resistance to some antifungal drugs, such as echinocandins. *C. parapsilosis* is in fact a complex composed of three closely related species: *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis*, which have important differences in antifungal drug sensitivity and virulence. Recent studies have reported that *C. orthopsilosis* and *C. metapsilosis* are responsible about 1-10% of *C. parapsilosis*-associated infections.

The fungal cell wall is the first point of contact between the fungus and host cells, and is a protective shield composed of polysaccharides and proteins. So far, it is unknown the cell wall composition and organization in these three species, but it is assumed it should be similar to that described for *C. albicans*, which has and internal layer of chitin and β-glucans and an external layer composed of mannoproteins. These glycoproteins along with β1,3-glucans are the main pathogen-associated molecular patterns recognised by the host immune system.

Here, we are aiming to study the cell wall composition and organization of *C. parapsilosis, C. orthopsilosis* and *C. metapsilosis*, and to assess the relevance of its components in the sensing by primary human peripheral blood mononuclear cells (PBMCs). Our preliminary results indicate that the cell wall phosphomannan content is significantly reduced in *C. parapsilosis* and *C. orthopsilosis.* This wall moiety is a key ligand recognised by macrophages during the phagocytic process. When human PBMCs were challenged with yeast cells, we found that the cytokine profiles elicited by members of the *C. parapsilosis* complex are significantly different of those stimulated by *C. albicans*. *C. parapsilosis sensu stricto* stimulated high levels of TNFα, IL-6 and IL-10, and we found that its cell wall has the β-glucan more exposed on its surface than *C. albicans*. *C. metapsilosis* is capable to produce significantly high IL-10 levels, which suggest it is a better stimulus for an anti-inflammatory state than other members of the complex. To our knowledge, this is the report indicating that members of the *C. parapsilosis* complex are differentially recognised by innate immune cells.

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Phenotypic characterization of the phytopathogenic fungi *Fusarium temperatum* and construction of a chimeric vector for the interruption of the histidine kinase gen

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The genus *Fusarium* counts with a big variety of species which causes a variety of diseases in plants as the vascular wilt in tomato, beans, cranberry, potato and stem rot of corn. Other genres produce mycotoxins that affect the quality of some grains. The plant – microorganism interactions begin with the interchange of chemical signals, this signals are processed by a signal transduction system emitting an environmental adapting answer. This environmental adapting response is known as the two-component system which has a histidine kinase protein, an intermediate protein and a response regulator protein. The aim of this study is mutate the histidine kinase protein gen *HK*I of *Fusarium temperatum* to analyze the role of this gene in the interaction fungi- plant.

Firstly we characterized the growth of the isolated fungi obtained from soil of Chapingo, State of Mexico in plates of PDA medium, obtaining hairy, flat, spreading colonies with a yellow pigment on the reverse at the three days of growth, but at the seven days the fungal colony turns purple with a dark purple pigment on the reverse. Then we made a slide culture to observe the microscope morphology of the fungi obtaining hyaline, septate mycelium and hyaline, macroconidia which are septate (3-4). To complete the identification of the fungi isolated of soil, we amplified two molecular markers, one the ITS (ITS1-5.8S rDNA-ITS2) and the elongation factor (EFα) with which find a 100% of identity with *Fusarium temperatum.* Also we did an enzymatic characterization in media with different carbone sources like xylan, pectin, cellulose and evaluate its phosphate solubilizing activity.

To create the chimerical plasmid that we will use to interrupt the gen, first we obtained the *HK* gene sequence of *Fusarium oxysporum* in the database FCGP (Fusarium Comparative Genomics Platform http://fcgp.fusariumdb.org), then we did an *in silico* analysis of the flanking sequences of the gen with the object to obtain primers which allowed the amplification of the selected fragments with the respective cleavage sites of restriction enzymes compatible with the pTef1/Zeo plasmid.

The next step in our research is to obtain the mutants and characterized them by enzymatic characterization and pathogenicity against tomato plants.

Antifungal and phytotoxic activity of endophytic fungi from *Gliricidia sepium* (Fabaceae)

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Endophytic fungi are microorganisms that live inside plant tissues without causing disease symptoms[1,2]. Their relationship with the host plant is sometimes considered beneficial because endophytes are able to produce bioactive metabolites that the host can use as defense mechanisms against pathogens and herbivores and that permit the survival of both organisms[2,3]. Because they have the ability to benefit the host plant, it is reasonable to think that the diverse bioactive compounds produced by the endophyte might exhibit potential applications in agriculture and medicine, and also serve as a source of inspiration for the discovery of new molecules[2,3].
The objective of this project was to determine the antagonist potential of endophytic fungi from *Gliricidia sepium* and to establish the chemical aspects related to endophyte-endophyte, endophyte-phytopathogen, and endophyte-host plant relationships through the evaluation of the antifungal and phytotoxic effect of their extracts with possible applications in agriculture as alternative agrochemicals.
We isolated 33 endophytic fungi from *G. sepium* leaves from the Sierra de Huautla Ecological Biosphere Reserve in Quilamula, Morelos, Mexico. The morphospecies were characterized macro- and micromorphologically. The fungi were paired opposite to each other in different combinations in Petri dishes with Potato dextrose agar (PDA) according to their growth rate against endophytes and four phytopathogenic fungi: *Pythium aphanidermatum*; *Phytophthora capsici*, *Phytophthora parasitica*, and *Fusarium oxysporum*. The most antagonistic fungi were cultivated in liquid (Potato dextrose broth, PDB) or solid media (PDA), and the crude extracts were obtained from the culture medium and mycelia. The culture-medium extracts were obtained with Amberlite XAD-16 or extracted with dichloromethane (CH_2Cl_2) followed by ethyl acetate (EtOAc); the mycelia were extracted with the same solvents. Antifungal and phytotoxic bioassays were carried out with phytopathogens *P. aphanidermatum*, *P. capsici*, *P. parasitica*, *F. oxysporum*, and two plants, *Amaranthus hypochondriacus* and *Solanum lycopersicum*[4]. One of the most active fungi was identified as *Nodulisporium* sp. by sequencing its ITS1–5.8S–ITS2 region[5].
Endophytic fungi produce antifungal and phytotoxic secondary metabolites that inhibit phytopathogens and plant growth. Thus, they can protect their host plant from pathogen attacks and can be indispensable for their survival inside the plant, and also in crop protection.
References:
[1]Rodríguez et al. 2009. New Phytol. 182:314-330. [2]Kusari et al. 2012. Chem. Biol. 19:792-798. [3]Gao, F. et al. 2010. Afr. J. Microbiol. Res. 4(13):1346-1351.[4]Macías-Rubalcava et al. 2008. Phytochem. 69:1185–1196. [5]Martin y Rygiewicz. 2005. BMC Microbiol. 5:28.

GENE EXPRESSION OF *glx-I* IN COLEOPTILES FROM MAIZE "RESISTANT TO AFLATOXIN CONTAMINATION" Sánchez-Medina M.A.[1], Coconi-Linares, L.N.[3], Pina-Canseco M.S.[2], Pérez-Santiago A.D.[1], Guzmán-Ortíz D.A.[3] [1]Instituto Tecnológico de Oaxaca, Av. Víctor Bravo Ahuja No. 125 Esq. Calz. Tecnológico, Oaxaca, Oax. C.P. 68030. [2]Universidad Autónoma Benito Juárez de Oaxaca, Carretera Antigua a San Felipe del Agua S/N. Oaxaca. C.P. 6820. [3]Laboratorio de Micotoxinas, Departamento de Biotecnología y Bioquímica, Centro de Investigación y Estudios Avanzados del IPN, Km 9.6, Libramiento Norte, Carretera Irapuato-León, Apdo. Postal 629, Irapuato, Gto., México. mmedinaito@gmail.com

Host resistance has become a viable strategy for controlling aflatoxin contamination in maize. Several genes have been identified which code for proteins that are present, in singly or in a greater proportion, in maize lines classified as resistant to aflatoxin production by *Aspergilli*. Glyoxalase I is a constitutive protein in the corn kernel embryo, which has been prove to have an important role in the resistance of maize to aflatoxin production by *Aspergilli.* In this study we analyzed the expression of *glx I* gene, in coleoptiles coming from four different maize races from Oaxaca previously identified: as resistant (3) and as susceptible (1) to aflatoxin production by *Aspergillus parasiticus*.
RNA extraction from coleoptiles, five days old, was performed by the method of Chomczynski and Sacchi and the *glx I* gene expression was determined by reverse transcription polymerase chain reaction (RT-PCR). It was found that the gene *glx I* was expressed in two out of three of the resistant maize races, in a greater proportion than in the susceptible race. The results show that *glx I*, whose protein has been only identified in maize grain, is also expressed during the early stages of germination of maize.

Biological evaluation of endophytic fungus PB3f isolated from *Haematoxylon brasiletto* (fabaceae)

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Plant endophytic fungi are defined as fungi that spend the whole or part of their life cycle colonizing inter- and/or intracellularly inside the healthy tissues of the host plants, typically causing no apparent symptoms of disease[1]. The host plant can supply plentiful nutrients and easy habitation for the survival of its endophytes. On the other hand, the endophytes would produce a number of bioactive compounds for helping the host plants to resist external biotic and abiotic stresses and to benefit in return the host's growth[2-4]. These bioactive metabolites possess a broad range of biological activities and could be the leading structures for the development of pharmaceutical or agrochemical products[4-6]
In the present work, we determined the allelochemical potential of the endophytic fungus PB3f over different species of phytopathogenic microorganisms through direct antagonism bioassays and evaluation of organic extracts from the culture medium and mycelium on growth inhibition of test phytopathogens and plants, in order to contribute to the chemical aspects involved in the relationships established between the host plants and the endophytic fungi.
Endophytic fungus PB3f was isolated from the healthy leaves of *Haematoxylon brasiletto* collected at the Ecological Biosphere Reserve in Morelos, Mexico. Allelochemical potential was evaluated quantitatively using direct antagonism bioassays against four economically important phytopathogenic microorganisms, and also the antifungal and phytotoxic activity of the organic extracts from the culture medium and mycelium against the growth of the following phytopathogens (*F. oxysporum, P. parasitica, P. capsici*, *P. aphanidermatum*) and the root length of seeds (*A. hypochondriacus* and *S. lycopersicum)* by dilution in agar[7].
In general, endophytic fungus PB3f shows a significant allelochemical effect against the test phytopathogenic microorganisms in both physical (mycelial barriers and growth inhibition) and chemical levels (metabolites present in the culture-medium organic extract). In the direct antagonism assay, fungus PB3f selectively inhibits the growth of all of the test oomycetes, >50%; the culture medium extract exerts a significant inhibitory effect mainly against the growth of oomycetes, predominantly against *P. capsici* (59%). The herbicidal potential exhibits a significant inhibitory effect against the growth of *S. lycopersicum* (69%). Active fungus PB3f possesses potential for isolation of bioactive compounds through bioassay-directed fractionation of the culture medium.

1) Rodríguez *et al.* (2009). New Phytol.182: 314-330; 2) Promputtha I. *et al.* (2007). *Microb. Ecol*. 53(4): 579-590; 3) Silvia F. *et al.* (2007). *Biologia*. 62: 251-257; 4) Gunatilaka L. (2006). J. Nat. Prod. 69: 509-526; 5) Zhou L. *et al.* (2009). New York: Nova SciencePublishers.91-119; 6) Schulz B. *et al.* (2002). Mycol. Res. 106: 996-1004; 7) Macías-Rubalcava *et al*. (2008). Phytochemistry 69: 1185–1196.

Effect of *Trichoderma* and *Bacillus* on the content of phenolic compounds and flavonoids, and antioxidant activity of onion

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The *Trichoderma* fungi and *Bacillus* bacteria are beneficial microorganisms used to biological control of plant diseases and growth promotion. However, these microorganisms may also affect the chemical composition of crops. Onion bulbs are rich in phenolic compounds and flavonoids with antioxidant activity and could be used as study model to know the effect of the microorganisms application on the chemical composition of crops. In this study was tested the effect of commercial products with *Trichoderma* and *Bacillus* in the production of phenolic compounds and flavonoids, and antioxidant activity of bulbs from three onion varieties.
The products tested with *Trichoderma harzianum* were PHC-T22 (T-22) and Spectrum Trico H, Ultraquimia (TUQ); and the product with *Bacillus subtilis* was PROBAC BS, Ultraquimia (BUQ). The onion varieties used were: Crystal White (CW), Red Satan (RS) and Mata Hari (MH). The products application was during the sow and the plant transplanting in pots, under greenhouse conditions. After eight weeks, the plants were collected and was evaluated the fresh and dry weight, the content of phenolic compounds (CF) and flavonoids (F), activity of phenylalanine ammonia lyase (PAL) and antioxidant activity.
The fresh and dry weight of bulbs increased from 2 to 3 time; but the protein amount did not change. In bulbs of CW variety, the PAL activity only increased with the BUQ product; while, the three products increased the PAL activity until 34 and 24.8% in bulbs of the RS and MH varieties, respectively. Bulbs of the RS variety showed the highest increase of phenolic compounds (57%); followed by bulbs of the CW (47.2%) and MH (35%) varieties. Flavonoids content also increased until 28.1, 35.6 and 41.3 % in bulbs of the MH, RS and CW varieties, respectively. These increases of the content of phenolic compounds and flavonoids were related with increases of antioxidant activity. The antioxidant activity increased until 28.6, 18.7 and 12.3% in bulbs of the MH, RS and CW varieties, respectively. The results show that the commercial products with *Trichoderma* and *Bacillus* promoted the growth, the production of phenolic compounds and flavonoids, the PAL activity and the antioxidant activity of onion bulbs. This biochemical response depended of the onion variety.

Genetic characterization of ant associated Ophiocordyceps

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Abstract

Entomopathogenic fungi have a great potential as biological control agents as they represent a diverse group of species which can provoke fungal infections of insect populations. *Ophiocordyceps unilateralis* (Ascomycetes: Hypocreales), is a specialized entomopathogenic fungus that infects, manipulates, and kills ants (also known as zombie ants). Previous work has shown that the ants of genus *Camponotus* (Hymenoptera: Formicidae), are the main host of this fungus.

Fungal parasites are likely to play an important role in structuring host populations. They manipulate host ant behavior, to great extent and can affect their distribution. Fifteen species of *Cordyceps*) parasitizing on insects have been recorded from Mexico of which *Cordyceps dipterigena* (from the State of Veracruz) and *C. pruinosa* (from Quintana Roo) were first reported in the country, while the species *Cordyceps miltaris* has the broadest distribution in the country.

We initiated a study of ants or other insects infected with entomopathogenic fungi, in Mexico, based on the samples from the states of Veracruz, Chiapas and Oaxaca. We optimized DNA extraction and purification procedures with several strains of entomopathogenic fungi cultivated in vitro, particularly of the genus *Cordyceps*, in order to establish the most adequate technique to apply directly in *Ophiocordyceps* infected samples from the field.

Fig. 1 (Infected ant with Ophiocordyceps)

Expression analyses of an endo-β-(1-6)-D-galactanase gene in two races of *Colletotrichum lindemuthianum* with different life style

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Endo-β-(1-6)-D-galactanase hydrolyze side chains of β-(1-6) galactosyl residues attached through O-6 of arabinogalactans (AGs) and arabinogalactans proteins (AGPs). It produced β-(1-6)-galacto-oligomers and β-(1-6)-galactobiose. AGPs are a family of complex proteoglycans found in all tissues of higher plants and localized in cell walls, plasma membranes and the extracellular matrix. Recent evidence suggests that they are related to defense in plants.

We evaluated the genetic expression of endo-β-(1-6)-D-galactanase gene (*Clebg)* by qPCR in mycelium grown in potato dextrose medium with glucose for 8 h, and Mathur´s medium containing arabinogalactan, xylan or cell wall from *Phaseolus vulgaris* as carbon source, at 0, 2, 6, 12, 24 and 48 h in two races from *C. lindemuthianum* with different life style, the pathogenic race (1472) and the saprophytic race (0).

The results showed that *Clebg* suffer catabolic repression in presence of glucose. When mycelium of both races grew with plant cell wall, an increase of relative expression of *Clebg* (~12 fold) was detected in the pathogenic race (1472) but not in saprophytic race (0). In culture medium with xylan or arabinogalactan, the pathogenic race (1472) showed major relative expression of *Clebg* (~6-7 fold respectively) compared with the saprophytic race (~3-4 fold respectively).

Since the plant cell wall induce expression of the endo-β-(1-6)-D-galactanase in pathogenic race but not in saprophytic race of *C. lindemuthianum*, this gene could be a pathogenesis factor.

Multilocus analysis of *Histoplasma capsulatum* associated with six molecular markers

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We analyzed 22 *Histoplasma capsulatum* isolates from different regions of Mexico. By means of the MEGA vers. 5.0 software we performed a phylogenetic multilocus analysis using six molecular markers [ARF, H-ANTI, OLE1, TUB1, $(GA)_n$, and ITS1-5.8S-ITS2 region] processed with the following phylogenetic methods: maximum likelihood (ML), neighbor-joining (NJ) algorithm using Kimura´s 2-parameter, and maximum parsimony (MP).The ML, NJ y MP trees of six concatenated markers defined two special groups of *H. capsulatum* isolates associated with the migratory bat *Tadarida brasiliensis* together with one isolate from *Mormoops megalophylla* (previously considered as a lineage). These results support the existence of two new genetic populations of *H. capsulatum* containing *T. brasiliensis* isolates, one of them represented by five isolates (EH-655P, EH-658H, EH-670H, EH-672B, and EH-672H) could be considered as new phylogenetic species and the other as a new lineage (EH-696P isolate).

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References. Kasuga T *et al*. J Clin Microbiol. 1999. 37:653-663; Kasuga T *et al.* Mol Ecol. 2003. 12:3383-3401; Taylor ML *et al*. Fungal Biol. 2012. 116:308-317; Taylor ML *et al*. FEMS Immunol Med Microbiol. 2005. 45:451-458.

Participation of Effector-Like Proteins in the Establishment of the Biological Relationship of *Trichoderma - Arabidopsis*

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Rizosphere is a dynamic region of biological interactions, establishing neutral, beneficial or harmful associations. Plant growth and development could be affected through interactions with pathogens. Plants possess various inducible defense mechanisms for protection against pathogen attack. An example of this is systemic acquired resistance (SAR), which is activated through pathogen recognition at the molecular level. The recognition molecules are collectively known as Microorganism-Associated Molecular Patterns, since the initial defense response may also be associated with a non-pathogenic microorganism, such that enable the plant to maintain a state of alert and provide an more efficient response to pathogen attack. It has been proposed that the establishment of the pathogen at the plant is facilitated through the release of small molecules known as effectors, which promote pathogenicity by modifications on the hormonal balance or by synthesis of hormone-like compounds[1]. It is common to find that an effector has many targets, globally affecting the innate immune response of the plant. In theory, each combination of an effector and host protein could be neutral, have negative or even promote beneficial results for the plant. Among soil microorganisms that have a beneficial impact over plants are species belonging to *Trichoderma* genera. Little is known about the function of effector proteins in the establishment of beneficial interactions, the mainly studied system corresponds to micorrhiza[2]. In *Trichoderma spp.* nine molecules with effector characteristics has been proposed[3], but only one has been characterized as effector-like protein: SM1, which is implicated in the establishment of plant-fungus interactions, activating SAR in cotton plants. Nowadays, in our work group we are searching for novel effector-like proteins in *Trichoderma* species interacting with the plant *A. thaliana*. By using bioinformatics tools, we have selected 37 genes that encode for possible effector-like proteins. By conventional RT-PCR we have found up regulated genes, down regulated genes and genes without expression changes when the fungus is co-cultivated with the plant. Additionally, for some of them we are analyzing their expression pattern in fungal cultures added with root exudates. We will confirm these results by Real Time RT-PCR in order to generate null *Trichoderma* mutants on these effector-like proteins and evaluate their participation during the *Trichoderma-Arabidopsis* beneficial interaction.

[1] Kamoun, S. (2006). A catalogue of the effector secretome of plant pathogenic oomycetes. *Annu Rev Phytopathol* 44, 41-60.
[2] Plett,J.M., Kemppainen,M., Kale,S D., Kohler,A., Legué,V., Brun,A., Tyler,B.M., Pardo,A.G., Martin,F. (2011) A secreted effector protein of *Laccaria bicolor* is required for simbiosis development. *Curr Biol* 21, 1197-1203.
[3] Hermosa, R., Viterbo A., Chet I., Monte E. (2012). Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology* 158, 17–25 .

Construction of a Bacterial Artificial Chromosome (BAC) Genomic Library for Physical Mapping and Genome Sequence Validation of a Brewing Yeast

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The most widely distributed beer in this industry is the *lager* style. This beer is produced by a hybrid species known as *Saccharomyces pastorianus*. The determination of its genome sequencing is one of the most promising approaches for its industrial exploitation. The characteristics of the Next-Generation Sequencing Technologies (NGS), such as reduced cost and high-throughput, have made possible the production of the first draft sequence of a brewing strain. Yet another feature of these technologies, particularly the short readings size, hinders the assembly process resulting in contigs whose relative position is unknown. Moreover, since the assembly is purely bioinformatic, there is no way of knowing whether the produced contigs are correctly assembled. For all the above is necessary to use alternative techniques that produce useful information for the comparison and validation of bioinformatic generated assembly. One way of obtaining this information is physical mapping which involves determining the order of the DNA fragments from a source. Most physical mapping techniques such as High-Information-Content Fingerprinting (HICF) and Whole Genome Profiling (WGP) are based on detection of partial overlaps between Bacterial Artificial Chromosome (BAC) clones. That is why the first step in the validation of sequencing assembly will be the construction of a BAC genomic library. The project will include: 1) obtaining the high molecular weight (HMW) DNA, 2) partial digestion of the DNA and selection of the appropriate size fragments, 3) ligation of these fragments to the vector, 4) transformation of the recombinant vectors in the host cells, and 5) quality estimation of the library generated (coverage, average insert size, etc.).

Functional characterization of Candida tropicalis *MNN4* and *OCH1*

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C. albicans and *C. tropicalis* are yeast-like organisms that can cause severe infections in immunocompromised patients. The cell wall structure and composition are critical for the interaction with host tissues and the immune system. In *Candida albicans* it is known that 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 during the glycosylation pathways. It is known that in *Candida albicans OCH1* is involved in the synthesis of the *N*-linked mannan outer chain, and is required for cell wall integrity and virulence. On the other hand, *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.

The *C. tropicalis* genome contains two putative genes with significant similarity to *C. albicans OCH1* and *MNN4*. 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* and *MNN4* is relevant to understand how the mannosylation and phosphomannosylation pathways contribute to the virulence and immune sensing of *C. tropicalis*.

In the present study, we are conducting the complementation of *C. albicans och1*Δ and *mnn4*Δ null mutants with the C. tropicalis ORF's under the control of *ACT1* promoter. This will help to demonstrate whether those genes are indeed the functional orthologs of *C. albicans OCH1* and *MNN4*.

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Analysis of nitrogen catabolite repression in *Lachancea kluyveri*

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ABSTRACT

Lachancea kluyveri (*Saccharomyces kluyveri*) is a budding yeast that was first isolated within the yeast flora from the intestinal canal of *Drosophila* in 1956. *S. kluyveri* shows a close phylogenetic relationship with a variety of species of other genera including *Kluyveromyces* and *Zygosaccharomyces*, so it was proposed to reassign this yeast among the *Lachancea* genus.

The ability to use preferentially 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, using a comparative approach, how different yeasts use diverse nitrogen sources and how this process is regulated.

In this work, we want to determine how *L. kluyveri* use different nitrogen sources. Using sequence analysis comparison, we found that *L. kluyveri* has orthologous genes encoding for the GATA transcription factors Gln3 and Gat1 and for the Ure2 protein. We have generated a collection of single mutant strains in these genes (*gln3Δ*, *gat1Δ* and *ure2Δ* strains) in order to evaluate its role in nitrogen assimilation when cells were grown on glutamine, ammonia or proline as sole nitrogen sources. Further work is underway to obtain double mutant strains (*gln3Δ gat1Δ*, *gln3Δ ure2Δ* and, *gat1Δ ure2Δ*) and the triple mutant *gln3Δ gat1Δ ure2Δ*. To elucidate if there is a NCR -like mechanism in *L. kluyveri* we are going to determine, by qRT-PCR, the transcription of some of the genes involved in the transport and catabolism of the nitrogen sources tested.

Isolation and functional characterization of *Sporothrix schenckii OCH1*

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S. schenckii is a dimorphic fungus that causes sporotrichosis, a granulomatous infection mainly found in humans and other mammals. The fungal cell wall plays an important role during host-fungus interaction, and in *S. schenckii* this organelle is composed of alkali-soluble and insoluble glucans, chitin and glycoproteins. The cell wall glycoproteins are synthesized by the protein glycosylation machinery localized within the endoplasmic reticulum and the Golgi complex. Och1 is an □1,6-mannosyl transferase that participates in the elaboration of *N*-linked glycans and has a key role in the elaboration of their outer chain. Thus far, there are not reports about the role of *OCH1* in cell wall integrity, virulence and glycosylation pathways of *S. schenckii.*

Here, we have isolated the putative *OCH1* encoding region, and to determine the function of its product we are complementing a *Caoch1Δ* null mutant. In order to achieve this, the ORF was cloned into the *HindIII–NheI* sites of the pACT1-GFP vector generating pACT1-*Ssoch1*, which was used to transform the null mutant. Strains obtained were utilized to determine the growth rate, morphology, cell wall phosphomannan content and status of the *N*-linked mannosylation pathway by EMSA.

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Cellular localization and gene transcriptional regulation have a role on the functional divergence of *Saccharomyces cerevisiae* Bat1 and Bat2 isozymes

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Gene duplication and divergence have been proposed as important evolutive forces of living beings offering genetic novelties and conferring adaptation to changing environments. These copy number gene alterations are widespread among the three major domains of life (eukarya, prokarya and archaea) and can affect not only to individual genes but also to several genes, chromosomes and to whole genome.

After gene duplication, both copies are under a relaxed selection pressure and both can accumulate mutations that could drive the evolutive fate to neofunctionalization, subfunctionalization, specialization or pseudogenization .

The yeast *Saccharomyces cerevisiae* belongs to a clade in which its members underwent a whole genome duplication event that occurred 100mya. An average of 500 duplicated genes was maintained in the *S. cerevisiae* genome. Some of them encode for pyridoxal – 5 – phosphate (PLP) – dependent aminotransferases (i.e. *ALT1*/*ALT2*, *AAT1*/*AAT2*, *ARO8*/*ARO9, BAT1*/*BAT2* among other duplicated genes). Bat1 and Bat2 of *S. cerevisiae* are transaminase isozymes which transfer the α – amino group from the glutamate to the α – keto acids precursors of the branched chain amino acids valine, isoleucine and leucine (VIL).

It has been shown that Bat1 and Bat2 have functional diverged, and that Bat2 has been specialized in branched chain amino acids catabolism. It has also been reported a possible contribution of the different cellular localization of these isozymes and the contrasting transcriptional regulation of the genes that encode them to the different metabolic roles in which these isozymes are involved.

In this work we tested subfunctionalization as an alternative evolutive model instead of specialization using *BAT1* gene of *Lachancea kluyveri* as ancestral model. In addition, we tested the contribution of the different Bat1/Bat2 cellular localization and *BAT1*/*BAT2* transcriptional regulation to the biosynthesis and catabolism of the branched chain amino acids (VIL).

Our data suggest that subfunctionalization would explain the evolutive fate of these duplicated genes. Also we observed that the presence of Bat2 in cytosol or sending Bat1 to cytosol are important factors for the efficient catabolism of (VIL). In addition, we confirmed that transcriptional regulation of these genes is also an important factor that contributes to the functional divergence of Bat1 and Bat2 isozymes of *S. cerevisiae*.

The GATA type factor Gln3 is involved in nitrogen uptake in the yeast *Candida glabrata*

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Fungi are ubiquitous free living cells and are able to use a wide range of compounds as carbon and nitrogen sources. The yeast *Saccharomyces cerevisiae*, in a mechanism known as *Nitrogen Catabolite Repression* (NCR), is able to preferably utilize good nitrogen sources (e. g. glutamine) instead of bad nitrogen sources (e. g. proline). A similar mechanism known as *Nitrogen Metabolite Repression* (NMR) has been reported for the filamentous fungi *Aspergillus nidulans*. When good nitrogen sources are available, NCR/NMR -sensitive genes are repressed. NCR/NMR -sensitive genes are regulated by zinc containing GATA domain factors and in the case of NCR by the Ure2 repressor, also.

Candida glabrata is a human pathogen yeast that is more phylogenetic related to *S. cerevisiae* than to the *Candida* species. However, a nitrogen repression mechanism has not been described in this pathogen yet. Here, we investigate the role of the *C. glabrata* GATA factors and the Ure2 protein when glutamine, ammonium or proline are available as nitrogen sources. When glutamine is available as nitrogen source, the doubling time of the wild type strain is similar to the observed in the *gat1Δ* strain (77 min). On the other hand, we observed a similar double time in the *gln3Δ*, *ure2Δ*, *ure2Δ gat1Δ*, *ure2Δ gln3Δ*, *gat1Δ gln3Δ* and *ure2Δ gat1Δ gln3Δ* mutant strains (approximately 30% higher than the wild type). On ammonium, the doubling time of the wild type strain is approximately 67 minutes and it is similar to the one observed for the *gat1Δ* mutant strain. In addition, the doubling time of the ure2Δ and *ure2Δ gat1Δ* mutant strains is 20% higher than the observed in the wild type strain. Remarkably, the doubling time of the *gln3Δ*, *ure2Δ gln3Δ*, *gat1Δ gln3Δ* and *ure2Δ gat1Δ gln3Δ* is two-fold higher than the observed for the wild type strain. When cells grow on proline as nitrogen source the doubling time of the wild type strain is approximately 105 minutes and it is similar to the observed in the *gat1Δ* and *ure2Δ* mutant strains. On the other hand, the doubling time calculated in the *gln3Δ*, *ure2Δ gat1Δ*, *ure2Δ gln3Δ* and *gat1Δ gln3Δ* is about 25 – 50 % higher than the calculated for the wild type strain. In addition, the *ure2Δ gat1Δ gln3Δ* strain have a doubling time 83% higher than the observed in the wild type strain. Altogether, the present data suggest that Gln3 but not Gat1, is involved in the nitrogen uptake of glutamine and proline. In addition, Gln3 has a principal role in ammonium uptake. In addition, the Ure2 protein is involved only when glutamine or ammonium are available as sole nitrogen sources. To elucidate if there is a NCR -like mechanism in *C. glabrata* we need to evaluate the transcription of some of the genes involved in the transport and catabolism of the nitrogen sources tested.

Functional characterisation *Candida tropicalis MNN4*

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Candida tropicalis is one of the most common pathogenic yeast species, when it invades host tissues, the first point of contact is the cell wall, which consists of an inner layer of chitin, β1,3- and β1,6-glucans and an outer layer of mannoproteins. These proteins are modified by oligosaccharides rich in mannose residues (mannans), enzymatically attached by the process known as protein glycosylation. Mannans can be modified with phosphomannan moieties (mannose residues attached to mannans by a phosphodiester link) that are present on the cell wall of a very limited number of fungal species, including *C. albicans* and *C. tropicalis*.
Phosphomannan synthesis in *Saccharomyces cerevisiae* is carried out by the phosphomannosyltransferase Mnn6 and its positive regulator Mnn4. The function of the latter is not fully understood yet, but disruption of its gene generated cells lacking phosphomannan, despite the protein does not have enzyme activity. Here, we isolated *MNN4* from *C. tropicalis* genome, cloned including its own regulatory regions in C. albicans *mnn4*Δ null mutant and demonstrated that this gene is the functional ortholog of *C. albicans MNN4*. These results indicate that *C. tropicalis* gene regulatory elements are functional in *C. albicans*.

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The Pep4Um vacuolar proteinase is involved in morphogenesis and pathogenesis of *Ustilago maydis*

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BACKGROUND

Ustilago maydis, the basidiomycota responsible for corn smut disease, displays a complex life cycle that requires the plant host for completion.

The gene encoding the vacuolar aspartic proteinase A (PrA) is involved in sporulation, vegetative growth, is essential for the vacuolar proteolytic system during nutritional stress, and implicated in the activation of other vacuolar hydrolases in other fungi. A homologue of this gene (*PEP4Um*) was identified in *U. maydis.* It encodes a protein containing 418 amino acid residues with high similarity to *Saccharomyces cerevisiae* PrA enzyme. Null Δ*PEP4* mutants were affected in the in the vitro pH-induced dimorphic transition, growth rate and pathogenicity, indicating their importance for the physiology of the organism.

AIM

To characterize the role of Proteinase A in *Ustilago maydis*

METHODS

Wild type strains FB1 (*a1b1*) and FB2 (*a2b2*) were used as genetic background; we obtained mutant strains by double homologue recombination by replacing the wild type gene with a hygromycin (Hyg) resistance *cassette.* Transformants were analyzed for gene replacement by PCR and confirmed by Southern blot analysis.

U. maydis populations with yeast or filamentous morphologies were obtained in synthetic MM medium with an initial pH of 7.0 or 3.0 respectively (Ruiz-Herrera *et al*. 1995). A mating assay on charcoal plates was performed as described by Holliday (1974). Plantlets of *Zea mays* were inoculated with cell suspensions (10^5 cells) of mixtures of sexually compatible *U. maydis* wild-type or mutant strains. Plants were monitored for symptoms and tumor formation during 15 dpi.

RESULTS

The *pep4Um:*:Hyg mutant strains have a lower growth rate in culture than wild type strains, and as expected, they showed an important decrease in PrA enzymatic activity.

Dimorphic transition induced by acid pH was affected in PrA mutants. Moreover, a similar effect was observed when mycelial induction was made by carbon source change replacing glucose by palmitic acid.

Mutant haploid strains FB1M and FB2M co-spotted on agar-charcoal plates displayed a severely reduced fuzz reaction in comparison to wild type controls. Accordingly, after 24 h remained in the yeast-form, in contrast to the wild type strains which at the same time formed filaments.

When inoculated in maize plants, the combination of mutant haploid strains showed severely reduced symptoms as compared to wild-type strains combinations.

CONCLUSIONS

Our data are evidence that *PEP4Um* is involved in cell growth, morphogenesis, and pathogenicity of *U. maydis*.

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Functional characterization of three mannosyltransferases of *Saccharomyces cerevisiae*

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Glycosylation is the major post-translational protein modification that starts in the endoplasmic reticulum and continues in the Golgi complex. In the yeast *Saccharomyces cerevisiae* have been studied the biosynthetic pathway of protein glycosylation. Thanks to these studies, now it is well known the identity of the main proteins involved in the elaboration of *O*-linked glycans, and most of the enzymes participating in *N*-linked glycan synthesis. The *KRE2/MNT1* gene family participates in these biosynthetic pathways and is composed of nine members whose products have the sequence signatures of mannosyltransferases. Until now, the majority of these members have been studied and their role in glycan elaboration has been established. Our group is interested in assign a role to the three uncharacterized members: Ktr4, Kt5 and Ktr7. Null mutants in these genes have no obvious phenotypes, so they have been useless in establishing the role of these genes in protein glycosylation. By the other hand no redundancy has been observed in *Candida albicans*, it is possible to use this fungus to know the function of the genes ktr4, kt5 and ktr7. We have null mutants that are non-functional in *O*- (Δmnt1 mnt2) and *N*- (Δmnt3 mnt5) glycosylation pathway and complementation experiments could us to assign a role of these uncharacterized genes.

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In silico analysis and expression profile of Candida albicans yeast glucanases

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Candida albicans is the most prevalent opportunistic infective agent in humans. It is normally found in the gastrointestinal tract as part of normal flora. However, it can cause an infection when the immune system is compromised. One structure of this fungus that protects against the host immune system is the cell wall. This structure is responsible for cell morphology and resistance to osmotic changes. The major structural component of the cell wall is glucan, a polysaccharide formed by D-glucose monomers linked by β-1,3 or β-1,6 glycosidic bonds. Glucanases are enzymes that hydrolyze the β-1,3-glucan. There are two types of glucanases, exoglucanases and endoglucanases. XOG1, SPR1 and ENG1 glucanases have been previously reported in *Candida alblicans* by other authors. In this work we searched the *C. albicans* genome for different glucanases. We found four genes encoding exoglucanases and two genes encoding endoglucanases. The *in silico* analysis revealed that Xog1(Exg1) glucanase has an identity of 44.6%, 40.3% and 22.7% identitiy to Exg2, Spr1(Exg3) and Exg4, respectively. On the other hand, Eng1 endoglucanase has 41.2% identity to Eng2. The four exoglucanases belong to the glycosylhydrolases family 5, while the two endoglucanases are part of the glycosylhydrolases family 81. We also determined the expression profile of these six genes in yeast cells by qRT-PCR and identified the residues that are important for the catalytic activity by *in silico* analysis. In addition, we have got the structure of three exoglucanases by homology modeling using the *C. albicans* Xog1 crystal structure as a template.

Transcriptional regulation of *GDH3* in glucose grown-cultures of *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae*, experimented a whole-genome duplication event about 100 million years ago. It has been suggested that genome duplication lead to speciation, because it promoted genome innovation and other possibilities that allowed the yeast to occupy new niches. The study of the conserved duplicated genes, known as paralogues, constitutes an interesting approach to understand their physiological roles that could explain their conservation.
The most common nitrogen sources for free-living microorganisms are ammonium and nitrate. These elements are assimilated into other compounds like amino acids constituting building blocks which are necessary for growth of the organism. The principal nitrogen donors are glutamate and glutamine this cellular nitrogen is needed to carried out biosynthetic reactions.
NADP-dependent synthesis of glutamate in *Saccharomyces cerevisiae* is mediated by the paralogues enzymes Gdh3 and Gdh1, they play a central role in nitrogen metabolism, and connect nitrogen metabolism to carbon metabolism through α-ketoglutarate.
Previous studies in our laboratory have shown that *GDH3* expression is repressed in glucose and derepressed when glucose is exhausted. This repression/derepression depends on the interaction of *trans* elements not identified yet, and *cis* elements in the gene promoter. Some candidates are the Nrg1/2 and Mig1 repressors and the HAP complex, Gln3 and Rtg3 activators. Proteins involved in chromatin remodeling as Hda1, Tup1 and Sir2, will also be tested.
The role of *NRG1/NRG2* as a repressor in the expression of *GDH3* was confirmed by Northern blot analysis; it was observed that in *nrg1Δ nrg2Δ* double mutant the expression of the *GDH3* transcript was 5-fold increased even in the presence of glucose. The analysis of *GDH3* expression in the *mig1Δ, sir2Δ, hda1Δ, tup1Δ* mutants is under way.
On the other hand the importance of *GDH3* native promoter was verified when it was replaced by a promoter insensitive to glucose (Ptet07). *GDH3* was expressed throughout the growth cycle even in the presence of glucose.

The *Trichoderma atroviride* photolyase-encoding gene is transcriptionally regulated by non-canonical light response elements

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The BLR-1 and BLR-2 proteins of *Trichoderma atroviride* are the *Neurospora crassa* homologs of white collar-1 and -2, two transcription factors involved in the regulation of genes by blue light. BLR-1 and BLR-2 are essential for photoinduction of *phr*-1, a photolyase-encoding gene whose promoter exhibits sequences similar to well-characterized light regulatory elements of *Neurospora*, including the albino proximal element and the light response element (LRE). However, despite the fact that this gene has been extensively used as a blue light induction marker in *Trichoderma*, the function of these putative regulatory elements has not been proved. The described LRE core in N. crassa comprises two close but variably spaced GATA boxes to which a WC-1/-2 complex binds transiently upon application of a light stimulus. Using 5′ serial deletions of the *phr*-1 promoter, as well as point mutations of putative LREs, we were able to delimit a ~50 bp long region mediating the transcriptional response to blue light. The identified light-responsive region contained five CGATB motifs, three of them displaying opposite polarity to canonical WCC binding sites. Chromatin immunoprecipitation experiments showed that the BLR-2 protein binds along the *phr*-1 promoter in darkness, whereas the application of a blue light pulse results in decreased BLR-2 binding to the promoter. Our results suggest that BLR-2 and probably BLR-1 are located on the *phr*-1 promoter in darkness ready to perform their function as transcriptional complex in response to light.

Expression divergence of *BAT1* and *BAT2 Saccharomyces cerevisiae* paralogous genes determines their functional diversification

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Expression divergence between duplicate genes has long been a subject of great interest to geneticists and evolutionary biologists, because it is considered an important step in the emergence of a new gene from a redundant duplicate. It is known that gene expression divergence following gene duplication could result in different expression profile, indicating functional evolution. To understand expression divergence of duplicate genes it is necessary to analyze their transcription regulation and chromatin organization. Here we examine an interesting example of expression divergence between the branched chain aminotransferases encoded by the paralogous genes *BAT1* and *BAT2* of the yeast *Saccharomyces cerevisiae*. These aminotransferases catalyze the first step in the biosynthesis and the last in the catabolism of branched chain amino acids. They transfer the amino group between valine, isoleucine, leucine and their corresponding α-keto acid. While *BAT1* codes for a mitochondrial protein, *BAT2* codes for a cytosolic protein, which has a 77% identity with Bat1. In this work we determined *BAT1* and *BAT2* expression profile in cultures grown in the presence of various nitrogen sources. The analysis revealed that *BAT1* expression is not determined by the quality of the nitrogen sources and is similar in both, repressing (glutamine) and non-repressing nitrogen sources (GABA). Conversely, *BAT2* expression depends of the quality nitrogen source. *BAT1* is expressed in glutamine, ammonium and GABA as nitrogen source, and repressed by the end product V I L, while *BAT2* expression is induced by V I L, and repressed by glutamine. We also analyze *BAT1* and *BAT2* expression in different mutants in order to determine which transcriptional regulators are involved in their expression profile. Then we analyze *cis-trans* interactions by qChIP assays, and finally we compared chromatin organization between *BAT1* and *BAT2* promoters by nucleosome scanning assays. Our main findings were; I) divergence of *cis*-elements between *BAT1* and *BAT2* promoters allows different *trans* interactions and expression profile, II) *BAT1* and *BAT2* have are coordinately expressed by action of the transcriptional modulator Gcn4, Gln3, Leu3 and Put3, III) Leu3 shows a repressor-activator character in glutamine as nitrogen source, IV) nucleosome positioning in *BAT1* and *BAT2* promoters could also play an important role in expression divergence. Transcriptional regulation, chromatin organization, and expression divergence of paralogous genes will be discussed in relation to their role *BAT1* and *BAT2*.

GDH1-GDH3 Paralogus Genes Show Divergent Transcriptional Regulation in *Saccharomyces cerevisiae*

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Gene duplication and the subsequent divergence of paralogous genes play a central role in the evolution of novel gene functions. *Saccharomyces cerevisiae* (*S. cerevisiae*) has two paralogous genes (*GDH1/GDH3*) which encode NADP-dependent glutamate dehydrogenases (NADP-GDHs). These two enzymes synthesize glutamate from ammonium and α-ketoglutarate. It has been proposed that *GDH1* and *GDH3* have been retained in *S. cerevisiae* since the encoded enzymes display different allosteric properties and rates of α-ketoglutarate utilization.
In regard to regulatory divergence, *GDH3* expression is repressed in glucose-grown cultures while *GDH1* is fully expressed.
GDH1 and *GDH3* expression is modulated by the *HAP* system. This regulator is implicated in the control of the expression of genes involved in carbon metabolism and respiratory function. *GDH1* expression is under regulation of transcriptional activators such as Gln3 which has been considered exclusive of nitrogen metabolism. In our laboratory we found that a hybrid complex composed by Hap2-3-5 and Gln3 determines transcripcional activation of *GDH1* in ethanol-glutamine; however, this complex does not regulate *GDH3* expression.
This work analyzed whether *GDH3* expression in glutamine was determined by a hybrid novel complex such as Hap2-3-5-X. We considered that the *RTG3*-encoded transcriptional factor could participate constituting a hybrid complex with Hap2-3-5, (Hap2-3-5-Rtg3) since *GDH3* expression is Rtg3-dependent in ethanol glutamine. Since Rtg3 nuclear localization is determined by the nature of the nitrogen source it could be considered that as has been found for Gln3, the low amount of Rtg3 found in the nucleus could be tethered to the Hap2-3-5 complex and recruited to the *GDH3* promoter. To analyze this matter Chip assays were performed. Results showed that Rtg3 binding to the *GDH3* promoter was independent of the *HAP* complex. It can thus be considered that Rtg3 is recruited to the *GDH3* promoter by a so far unidentified regulator. Northern analysis indicated that *RTG3* is a positive regulator of *GDH3* expression, while it does not regulate that of *GDH1*.
To analyze the nature of the negative regulator determining *GDH3* repression in glucose, *GDH3 cis* elements were analyzed and three potential binding sites for Nrg1 in *GDH3* promoter and two in *GDH1* promoter were found. Chip analysis showed that Nrg1 bound both promoters. Northern studies demonstrated that Nrg1 acts as a negative regulator for *GDH3* and a positive regulator for *GDH1*. We will discuss the role of differential regulatory patterns in *GDH3-GDH1* functional divergence.

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Dosage matters: Revealing the fitness effects of experimental gene duplication

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Duplicate genes are widely distributed at high frequencies across eukaryotic genomes. Within the hemiascomycete yeasts, certain genes are often been retained in duplicate, while others are universally present as single-copy genes. What is the role of natural selection in determining such duplication frequencies? Since gene redundancy is known to be evolutionary unstable, it is likely that positive selection has been determinant for the immediate fixation of newly arisen duplicate genes. On the other hand, the duplication of dosage-sensitive genes could have been prevented by immediate fitness disadvantages of such duplication events.

Here, we experimentally duplicate over 900 essential genes in *Saccharomyces cerevisiae* using centromeric (monocopy) constructs bearing these yeast genes controlled by their native promoter and terminator sequences. We measure the immediate phenotypic consequence of such genetic perturbation by quantifying the relative fitness of single-copy vs double-copy strains under normal or stressful laboratory conditions using a high-resolution phenotyping method.

Our results show that 13% of the strains bearing an additional gene copy grow significantly slower than the wild-type, indicating that gene duplication of certain genes has an immediate deleterious fitness consequence. Surprisingly, experimental gene duplication results in an immediate fitness advantage for 9% of the strains, and this fraction increases to 12% when yeast is grown under osmotic stress. Gene duplications with a fitness disadvantage are enriched among genes that encode for members of protein complexes and important cell processes like structural organization and ribosome biogenesis. Intriguingly, being part of a protein complex is also a hallmark of beneficial gene duplications, which is the case for regulators DNA and RNA metabolism.

Our results suggests that natural selection of duplicated gene dosage influences the presence or absence of redundant gene copies in eukaryotic genomes, which may represent an initial step for the further diversification of gene function.

"Regulatory divergence in paralogous genes *ALT1* and *ALT2* in *Saccharomyces cerevisiae*"

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Genic duplication is an important process for the evolution of living organisms Duplicated genes can result in the generation of a new function not present in the ancestor or the original function can be distributed in the two genes through subfunctionalization. *Saccharomyces cerevisiae* genome went through complete genome duplication known as: Whole Genome Duplication (WGD). *ALT1* and *ALT2* are two paralogous genes which arose after WGD. Previous results from our laboratory confirmed that *ALT1* encodes an alanine aminotransferase that translocates amino group from alanine to α-oxoglutarate to form glutamate and pyruvate; surpringsinly, no function has been determined for Alt2 even when these two enzymes share 67% of identity and both present 11 invariable residues characteristic of aminotransferases. Previous results in our laboratory showed that the expression profile of *ALT1* and *ALT2* is contrasting. Northern Blots were carried out from cells grown in two different media, glucose-ammonia (biosynthetic conditions) and glucose-alanine (catabolic conditions), *ALT1* is predominantly expressed in RNA samples prepared from yeast grown on alanine as nitrogen source, and is poorly expressed in glucose-ammonia. The opposite occurs with *ALT2* expression, this gene is only expressed on biosynthetic conditions during the first hours, as culture grows, expression is decreased. On glucose-alanine *ALT2* expression is completely repressed. These results suggest alanine is co-inductor for *ALT1* and co-repressor for *ALT2.* In this work we have analyzed the transcription factors which are involved in regulation of *ALT1* and *ALT2* expression. We found that Gcn4 participates as an activator of the two genes*.* Unexpectedly, the positive regulators Gln3 and Rtg3 function as *ALT2* repressors. Gln3 represses *ALT2* just in glucose-ammonia, but not on glucose-alanine, conversely, Rtg3 functions as repressor under both conditions: ammonia and alanine. The negative regulator Nrg1 regulates negatively both genes. Since Hda1 and Tup1 often participatre as Nrg1 correpresors we analyzed whether these could participate as modulators. It was found that Hda1 negatively modulates *ALT1* and *ALT2*, while Tup1 only exerts its negative role on *ALT2* expression. Thus, our studies reveal there are five negative regulators for *ALT2* and a single activator. While *ALT1* expression is determined by one activator and two negative modulators.

Inhibition of local silencing increases the resistance of *Candida glabrata* to fluconazole and oxidative stress

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In *Candida glabrata*, the sirtuins Sir2 and Hst1 control the expression of a wide number of genes including adhesins required for host colonization and niacin transporters needed for growth. Given that these sirtuins can be inactivated during infection, we asked if their inhibition could modify the response of *C. glabrata* to other stressful conditions. Here, we found that deletion of *HST1* decreases susceptibility of *C. glabrata* to fluconazole and hydrogen peroxide. The transcription factor Pdr1 and the ABC transporter Cdr1 mediated the fluconazole resistance phenotype of the *hst1*Δ cells, whereas the transcriptional activator Msn4 and the catalase Cta1 are necessary to provide oxidative stress resistance. We show that the transcription factor Sum1 interacts with Hst1 and participate in the regulation of these genes. Interestingly, even though *C. glabrata* and *Saccharomyces cerevisiae* are closely related phylogenetically, deletion of *HST1* decreased susceptibility to fluconazole and hydrogen peroxide only in *C. glabrata* but not in *S. cerevisiae*, indicating a different transcriptional control by two similar sirtuins. Our findings suggest that Hst1 acts as a regulator of stress resistance associated-genes

Aspergillus terreus HIGHER LOVASTATIN PRODUCTION IN SOLID-STATE FERMENTATION CORRELATES WITH HIGHER EXPRESSION OF GENES *laeA* AND *lovE*

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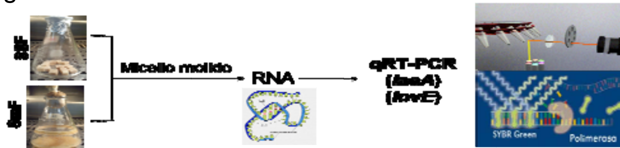
Keywords: laeA, lovE, lovastatin

Introduction. In a previous work we developed a high lovastatin producing system based on solid-state fermentation (SSF) on artificial inert support. In this culture system, *A. terreus* showed higher production, in relation to submerged fermentation (SmF) (1). Recent studies indicate that the fungus receives environmental signals, indicating the culture system, which generate a differential gene expression (2, 3 and 4). LaeA is a global regulator of secondary metabolism in *Aspergillus sp.* and other filamentous fungi, and part of the cAMP-PKA signaling pathway. This pathway transduces environmental stimuli, such as nutrient availability and others, and regulates genes associated with growth. Under stress conditions, this pathway activates genes related to secondary metabolism, sporulation, and stress resistance (5).
In the present work we studied the behavior of *laeA* and *lovE* regulatory genes during lovastatin SSF and FL, to determine molecular differences that help explain the higher production of lovastatin obtained in FS, in relation to the FL.

Methodology. Expression of genes *laeA* and *lovE* was determined (by qRT-PCR) during SSF with polyurethane as inert support, impregnated with liquid medium, and in SmF with *Aspergillus terreus* TUB F-514, a lovastatin producing strain (1). Total RNA was extracted using Trizol and treated with RQ1 RNase-Free DNase. The qRT-PCR was carried out using One-Step kit EXPRESS SYBR®GreenER™. A relative quantification was performed using a standard curve of *laeA* and *lovE* gene cDNA cloned in pGEM-T. These values were normalized with *H4* gene expression, which was used as endogenous control.



In SmF, biomass was quantified by dry weight. During SSF, biomass was determined by glucosamine content through a colorimetric method. Lovastatin concentration was quantified by HPLC (1).

Results. As in previous works, we observed higher lovastatin production in SSF, in relation to the SmF. On day 5, the SSF specific production was about 9 times higher than SmF (Fig 1).
To study the potential role of genes *lovE* y *laeA* in the higher lovastatin production observed in SSF, their expression was quantified during SSF and SmF. As expected, no *lovE* transcripts were detected during growth phase (trophophase). However, in idiophase its expression was 3.2- and 2.4-fold higher in SSF (42 h and 72 h), in relation to SmF.

Surprisingly, *laeA* expression started during trophophase (18 h), and continued through out idiophase, in both culture systems. As in the previous case, at 72 h, *laeA* expression was 6.2-fold higher in SSF than in SmF.

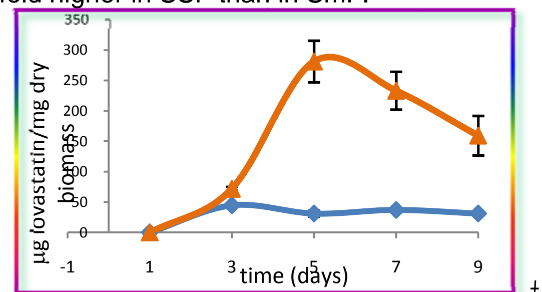


Figure 1. Lovastatin specific production by *A. terreus*, in SSF(▲) and SmF (◆).

This is consitent with the higher *lovE* expression and higher lovastin production found in SSF. These results also agree with the report of Barrios-González *et al.* (2008) (3), where the authors calculated a 4.6-fold higher *lovE* expression in SSF, by using Northern analysis.

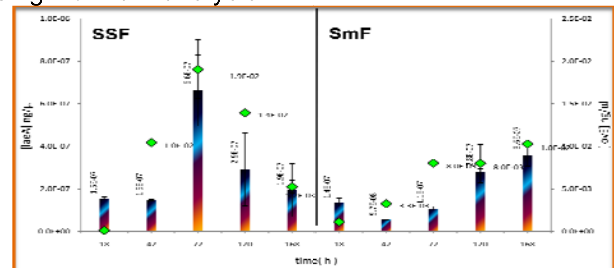


Figure 2. qRT-PCR analysis showing *laeA* (■) and *lovE* (◆) expression during the course of lovastatin SSF and SmF.

Conclusions. Results show that higher lovastatin production in SSF is, at least partially, due to higher expression levels of regulatory genes *laeA* and *lovE*. Results also indicate a role of signaling pathway cAMP-PKA in transducing environmental cues indicating it is a solid medium. This, in turn, results in differential genes expression, giving rise to a different physiology in SSF.

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References.

1) Baños J., Tomasini A., Szakács G, Barrios-González J. (2009). *J of Bioscience and Bioeng.* 108(2): 105-110.
2) Hisada H., Hata Y., Kawato A., Abe Y., Akita O. (2005). *J of Bioscience and Bioeng.* 99(6): 562-568.
3) Barrios-González J., Baños J., Covarrubias A., Garay-Arroyo A. (2008). *Appl Microbiol Biotechnol.* 79: 179–186.
4) Barrios-González J. (2012). *Process Biochemistry.* 47: 175–185.
5) Bok J. W., Keller N. P. (2004). *Eukaryot. Cell.* 3:527-35.

Lovastatin production, ROS accumulation and sporulation in *Aspergillus terreus* with a silenced gene *Atyap1*

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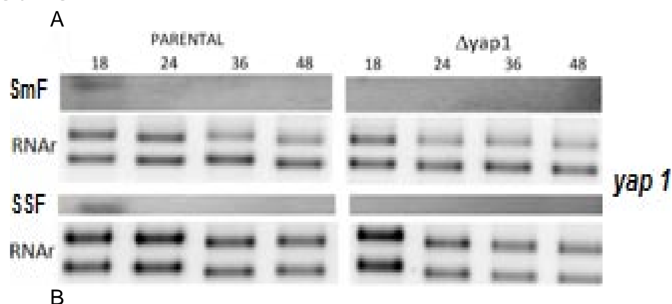
Introduction. Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS), and the intracellular antioxidant defenses. To keep healthy ROS levels, the cells have developed mechanisms to detect and respond to oxidative events (1). Yap1 is a transcription factor that acts as a redox sensor and is activated directly by increased levels of ROS. In a previous work, we showed that ROS contribute to the regulation of lovastatin biosynthesis in *Aspergillus terreus* (1). An *in silico* analysis of the promoter of transcription factor of lovastatin biosynthetic pathway *LovE*, revealed potential binding sites for Yap1(1) . In order to study the molecular mechanism by which ROS regulate lovastatin biosynthesis (2), *yap1* gene was silenced and transformants were characterized.

Methodology. Vector *pGdpPki-RNAi-yap1* was constructed and transformed into *A. terreus* TUB F-514, a high lovastatin biosynthesis strain. Transformants were characterized for their sensitivity to H_2O_2, ROS accumulation, and lovastatin production. Sporulation kinetics were obtained by quantifying with Neubauer chamber. Lovastatin was determined by HPLC in ACN extracts samples from solid-state (SSF) and liquid submerged fermentations (SmF). ROS accumulation over time was determined using the technique described by U. Miranda, 2008. The analysis of gene expression of *yap1* and *lovE* was performed by Northen Blot technique.

Results.



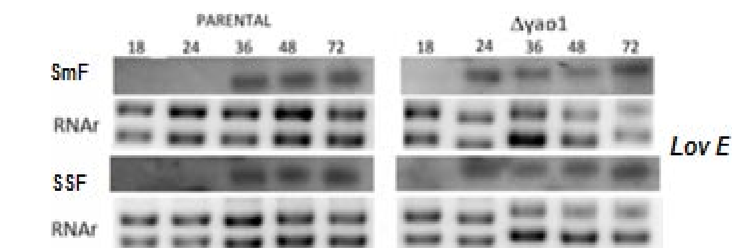


Figure 1. Northen Blot analysis comparing expression of *yap1* (A) and lovE (B) in the transformant (silenced *yap1*) and the parental strain, in SSF and SmF.

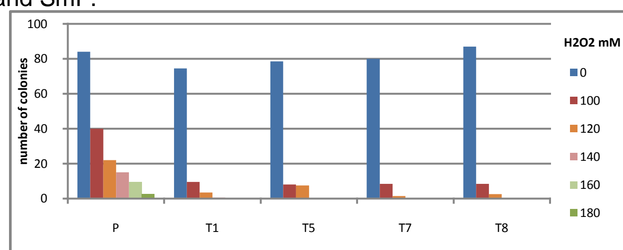


Figure 2. Sensitivity of spores to H_2O_2, of P (parental) and transformants:T1toT8.

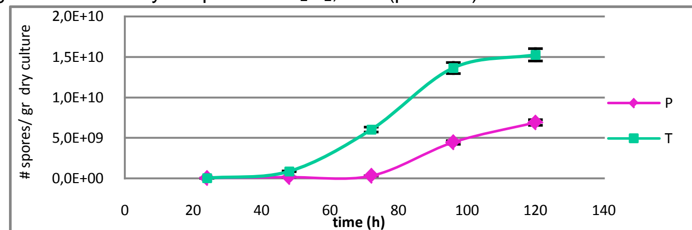


Figure 3. Sporulation kinetics of P (parental) and T7 (transformant) during lovastatin SSF.

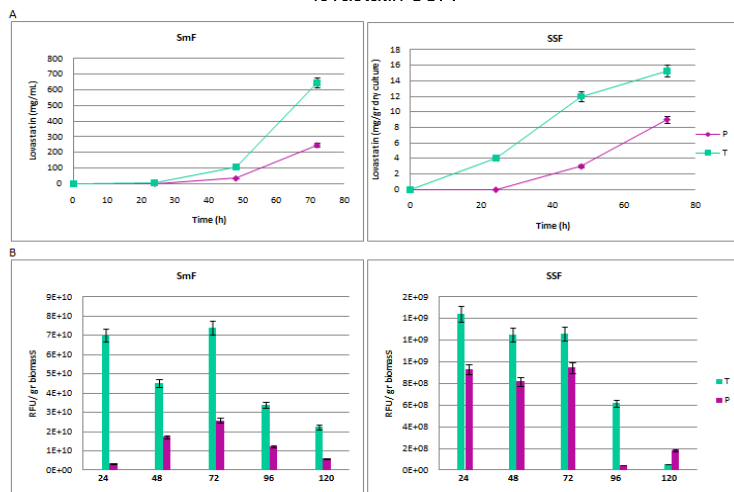


Figure 4. Lovastatin (A) and ROS (B) kinetics in SmF and SSF using P (parental) and selected transformant (T7).

Conclusions. *yap1* silencing caused a marked sensitivity to H2O2 in the transformants. The transformant showed a precocious increase in ROS accumulation, which provoked an early onset of lovastatin biosynthesis (and early *lovE* expression) and conidiation. This suggests that *yap1* negatively regulates *lovE*, as well as sporulation-related genes in *Aspergillus terreus* sporulation but upregulated genes to combat the accumulation of ROS.

References.

1. U. Miranda, Barrios-González, (2013a), Oxidative state in idiophase links reactive oxygen species (ROS) and lovastatin biosynthesis: Differences and similarities in submerged and solid state fermentations. Fungal Biology.

2. Miranda RU, Gómerz LE, Fierro F, Barrios-Gonzalez J. (2013b), Reactive oxygen species participate in the regulation of lovastatin biosynthesis in Aspergilus terreus. Submitted for publication. Fungal Biology.

Regulation of the expression of the acidic ribosomal proteins in *Saccharomyces cerevisiae*

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ABSTRACT

The expression of all ribosomal proteins is highly regulated in yeast, as the rest of superior eukaryotes, so that is not possible to detect “free” ribosomal proteins in cytoplasm. In the case of *S. cerevisiae* it has been observed that regulation occurs more frequently in a transcriptional level. The only ribosomal protein components that could be found in a free manner in the cytoplasm are the proteins that form the ribosomal stalk. The ribosomal stalk is a structure that is present in the large subunit from ribosome; it is evolutionarily conserved in the 3 domains. In *S. cerevisae* is formed by four acidic phosphoproteins (or acidic ribosomal proteins) P1A, P1B, P2A and P2B of an approximate weight of 12kDa and that along the protein P0 form a pentameric structure. The acidic ribosomal proteins (ARP) are the only ribosomal proteins that are present with more than a copy in the ribosome and interact with soluble factors of translation, affecting its function during the initiation and elongation events. A cytoplasmic pool of the 4 ARP has been described en yeast and other organisms. Additionally, there is an active refill between the cytoplasmic pool and de ribosomal stalk, the existence of a cytoplasmic pool and the presence of the ARP in more than a copy in the ribosome, make suppose that mechanisms responsible of the regulation of these proteins must be, at least in part, different from the rest of ribosomal proteins. The inherent mechanisms to gene regulation of acidic proteins are unknown. The control of a gene expression occurs in different levels as: transcription, post-transcriptional process, mRNA stability, translation, post-translational and protein degradation. Some mRNA whose products form part of the same physiological events, show coordination in their stability and therefore, a decay rate; this observation allows to suppose the existence of “decay regulons” as a post-transcriptional modulation event. During a medium heat shock event from 23 to 36 degrees Celsius, there is a decrease in the synthesis of a protein group, ribosomal proteins specifically; in contrast, in the rest of proteins there isn’t a significant change in the expression of the rest of proteins, except for those of thermal shock response. Therefore, this work pretends to study the behavior of ARP during a thermal shock event and observe, thus, its regulation. This will be realized characterizing the behavior of decay kinetic from mRNA and the 4 ARP and their correlation with the expression of their protein products.

Analysis of *xyl3* gene expression in *Fusarium oxysporum* f. sp. *lycopersici* strains

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Abstract

Fusarium oxysporum f.sp. *lycopersici* is a phytopathogen fungus causing vascular wilt disease on tomato, known as fusariosis. In 2008, Martinez-Rocha *et al.* generated mutants with an inactivated copy from the gene *Rho1* (*rho1::hyg,)* which loses the ability to infect tomato plant. This group also generated the mutant *rho1::hyg+rho1G14V* from *F. oxysporum,* which expressed the active form of the protein Rho1 and retains its pathogenicity. A comparative proteomic analysis of the secretory vesicles of the wild strain 4287, *rho1::hyg* and *rho1::hyg+rho1G14V* from *F. oxysporum* carried out by Macías-Sánchez *et al.* in 2011*,* suggests a differential expression of the proteins laccase 5, xyl3, Fga2, among others. In this work, we analyze of *xyl3* gene expression the wild strain 4287, *rho1::hyg* and *rho1::hyg+rho1G14V.* The three strains from *F. oxysporum* were grown for 24 h in synthetic medium supplemented with xylan 1% (from beechwood) as carbon source. Total RNA obtained from mycelia was used to generate cDNA using transcriptase reverse. PCR conditions were as follows: 40 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 1 min. An initial denaturation step of 5 min at 94°C and a final elongation step at 72°C for 7 min were included. A transcript of 629 pb was detected in mycelia obtained from *rho1::hyg* and *rho1::hyg+rho1G14V,* while in the wild strain there is a lower expression. These results could indicate that the carbon source may play an important role in the regulation of this gene, as obtained by Ruiz-Roldan *et al.*, 1999, which employed different carbon sources. They observed that *xyl3* were induced on oat spelt xylan but not on larchwood xylan. This is because the two commercial sources may differ in their carbohydrate composition, indicating a considerable degree of specificity in the regulation of expression of this gene. Also the expression of *xyl3* may be influenced by the expression and state of the protein Rho1.

Quantitative expression analysis in *Sporothrix schenckii*: identifying constitutively expressed genes for data normalization

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Sporothrix schenckii is a dimorphic fungus that causes sporotrichosis, a lymphocutaneous disease of cosmopolitan distribution, more prevalent in tropical and subtropical areas. The infection generally occurs by traumatic inoculation of soil, plants, and organic matter contaminated with the fungus. Nowadays there are few basic studies about this organism due to limited availability of molecular tools and a genomic sequence database. Recently, a gene silencing methodology was established, but gene expression analyses are required to validate it. In addition, the gene expression assays are also important to assess how cells respond to external stimuli, and thus to determine its adaptation to several environmental conditions. There are different methods to quantify mRNA abundance, such as Northern blotting, RNAse protection assays, microarrays and qRT-PCR. Regardless the chosen methodology, it is required a result normalization using endogenous control genes, which are expected to have minimal expression variation under different experimental conditions.

Housekeeping genes are commonly used as controls for expression normalization, because it is assumed that they do not have significant variations in their expression. However, there are recent reports indicating that some “housekeeping genes” may have variations in their expression when cells are grown in different environmental conditions. Furthermore, this group of genes are organism-specific.

Here, we are aiming to identify genes whose expression is not significantly different during the dimorphic transition of *S. schenckii*, and during its growth in different experimental conditions. Thus far, we have identified potential housekeeping genes with no significant changes in their expression in conidia, yeast cells and hyphae.

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Expression analysis of information contained in the *Candida glabrata MTL* loci: Possible heterodimeric molecules a1/alpha2, a1/alpha3 and a1/alpha4

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Sexual reproduction and genetic recombination increase biodiversity, but several human pathogenic fungi present mainly asexual reproduction; this is the case of *Candida glabrata*, an opportunistic pathogenic fungus which has no known sexual cycle. *C. glabrata* has three mating type loci: *MTL1* may contain type **a** information (**a1** gene) or type alpha information (alpha1 and alpha2 genes). The *MTL2* locus contains type **a** information and the *MTL3* locus contains alpha information. Both *MTL1* and *MTL2* loci are transcriptionally active, while *MTL3* presents an incomplete silencing. Interestingly, alpha information from *MTL3* locus is not identical to alpha information present in *MTL1* locus in the sequenced strain, so that alpha2 gene at *MTL3* locus (called alpha3) is a longer variant of the alpha2 gene present in *MTL1* locus. In some clinical isolates we found another variant (alpha4) at *MTL2* locus. In *S. cerevisiae* and *C. albicans* cells expressing both **a** and alpha information, the **a1**/alpha2 heterodimer repressor is found, this hetrodimeric molecule regulates cell identity. In *C. glabrata* expression of **a** information from *MTL1*, alpha4 information from *MTL2* in some clinical isolates and alpha3 from *MTL3*, could result in the formation of heterodimers a1/alpha2, a1/alpha3 or a1/alpha4, similar to a1/alpha2 hetrodimer found in *S. cerevisiae* and *C. albicans*. We will generate strains containing each protein fused to fluorescent protein genes (YFP, CFP and Cherry) as well as to different epitope tags (c-Myc, HA, FLAG) to determine whether all these genes are translated, and wheter the heterodimers or homodimers are formed by CoIP. We have developed a set of integrative and replicative vectors to generate translational fusions of any gene with each of the three different fluorophores or the three epitopes. Integrative vectors will allow homologous recombination of the fusion protein into its normal locus in the genome in one step. With these new vectors we will perform the experiments described to understand the role the genes encoded in the *MTL* loci play in this organism and why they have been conserved throughout evolution.

Recombinant Expression of an immunodominant antigen from *Mycobacterium tuberculosis* in *Pichia pastoris*

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Introduction: Tuberculosis is a global health issue, affecting 9 million of people each year around the world. It has been reported that current BCG vaccine does not confer full protection against the mycobacterium, thus the necessity to improve novel ways of vaccination. Antigens from *M. tuberculosis (Mtb)* were assayed previously, and the secreted glycoprotein APA (45/47 kDa) was described as an immunodominant antigen with an *O*-mannosylation pattern in 4 residues of Threonine, conferring antigenic properties to the molecule. Mannoses added in the C-terminal is suspected to influence T-cell proliferation as well as delayed-type hypersensitivity (DTH) reaction when expressed in *M. smegmatis* and *M. bovis,* unlike APA unglicosilated from *E. coli*. Recently, the expression of APA in *S. lividans* proved to mediate a stronger lymphoproliferative response compared with native protein from *Mtb*. In this project a novel bioprocess was designed to express recombinant APA in the fungal yeast *P. pastoris* to facilitate induction, expression, purification, culture time and media. Further characterization of the C-terminal and *in-vitro* immunoassays will determine the properties of rAPA compared with previous expression systems.
Objective: Design, express and characterize rAPA produced and secreted in the methylotrophic host *P. pastoris*
Methodology: According to databases, gene *modD* was optimized for *P. pastoris* system and unwanted glycosilation sites mutated. The sequence was synthesized and ligated into the expression vector, transformed *E. coli* and selected with LB+Antiobiotic media. The plasmid was propagated, purified and sequenced, then electroporated in *P. pastoris* X-33 and grown in YPD+Antibiotic until colonies formed. PCR was performed to confirm correct insertion and genome number integration. Fermentation was perfomed in BMMY media and induction was done with methanol. Protein was separated in a 15% SDS-PAGE and Western-blots were assayed with anti-APA. Subsequently, rAPA will be purified by Con-A, and digested with Lys-C. The C-terminal fragment will be analyzed by MALDI-TOF to observe mannosylation patterns.
Results: PCR and DNA electrophoresis of plasmid from an isolated colony of *E. coli* successfully transformed with pAPADJNV proved the desirable plasmid size. Sequentiation proved the correct insertion of the gene into an ORF. The sequence backtranslated into corresponding aminoacid and Blast confirmed 100% homology to native APA. Transformation of *P. pastoris* was achieved and amplification of the *modD* fragment proved the genome site insertion. Supernatants of induced cultures are currently being analyzed by SDS-PAGE and Western-Blots.
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Caracterización de los genes de *Kluyveromycesmarxianus* involucrados en la producción del 2-feniletanol mediante la Vía de Ehrlich

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Las cepas de *Kluyveromycesmarxianus*han sido descritaen la literatura como uno de los mejores productores de 2-feniletanol, el cual posee un agradable aroma a rosas y es el segundo alcohol más importante comercialmente. Mediante la vía de Ehrlich, la fenilalanina es transaminada a fenilpiruvato, luego descarboxilado a fenilacetaldehido y finalmente reducido a 2-feniletanol. La vía de Ehrlich es un proceso complejo, ya que las levadurasutilizan al menos tres aminotransferasas, cinco descarboxilasas y seis deshidrogenasas. ARO8 y ARO9 fueron caracterizados como aminotransferasas de aminoácidos aromáticos I y II respectivamente.Los genes PDC1, PDC5, y PDC6 codifican piruvatodescarboxilasas, mientras que ARO10 es un gen candidato alternativo para descarboxilasas de la vía de Ehrlich. El objetivo de este trabajo es caracterizar los genes de *K.marxianus* involucrados en la producción del 2-feniletanol mediante la vía de Ehrlich, por lo que se realizaron fermentaciones a 30 y 37°C con 9 g/L de L-fenilalanina, durante 72h de cultivo, por *Kluyveromycesmarxianus* ITD0090. La mayor producción se obtuvo a 37°C. Se realizó la búsqueda de los genes que participan en la vía de Ehrlich, reportados para *S. cerevisiae,* los cuales fueron utilizados como sondas para incursionar en el genoma de *K. marxianus.* Apartir de las secuencias obtenidas se realizó el diseño de oligonucleótidos, con los cuales se logró la amplificación génica de dosaminotrasnferasas ARO8 y ARO9 y, una descarboxilasa ARO10. Tres genes de *Kluyveromycesmarxianus*involucrados en la vía de Ehrlichfueron secuenciados.

Palabras clave: Aminotrasnferasas, descarboxilasas, *Kluyveromycesmarxianus,* 2-feniletanol.

A Novel and Highly Efficient Method for Genetic Transformation of Fungi Employing Shock Waves

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Filamentous fungi have been used to study metabolic processes and genomic organization of eukaryotes for many decades. Filamentous fungi are attractive organisms for the production of diverse compounds such as antibiotics, enzymes, therapeutic agents, secondary metabolites and industrial polymers. Furthermore, filamentous fungi are the ideal organisms for heterologous protein expression because they are capable of secreting large amounts of enzymes. The genus Aspergillus can secrete up to 40 grams per liter of native enzymes respectively, which is at least twice the production of the best production systems (CHO cells). Introduction of heterologous sequences by genetic transformation has been a necessary requirement to obtain fungal strains that produce compounds with high yields as well as to study particular aspects of fungal metabolism. However, the current methods for genetic transformation of filamentous fungi (protoplasts, Agrobacterium-mediated transformation or biolistics) are costly, very inefficient and have low reproducibility. There are some fungal species that have been deemed recalcitrant to genetic transformation. . Cklearly, more efficient methods for genetic manipulation of filamentous fungi are needed.
The methods was successfully tested in Aspergillus niger, Fusarium oxysporum, Trichoderma reesei and Phanerochaete chrysosporium, among other species. The transformation frequency per number of conidia was between two and four orders of magnitude higher in comparison to previously published methods. For example, the frequency of transformation in A. niger was improved up to 5,400-fold as compared with Agrobacterium-based protocols. Transformation was verified by expression of the green fluorescent protein, PCR and Southern blot. Our method offers new possibilities for fast, easy and efficient genetic manipulation of diverse fungal species.