XIV CONGRESO DE BIOLOGÍA MOLECULAR Y CELULAR DE HONGOS

Academic Program

Sociedad Mexicana
de Bioquímica A.C.

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Jorge Verdín, CIATEJ

Guadalajara, Jalisco, México. Hotel Camino Real 15 al 19 de octubre de 2023

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ACKNOWLEDGEMENTS

We would like to express our gratitude to the Institutions and Companies that provieded financial support to this Congress

Academic Program

SUNDAY October 15

11.00 - 16:30	Registration
16:45-17:00	OPENING CEREMONY
17:00 - 18:00	OPENING KEYNOTE ***Saccharomyces* variation across the world** *Gianni Liti* Institute for Research on Cancer and Ageing of Nice Université Côte d'Azur. France Chair: Lina Riego. IPICyT
18:00 – 19:00	CULTURAL TALK **La ciencia de la Luz y el color detrás de la cámara en Pinocchio, de Guillermo del Toro** *Michel Amado* Artista. Cinefotógrafo / Fotógrafo Chair: Alicia González. IFC. UNAM
19:30 – 21:30	WELCOME RECEPTION. GRAN ALAMEDA ROOM

MONDAY October 16

9:00 - 10:00	KEYNOTE 2 **How a fungus protects itself when producing a toxic secondary metabolite** *Gustavo H. Goldman* Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil Chair: Alfredo Herrera. UGA. Cinvestav Unidad Irapuato
10:00 - 11:30	SYMPOSIUM 1 **Dr. José Ruiz Herrera. *In Memoriam*** **Las contribuciones mas sobresalientes de José Ruiz Herrera a la biología celular de los hongos** *Salomon Bartnicki García* Centro de Investigación Científica y de Educación Superior de Ensenada

	Lo aprendido en el estudio del efecto del estrés en las células. Lo bueno, lo extraño y lo impresionante: notas del modelo de enseñanza/aprendizaje de un gran maestro mexicano *Elva Aréchiga* Universidad Autónoma de Nuevo León **José Ruiz, el ser humano detrás del científico** *Claudia Gerladine León Ramírez* Cinvestav Irapuato Chair: Alfredo Herrera. UGA. Cinvestav Unidad Irapuato
11:30 – 12:00	COFFEE BREAK
12.00 - 13:30	SYMPOSIUM 2 **Biotechnology** **Plant growth promotion, stress tolerance and pathogen resistance in chili plants inoculated with a *Trichoderma atroviride* strain expressing the cell wall remodeling protein SWO1.** *Jorge Luis Folch Mallol* Centro de Investigación en Biotecnología, UAEM **Proteomic comparison of the toxic effect of aurofusarin from *Fusarium graminearum* on azole-resistant *Candida albicans* strains** *Ana Lilia Martínez Rocha* Departamento de Biología, DCNE, Universidad de Guanajuato. **CRISPRi for transcriptional regulation of *IAH1* gene and its impact on volatile compounds profile in *Kluyveromyces marxianus* DU3.** *Luis Alfonso Muñoz Miranda*, Alejandro Pereira Santana, Leticia Casas Godoy, Andrés Méndez Zamora, Iliana del Carmen Barrera Martínez, Andrea Catalina Zepeda Peña, Anne Christine Gschaedler Mathis, Luis J. Figueroa Yáñez. CIATEJ-Zapopan **Al and Zn binding peptides displayed on the surface of *Neurospora crassa* to build biofilters.** *Francisco Anguiano*, Paul Montaño Silva, Leticia Casas Godoy, Luis Arellano García, Jorge Verdín. CIATEJ Zapopan Chair: Jorge Verdín. CIATEJ
13:30 – 15:00	*LUNCH.* (for your own)
15:00 – 15:30	FLASH TALKS FOR POSTERS ADVERTISING **Blood serum stimulates the virulence through the enhancement of the mitochondrial oxidative metabolism and rhizoferrin production in Mucorales.** J. *Alberto Patiño Medina,* Viridiana Alejandre Castañeda, David Vargas Tejeda, Marco I. Valle Maldonado, Rafael Ortiz Alvarado, Joel Ramírez Emiliano, Martha I. Ramírez Díaz, Karla V. Castro Cerritos, Victoriano Garre, Ulrike Binder, Víctor Meza Carmen. Universidad Michoacana de San Nicolás de Hidalgo

CFEM proteins in *Neofusicoccum parvum* and their role in the pathogenicity process. *Edgar D. Carrillo Hernández*, Nohemí Carreras Villaseñor, Javier Plasencia de la Parra, Eric E. Hernández Domínguez y Diana Sánchez Rangel. Instituto de Ecología, A.C.

The volatile 6-pentyl-2Hpyran- 2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root meristem via an ethylene-mediated phosphate (Pi) deficiency response. *Saraí Esparza-Reynoso*, José López-Bucio, Alfredo Herrera-Estrella. UGA-LANGEBIO Cinvestav-Irapuato

Evolution of *Candida glabrata* throughout the course of an infection. *Ana Lizbeth López Marmolejo,* Guadalupe Gutiérrez Escobedo, M. Selene Herrera Basurto, Alejandro De la Peñas, Irene Castaño. IPICYT

LOX1 and PLP1 lead a transcriptional reprogramming essential for injury-induced conidiophore development in *Trichoderma atroviride*. *Martín O. Camargo Escalante*, Edgar Balcázar López, Exsal M. Albores Méndez, Robert Winkler and Alfredo Herrera Estrella. Unidad de Genómica Avanzada, Cinvestav

Effects of culture time and carbon source on the proteome of *Neurospora crassa* extracellular vesicles. *Daniel Alfonso Salgado Bautista*, Rubén Cadena Nava and Meritxell Riquelme Pérez. Departamento de Microbiología. Centro de Investigación Científica y de Educación Superior de Ensenada

Effects of human Tau protein expression on the yeast mitochondrial physiology. *Yaisa Castillo Casaña*, Laura Kawasaki, and Roberto Coria. Departamento de Genética Molecular, Instituto de Fisiología Celular. UNAM

Gene co-expression network and the regulation by the RNAi machinery during mycoparasitism in *Trichoderma atroviride*. *Camilo Pérez Salazar*, Eli Efrain Enriquez Felix, José Guillermo Rico Ruiz, José Manuel Villalobos Escobedo and Alfredo Herrera Estrella. UGA-LANGEBIO Cinvestav-Irapuato

***Trichoderma* requires the Arabidopsis DICER-LIKE 3 and ARGONAUTE 9 proteins to modulate the expression of the Nitrile Specifier protein 4 (NSP4) gene**. *Maria Montserrat Rosendo Vargas*, Oscar Guillermo Rebolledo Prudencio, and Sergio Casas Flores. Division de Biología Molecular. IPICYT

Transcriptomic profile of *Colletotrichum lindemuthianum* pathotypes. *Ma. Irene Morelos Martínez*, Horacio Cano Camacho, Karla Morelia Díaz Tapia, Everardo López Romero, June Simpson, María Guadalupe Zavala Páramo. Centro Multidisciplinario de Estudios en Biotecnología, FMVZ, Universidad Michoacana de San Nicolás de Hidalgo

Chair: Alexander de Luna. UGA LANGEBIO

15:30 – 17:00	SYMPOSIUM 3 **Fungi-Host Interaction** **Effector prediction, a challenging task in a world in constant evolution** *Blondy Beatriz Canto Canché*. Centro de Investigación Científica de Yucatán **Characterizing micro-transcriptomic response during plantbeneficial and -pathogenic fungal interactions** *Mario Serrano* Centro de Ciencias Genómicas, UNAM **The *DNA-PrimL* gene of *Arabidopsis thaliana* a potential target of the *Trichoderma atroviride* small RNA1 during their mutualistic relationship**. *Eyra Judith Hernández Hernández*, Mitzuko Dautt Castro and Sergio Casas Flores. IPICYT **Bacterial Guests and Fungal Love: Unraveling the Secrets of a Holobiont Model**. *José Francisco Cabrera Rangel*, Gonzalo Córdova López, J. Roberto Bermúdez Barrientos, Raúl Alcalde Vázquez, Robert Winkler and Laila P. Partida Martínez. Cinvestav Irapuato Chair: Laila Partida. Cinvestav Irapuato
17:00 – 17:30	COFFEE BREAK
17:30 - 18:30	KEYNOTE 3 **Genome Evolution of *Saccharomyces* Yeasts and Their Interspecies Hybrids** *Lucía Morales* Laboratorio Internacional de Investigación sobre el Genoma Humano. UNAM Chair: Lina Riego. IPICyT
18:30 – 20:00	POSTER SESSION I Even Numbers

TUESDAY October 17

9:00 - 10:00	KEYNOTE 4 **Fungal responses to global climate change in a dryland ecosystem and potential impacts to public health** *Adriana Romero Olivares* New Mexico State University. USA Chair: Ramón Batista. UAEM

10:00 - 10:30	SPONSOR TALK **Molecular Biology Applications in Fungi** *David Sánchez Marín* UNIPARTS Chair: Alfredo Herrera. UGA LANGEBIO Cinvestav
10:30 - 12:00	HAVING COFFEE WITH&..
10:30 – 11:30	BUSSINESS SESSION
11:30 – 12:00	COFFEE BREAK
12:00 – 13:30	SYMPOSIUM 4 **C e l l B i o l o g y** **New views on fungal morphology** *Braulio Gutiérrez Medina* Instituto Potosino de Investigación Científica y Tecnológica, IPICYT **Unveiling Microtubule Architecture and Functions in Filamentous Fungi: Insights into Cellular Dynamics** *Rosa Mouriño Pérez* Centro de Investigación Científica y de Educación Superior de Ensenada **Role of LST-1 during the formation of ERV-14 dependent COPII vesicles in *Neurospora crassa*.** *Mónica Esther Cante Paz,* Meritxell Riquelme Pérez. Centro de Investigación Científica y de Educación Superior de Ensenada. **The nucleolar structure in *Ustilago maydis*.** *Oscar Said Quiroz Zerecero*, Reyna Lara Martínez, Claudia Geraldine León Ramírez, José Ruiz Herrera, María de Lourdes Segura Valdez, Luis Felipe Jiménez García. Facultad de Ciencias, Universidad Nacional Autónoma de México Chair: Leonardo Peraza. IFC. UNAM
13:30 – 15:00	*LUNCH.* (for your own)
15:00 – 15:30	FLASH TALKS FOR POSTERS ADVERTISING **Yeasts from open agave fermentation show geographic populations structure with sparse conservation of introgressions**. *J. Abraham Avelar Rivas*, Luis Fernando García Ortega, Iván Sedeño, Claudio López, Antonio Urbán Aragón, Eugenio Mancera, Alexander de Luna, Lucía Morales. UGA-LANGEBIO, CINVESTAV Irapuato ***Trichoderma brevicompactum* 2IG2102 has features of biological control agent and produces secondary metabolites that contribute to its antagonism towards fungal phytopatogens**. *Luis David Maldonado Bonilla*, Xuxani Juquila Moreno López, José Luis Villaruel Ordaz, Aneliz de Ita Zárate Ortiz, Humberto Valenzuela Soto, Rommel Carballo-

	Castañeda, Aldo Moreno Ulloa, Ana Lilia Torres Machorro. Universidad del Mar, Puerto Escondido **Molecular and sensitive specific detection of *Candida glabrata*.** *Marco Josué Hernández Chávez*, Enrique Merino Pérez, Irene Castaño. IPICYT **Characterization of GPI-proteins ACW-1 and CCG-6, and CBM-52 protein NCW-3 in *Neurospora crassa* as possible anchors for a surface display system.** *Ana Sofía Ramírez Pelayo*, Lorena Amaya Delgado, Jorge Rodríguez, Jorge Verdín. CIATEJ-Zapopan **The unexplored apical and subapical organization of the endoplasmic reticulum in growing hyphae of *Neurospora crassa*.** *Martínez Andrade J.*, Roberson RW. and Riquelme M. CICESE **Pseudogenization-driven gene loss shapes genome evolution in Hanseniaspora.** *Luis Fernando García-Ortega*, Ángela María García Acero, Alexander De Luna, Lucía Morales, Luis Delaye, Eugenio Mancera. Cinvestav-Irapuato **The vacuolar proteases of the yeast multi-resistant to antifungal *Candida auris* in autophagy conditions**. *Heriberto Daniel Clark Flores*, Alvaro Vidal Montiel, Margarita Juárez Montiel, Juan Alfredo Hernández García, César Hugo Hernández Rodríguez, María de Lourdes Villa Tanaca. Escuela Nacional de Ciencias Biológicas. IPN **Secreted Rich Cystein Protein 1 (SCP1) from Trichoderma atroviride is a new effector candidate involved in plant interaction process**. *Francisco Vargas Gasca*, Juan Ignacio Macías Segoviano, Alfredo Herrera Estrella & Vianey Olmedo Monfil. División de Ciencias Naturales y Exactas. Universidad de Guanajuato ***Candida glabrata* secretome molecules from stationary-phase induce a quiescence-like state in growing cells.** Carlos Ricardo González Ruiz, Javier Montalvo Arredondo, Juan Ernesto López Ramos, Guadalupe Gutiérrez Escobedo, Irene Castaño and Alejandro De Las Peñas. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica **The yapsins of the yeast multi-resistant *Candida auris* in osmotic and cell wall stress conditions.** *Alvaro Vidal Montiel*, Daniel Clark Flores, Lourdes Villa Tanaca, Margarita Juaréz Montiel, César Hernández Rodríguez. Escuela Nacional de Ciencias Biológicas. IPN Chair: Jorge Verdín. CIATEJ
15:30 – 17:00	SYMPOSIUM 5 **Transcriptional regulation and epigenetics** **Deciphering the biological relevance of a KilA-N/APSES Transcription Factor in Fusarium sp. associated with the ambrosia beetle *Xylosandrus morigerus*** *Diana Sánchez Rangel* Instituto de Ecología, A.C. Red de Estudios Moleculares Avanzados (REMAv)

	The epigenetic dialogue in plant-Trichoderma interaction. *Sergio Casas Flores***,** División de Biología Molecular IPCyT **Chromatin architecture in *Candida glabrata* plays a role in the regulation of EPA genes.** *Grecia Hernández Hernández,* Ma. Guadalupe Gutierrez Escobedo, Alejandro De Las Peñas and Irene Castaño. IPICyT **The yeast response regulator Skn7 is necessary for modulating the transcription of genes that respond to endoplasmic reticulum stress induced by the N-glycosylation inhibitor, tunicamycin**. *Diana Iris Hernández Rojas,* Laura Kawasaki and Roberto Coria Ortega. Instituto de Fisiología Celular, UNAM Chair: Vianey Olmedo. Universidad de Guanajuato
17:00 – 17:30	COFFEE BREAK
17:30 – 18:30	KEYNOTE 5 **Genomics gives unprecedented insights on adaptive and evolutionary processes driving the success of Black Fungi in the extremes** *Laura Selbmann* Department of Ecological and Biological Sciencias, University of Tuscia. Italia Chair: Ramón Batista. UAEM
18:30 – 20:00	POSTER SESSION 2 Odd numbers

WEDNESDAY 11

9:00 – 15:00	FREE MORNING
15:00 – 16:30	SYMPOSIUM 6 **S t r e s s** **Unraveling the mechanisms of lifespan extension by metformin in aging yeast cells** *Alexander De Luna* Cinvestav, Unidad de Genómica Avanzada. Langebio. Guanajuato **Exploring the fungal syntenome: genomic duplications and trait diversification**. *Alejandro Pereira Santana* Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Unidad Sureste **Cellular response of black yeasts of the genus Neophaeotheca, isolated from the Gulf of Mexico to nutrient scarcity and salinity.** *Ma. Dolores Camacho López*, Meritxell Riquelme. Ensenada Center for Research and Higher Education. CICESE

	The Osmotolerant Yeast *Debaryomyces hansenii* Under Nitrogen Limitation: Assessing the Role of Hog1 in Lipid Accumulation. *Diana Villarreal Huerta*, Lucero Romero Aguilar, Oscar Said Quiroz Zerecero, Luis Felipe Jiménez García, Norma Silvia Sánchez, Mohammed El Hafidi, Claudia Segal Kischinevzky & James González. Departamento de Biología Celular, Facultad de Ciencias, UNAM Chair: Elva Aréchiga. Universidad Autónoma de Nuevo León
16:30 – 17:00	COFFEE BREAK
17:00 – 18:30	SYMPOSIUM 7 **D e v e l o p m e n t** **Discovering the role of genes in developmental and metabolic processes in filamentous fungi with a genome-wide loss-of-function approach** *José Manuel Villalobos Escobedo* Plant and Microbial Biology, University of California, Berkeley. The Environmental Genomics and Systems Biology Div., The Lawrence Berkeley Nat. Lab. **Not all roads lead to Rome: the different secretory routes in *Neurospora crassa* hyphae** *Meritxell Riquelme Pérez* Departamento de Microbiología Centro de Investigación Científica y de Educación Superior de Ensenada CICESE **Evolution of the transcription circuit that regulates filamentation in two closely related species of Candida.** *Teresa Meza Davalos*, Luis F. García Ortega, Eugenio Mancera. Departmento de Ingeniería Genética, Cinvestav Irapuato **Using experimental evolution of hybrid genomes to identify genetic incompatibilities in yeast**. *Artemiza A. Martínez Medina* and Gregory I. Lang. Lehigh University. Bethlehem. Pennsylvania Chair: Eugenio Mancera. Cinvestav Irapuato
18:30 – 19:30	CLOSING KEYNOTE **Nitrate utilisation in the fungi, from replica plating to alpha-fold** Claudio Scazzocchio Université Paris-Saclay and Imperial College London Chair: Alicia González. Instituto de Fisiología Celular. UNAM
19:30 – 20:00	FINAL ANNOUNCEMENTS AND CLOSING CEREMONY
21:00 – 2:00	FAREWELL DINNER. GRAN ALAMEDA ROOM

POSTER SESSION I. Even Poster Presentation: Monday October 16th

POSTER SESSION II. Odd Poster Presentation: Tuesday October 17th

1.	**The peroxisome protein translocation machinery is developmentally regulated in the fungus *Podospora anserine*.** Beatriz Aguirre López, Fernando Suaste Olmos, Leonardo Peraza Reyes. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
2.	**Effects of human Tau protein expression on the yeast mitochondrial physiology.** Yaisa Castillo Casaña, Laura Kawasaki, and Roberto Coria. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
3.	**Identification of molecules with antibacterial activity from different fungal pathogens.** Jesús Antonio Arroyo García, Guadalupe Gutiérrez Escobedo and Alejandro De Las Peñas IPICyT, Instituto Potosino de Investigación Científica y Tecnológica
4.	***ALT2* and prostaglandin biosynthesis: a new metabolic pathway in the yeast *Saccharomices cerevisiae*.** Sheyla Sabel Cruz Cruz, José Carlos Campero Basaldua, Gabriel Del Río Guerra, Gabriel Marcelino Pérez, Alicia González Manjarrez. Instituto de Fisiología Celular. UNAM
5.	**Differential CWDEs secretion capacity among *Colletotrichum lindemuthianum* pathotypes.** Karla Morelia Díaz Tapia, María Guadalupe Zavala Páramo, Ma. Irene Morelos Martínez, Everardo López Romero, June Simpson, Horacio Cano Camacho. FMVZ, Universidad Michoacana de San Nicolás de Hidalgo
6.	**Establishment of growth conditions to increase the production of fungal dyes**. Lara Bravo I., Vázquez Martinez J., Alvarez Mejia C. and López Ramírez V. Tecnológico Nacional de México / ITS de Irapuato
7.	**The Role of Subcellular Localization on the Functional Diversification of the Paralogous Proteins Bat1 and Bat2**. Yamile Paredes Chiquini, Daniela Trejo Zambrano, Alicia Gonzalez Manjarrez. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
8.	**Amyloid beta peptide expression' effect in the physiology of the endoplasmic reticulum of yeast.** Laura María Reyes Fermín, Laura Kawasaki and Roberto Coria Ortega. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
9.	**Heterologous expression and characterization of hydrophobins from the polyurethanolytic fungus *Cladosporium tenuissimum* A3.I.1 for their potential application in plastic biodegradation.** Zaizy Rocha Pino, Ana Paulina García Bernal, Brenda Sofía Jiménez Arreola, Martín Vargas Suárez, Luigui Gallardo Becerra, Adrián Ochoa Leyva, and Herminia Loza Tavera. Facultad de Química, UNAM
10.	**The Mitochondrial Alternative Oxidase in *Ustilago maydis* Is Not Involved in Response to Oxidative Stress.** Romero Aguilar Lucero, Vázquez Meza Héctor, Luqueño Bocardo Oscar Ivan, Guerra Sánchez María Guadalupe, Pardo Juan Pablo. Facultad de Medicina, Universidad Nacional Autónoma de México
11.	**Evaluation of kinetic growth parameters of parental, reconstituted, and hybrid strains of *Pleurotus eryngii.*** Erick Daniel Álvarez Ramírez, Hermilo Leal Lara, Gustavo Valencia Del Toro, Leticia Aguilar Doroteo. UPIBI, IPN
12.	***Mucor circinelloides* and *Coprinopsis cinerea* has genes are *bona fide* hyaluronic acid synthases?** Mariandrea Aranda Barba, Laura Marina Franco Herrera, Eddy Sánchez León, Rosa María Camacho Ruiz, Jorge Verdín. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
13.	**Pectinase enzymes produced from yeast using citrus waste.** Rut Mara Arteaga Ojeda, María del Socorro Ramírez-González, José Alberto NarváezZapata, y Claudia Patricia Larralde Corona, Centro de Biotecnología Genómica, IPN
14.	**Remotion of Cr (VI) with biomass of water Kefir and SCOBY of Kombucha.** Daniela Ayala Camarena, Adán Topiltzin Morales Vargas, Vicente Peña Caballero. Universidad de Guanajuato
15.	**Lacasse from *Trametes sanguineus*: A biocatalyst for modifying quercetin.** Iliana Barrera Martínez, David Alejandro Macias Martín, Israel Ramos Torres, Ricardo Cerón Camacho. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco

16.	**Expression analysis of the polyketide synthase gene *adaA* in a chromium reducer environmental strain and two collection strains of *Aspergillus***. Juan Carlos Bautista_Bautista, Ana Lilia Martínez Rocha, Adolfo López Torres, Fernando Santos Escobar y J. Félix Gutiérrez Corona. Universidad de Guanajuato
17.	**A new terpene from *Trichoderma virens* activates JA plant defense pathway and controls growth of *Sclerotium cepivorum*** Jonathan Duran Palmerin, César Alejandro García Morales, Carla V. Sánchez Hernández, Vianey Olmedo Monfil. División de Ciencias Naturales y Exactas, Universidad de Guanajuato
18.	**The action of chitosan on the development of soil isolates from the desert of Baja California Norte, Mexico**. Karina García Gutiérrez, Adriana Jazmín Legorreta Castañeda, Thabata Montserrat Hernández Cruz, Darío Rafael Olicón Hernández, María Guadalupe Guerra Sánchez. Escuela Nacional de Ciencias Biológicas, IPN
19.	**Utilization of Ureasa from *Ustilago maydis* of three strains in biocement production.** Nimsi Merari Gil Martínez, Dario Rafael Olicón Hernández, Maria Guadalupe Guerra Sánchez. Escuela Nacional de Ciencias Biológicas. IPN
20.	**Characterization of *Periconia macrospinosa* HAgJ2 isolated from *Agave tequilana* as a biocontrol agent.** Ingrid Melissa Gómez Vázquez, Fernando Uriel Rojas Rojas, Julio C. Vega Arreguin. Escuela Nacional de Estudios Superiores Unidad León UNAM
21.	**Evaluation of the antifungal activity of silver nitrate against the fungus that causes in *Agave salmiana* black mold.** Issela Granados Zamora, Teresa Romero Cortes, Martin Peralta Gil, Jaime Alioscha Cuervo Parra, Mónica Ivette Sánchez Contreras, María Magdalena Armendáriz Ontiveros, Víctor Hugo Pérez España. Escuela Superior de Apan. Universidad Autónoma del Estado de Hidalgo
22.	**A comparison study of pellet capacity formation between *Rhizopus stolonifer* and *Pleurotus ostreatus* and the effect of Methotrexate on spore germination.** Thabata Montserrat Hernández Cruz, Adriana Jazmín Legorreta Castañeda, Karina García Gutiérrez, Darío Rafael Olicón Hernández, María Guadalupe Guerra Sánchez. Instituto Politécnico Nacional
23.	**Degradation of chlorpyrifos and lambda-cyhalothrin by rhizospheric fungi from *Typha domingensis* plant in the Turbio River**. Daniella MJ. Hernández Pérez, José Francisco Cabrera Rangel, Juan Vázquez Martínez, Laila P. Partida Martínez y David A. Camarena Pozos. Centro de Innovación Aplicada en Tecnologías Competitivas
24.	**Isolation and selection of thermotolerant filamentous fungi producing amylases obtained from the Tolantongo caves.** Adriana Jazmín Legorreta Castañeda, Karina García Gutiérrez, María Guadalupe Guerra Sánchez, Darío Rafael Olicón Hernández. Escuela Nacional de Ciencias Biológicas, IPN
25.	***Trichoderma brevicompactum* 2IG2102 has features of biological control agent and produces secondary metabolites that contribute to its antagonism towards fungal phytopathogens.** Luis David Maldonado Bonilla, Xunaxi Juquila Moreno López, José Luis Villarruel Ordaz, Aneliz de Ita Zárate Ortiz, Humberto Valenzuela Soto, Rommel Carballo Castañeda, Aldo Moreno Ulloa, Ana Lilia Torres Machorro. Instituto de Genética, Universidad del Mar campus Puerto Escondido
26.	**Characterization of *Metarhizium* response to different abiotic factors.** Guadalupe Araceli López Andrade, Melissa Fernández García, Juan Carlos Torres Guzmán, Israel Enrique Padilla Guerrero, Olga Callejas Negrete, Eduardo Salazar Solís, Gloria Angélica González Hernández. Departamento de Biología, Universidad de Guanajuato
27.	**Exploring *Neurospora crassa* PIR proteins as molecular anchors for cellsurface protein display.** Paul Montaño Silva y Jorge Verdín. Biotecnología Industrial. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
28.	**Multiple display of peptides with affinity to Al and Zn on the cell surface of *Neurospora crassa* using the hydrophobin class 1, EAS, as an anchor.** Pablo Valentín Navarro Enguilo, Wilhelm Hansberg, Mauricio Flores Moreno and Jorge Verdín. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
29.	**Identification and characterization of a filamentous fungus isolated from plastic bags in an estuary in Mazatlán, Sinaloa.** Sandra Abigail Ortega Molinar, Israel Benítez García, Alfredo Herrera Estrella, Edgar Balcázar López. Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara

30.	**Evaluation of the Micellar Invasion Efficiency of Pleurotus ostreatus on Lignocellulosic Residues in Agar Matrix.** Tania Marieel Ovalle Ruiz, Nivia Iracemi Escalante García y Pamela Romo Rodríguez. Tecnológico Nacional de México. Campus Pabellón de Arteaga
31.	**Phenanthrene and Anthraquinone biodegradation by *Aspergillus spp.* isolated from contaminated soil in Reynosa, Mexico.** Bryan G. Pineda Cagua, Karen A. Cavada Prado, Timoteo Delgado Maldonado, Edgar E. Lara Ramírez, Gildardo Rivera Sanchez, Alma D. Paz González. Centro de Biotecnología Genómica. IPN
32.	**Análisis del potencial de degradación de naproxeno y ampicilina de cepas de hongos aisladas en Reynosa, México.** Mailyn Stephany Porras Garcia, Nancy Carolina Zuñiga, Timoteo Delgado Maldonado, Ana Verónica Martínez Vázquez, Gildardo Rivera Sanchez, Alma Delia Paz Gonzalez. Laboratorio de Biotecnología Farmacéutica, Centro de Biotecnología. IPN
33.	**Characterization of GPI-Proteins ACW-1 and CCG-6, and CBM-52 Protein NCW-3 in *Neurospora crassa* as possible anchors for a surface display system.** Ana Sofía Ramírez Pelayo, Lorena Amaya Delgado, Jorge Rodriguez y Jorge Verdín. Centro de Investigación y asistencia en tecnología y diseño del Estado de Jalisco, Unidad Zapopan
34.	**Diversity of secondary metabolites produced by *Beauveria bassiana* grown on different nutrients.** Yvonne Ramírez Cáceres, Rubén Santiago Adame, Ninfa M. Rosas García. Centro de Biotecnología Genómica. IPN
35.	**Caracterización morfológica de levaduras nativas aisladas de bebidas alcohólicas tradicionales y su potencial para fermentar azucares.** Jorge Oscar Rivera Hernández, Darío Rafael Olicón Hernández, Guadalupe Guerra Sánchez, Erika Yanet Tapia García. Instituto Politécnico Nacional
36.	**Enhancing pet degradation through tannase overexpression in *trichoderma atroviride*.** Andrea Román Delgado, Mario Iván Alemán Duarte, Alfredo Herrera Estrella, Edgar Balcázar López. Centro Universitario de Ciencias Exactas e Ingenierías. Universidad de Guadalajara
37.	**Detoxification of tequila vinasse by *T. sanguineus* for water reuse in seedling growth.** Karla Verónica Teymennet Ramírez, Soledad García Morales, Iliana Del Carmen Barrera Martínez. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
38.	**Potential degradation of LDPE using enzymatic extract from *trametes sanguínea*.** Jesús Urbar Ulloa, Iliana Barrera Martínez, Karla Verónica Teymennet Ramírez, Leticia Casas Godoy. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
39.	**Isolation and characterization of yeasts from a wastewater valorization plant.** Vázquez de los Reyes Dana Lorena, Escobar Sánchez Viviana, González James, Segal Kischinevzky Claudia. Facultad de Ciencias, Universidad Nacional Autónoma de México
40.	**Does CURDX, a putative cupredoxin, localize to the cell wall of *neurospora crassa*?** Claudia Cecilia Vega García, Jesús Urbar Ulloa, and Jorge Verdín. Center for Scientific Research and Assistance in Technology and Design of the State of Jalisco
41.	**Generation of a FRET system for *Saccharomyces cerevisiae* with the potential to detect the formation of oligomers of the hyaluronic acid synthase, CPS1, of *Cryptococcus neoformans*.** Yazmin Jeanette Velázquez López, Ana Sofía Ramírez Pelayo, Mariandrea Victoria Aranda Barba and Jorge Verdín. **C**entro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
42.	**Stoichiometric balance in *Pleorotus dijamor* on agave substrate (*Agave* sp.).** José Luis Zárate Castrejón, Adán Topiltzin Morales Vargas, Talina Olivia Martínez Martínez, Vicente Peña Caballero. Universidad de Guanajuato
43.	**A new species of talaromyces produces secreted antifungal metabolites.** Aneliz de Ita Zárate Ortiz, Luis David Maldonado Bonilla, José Luis Villarruel Ordaz, Rommel Carballo Castañeda, Aldo Moreno Ulloa. Instituto de Genética, Universidad del Mar. Campus Puerto Escondido
44.	**LOX1 and PLP1 lead a transcriptional reprogramming essential for injury-induced conidiophore development in *Trichoderma atroviride.*** Martín O. Camargo Escalante, Edgar Balcázar López, Exsal M. Albores Méndez, Robert Winkler and Alfredo Herrera Estrella. Laboratorio Nacional de Genómica para la Biodiversidad. Unidad de Genómica Avanzada, Cinvestav
45.	**Intracellular organization, cell wall integrity and endocytosis are dependent of Actin Cytoskeleton in *Metarhizium brunneum*.** Olga Alicia Callejas Negrete, Edwin Abraham Hernández Reyes, Gloria Angélica González Hernandez, Juan Carlos Torres Guzmán and Rosa Reyna Mouriño Pérez. Centro de Investigación Científica y de Educación Superior de Ensenada

46.	**Nanoscopic dynamics of endocytic patches during hyphal growth of *Neurospora crassa.*** Diego Luis Delgado Álvarez, Adán Oswaldo Guerrero Cárdenas, Rosa Reyna Mouriño Pérez. Centro de Investigación Científica y de Educación Superior de Ensenada
47.	**Role of the endoplasmic reticulum-shaping proteins YOP1 and RTN1 in *Podospora anserina* mitochondrial dynamics.** Mariana Aline González Manríquez, Leonardo Peraza Reyes. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
48.	**Organelle dynamics in absence of kinesin-3 motor proteins in *Podospora anserina*.** Fernando Hernández Sánchez, Sara K. Schroder and Leonardo Peraza Reyes. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
49.	**The unexplored apical and subapical organization of the endoplasmic reticulum in growing hyphae of *Neurospora crassa*.** Martínez Andrade J., Roberson RW. and Riquelme M. Centro de Investigación Científica y de Educación Superior de Ensenada
50.	**Role of the small GTPase MIRO1 in *Podospora anserina* organelle dynamics.** Karen Abigail Moreno García, Yovanna Alexandra Pardo Fermín, Raful Navarro Espíndola, Leonardo Peraza Reyes. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
51.	**El papel del complejo de unión entre retículo endoplásmico y mitocondria (ermes) en la regulación de la dinámica del retículo endoplásmico en podospora anserina.** Matías Ramírez Noguez, Melisa Selene Álvarez Sánchez, Carlos Leonardo Peraza Reyes. Instituto de Fisiología Celular**,** Universidad Nacional Autónoma de México
52.	**Effects of culture time and carbon source on the proteome of *Neurospora crassa* extracellular vesicles**. Daniel Alfonso Salgado Bautista, Rubén Cadena Nava and Meritxell Riquelme Pérez. Centro de Investigación Científica y de Educación Superior de Ensenada
53.	**Mycotoxin-producing fungi presence in medicinal and aromatic plants; eucalyptus (*e. melliodora* l.), horsetail (*e. hyemale*), and skunkweed (*p. alliacea* l.).** Nancy Dinorah Ruelas Hernández, Rocío Guadalupe Barcelos García, Carlos Eduardo Covantes Rosales, Guadalupe Herminia Ventura Ramón, Adela Yolanda Bueno Durán. Universidad Autónoma de Nayarit
54.	**Role of Auxins Produced by *Trichoderma atroviridein* Its Growth and Development.** Ruvalcaba Villagrán Melanie Lizbeth y Herrera Estrella Alfredo Heriberto Laboratorio Nacional de Genómica para la Biodiversidad-Unidad de Genómica Avanzada, Cinvestav
55.	***Candida glabrata* secretome molecules from stationary-phase induce a quiescence-like state in growing cells.** Carlos Ricardo González Ruiz, Javier Montalvo Arredondo, Juan Ernesto López Ramos, Guadalupe Gutiérrez Escobedo, Irene Castaño y Alejandro De Las Peñas. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica
56.	**Functional role of the metalloprotease Ermp1 from *Schizosaccharomyces pombe* in the stress response in endoplasmic reticulum.** González Esparragoza Dalia, Carrasco Carballo Alan, Rosas Murrieta Nora, Millán Pérez Peña Lourdes, Luna Morales Félix, Herrera Camacho Irma. Centro de Química del Instituto de Ciencias. BUAP
57.	**Production of mannosylerythritol lipids as an antimicrobial agent, secreted by strain FMA2, genotype NRG1 of *Ustilago maydis*.** Julia Alexsandra González Villarreal, Licet Villarreal Treviño, Carlos Eduardo Hernández Luna, Susana De la Torre Zavala, Mauricio Andrés Salinas Santander, Elva Teresa Aréchiga Carvajal. Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León
58.	**Trx3 (thioredoxin), Prx1 (peroxiredoxin), and glutathione (GSH) participate in the mitochondrial thiol redox balance in *Candida glabrata.*** Ma. Guadalupe Gutiérrez Escobedo, Ana Lizbeth López Marmolejo, Grecia Hernández Hernández, Irene Beatriz Castaño Navarro y Alejandro De Las Peñas Nava. Instituto Potosino de Investigación Científica y Tecnológica. División de Biología Molecular
59.	**OxrA protein as part of the antioxidant response and its possible role on mitochondrial dynamics in *Aspergillus nidulans.*** Hernández Gutiérrez Laurel, Aguirre Jesús. Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, UNAM
60.	**Quorum sensing in *Candida glabrata*.** Javier Montalvo Arredondo, Carlos Ricardo González Ruiz, Juan Ernesto López Ramos, Guadalupe Gutiérrez Escobedo, Yazmin Contreras Bravo, Irene Castaño y Alejandro De Las Peñas. Universidad Autónoma Agraria Antonio Narro

61.	**Tolerance to Cu, Cr and Pb of *Trichoderma asperellum* and *Trichoderma longibrachiatum.*** Diego Helman Zapata Sarmiento, Mario Rodríguez Monroy, Gabriela Sepúlveda Jiménez. Centro de Desarrollo de Productos Bióticos. IPN
62.	**Unraveling the functional role of fungi isolated from human milk.** Mario Iván Alemán Duarte, Luz Elena Ruiz Ibarra, Felipe de Jesús Ramírez Salazar, Angela Citlalli Peña García, Edgar Nathaniel Villarreal Amézquita, Blanca Rosa Aguilar Uscanga, Josué Raymundo Solís Pacheco, Edgar Balcázar López. Centro Universitario de Ciencias Exactas e Ingenierías. Universidad de Guadalajara
63.	**Yeasts from open agave fermentation show geographic population structure with sparse conservation of introgressions.** J Abraham Avelar Rivas, Luis Fernando García Ortega, Iván Sedeño, Claudio López, Antonio Urban Aragón, Eugenio Mancera, Alexander De Luna, Lucía Morales. UGA. LANGEBIO. Cinvestav Unidad Irapuato
64.	**High fertility of yeast hybrids associated with agave fermentations**. Vanessa Arellano, Eugenio Mancera, Alexander De Luna. Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados. IPN. Unidad Irapuato
65.	**The vacuolar proteases of the yeast multi-resistant to antifungal *Candida auris* in autophagy conditions**. Heriberto Daniel Clark Flores, Alvaro Vidal Montiel, Margarita Juárez Montiel, Juan Alfredo Hernández García, César Hugo Hernández Rodríguez, María de Lourdes Villa Tanaca. Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas. IPN
66.	**Genetic modification of *Candida maltosa*, a nonpathogenic CTG species.** Marco A. Chávez Tinoco, Luis F. García Ortega, Eugenio Mancera. Centro de Investigación y de Estudios Avanzados. IPN
67.	**A landscape of evolution in the Fungal Tree of Life through syntenic blocks.** Escobar Turriza PJ, Muñoz Miranda L, Pereira Santana A. CONACYT-Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco. Subsede Sureste
68.	**Search for hyaluronic acid synthases in Fungi: Do filamentous fungi synthesize hyaluronic acid?**. Laura Marina Franco Herrera, Mariandrea Aranda Barba, Paul Montaño Silva, Jorge H. Ramírez Prado, Jorge Verdín. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
69.	**UstilagoNet: a database of gene regulatory and co-expression interactions in *Ustilago maydis.*** Edgardo Galán Vásquez, Cinthia Valentina Soberanes Gutiérrez, Ernesto Pérez Rueda. Instituto de Investigaciones en Matemáticas Aplicadas y en Sistemas. UNAM
70.	**Transcriptomic profile of *Colletotrichum lindemuthianum* pathotypes**. Ma. Irene Morelos Martínez, Horacio Cano Camacho, Karla Morelia Díaz Tapia, Everardo López Romero, June Simpson, María Guadalupe Zavala Páramo. FMVZ, Universidad Michoacana de San Nicolás de Hidalgo
71.	**Pseudogenization-driven gene loss shapes genome evolution in *Hanseniaspora***. Luis Fernando García Ortega, Ángela María García Acero, Alexander De Luna, Lucía Morales, Luis Delaye, Eugenio Mancera. Centro de Investigación y de Estudios Avanzados. IPN. Unidad Irapuato
72.	**Whole deep genome analyses of an undescribed strain of *Trichoderma*, isolated from a Milpa in Morelos, Mexico**. Iza Arteaga ML., Zúñiga Silgado D, Balcázar López E, Sánchez Reyes A, y Folch Mallol JL. Centro de Investigación en Biotecnología. Universidad Autónoma del Estado de Morelos
73.	**Genomic analysis of new bacterial species from extreme environments.** Martínez Yesenia y Dr. Kyndt John. Bellevue University
74.	**The role of the transcription factor Gln3 in fluconazole resistance in *Candida glabrata.*** Karina Asyade Robledo Márquez, Lina Raquel Riego Ruiz. Instituto Potosino de Investigación Científica y Tecnológica A. C.
75.	**Prediction of Biosynthetic Gene Clusters in Mexican Isolated Fungi from Strawberry Fields.** Nelly Sélem Mojica, Pedro Martínez Hernandez, José Manuel Villalobos Escobedo, Antón Pashkov, Alfredo Herrera Estrella. Universidad Nacional Autónoma de México
76.	**Immunomodulatory effect of an NSAID-antifungal therapy for the treatment of *eumycetoma* in mice.** Yordi Alejandro Apresa Morales, Jaqueline Reynoso Sánchez, José David Torres Tirado, Gabriela Pérez Flores, Mónica Jasso Romo, Luis Antonio Pérez García. Facultad de Estudios Profesionales Zona Huasteca. Universidad Autónoma de San Luis Potosí

77.	**Identification of strawberry pathogens and their biological control.** Gretel Clara Campos Torres, Omar Said Juárez Becerril, Francisco Vargas Gasca y Vianey Olmedo Monfil. Departamento de Biología. División de Ciencias Naturales y Exactas. Universidad de Guanajuato
78.	**CFEM proteins in *Neofusicoccum parvum* and their role in the pathogenicity process**. Edgar D. Carrillo Hernández , Nohemí Carreras Villaseñor , Javier Plasencia de la Parra, Eric E. Hernández-Domínguez y Diana Sánchez-Rangel. Instituto de Ecología, A.C. Red de Estudios Moleculares Avanzados
79.	**Role of two secreted proteins in development and pathogenesis of *Fusarium* sp. associated with the ambrosia beetle *Xylosandrus morigerus***. Nohemí Carreras Villaseñor y Diana Sánchez Rangel. Instituto de Ecología, A.C. Red de Estudios Moleculares Avanzados
80.	**The volatile 6-pentyl-2H-pyran-2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root meristem via an ethylene-mediated phosphate (Pi) deficiency response.** Saraí Esparza Reynoso, José López Bucio, Alfredo Herrera Estrella. UGA LANGEBIO Cinvestav Irapuato
81.	**Small RNAs during mycoparasitism in *Trichoderma atroviride.*** Eli Efrain Enriquez Felix, Camilo Pérez Salazar, José Guillermo Rico Ruiz, José Manuel Villalobos Escobedo y Alfredo Herrera Estrella. UGA LANGEBIO Cinvestav Irapuato
82.	**Analysis of the influence of genes encoding for proteins with nitronate monooxygenase activity on different *Metarhizium* lifestyles.** Ximena Esquivias Varela, Diana Laura Herrera Lino, Israel Enrique Padilla Guerrero, Angélica González Hernández y Juan Carlos Torres Guzmán División de Ciencias Naturales y Exactas. Universidad de Guanajuato
83.	**The role of *Trichoderma atroviride* small RNA 2 in plant immunity.** Emily Daniela Gallardo Andrade, Saúl Jijón Moreno, and Sergio Casas Flores. Instituto Potosino de Investigación Científica y Tecnológica
84.	**SCP3 as an effector candidate from *Trichoderma atroviride.*** Cesar Alejandro Garcia Morales, Francisco Vargas Gasca, Vianey Olmedo Monfil. División de Ciencias Naturales y Exactas, Universidad de Guanajuato
85.	**Exploring the interaction between obligate and facultative mangrove marine fungi isolated from Pichilingue. La Paz, Baja California Sur.** María C. González, Karime Álvarez Luque, Mónica Lara, Eduardo Camacho Tejeda. Instituto de Biología. UNAM
86.	**Functional analysis of a predicted lysozyme encoded by the scp2 gene from *Trichoderma atroviride*** Samantha Gudiño Vallejo, Francisco Vargas Gasca y Vianey Olmedo Monfil. División de Ciencias Naturales y Exactas. Universidad de Guanajuato
87.	**A potential role of effector proteins during *Trichoderma atroviride* and plant growth-promoting bacteria interactions.** Paulina Guzmán Guzmán, María Fernanda Valencia Marín, Salvador Chávez Ávila, Eduardo Valencia Cantero, Gustavo Santoyo. Institute of Biological and Chemical Research, Universidad Michoacana de San Nicolás de Hidalgo
88.	**Identification of *Lasidioplodia theobromae* peptides putative implicated in pathogenesis.** Eric Edmundo Hernández Domínguez, Luz Clara Gómez Montiel y Luis A. Martínez Rodríguez. Instituto de Ecología, A.C. Red de Estudios Moleculares Avanzados
89.	**Antimicrobial effect of SCP1 from *Trichoderma atroviride* over beneficial and pathogenic rhizosphere bacteria.** Juan Mauricio Ibarra Chavira, Francisco Vargas Gasca, Vianey Olmedo Monfil. División de Ciencias Naturales y Exactas. Universidad de Guanajuato.
90.	**The photorespiratory enzyme glutamate: glyoxylate aminotransferase 1 (GGAT1) plays a key role in the regulation of plant defense responses induced by *Trichoderma* in Arabidopsis.** Saúl Jijón Moreno, Daniel David Pacheco Rodríguez y Sergio Casas Flores. Laboratorio de Genómica Funcional y Comparativa, División de Biología Molecular. Instituto Potosino de Investigación Científica y Tecnológica.
91.	**Abundancia de especies de *Candida* presentes en mujeres adultas jóvenes con sobrepeso y que realizan diferente actividad física.** Claudia Gabriela Mata Hernández, Mónica Flores Gutiérrez, Janely Rivera Ramírez, Luis Ernesto fuentes Ramirez, Ricardo Carreño Lopéz, Marcos Flores Encarnación, Silvia María del Carmen García García. Centro de Investigaciones en Ciencias Microbiológicas. Benemérita Universidad Autónoma de Puebla
92.	**Influence of bacterial endosymbionts on the pathogenic capacity of *Metarhizium.*** Aida Gabriela Mora Acebedo, Azul Martínez Vázquez, Israel Enrique Padilla Guerrero, Angélica González

	Hernández y Juan Carlos Torres Guzmán. División de Ciencias Naturales y Exactas. Universidad de Guanajuato
93.	**Peptidases involved putatively in the pathogenicity of INECOL_BM-04 and INECOL_BM-06 *Fusarium* sp. strains isolated from the ambrosia beetle *Xylosandrus morigerus.*** Jire A. Muñoz Jaimes, Eric Edmundo Hernández Domínguez, Sobeida Sánchez Nieto, Diana Sánchez Rangel. Red de Estudios Moleculares Avanzados, Instituto de Ecología A.C
94.	**Blood serum stimulates the virulence through the enhancement of the mitochondrial oxidative metabolism and rhizoferrin production in Mucorales.** J. Alberto Patiño Medina, Viridiana Alejandre Castañeda, David Vargas Tejeda, Marco I. Valle Maldonado, Rafael Ortiz Alvarado, Joel Ramírez Emiliano, Martha I. Ramírez Díaz, Karla V. Castro Cerritos, Victoriano Garre, Ulrike Binder, Víctor Meza Carmen. Instituto de Investigaciones Químico Biológicas. Universidad Michoacana de San Nicolas de Hidalgo
95.	**Identification and characterization of *Neofusicoccum parvum* peptides potentially involved in its pathogenesis.** Ismael Pérez García, Jesús A. Zamora Briseño, Ana P. Barba de la Rosa, Diana Sánche Rangel y Eric E. Hernández Domínguez. Instituto de Ecología A.C. Red de Estudios Moleculares Avanzados
96.	**Mass spectrometry detection of molecules related to the interaction between mycotoxin-producing fungi and commercial coffee beans.** Ramos Aboites Hilda Eréndira, Martínez Flores José Jovani, Pérez Salgado Luis David, Buendía Corona Isabel , Winkler Robert . UGA-Langebio Cinvestav
97.	**Effect of combined NSAID-antifungal therapy on the reduction of eumycetic grains.** Jaqueline Reynoso Sánchez, Yordi Alejandro Apresa Morales, José David Torres Tirado, Gabriela Pérez Flores, Mónica Jasso Romo, Luis Antonio Pérez-García. Facultad de Estudios Profesionales Zona Huasteca. Universidad Autónoma de San Luis Potosi
98.	***Trichoderma* requires the Arabidopsis DICER-LIKE 3 and ARGONAUTE 9 proteins to modulate the expression of the Nitrile Specifier protein 4 (NSP4) gene.** Maria Montserrat Rosendo Vargas, Oscar Guillermo Rebolledo Prudencio Y Sergio Casas Flores. IPICYT, Division de Biología Molecular
99.	**Dissecting AGO-sRNA binding affinities between kingdoms of life through RNA-Protein docking analyses**. Daniel Rafael Saldaña Torres, Cesaré Moisés Ovando Vázquez, J. Sergio Casas Flores. Centro Nacional de Supercomputo. IPICYT
100.	**Identification of interactor proteins of FkCER1 and FkCER2, two Cerato platanin proteins from ambrosial fungus *Fusarium kuroshium*.** Nidia Sánchez León, Eric Edmundo Hernández Domínguez. Instituto de Ecología, A.C. Red de Estudios Moleculares Avanzados
101.	**Establishment of *Candida glabrata* biofilm on epithelial cells.** Serna Pérez Amanda Belén, Hernández Benítez José Alejandro, García Pérez Blanca Estela, Rodríguez Tovar Aida Verónica. Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas. IPN
102.	**Proteinase A is involved in the cell death of *Ustilago maydis.*** Cinthia V. Soberanes Gutiérrez, Edgardo Galán Vásquez, Julio Vega Arreguín. Comisión Intersecretarial de Bioseguridad de Organismos Genéticamente Modificados. CONAHCYT.
103.	**Secreted Rich Cystein Protein 1 (SCP1) from *Trichoderma atroviride* is a new effector candidate involved in plant interaction process.** Francisco Vargas Gasca, Juan Ignacio Macías Segoviano, Alfredo Herrera Estrella y Vianey Olmedo. División de Ciencias Naturales y Exactas, Universidad de Guanajuato
104.	**Interaction networks between yeasts from agave fermentations.** Carolina G. Torres Lagunes, Eugenio Mancera y Lucía Morales. Laboratorio Internacional de Investigación sobre el Genoma Humano. UNAM
105.	**Characterization of the response of *Candida glabrata* clinical isolates to immune cells.** Ana Belén Vargas Antillón, Ma. Guadalupe Gutiérrez Escobedo, Alejandro de las Peñas Nava, Irene Beatriz Castaño Navarro. Instituto Potosino de Investigación Científica y Tecnológica
106.	**Determinación de Ocratoxina A en café soluble.** Oswaldo Alberto Alvarez, Carlos Eduardo Covantes Rosales, Ansony Jhovanny Odeja Duran, Adela Yolanda Bueno Durán. Universidad Autónoma de Nayarit
107.	**Evaluation of 4H-chromenes in P-glycoprotein (P-gp) of *Candida spp*: phylogeny, modelling, molecular docking and enzymatic activity.** Dulce María Andrade Pavón, Jorge Omar Gómez García, Lourdes Villa Tanaca. Escuela Nacional de Ciencias IPN

108.	**Soil, rhizosphere and phyllosphere meta-taxonomics for the evaluation of the cessation of the use of glyphosate in orange cultivation in northern Veracruz.** Diana Baeza, Pablo Valentín Navarro Enguilo y Jorge Verdín. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco
109.	**Circular economy in a brewery: The use of Brewer's Spent Grain for the growth *of Pleurotus ostreatus* and *Pleurotus djamor*.** Luis Alejandro Castillo González, Rubén Darío Cárdenas Duarte, José Luis Martínez Salgado, Luz María Teresita Paz Maldonado. Facultad de Ciencias Químicas. Universidad Autónoma de San Luis Potosí
110.	**Using Yeast to Study Human Gene Variants Associated with Mitochondrial Disorders.** Maritrini Colón González, Luis Enrique Dávila Juárez, José Héctor Gibrán Fritz García y Lucía Morales. Laboratorio Internacional de Investigación sobre el Genoma Humano UNAM
111.	**Microevolution of *Candida glabrata* cells in the presence of antifungals.** Rosa Lilian Díaz Chávez, Alejandro Juárez Reyes, Irene Castaño. Instituto Potosino de Investigación Científica y Tecnológica
112.	**Evaluation of the antifungal effect of the extract of *Pleurotus ostreatus* on *Aspergillus parasiticus.*** Edmar de Jesus Díaz García, Cecilia Pérez Cruz, María del Socorro Pina Canseco, Marco Antonio Sánchez Medina, Alma Dolores Pérez Santiago. CONAHCYT. Universidad Autónoma Benito Juárez de Oaxaca
113.	**Molecular and sensitive specific detection of *Candida glabrata.*** Marco Josué Hernández Chávez, Enrique Merino Pérez, Irene Castaño. Instituto Potosino de investigación científica y tecnológica A.C.
114.	**Evolution of *Candida glabrata* throughout the course of an infection.** Ana Lizbeth López Marmolejo, Guadalupe Gutiérrez Escobedo, M. Selene Herrera-Basurto, Alejandro De la Peñas, Irene Castaño. Instituto Potosino de Investigación Científica y Tecnológica
115.	**Evaluation of internal region spacer of ribosomal DNA sequence for molecular identification of filamentous fungi in the Colección Nacional de Cepas Microbianas y Cultivos Celulares (CINVESTAV-Mexico).** Daniel Alfonso Estrada Bárcenas, Juan Carlos Estrada Mora. Colección Nacional de Cepas Microbianas y Cultivos Celulares. Cinvestav
116.	***Fusarium* metallothioneins: a study in *Fusarium kuroshium.*** Mónica Janett Muñoz Contreras, Rogelio Rodríguez Sotres, Eliel Ruiz May, Diana Sánchez Rangel, Eric Edmundo Hernández Domínguez. Instituto de Ecología A.C.
117.	**Study of human gut mycobiota in dysbiosis and their metabolic profile.** Teresa Cruz Yanet, Fajardo-Hernández Carlos A., Figueroa-Saldivar Mario A., Romero-Rodríguez Alba I. Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas. U.N.A.M.
118.	**Design of a novel multiplex qPCR system to detect pathogenic *Mucoralean* fungi from clinical human samples.** Marco Iván Valle Maldonado, José Alberto Patiño Medina, Viridiana Alejandre Castañeda, Rafael Ortiz Alvarado, Martha Isela Ramírez Díaz, Victor Meza Carmen. Instituto de Investigaciones Químico Biológicas.Universidad Michoacana de San Nicolás de Hidalgo
119.	**Neo-functionalization in *Saccharomyces cerevisiae*: A Novel Nrg1-Rtg3 chimeric transcriptional modulator is essential to maintain mitochondrial DNA integrity**. José Carlos Campero Basaldua, James González Flores, Janeth Alejandra García Rodríguez, Edgar Adrián Ramírez González, Hugo Antonio Hernández Pérez, Beatriz Aguirre López, Nayelli Torres Ramírez, Dariel Márquez Gutiérrez, Norma Silvia Sánchez Sánchez, Nicolás Gómez Hernández, Lina Raquel Riego Ruiz, Claudio Scazzocchio y María Alicia González Manjarrez. Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, U.N.A.M.
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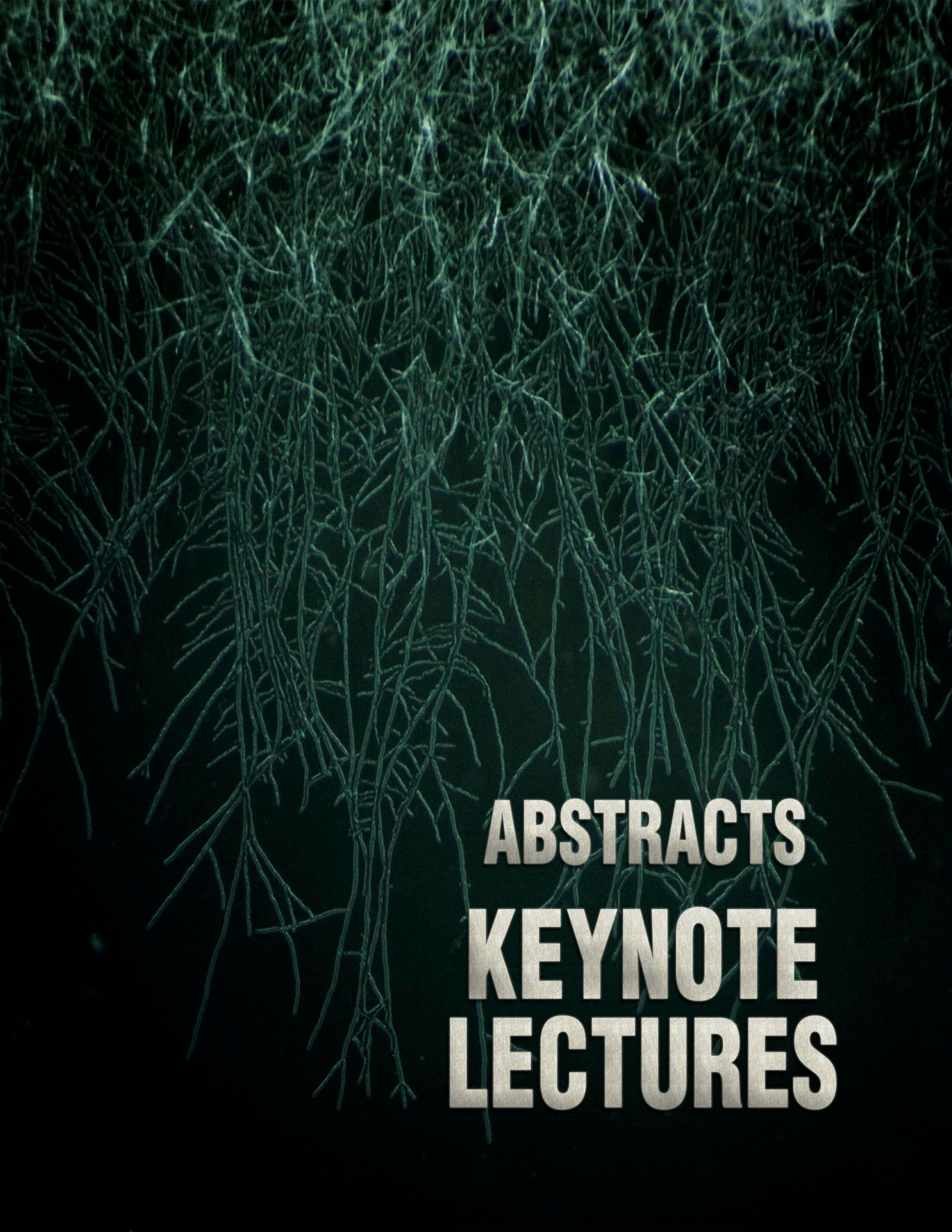

ABSTRACTS
KEYNOTE LECTURES

Saccharomyces variation across the world

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An understanding of natural variation is crucial to efforts in current biology and to decipher the dynamics of genome evolution. The budding yeast, *Saccharomyces* cerevisiae, has emerged as a leading system for population genomics studies due to it's small, well-characterized genome and experimental tractability. In the past decade, we assembled a large collection of natural isolates of S. cerevisiae and its closest relative S. paradoxus strains and characterized them at the genomic and phenotypic levels. We applied different sequencing and computational approaches to investigate origin, evolution, secondary contacts, and domestication of the species. These data provide a comprehensive view of genomic diversity in budding yeast and expose pronounced population-level differences.

Michel Amado
(León, México. 1977)
Artista. Cinefotógrafo / Fotógrafo

Breve reseña
Director de fotografía especializado en la técnica de animación *stop motion* donde ha colaborado en más de 60 comerciales, varios cortometrajes y un largometraje. En el 2020 inicia su carrera en Estados Unidos como *Lighting Cameraperson* (LC) para la multipremiada película 'Guillermo del Toro's Pinocchio' (Netflix, 2022), co-dirigida por Guillermo del Toro y Mark Gustafson. Actualmente dirige la fotografía del *sit-com* `In The Know´ (Peacock, 2023), escrito y dirigido por Mike Judge, Zach Woods y Brandon Gardner.

Director de fotografía de dos producciones ganadoras del Ariel: 'Bajo la Sal', 2008, por 'Mejores efectos especiales´ entregado a René Castillo por las secuencias de animación; y 'Viva el Rey', 2019, por `Mejor cortometraje de animación´ entregado al director Luis Téllez. Premio Jalisco de Periodismo 2016 en la categoría de fotografía por la serie documental 'Pueblo Quieto' y ganador de la Pantalla de Cristal en el 2014 por mejor fotografía por el videoclip 'El Aire' (dirigido por Carlos Cruz), de Sidarttha.

Michel Amado es profesor de fotografía y cinefotografía, y además es un artista sistemático, sensible y seducido por los contenidos gráficos resultado de la combinación de la técnica, la ciencia, la imaginación, la tradición pictórica y la magia; cree en la capacidad sorpresiva de la imagen, en el poder narrativo y dramático de la luz, y en la expresividad emotiva del color. Sus intereses rondan el universo reflexivo de las personas y su interacción con el mundo. En su obra responde a preguntas sobre la memoria, la nostalgia y la evocación de la intimidad de los seres humanos.

Más referencias profesionales:
IMDb. http://www.imdb.me/michelamado
IMDb Pro. https://pro.imdb.com/name/nm3020853?ref_=nm_nv_usr_profile

Video:
Vimeo. Michel Amado / Link. http://vimeo.com/user6126964

Fotografía de obra:
Selección. Galería Bluestreets. http://bluestreets.org/michelamado

Redes sociales:
Instagram. Obra de autor @michelamado
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Facebook fan page. https://www.facebook.com/pages/Michel-Amado/112623255481270

How a fungus protects itself when producing a toxic secondary metabolite

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Aspergillus fumigatus causes a range of human and animal diseases collectively known as aspergillosis. *A. fumigatus* possesses and expresses a range of genetic determinants of virulence, which facilitate colonisation and disease progression, including the secretion of mycotoxins. Gliotoxin (GT) is the best studied *A. fumigatus* mycotoxin with a wide range of known toxic effects that impair human immune cell function. GT is also highly toxic to *A. fumigatus* and this fungus has evolved self-protection mechanisms that include (i) the GT efflux pump GliA, (ii) the GT neutralising enzyme GliT, and (iii) the negative regulation of GT biosynthesis by the *bis*-thiomethyltransferase GtmA. We identified a transcription factor (TF), RglT, important for *A. fumigatus* oxidative stress resistance, GT biosynthesis and self-protection, and virulence. RglT regulates the expression of several gli genes of the GT biosynthetic gene cluster, including the oxidoreductase-encoding gene gliT, by directly binding to their respective promoter regions. RglT is the main regulator of GliT and this GT protection mechanism also occurs in the non-GT producing fungus *A. nidulans*. However, *A. nidulans* does not encode GtmA and GliA. This work aimed at analysing the transcriptional response to exogenous GT in *A. fumigatus* and *A. nidulans*, and to identify additional components required for GT protection in *Aspergillus spp*. RNA-sequencing shows a highly different transcriptional response to exogenous GT with the RglT-dependent regulon also significantly differing between *A. fumigatus* and *A. nidulans*. However, we were able to observe homologues whose expression pattern was similar in both species (43 RglT-independent and 11 RglT-dependent). Based on this approach, we identified a novel RglT-dependent methyltranferase, MtrA, involved in GT protection. Taking into consideration the occurrence of RglT-independent modulated genes, we screened an *A. fumigatus* deletion library of 484 TFs for sensitivity to GT and identified 15 TFs important for GT self-protection. Of these, the TF KojR, which is essential for kojic acid biosynthesis in *Aspergillus oryzae*, was also essential for virulence and GT biosynthesis in *A. fumigatus*, and for GT protection in *A. fumigatus*, *A. nidulans*, and *A. oryzae*. KojR regulates *rglT*, *gliT*, *gliJ* expression and sulfur metabolism in *Aspergillus* spp. Together, this study identified conserved components required for GT protection in *Aspergillus* species.

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Genome Evolution of *Saccharomyces* Yeasts and Their Interspecies Hybrids

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Interspecific hybridization events have shaped the genomes of various organisms across Eukarya. Yeast hybrids are valuable models for understanding the impacts of hybridization on speciation, adaptation, and genome evolution due to their weak prezygotic barriers and high prevalence in human-associated environments. In this study, we isolated and sequenced over 250 *Saccharomyces* strains from traditional agave distilleries and their natural surroundings across 12 states in Mexico. The analysis of these genomes revealed the coexistence of a divergent and highly structured population of *S. cerevisiae* and a new subpopulation of *S. paradoxus*. Remarkably, approximately 10% of the *Saccharomyces* sequenced strains were identified as full hybrids, representing up to 40% in some of the sampling regions. The analysis of these hybrid genomes suggests hybridization events between these two species happening recurrently in distilleries, but not in natural reservoirs. Additionally, most *S. cerevisiae* isolates carried around 150 genes derived from the sister species *S. paradoxus*, likely resulting from an ancient and eroded interspecific hybridization event. Comparative analyses of nuclear and mitochondrial genomes unveiled at least 13 distinct genomic architectures, suggesting multiple independent hybridization events at different stages of genome stabilization. In summary, here we present the genomic diversity of *Saccharomyces* yeast subpopulations in a human-associated scenario in which two sister species coexist and hybridize recurrently. Overall, this work is proving a valuable resource to study the impact

Fungal responses to global climate change in a dryland ecosystem and potential impacts to public health

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Global climate change is projected to increase the spread of soil-borne infectious diseases due to persistent stressful environmental conditions, such as drought and soil disturbances. Because pathogenic fungi are good at tolerating stress, they may thrive under global climate change compared to other non-pathogenic fungi. In this project we investigated if global change drivers, drought and disturbance, represent a threat to public health by examining if global climate change favors the increase in abundance of pathogenic soil fungi and the transcription of pathogenicity-related genes. We sampled soils from a global change experiment in the Chihuahuan desert, in the Southwest of the United States, where soils are experimentally droughted as predicted for this area under continued global climate change and mechanically disturbed to mimic impacts from vehicle traffic and overgrazing. We extracted DNA and RNA from control and treatment soils. Within each plot, we collected samples from under perennial vegetation and interspace (i.e., baren soil without vegetation). Soil nutrients, moisture, and temperature are vastly different between these two micro-sites and can consequently affect the community of microbes. We carried out ITS metabarcoding and metatranscriptomics to identify fungi at the taxonomical level and at the functional level, and to examine the abundance of pathogenicity-related genes. Understanding the complex causal relationship between global climate change, shifts in the fungal community in terms of community and activity, and potential public health threats, will allow for better predictions of future infectious disease hot spots and outbreaks. Moreover, our work is providing information for policy makers on the public health threat potential that climate change has on soil fungal communities in the Chihuahuan desert.

Genomics gives unprecedented insights on adaptive and evolutionary processes driving the success of Black Fungi in the extremes

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The terms “Black fungi” indicate a polyphyletic and morpho-ecological group within Ascomycota, mainly in the classes Eurotiomycetes and Dothideomycetes and include some of the most successful extreme-tolerant organisms on Earth, displaying exceptional skills to exploit all kinds of extremes, including exposed rocks in Polar deserts and high altitudes in Alpine regions. Some species are also human opportunists, and serve as important model organisms to clinical mycology. Shared characters such as melanin-like pigments, thick cell walls, flexible morphology, and meristematic growth are key adaptations to cope with different stressors as expressions of convergent evolution; all together facilitate their persistence and diversification up to the edge for life on Earth; they also inform on the possibility for life beyond, being able to survive even space and simulated Martian conditions. However, due to the scarcity of genomic data available, we are still far from fully unravelling their adaptation strategies. In this respect, we herein present the first wide comparative genomic analysis of over 100 strains of black fungi. We found that genomes of *Dothideomycetes*, much more common in natural cold-dry environments, were significantly enriched in genes for cold and ultraviolet (UV) radiation resistance and DNA damage repair. Conversely, *Eurotiomycetes*, spreading mainly in hot and human impacted and polluted sites, were enriched in genes related to hot tolerance and hydrocarbons degradation. Genome size variability was wider in *Dothideomycetes*, varying from 23.49 to 121.19 Mbp, while never exceeds 57.34 Mbp in *Eurotiomycetes*. Largest genomes with higher number of genes and CG content were associated with harshest conditions as the Antarctic deserts or highest mountain peaks and include potential diploid and triploid lineages, supporting the idea that polyploidy may be a key for the success in the extremes. These genomes were also positively correlated with UV index, which strongly impact such environments. Yet, *Dothideomycetes* from the harshest environments included also the most ionizing-radioresistant strains; we hypothesize that this ability can be a bioproduct of adaptation to multiple stresses and be regulated by a plethora of genomic features.

Nitrate utilisation in the fungi, from replica plating to alpha-fold

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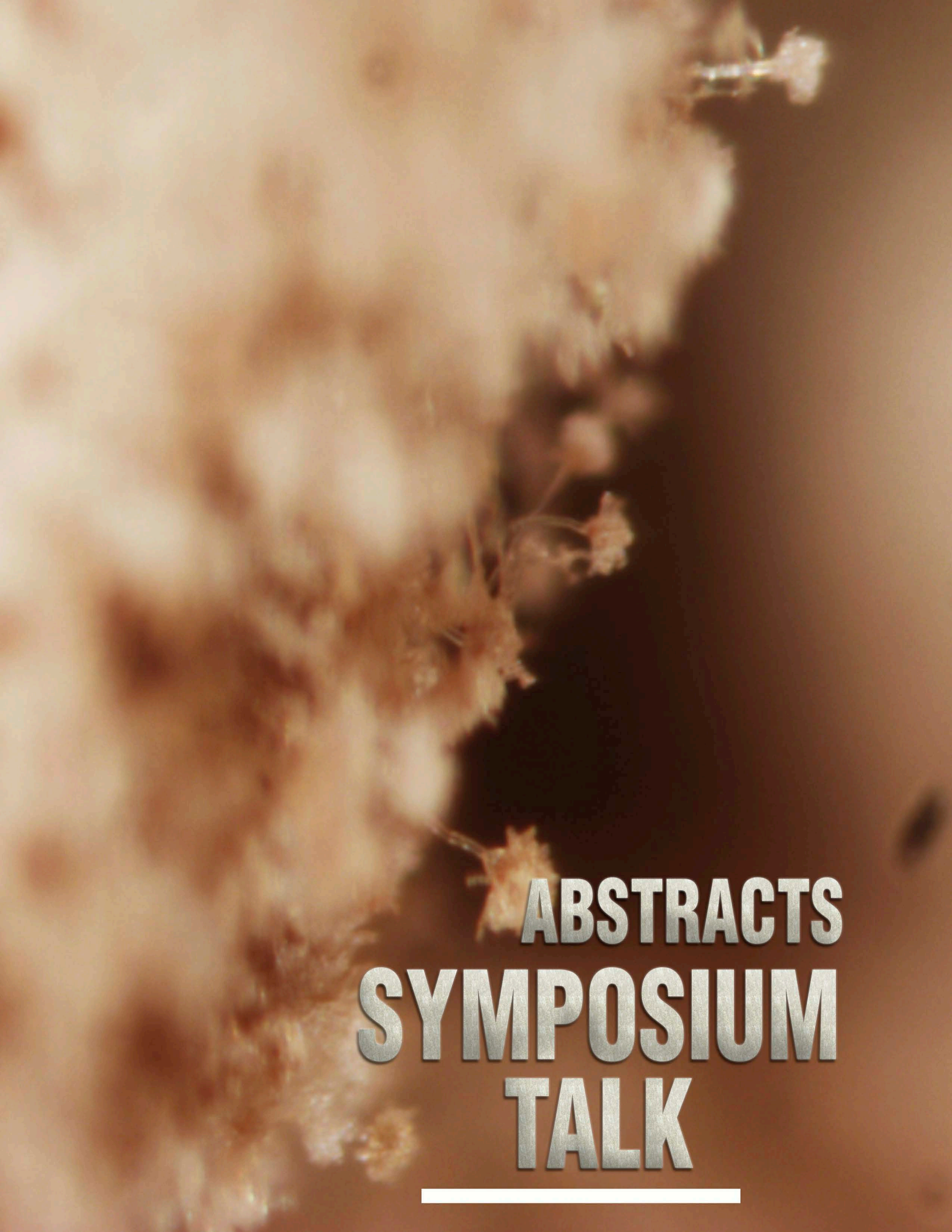
In 1962, a simple replica plating experiment carried by out by David Cove using the ascomycete *Aspergillus nidulans* gave, in a world then dominated by the "one gene one enzyme" paradigm, a paradoxical result. Mutations unable to utilise nitrate map at, at least seven loci. The paradox was resolved when it was established that six of the loci determined steps in the synthesis of a Molybdopterin cofactor (MOCO), common to at least two enzymes in *A. nidulans*. This cofactor is universal, from bacteria to us, including plants. Work using widely different organisms (including *Neurospora crassa* and *A. nidulans*) established a completely conserved biosynthesis. It was found that the three genes involved in nitrate utilisation, encoding respectively nitrate reductase, nitrite reductase and a nitrate transporter are tightly clustered in *A. nidulans*. The genes are strictly coregulated, induced by nitrate and controlled by a specific Cys6Zn2 transcription factor (called NirA/Nit4) together with a wide domain GATA factor responding to the nitrogen status of the cell. Further work, now ongoing in the laboratory of Joseph Strauss, is unravelling the cooperation between the two factors at the chromatin level and a surprising mechanism of nitrate induction.

Evolutionary studies carried out together with the laboratory of Iñaki Ruiz-Trillo have established the origin of the nitrate assimilation cluster in eukaryotes, including the independent and convergent origin of the nitrate reductase enzyme and a complex pattern of horizontal transfer events (Ocaña-Pallarés et al. 2019), which *inter alia* establishes that MOCO must pre-exist the nitrate pathway.

This work posits new questions. Why is the cluster strictly maintained in some organisms (some fungi such as *A. nidulans*, green algae) and scattered in others (*N. crassa*, flowering plants)?

In phylogenetically distant organisms widely different transcription factors (TFs) are recruited to mediate nitrate induction. The independent recruiting of different specific factors in organisms that range from Ichthyosporea (Holozoa, unicellular relatives of animals) to plants, posits an even more interesting evolutionary problem.

In the Pezizomycotina, the specific transcription factor is unlinked to the metabolic genes. In *Ogataea angusta* (Pichiaceae, Saccharomycotina) two Cys6 Zn2 interacting factors are necessary for transcription and are included in the gene cluster (Severio and Strauss groups), neither TF is orthologous to well-studied NirA/Nit4 factor. Work by Antony Rokas (Shen et al., 2018) group has shown a very patchy distribution of the nitrate reduction pathway in the Saccharomycotina. We (Ekaterina Shelest, University of Portsmouth, UK, and myself) are now addressing the following questions. Is the clustering of two TFs with the metabolic genes general in the nitrate utilising Saccharomycotina? Are those TFs monophyletic or polyphyletic? Are these factors acting as dimers? For those that are, how conserved are the inter-molecular contacts? We hope that this work could contribute to the general problem of the recruiting of TFs within a specific taxon.



ABSTRACTS SYMPOSIUM TALK

EL SPITZENKORPER- MOTOR Y GUIA DEL CRECIMIENTO DE LAS HIFAS

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Descubierto hace exactamente 100 años, fue solo hasta 1959 cuando M. Girbardt pudo demostrar que el Spitzenkorper (SPK) era una estructura real y tenía una función esencial en el crecimiento de las hifas. La microscopia electrónica dio a conocer que el SPK consistía de dos acúmulos de vesículas de distinto tamaño, las pequeñas en el centro y las grandes en la periferia. Posteriormente por microscopia confocal e ingeniería genética se descubrieron sus funciones: las microvesículas acarrean quitina sintasa (i.e. son los quitosomas descubiertos in vitro en 1974), las macrovesículas transportan glucana sintasa. ¿Por qué es necesaria esta dualidad? Un análisis matemático cibernético sugirió que el SPK funciona como "*VSC vesicle supply center*" o sea centro suministrador de vesículas cuyo avance produce una descarga continua de vesículas, creando así un patrón de exocitosis que genera el gradiente de construcción de pared que existe en la punta de la hifa y el cual le da una forma muy peculiar (hifoide). Hay nueva evidencia de que las vesículas son transportadas del SPK a la membrana citoplasmática por un esqueleto de actina que emana del SPK. También hay evidencia de que la formina dispara el ensamble del esqueleto de actina del VSC y esto probablemente ocurre desde que nace el tubo germinal de la espora. Queda por demostrar uno de los puntos más críticos del SPK ¿Qué es lo que mueve/desplaza al SPK dentro de la hifa?

Lo aprendido en el estudio del efecto del estrés en las células; Lo bueno, lo extraño y lo impresionante: Notas del modelo de enseñanza/aprendizaje de un gran Maestro Mexicano.

Aréchiga-Carvajal, E.T.

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Por allá de de los 90s descubrí a los hongos, el modelo perfecto de estudio de células eucariotas. Además de la ad que existe de atacar una infección micó ca sin dañar al ser humano. Aun no sabía la cercanía evolu a que este con los humanos. Pero descubrí que en México pa nte en Irapuato Guanajuato trabajaba un inves ador super reconocido. Y me inscribí en el programa de Verano de la Inves ación Cien ca de la Academia Mexicana de Ciencias. Afortunadamente el Dr. Ruiz me aceptó y aunque fue con La Maestra Claudia León con quien trabajé, el Dr. Ruiz me hablaba todos los días para darme algún capítulo de libro o algún lo para leer. Sen que era un apersona que apreciaba mi gusto por la ciencia y más allá de lo que se decía del siempre fue un caballero muy amable (si, muy exigente) conmigo. El hecho de que descubriese que el pH es el d smo en *Us o maydis* me enganchó, pero ya El Dr. Sco Gold inves ador de Estados Unidos estaba describiendo que era a través de la vía de pKa por la cual se respondía a este es generando el cambio dimó o. Cuando terminé ese verano regresé a la Facultad de biología en Monterrey con la invitación del Doctor a entrar al programa de Doctorado Directo bajo su dirección. Eso me dio mucha fuerza para estudiar y esmerarme y terminar pronto mi carrera. Durante mi Doctorado y después de haberme graduado el Doctor y yo colaboramos en el descubrimiento y descripción del sistema de respuesta a estrés PACC/Pal, Patentamos una B glucana con ac ad in ulante secretada por la mutante Rim101 en *Us go*, fui tes o del hallazgo y caracterización del endosimbionte dor de nitrógeno *de Us maydis,* lo cual me ayudo a elucidar lo que pasaba con levaduras de *Kluyveromyces marxianus* aisladas por ins o tecnológico de Durango que posee más de 16 s diferentes de bacterias dentro. El doctor celebró conmigo el hallazgo y nos apoyó para describir el fenómeno (aún en marcha) Los seminarios de avance siempre me dejaron enseñanzas que me han servido toda la vida tanto en la vida como en el aula y hoy puedo decir que ya se me otorgaron dos patentes y solicite protección de dos más (una relacionada con *Us go* también). En mi presentación les hablare de estos hallazgos y como se relacionaron con alguna cosa aprendida con el Dr. Ruiz, un maestro de vida.

Plant growth promotion, stress tolerance and pathogen resistance in chili plants inoculated with a *Trichoderma atroviride* strain expressing the cell wall remodeling protein SWO1.

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Chili is a worldwide consumed vegetable, being Mexico its main producer and exporter. However, farmers must often confront adverse climatic conditions or plagues which undermine chili production. As an alternative to the use of agrochemicals to confront the later situation, attention has focused on Plant Growth Promoting Microorganisms (PGPM), since they can reduce the use of chemical fertilizers and pesticides due to a genetic reprogramming of the plants' gene expression that leads to modification of the root architecture, induce the systemic defense mechanisms against pathogens, and activate stress tolerance mechanisms, among others. The genus *Trichoderma* has been widely studied regarding these traits when colonizing plant roots in a wide range of different plant species.
The first barrier that *Trichoderma* spp. must overcome is the root plant cell wall, which is composed mainly by cellulose, hemicellulose, and lignin. So, to colonize the root, these polymers must be degraded or modified to allow hyphae to penetrate to the root cortex cells. It is well documented that *Trichoderma* spp. produce enzymes that can perform such activities. However, a protein which remodels (but does not hydrolyze) cellulose was described originally in *T. resseii* and found also in the *T. atroviride* genome The protein was named swollenin (SWO1), since it swelled cotton fibers. Data from Reihtner *et al*., 2011, showed that SWO1 was induced very early (before contact) during the confrontation of *T. atroviride* with several fungal pathogens. This fact led us to propose a role of SWO1 in the colonization of root cells during plant interaction. This was shown to be truth in the *T. asperellum*-cucumber association (Brotman, et al., 2008), although these authors did mainly *in vitro* experiments. In this work, the *SWO1* gene of *T. atroviride* was overexpressed or deleted and the resulting strains were tested for their plant growth promotion activities, stress tolerance and pathogen resistance in chili plants. All these traits were enhanced when the overexpressor strains were inoculated, but, curiously, the deletion mutants behaved very similar to the wild-type strain indicating that SWO1 is not essential for root colonization. Pathogen resistance in chili leaves achieved intermediate levels when using the deletion mutants, indicating also that another mechanism different from the well-studied Induced Systemic Resistance or Acquired Systemic Resistance mechanisms must be operating. Drought stress provoked differential compatible osmolyte production in the different strains.

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Proteomic comparison of the toxic effect of aurofusarin from *Fusarium graminearum* on azole-resistant *Candida albicans* strains

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Introduction. *Fusarium graminearum* is the causal agent of Fusarium Head Blight in wheat, and Cob rot in maize. *F. graminearum* produces several secondary metabolites such as aurofusarin (AUR). AUR is a dimeric metabolite belonging to the group of naphthoquinone polyketides derived from the *PKS12* polyketide synthase. The ∆*PKS12* null mutant is unable to produce AUR. A recent study showed the toxicity of AUR in fungi such as *Candida albicans*, the main etiologic agent of candidiasis in humans, using mycelium extracts of a wild type (WT) strain and *ΔPKS12* mutant as control. The resistance of *Candida* spp. to conventional antifungals is increasing. Therefore, AUR could be a lead substance to synthesize new antifungal compounds. In this study, we determine the toxic effect of AUR on fluconazole-resistant or susceptible *C. albicans* strains. Additionally, we identify *C. albicans* differentially expressed proteins in response to AUR.
Methods. Supernatants from mycelia grown in PDB medium at 26 °C, 180 rpm for 5 days from *F. graminearum* WT (PH1) and *ΔPKS12* strains were used as AUR extract or control. To determine toxicity, increasing concentrations of AUR were used during the growth of fluconazole sensitive (ATCC 10231 and 1191-H) and resistant (285-H) strains of *C. albicans*. Proteins of *C. albicans* inhibited with or without AUR were extracted, analyzed on SDS-PAGE, and sequenced by LC-MS.
Results. Extracts from *F. graminearum* WT strain (containing AUR) and *ΔPKS12* mutant as a control (without AUR) were prepared and used for inhibition assays. All *C. albicans* strains were susceptible to AUR at a concentration of 2.8 µg/µL, with no effect observed with the *ΔPKS12* mutant extract. Strain 285-H resistant to azoles was the most susceptible revealing inhibition at a concentration of 0.5 µg/µL of AUR, 37°C, 150 rpm for 3 h. Therefore, we used such conditions to extract proteins, which were analyzed and sequenced. We identified 2098 total proteins, of which four were expressed only in the presence of AUR, and three were expressed exclusively in the absence of AUR. The differentially expressed proteins were 215, of which 86 were expressed with, and 129 were expressed without AUR. Of the differentially expressed proteins, six corresponded to heat shock proteins (Hsps) that were overexpressed in the AUR condition.
Conclusions. Aurofusarin showed increased toxicity in fluconazole-resistant *C. albicans* strain 285-H at a minimal inhibitory concentration of 0.7 µg/µL and a minimal lethal concentration of 1.4 µg/µL. Overexpressed Hsps proteins were identified in the AUR condition, these proteins could be explored as targets to combat antifungal resistance, as Hsps are known to confer resistance to antifungal drugs to *C. albicans*.

CRISPRi for transcriptional regulation of *IAH1* gene and its impact on volatile compounds profile in *Kluyveromyces marxianus* DU3

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Introduction: Yeasts have been associated with the production of esters in fermented beverages, such as mezcal, contributing essential organoleptic characteristics to the final product. Among the yeasts isolated from mezcal fermentations, *Kluyveromyces marxianus* stands out for its involvement in ester synthesis, which significantly influences the flavor and aroma of the beverage. These compounds depends on the availability of the respective alcohols, acetyl-CoA, and the expression and activity of alcohol acetyltransferase enzymes. The activity of ester-synthesizing and ester-hydrolyzing enzymes plays a crucial role in ester accumulation. The *IAH1* gene encodes a hydrolyzing isoamyl acetate esterase, and its esterase activity is believed to influence ester concentration.

Methodology: In this study, we employed CRISPRi technology in *K. marxianus* strain DU3, utilizing dCas9 fused with the Mxi1 repressor domain. Two sgRNAs were designed in the promoter region of the *IAH1* gene for its down-regulation. Through gene expression analysis, we investigated the impact of *IAH1* downregulation on the metabolic profile of volatile compounds through gas chromatography. Additionally, we quantified the expression of other genes (*ATF1, EAT1, ADH1* and *ZWF1*) involved in the biosynthesis of volatile compounds by RT-qPCR.

Results: Our results demonstrated successful downregulation of *IAH1* expression in the *K. marxianus* strain DU3 using the CRISPRi system. Modulating *IAH1* gene expression resulted in changes in volatile compound production, particularly ethyl acetate, a key contributor to the beverage's aroma. Further analysis revealed altered expression levels of other genes involved in ester biosynthesis, suggesting potential regulatory interactions among these genes.

Conclusions: These results demonstrated successful down-regulation of *IAH1* expression in *K. marxianus* strain DU3 using the CRISPRi system. The utilization of CRISPRi technology opens up new possibilities for targeted gene expression modulation, synthetic biology, and metabolic engineering strategies in this yeast strain.

Al- and Zn binding peptides displayed on the surface of *Neurospora crassa* to build biofilters

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Contamination of surface water bodies, such as the Santiago River, is one of the major problems of our time. Metals are among the main hazardous wastes discharged by industries. Current conventional processes to remove metals are expensive, and their use is not widespread. An alternative for metal removal is the use of genetically engineered functionalized biological surfaces. Filamentous fungi are an ideal choice due to their natural metal adsorption capabilities and the high specific area of their mycelium that can be further functionalized by protein display strategies. The latter, scarcely developed for filamentous fungi, consists of the co-translational fusion of a host cell wall resident protein to a metal-binding peptide, such that the engineered microorganism increases its metal removal capacity. In this work, we report the design and development of functionalized surfaces of the filamentous fungus *Neurospora crassa* with Al- and Zn-binding peptides, using the native cell wall resident protein PIR-1 (NCU04033) as a molecular anchor. After strains molecular characterization and immunolocalization of the surface displayed peptides, the *N. crassa* pFA4h (*Pccg-1::pir-1ΔGPI::V5::Albp*) and *N. crassa* pFA5h (*Pccg-1::pir-1ΔGPI::V5::Znbp*) strains showed a significant increase in their Al (1.32 mg Al per g dry mycelium) and Zn (1.20 mg Zn per g dry mycelium) adsorption capacity, respectively, compared to the wild-type strain (0.80 and 0.85 mg Al and Zn per g dry mycelium, respectively). In addition, both strains also improved their growth capacity in the presence of the metals (Al, 9%; Zn, 32%). This work was supported by FODECIJAL-COECYTJAL, grant 8186-2019.

Effector prediction, a challenging task in a world in constant evolution

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The term, “effector”, is usually related to pathogenicity. Effectors are molecules, mostly proteins, that play key roles as virulence-promoting molecules that manipulate a variety of host processes, for example, actin polymerization, mimicry of host transcriptional activators and targeting host transcription factors, inhibition of phytoregulator biosynthesis, etc. Effectors have been described in bacteria, fungi, oomycetes, nematodes, insects, and parasites, among other organisms. While their roles in pathogenicity have been largely studied, it is now evident that effectors are present not only in pathogens, but also in beneficial microorganisms, where they modulate various interactions between microorganisms as well as microorganisms and their plant hosts.

In fungi, most effectors have been described as cysteine-rich, small proteins ($\leq$400 amino acids in length) which have a signal peptide for secretion and lack a transmembrane domain. Proteins that meet these criteria are termed canonical or classical effectors, but many effectors that do not meet these features (non-canonical effectors) have also been discovered.

Fungal effectorome identification is a complicated task since different authors have followed different pipelines in effectoromics. In order to formulate an appropriate strategy for fungal effector identification, we compiled a list of experimentally validated effectors to understand their characteristics; we found that non-canonical effectors occur much more frequently than previously believed. Based on our findings, we developed the EffHunter and WideEffhunter algorithms, which are able to identify canonical and non-canonical fungal effectors, respectively. While other algorithms are dedicated to effector identification only in pathogenic or in non-pathogenic fungi, WideEffHunter can identify effectors in both types of fungi and also in oomycetes. EffHunter and WideEffHunter have larger F1 scores in comparison with other fungal effector predictors, making them amenable tools for fungal effectoromics studies.

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Characterizing micro-transcriptomic response during plant-beneficial and -pathogenic fungal interactions

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The model plant *Arabidopsis thaliana* interacts with a diverse community of bacteria, yeasts, fungi, viruses, and mites. Existing beneficial and pathogenic interactions such as that with the endophytic root-colonizing fungi *Serendipita indica* and that with the necrotrophic fungi *Botrytis cinerea*, respectively. To respond to these complex interactions, *A. thaliana* have evolved inducible defense responses, including the so-called plant innate immunity. This immune response includes a broad spectrum of defenses such as accumulation of reactive oxygen species (ROS), mitogen-activated protein kinase (MAPK)-dependent signaling cascades, induction of defense genes, synthesis of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) and the accumulation of small RNAs (sRNAs) either, from the plant and form the microorganism. sRNAs have been described as key regulators of plant development, growth, abiotic and biotic responses and to mediate cross-kingdom communication between plants and microorganisms. Despite the importance of this communication, to our knowledge, there are currently no transcriptome-wide studies on the regulation mediated by sRNAs during beneficial nor pathogenic plant-microbe interactions in this model plant. Moreover, most of the previous reports are limited to the analysis of specific plant miRNAs, often neglecting the other classes of sRNAs. In this talk, we will present a transcriptome-wide small RNA expression analysis, on these beneficial and pathogenic interactions. We performed a computational search for putative targets for the differentially expressed sRNAs between the interactions. The identification of such sRNA-mediated regulatory circuits could help to develop sustainable strategies to improve or mitigate fungal interactions, which, ideally, could be applied not only in *A. thaliana* but also in other plants.

The *DNA-PrimL* gene of *Arabidopsis thaliana* a potential target of the *Trichoderma atroviride* small RNA1 during their mutualistic relationship

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Abstract

Plants are constantly exposed to biotic and abiotic factors, which affect their growth and development. In their natural settings, plants are invariably interacting with both, pathogens, and beneficial microorganisms; however, plants have developed several lines of defense, including basal chemical defenses, structural barriers, and innate immunity to counteract pathogens. When pathogens surpass these defense layers, plants trigger sophisticated molecular mechanisms to neutralize pathogen attack, which initiates with the detection of pathogen-associated molecular patterns (PAMP) to activate PAMP-triggered immunity (PTI) that limits the pathogen spreading from the original site of infection. However, some pathogens have developed effector molecules to suppress the PTI. To hinder pathogens, plants employ resistance (R) proteins, a kind of intracellular receptors that perceive pathogenic effectors. This defense system is termed effector-triggered immunity (ETI). It has been shown that small RNAs (sRNAs) synthesized by pathogens can act as effectors molecules to suppress plant immunity. *Trichoderma* spp. are plant beneficial fungi that colonize plant roots, conferring beneficial effects by promoting plant growth and inducing the systemic disease resistance. Here, it was predicted that *Arabidopsis DNA-PrimL* (DNA primase large subunit) gene is a putative target of *Trichoderma atroviride* small sRNA1 (*Ta*_sRNA1) during their interaction with the plant. Stem- loop RT-qPCR showed that the *Ta*_sRNA1 is accumulated in the presence of Arabidopsis and the expression of the *DNA-PrimL* gene is downregulated in in the plant at different times of interaction with the fungus, compared with the uninoculated plants. Arabidopsis overexpressing lines of *Ta*_sRNA1 showed different accumulation levels of the *Ta*_sRNA1, which correlated with the downregulation levels of *DNA-PrimL*. On the other hand, *Agrobacterium*-mediated transient co-expression assays in *Nicotiana benthamiana* revealed that *Ta*_sRNA1 co-expression with *DNA-PrimL* WT resulted in decreased accumulation of its transcript. *A. thaliana* transgenic lines bearing a short tandem target mimic (STTM), to inhibit function of the *Ta*_sRNA1, exhibited increased expression of *DNA-PrimL* during its interaction with *T. atroviride* compared with control plants treated with *Trichoderma*.

Bacterial Guests and Fungal Love: Unraveling the Secrets of a Holobiont Model

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The concept of “Holobiont” considers the assembly of interactive species as an evolutionary and ecological functional unit. The associated microbiota can have a significant impact on the physiology, morphology, development, and fitness of their hosts. The heterothallic fungus *Rhizopus microsporus* (Mucoromycota) has emerged as a study model for the fungal holobiont. It is associated with endofungal bacteria from the genus *Mycetohabitans* (*Burkholderia* sensu lato), which significantly affect its development. These bacterial guests can utilize both sexual (zygospores) and asexual (sporangiospores) fungal spores to vertically transmit themselves to the next host generations. The elimination of these bacterial symbionts through antibiotic treatments resulted in a complete absence of sporangiospores production, a decrease in sexual reproduction events, and a reduction in the number of zygospores produced. In heterothallic mucoralean fungi, the co-growth of both complementary mating types, plus and minus, is necessary to initiate the chemical communication that leads to the synthesis of trisporic acid (TA), a fungal sex hormone. TA induces the initial steps of sexual differentiation and the development of zygospores in Mucorales. In this study, we employed various techniques such as transcriptomic analyses, bacterial exchange between fungal hosts, and C13 metabolite labeling to reveal how endobacteria stimulate the synthesis of isoprenoids, carotenoids and trisporoids, which are the precursors of TA. Additionally, we compared several loci of *Mycetohabitans* and *Rhizopus* genomes to identify potential differences that could be important for sexual reproduction in *R. microsporus*. In summary, our study adds a new layer of fungal-bacterial metabolic integration, triggering the production of the sexual hormone TA.

New views on fungal morphology

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Abstract

The evaluation of morphology is fundamental to comprehend how fungi grow, develop, and interact with the environment. Although fungal growth has been extensively studied associated to two-dimensional geometries, the complex three-dimensional (3D) structure exhibited by mycelia has been little explored. In this talk, I report the development of tools for fungal visualization in 3D. We built a light-sheet fluorescence microscope capable of performing time-lapse visualization of 3D biological structures (4D microscopy). We have used this instrument to image *Trichoderma atroviride* and *Neurospora crassa* strains growing in liquid media, over extended times (~12 h) and volumes (~400×1500×800 μm^3) at single-hypha resolution, revealing interactions among hyphae and enabling measurement of 3D apical extension rates. Our experiment allowed study of hyphal mechanics in 3D, as we observed direct collision-deformation events among hyphae, from where we estimate that the force developed by hyphae during tip elongation is at least 260 pN. I also report the invention of a new type of microscopy, optical sectioning in bright-field microscopy (OSBM), a 3D image reconstruction method based on conventional light microscopy, that requires no fluorescence and can reveal hyphal structure. Lastly, I will describe our nascent efforts to approach the mycelium as a physical network with emergent properties.

Keywords: fungal morphology, three-dimensional microscopy, hyphal growth

Unveiling Microtubule Architecture and Functions in Filamentous Fungi: Insights into Cellular Dynamics

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The coordinated movement of elements required for polarized growth through actin or microtubules is pivotal for the development and morphogenesis of filamentous fungi. Microtubules (MTs), being the larger components of the cytoskeleton, are subject to regulation by a diverse array of proteins that facilitate various processes at either the MT plus or minus-ends. In particular, MT nucleation occurs at the minus end, while polymerization and depolymerization occur at the MT plus-end. While the fundamental processes regulating MT are conserved, differences exist among fungal species. Our work was focused on two groups of proteins associated with MTs: the TEA (Tip Elongation Aberrant protein) complex, linked to the MT plus-end, and the complex associated with microtubule organizing centers (MTOCs) positioned at the minus end. Notably, TEA-1-GFP and TEA-4-GFP accumulated as bright spots where the germ tube would emerge. Subsequently, after germination, both were localized in the apical dome, avoiding the Spitzenkörper region. GFP-TEA-5 was observed in the apical dome of mature hyphae. Additionally, TEA-1-GFP, TEA-4-GFP, and GFP-TEA-5 were identified in forming septa as double rings flanking the plasma membrane. Our findings indicated that tea-1 and tea-4 were not indispensable, but the absence of tea-5 was lethal. Regarding the Spindle Pole Bodies (SPBs) associated with the MT minus-end in Neurospora crassa, they were embedded within the nuclear envelope. The γ-TuRC targeting proteins PCP-1 (Pcp1/PcpA) were located on the inner plaque, while APS-2 (Mto1/ApsB) resided on the outer plaque of the SPB. PCP-1 specifically constituted a component of nuclear MTOCs, while APS-2 was also present at the septal pore. Interestingly, although γ-tubulin was exclusively detected in the nucleus, spontaneous MT nucleation occurred in the apical and subapical cytoplasm during recovery from benomyl-induced MT depolymerization experiments. No evidence of MT nucleation at septa was observed. Nevertheless, in the absence of benomyl treatment, MT plus-ends were organized in the septal pore through MTB-3 (EB1), and these septal MT plus-ends facilitated MT polymerization from septa in interphase cells. In summary, the behavior of proteins associated with either the plus or minus ends of MTs in *N. crassa* differs from other organisms. The SPBs serve as the exclusive MT nucleation site, while the septal pore contributes to MT network arrangement by anchoring MT plus-ends through a pseudo-MTOC. Furthermore, TEA-5 was found to be indispensable for this fungus, and the evidence suggests that TEA proteins do not play a role in dictating polarized growth direction but rather contribute to breaking symmetry during conidial germination, conidial formation, and the organization of actin filaments in the apical region.

Role of LST-1 during the formation of ERV-14 dependent COPII vesicles in *Neurospora crassa*

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The growth of filamentous fungi requires the continuous synthesis of cell wall and plasma membrane proteins. This process takes place in the endoplasmic reticulum (ER), where the newly synthetized proteins must be incorporated into Coat Proteins II (COPII) vesicles, which bud off from the ER membrane and follow the secretory pathway to their destination site. Some of the proteins involved in this vesicle formation process are the inner and outer coat proteins, cargo receptors, and assembly regulators, to mention a few. In *Neurospora crassa*, we have identified ERV-14 as an essential transmembrane cargo receptor of the ER, belonging to the cornichon protein family. This protein is an orthologue of Erv14 of *Saccharomyces cerevisiae*, where it works as a cargo receptor of some plasma membrane proteins. The experimental evidence has shown that Erv14 binds simultaneously to the cargo protein and to Sec24, an inner coat protein of COPII, improving the export of the vesicle. Previous work in the lab showed partial co-localization of fluorescently tagged versions of ERV-14 and SEC-24 at a network of membranes in hyphae of *N. crassa*. We identified LST-1 as a SEC-24 isoform. Here, the distribution and dynamics of LST-1-GFP in growing hyphae of *N. crassa* was analyzed by laser scanning confocal microscopy and spinning disk confocal microscopy. LST-1-GFP is found throughout the cell, although it is more predominant in subapical and distal regions, and in the form of puncta close to nuclei. Movement analysis reveals fluorescent puncta being translocated to distal cortical regions, suggesting that LST-1-GFP could participate in a sub-population of COPII vesicles that travels directly to the plasma membrane. Co-expression of LST-1-GFP and ERV-14-mCherry showed lack of co-localization, suggesting that LST-1 may interact with other cargo receptors, and not with ERV-14. Co-expression of LST-1-GFP and SEC-24-mCherry revealed a different distribution for both proteins. LST-1-GFP is found mainly in distal regions, whereas SEC-24-mCherry is found primarily in regions near the apex. However, a partial co-localization was observed at a few puncta. This suggests that LST-1-GFP and SEC-24-mCherry transiently converge in the same organelle. This suggests that LST-1 and SEC-24 participate in the biogenesis of different sub-populations of COPII vesicles. Pulldown experiments of ERV-14-GFP and LC/MS, are under way to identify putative interacting proteins during COPII vesicle formation.

The nucleolar structure in *Ustilago maydis*

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The nucleolus is the most prominent membrane-less organelle, ubiquitous in eukaryotic cells. It is mainly composed of ribonucleoproteins and nucleic acids. The nucleolar structure and function is closely related to RNA metabolism. In mammals, three morphological components have been described and each one is associated with different steps in the essential pre-rRNA processing. The rRNA synthesis takes place between fibrillar centers and dense fibrillar component, pre-rRNA processing in dense fibrillar component and pre-ribosome particle assembly at granular component. In addition, the nucleolar structure in the budding yeast *Saccharomyces cerevisiae* has also been studied and it has been described with two or three ultrastructural elements. However, morphological and cytochemical nucleolar properties are unclear across the fungal kingdom. Here we present a systematic *in situ* study of the nucleolar ultrastructure in the basidiomycete *Ustilago maydis* the agent causing corn smut disease or “Huitlacoche” in maize (*Zea mays*). We employed standard and cytochemical methods for transmission electron microscopy. Our morphological results showed a prominent spheroid shape peripherical intranuclear nucleolus with fibro-granular elements and some chromatin clumps in the nucleoplasm. Furthermore, by cytochemistry, we observed a nucleolar structure composed of ribonucleoproteins, positive to specific silver staining for nucleolar Ag-NOR proteins and partially surrounded by DNA. Our results in *U. maydis* improve our knowledge to understand the nucleolar and nuclear structure in basidiomycete cell.

Deciphering the biological relevance of a KilA-N/APSES Transcription Factor in *Fusarium* sp. associated with the ambrosia beetle *Xylosandrus morigerus*

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Ambrosia beetles are causal agent of important phytosanitary problems and have been considered as a threat to both forest and agroeconomic ecosystems. They are xylem-borers of mainly dead and stressed trees and perform fungus farming as source of food, creating a beetle-fungus highly specific nutritional symbiosis. Also, exists an association of beetles with fungi acquired from the environment, including phytopathogens.
The phytopathogenic *Fusarium* sp. INECOL-BM-06 was isolated from the ambrosia beetle *Xylosandrus morigerus,* denoting the potential of this insect as vector of phytopathogenic fungi. To get insight in the pathogenesis molecular mechanism of *Fusarium* sp, we characterized *Fusarium* sp. TF65-6, a CRISPR/Cas9 edited strain in Fsp*tf,* which encode a KilA-N/APSES Transcription Factor (TF), homologous to Bqt4, the less studied member of this TF family. The phenotypic analysis revealed that TF65-6 increased its mycelial growth rate, decreased the conidia production, and increased conidia germination rate. In addition, the edited strain showed affectation in mycelia and culture pigmentation, and the response to certain stress conditions was different in comparison with WT. Interestingly, the plant infection process was compromised, but the magnitude depend on the plant host. By untargeted metabolomic and transcriptomic analysis, was clearly showed the regulation mediated by FspTF of different biological processes such as secondary metabolism with emphasis in pigments and toxins production, transmembrane transport related with detoxification of fungicides and transcriptional regulation of metabolic pathways. Also, its relatedness with virulence and other processes was encountered. These data established for first time the biological relevance of an orthologue of Bqt4 in a phytopathogenic fungus such as *Fusarium* sp. associated to an ambrosia beetle.

The epigenetic dialogue in plant-Trichoderma interaction

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Abstract

Trichoderma spp. are ascomycete filamentous fungi widely distributed worldwide that establish mutualistic relationships with plants by antagonizing phytopathogens in the rhizosphere and colonizing the plant roots, hence, promoting plant growth and triggering the systemic resistance against phytopathogens. It has been shown that small RNAs (sRNAs) synthesized by pathogens can act as effectors molecules to suppress plant immunity. In this presentation I will show data from our Lab that demonstrate that *Trichoderma atroviride* send sRNAs to Arabidopsis to modulate its immunity to establish a beneficial relationship. Furthermore, we have shown that *Trichoderma* fine tunes plant epigenetics to establish a mutualistic relationship.

Keywords: plant-microbe interaction, mutualism, epigenetics.

Chromatin architecture in *Candida glabrata* plays a role in the regulation of *EPA* genes

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Chromatin organization in eucaryotic organisms play an important role in many process that involved DNA metabolism, such as replication, transcription, repair, and recombination. In the human pathogen *Candida glabrata*, most of the *EPA* genes (Epithelial Adhesin) which confers adhesion to epithelial cells are localized in subtelomeric regions and regulated by subtelomeric silencing, and depends on the silencing proteins Rap1, Abf1 and SIR complex. *In vitro* adhesion to epithelial cells is primarily mediated by Epa1. We previously found important *cis*-acting elements at the telomere E-R where *EPA1*, *EPA2* and *EPA3* form a cluster, and are crucial to repress these genes through the formation of chromatin loop structures determine by 3C assays. In this work, we determined using ChIP-qPCR assays, that Abf1 binds to the promoter region of *EPA1* at two different positions. Additionally, we found that in a mutant *abf1-43* that lacks the last 43 amino acids important to mediate silencing have a higher adhesion percentage to epithelial cells *in vitro* than the parental strain, which suggest that Abf1 may have a direct role in the negative regulation of *EPA1* expression. Furthermore, we define the sequence of the binding site for Abf1 and Rap1 in *C. glabrata*. Finally, we found that Sir4 and Ku70 that also play a role in subtelomeric silencing in *C. glabrata* are distributed along the nuclear periphery which suggest that silent chromatin could be associated to this localization within the nuclei.

Keywords: Chromatin, Abf1, Rap1, adhesins, Sir4, Ku70, nuclear periphery.

The yeast response regulator Skn7 is necessary for modulating the transcription of genes that respond to endoplasmic reticulum stress induced by the N-glycosylation inhibitor, tunicamycin.

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In eukaryotic cells, membrane proteins and those involved in the secretion pathway undergo folding and modification within the Endoplasmic Reticulum (ER) lumen. ER stress occurs when there is an accumulation of misfolded proteins in the ER lumen, and it can be experimentally induced using the antibiotic tunicamycin (Tn), which specifically inhibits the N-glycosylation process. In addition to the Unfolded Protein Response pathway (UPR), several parallel pathways are activated to collectively alleviate ER stress. Skn7 is a conserved response regulator connected to the Sln1-dependent phosphorelay system, and acts as a transcription factor under oxidative stress in fungi. It has been described that Skn7 associates with other transcription factors to mount responses to several stresses including cell wall stress, oxidative stress, and calcium stress. In this work we describe that Skn7 plays an important role in cells exposed to ER stress, and similar to its activity in oxidative stress, the phospho-acceptor Asp(427) residue is no required for the Tn response. In addition, we found that Skn7 regulates, under ER stress, the transcription of the AHP1 and CWP2 genes, which have been implicated in the response to oxidative stress and cell wall integrity pathway, respectively. We detected that an intact DNA-binding domain of Skn7 is necessary but not sufficient to regulate transcription of AHP1 and CWP2. Skn7 also requires its protein-protein interaction domain to fulfil its transcriptional activity. In this work we described for the first time that Skn7 acts as a transcription factor to modulate the response to ER stress inducers.

This work was supported by CONACyT project CF-58550 and PAPIIT-DGAPA-UNAM project IN207223. DI, H-R is a PhD student of the Biochemical Science Program, UNAM and received a PhD fellowship from CONACyT (No:52046130-1) and was support by PAEP, UNAM to attend this meeting.

Unraveling the mechanisms of lifespan extension by metformin in aging yeast cells

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Metformin, a drug commonly prescribed for Type 2 diabetes treatment, has demonstrated remarkable longevity benefits across various species, including yeast, nematodes, and mice. There are indications that metformin may also extend life expectancy in humans. However, a comprehensive understanding of the specific genes involved in its lifespan-extension effect remains elusive. To investigate the genetic factors contributing to metformin's lifespan extension effects in the budding yeast *Saccharomyces cerevisiae*, we employed a robust functional genomics assay. Our approach involved measuring the chronological lifespan of 1,414 knockout strains, each lacking a single gene with a human ortholog. Additionally, we used high-resolution flow cytometry to examine metformin-induced changes in the proteome, aiming to obtain a comprehensive understanding of the drug's mechanisms of action. In this presentation, we will elucidate the key biological mechanisms and pathways responsible for metformin's ability to extend lifespan in yeast. Intriguingly, we discovered that metformin and inactivation of the Set3-deacetylation complex impact shared pathways that promote longevity, uncovering unprecedented insights into the intricate workings of this drug. Our findings demonstrate that a complex cellular crosstalk involving mitochondrial function, Ty retrotransposition, and transfer-RNA abundance underlies the lifespan extension by metformin and Set3-mediated chromatin modification. Our genome-wide functional analyses provide an unbiased and global perspective on the genetic determinants and pathways that aging cells use to harness the life-extending power of metformin.

Exploring the fungal syntenome: genomic duplications and trait diversification

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Área: Genómica Funcional y Comparativa

Fungi are important organisms that play a variety of roles in our lives, from being crop pests and causing health problems, to providing food and beverages, and contributing to the ecology of the planet. They are ancient organisms with a large number of species and diverse characteristics. Unlike plants and animals, fungi do not have a consistent genomic structure.This study aims to analyze the evolution of fungi by looking at the conservation of key genes through synteny analysis. We aim to understand how genomic duplications, which have been studied in plants, affect fungal evolution.

To achieve these objectives, we analyzed more than 600 complete genomes of different fungal phyla. We focused on characterizing genes that are conserved among phyla, classes, orders, families, and taxonomic groups with functional relationships that may be key to evolutionary diversification among groups. The analysis involves the detection of multiple syntenic genes, indicating evolutionary constraints to conserve gene order in duplicated blocks that may be correlated with metabolic functions, adaptation, and diversification. These genes are critical because they are the result of evolutionary selection pressures to maintain metabolic functions, create specializations of molecular machinery within taxonomic groups, address different lifestyles (plant, animal, human pathogens; saprophytes, necrotrophs, etc.), produce metabolites essential for survival in specific niches, and conserve biosynthetic groups (for metabolites of interest) in specific clades.

In this work we present different evolutionary hypotheses related to the importance of genome duplication in fungi and the conservation of gene clusters across taxonomic groups as a key driver of metabolic diversification, functional novelty in different clades and adaptation to different fungal lifestyles. In this work we use synteny networks to study trait evolution in fungi, an analysis never before applied to the Fungal Tree of Life.

Keywords:
Synteny, Genome duplication, Evolution

Cellular response of black yeasts of the genus *Neophaeotheca,* isolated from the Gulf of Mexico to nutrient scarcity and salinity

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Neophaeotheca is a fungal genus that comprises two species: *N. triangularis and N. salicorniae*. It occupies the basal position as one of the first lineages of the Capnodiales considered within the group of black yeasts, which have frequently been isolated from saline environments. A relevant characteristic of black yeasts is that they produce melanin and have an unconventional cell division, features that are probably an adaptation to the extreme environments they inhabit. We have been studying the fungal diversity of sediments samples from the Gulf of Mexico as part of a Gulf of Mexico Research Consortium (CIGOM) initiative aimed to set the baseline of biological diversity of this ecological niche. In the present work, we report the isolation of the two species of *Neophaeotheca* from deep sediments of the Gulf of Mexico. To determine whether nutrient, salinity and melanin production affect cellular growth and morphogenesis in these black yeasts, assays were carried out in modified Czapek Dox medium, containing different concentrations of glucose (0.4 and 20 $g.L^{-1}$), salt (0, 3.5% sea salt, 10 and 20% NaCl) and melanin inhibitor (Phthalide $1g.L^{-1}$). Cells were grown in solid and liquid media to analyze morphology and cell division changes, respectively. These species belong to the same endoconidiogenesis taxon. However, under the same growth conditions, some differences were observed. Regardless of glucose concentration, *N. triangularis* grew as yeast-like in 0 and 3.5% sea salt concentrations, and as filamentous in 10 and 20% NaCl. In contrast, *N. salicorniae* grew dimorphically in all salt concentrations evaluated. Additionally, in both species cell division occurred faster under low glucose concentration (0.4 $g. L^{-1}$). *N. triangularis* began to release endoconidia at 35, 40, and 53 h of growth in 3.5% sea salt, 10% NaCl, and medium without salt, respectively; whereas *N. salicorniae* began to release endoconidia at 29 and 44 h of growth in 3.5% sea salt and 10% NaCl, respectively, and at 48 h in 3.5% sea salt and 20 $g.L^{-1}$. In the presence of phthalide, almost null formation of hyphae occured. With this information it can be concluded that salinity, nutrient restriction, and melanin depletion induce changes at the cellular level in these species. With this work we expand our knowledge on the diversity of fungi in the Gulf of Mexico and on the cell biology of halotolerant black yeasts.

Key words: cell division, morphology, endoconidial taxa.

The Osmotolerant Yeast *Debaryomyces hansenii* Under Nitrogen Limitation: Assessing the Role of Hog1 in Lipid Accumulation

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Lipid synthesis by yeast has gained attention as a sustainable alternative for producing oleochemicals. Nonetheless, the mechanisms involved in the regulation of lipid metabolism in oleaginous yeasts (OY) in stressful conditions still need to be better understood. In the non-oleaginous yeast *Saccharomyces cerevisiae*, the MAP kinase Hog1 is required for neutral lipid (NL) accumulation, while in the opportunistic pathogen yeast *Candida albicans*, Hog1 down-regulates lipid synthesis. However, it is unknown whether Hog1 is involved in the *de novo* lipid synthesis of *Debaryomyces hansenii*, an osmotolerant yeast that stores NL under nitrogen-limited conditions. This study aims to characterize the synthesis of NL in response to nitrogen limitation and determine if *Dh*Hog1 contributes to lipid accumulation in *D. hansenii* by comparing a wild-type (WT) strain with a *Dhhog1Δ* mutant. We determine the fatty acid (FA) profile and quantify NL by GC-MS and flow cytometry, respectively. The expression of several genes involved in the *de novo* lipid synthesis pathway is also analyzed by RT-qPCR. Using an enzymatic assay, we look up whether the ATP-citrate lyase enzyme (Acl) is present or absent in *D. hansenii*, as Acl provides the necessary acetyl-CoA for lipid synthesis in many OY under lipogenic conditions. We also examine stress-associated morphological changes in individual cells by TEM. Our results suggest that the most significant accumulation of NL occurs in the *Dhhog1Δ* during the stationary phase under nitrogen-limited conditions, in which α-linoleic acid is overrepresented with respect to WT. The genes involved in lipogenesis *ACC1*, *FAS1* and *FAS2* are overexpressed in a *Dhhog1Δ* under different conditions. Moreover, the *Dhhog1Δ* mutant shows morphological modifications possibly associated with endoplasmic reticulum stress under nitrogen limitation. We will discuss the role of *Dh*Hog1 in the lipid accumulation of *D. hansenii*, which could help understand the relationship between stress and lipid metabolism.

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Discovering the role of genes in developmental and metabolic processes in filamentous fungi with a genome-wide loss-of-function approach

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There is limited experimental evidence regarding the function of genes in most filamentous fungi. In bacteria, a technique based on insertional mutagenesis by an engineered transposon (RB-TnSeq) containing unique barcodes allowing the identification of insertions across the genome. Subsequent sequencing of these mutant libraries via barcodes in fitness experiments (BarSeq), allowed the characterization of hundreds of genes in a massive way. However, in filamentous fungi, large-scale transformation has been challenging to apply this technique.

In this study, we conducted several transformation tests on conidia of *Aspergillus flavus, Neurospora crassa*, and *Trichoderma atroviride*, using *Agrobacterium tumefaciens* infection. By varying more than 10 conditions of the conventional transformation protocol, we obtained a highly efficient transformation method. In all models, we were able to increase the transformation efficiency by up to 200 times. This development allowed us to carry out whole-genome insertional mutagenesis experiments, resulting in mutant populations encompassing over 50% of all genes encoded in each genome. Particularly in *T. atroviride* we were able to obtain a library with more than 7,000 genes with at least one insertion and with insertions throughout all contigs of the genome, with over 31,000 unique barcodes.

Using these *T. atroviride* insertion libraries, we conducted hundreds of experiments using BarSeq to describe the change in fitness of mutants in the population under different experimental conditions. By combining BarSeq and RNA-seq data obtained for *T. atroviride* under similar conditions, we have begun to reveal the function of multiple previously unannotated genes in developmental and metabolic processes.

These results demonstrate the enormous potential of this technique to assess the function of fungal genes under different conditions using a genome-wide approach. Development of RB-TDNA-seq for filamentous fungi opens multiple opportunities in research with industrial, medical and ecological applications.

Not all roads lead to Rome: the different secretory routes in *Neurospora crassa* hyphae

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A vast amount of work in our lab has been directed toward understanding hyphal morphogenesis in the filamentous fungus *Neurospora crassa*. We have shown that the polarized apical growth of hyphae is supported by the directional transport of secretory vesicles to the apex, where they accumulate at the Spitzenkörper (SPK). In the last stages of the secretory pathway, vesicles fuse with the plasma membrane (PM) providing all the materials and enzymes necessary for cell wall expansion. Chitin synthases (CHS) travel to two main destinations after exiting the endoplasmic reticulum (ER); the hyphal apex, where they concentrate at the core of the SPK, and regions of septum formation. Other proteins, such as proton pumps, ion channels, amino acid transporters, and pH sensors are transported to the lateral PM, independently of the SPK. For instance, the H^+-translocating ATPase PMA-1 gets incorporated directly at the PM in distal hyphal regions and in completely developed septa. The mechanisms that govern vesicular packaging and distribution of proteins to different cellular sites are very complex and largely unknown. Our most recent efforts aim to identify and analyze the key players of the secretory processes that determine the biogenesis of the different types of vesicles that are distributed to the different delivery sites. We have identified two ER chitin synthase export receptors, CSE-7 (NCU05720) and CSE-8 (NCU01814), responsible for the exit of chitin synthases from the ER. Both CSE-7 and CSE-8 are highly conserved within filamentous Ascomycota, whereas in *S. cerevisiae* there is only one copy of these chitin synthase export receptors. Some of the current challenges we are addressing and that will be discussed are: 1. Identification of the determinants of receptor-cargo interaction and vesicle biogenesis, 2. Characterization of the different subdomains of the ER, and 3. Correlation of the live imaging data with the ultrastructural data.

Evolution of the transcription circuit that regulates filamentation in two closely related species of *Candida*

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The yeast *Candida dubliniensis* is the most closely related species to *C. albicans,* one of the most important human fungal pathogens. Despite sharing many phenotypic characteristics, *C. dubliniensis* is significantly less pathogenic than *C. albicans*. To better understand the molecular changes underlying their pathogenicity dissimilarities we focused on the regulation of filamentation, a developmental trait known to contribute to host invasion in these yeasts. While *C. dubliniensis* forms filaments under nutrient-poor conditions, *C. albicans* does it under a much wider range of settings. To investigate these differences, we generated a collection of 45 *C. dubliniensis* null mutants of transcription factors whose orthologs in *C. albicans* had been previously implicated in filamentous growth. These transcription factors are very similar at the amino acid sequence level making it difficult to prioritize possible causes of the filamentation differences between the species through computational analyzes. Phenotypic characterization of the collection of transcription factor mutants showed that under nutrient-poor conditions almost 45% of the regulators are involved in filamentous growth in *C. dubliniensis.* Detailed temporal profiling identified four mutants that displayed particularly strong differences in filament development through time between the two species. Three of these mutants displayed decreased filamentous growth in one species, while in the other filamentation was similar to that of the WT strain. The fourth mutant filamented even before induction in one species while in the other the filamentation defect was only apparent in latter stages. Transcriptional profiling of one of these mutants showed considerable interspecific diversification of the target genes and *in silico* prediction of target genes for several filamentation transcription factors was consistent with this finding. Overall, our results suggest that several regulatory pathways are involved in the filamentation differences between these two yeasts, despite their phylogenetic proximity. Furthermore, the diversification in filamentation between *C. dubliniensis* and *C. albicans* seems to be mostly due to changes in the target genes rather than in the transcription factors themselves. Our work sheds light on the molecular determinants of essential developmental differences between these two species of medical relevance for humans.

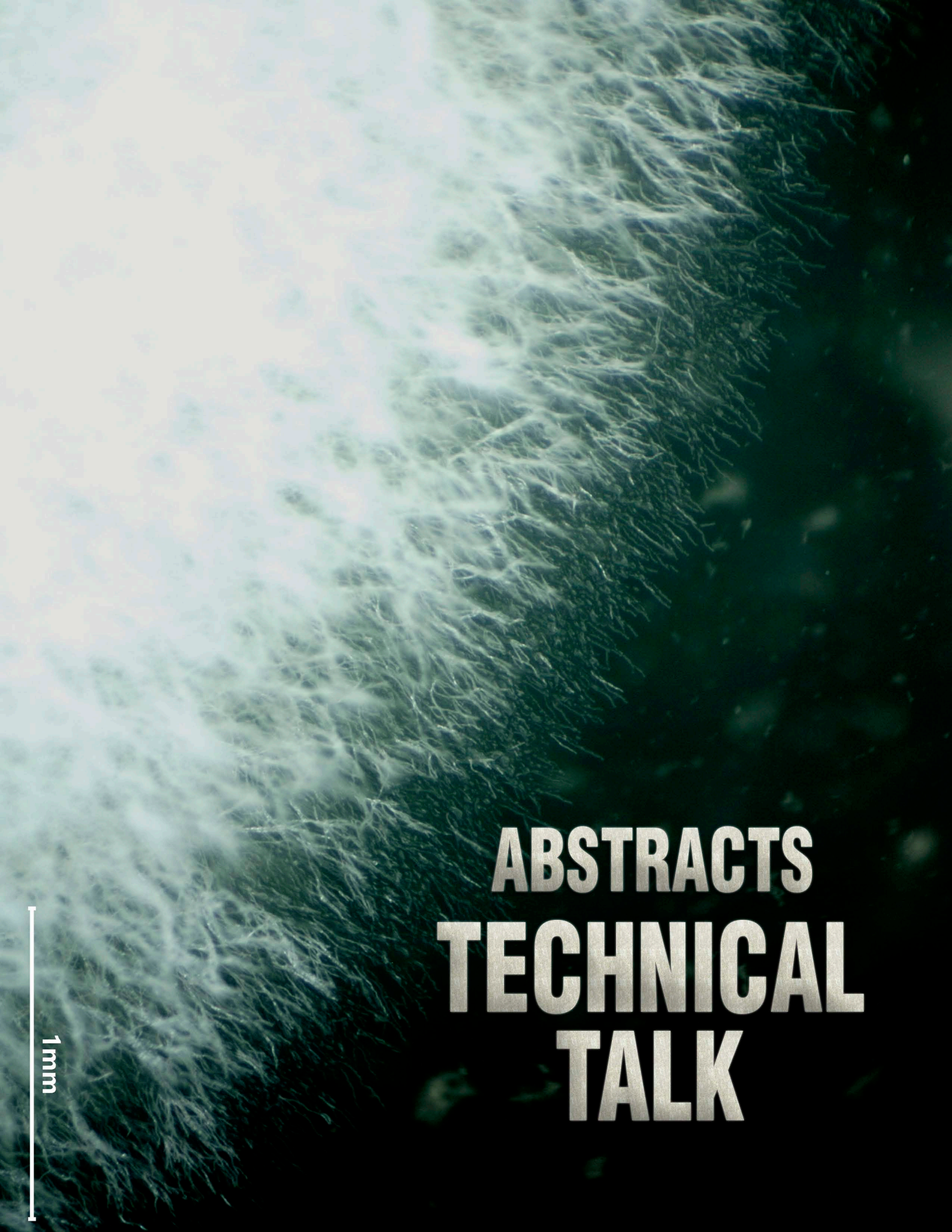
Title: Using experimental evolution of hybrid genomes to identify genetic incompatibilities in yeast

Authors: Artemiza A. Martinez Medina and Gregory I. Lang

Saccharomyces yeast species exhibit low pre-zygotic barriers to mating, and interspecific hybridization readily occurs in nature and in the lab. Diploid hybrids, however, are sterile because high sequence divergence among *Saccharomyces* yeasts prevents proper chromosome segregation during meiosis. Although other factors such as chromosomal rearrangements and genetic incompatibilities may also contribute to hybrid sterility, there is no evidence of strong genetic incompatibilities outside of a few nuclear-mitochondrial interactions.

Here we are using experimental evolution to test for the presence of weak but pervasive negative-genetic interactions between nuclear genes in the genomes of the sibling species *S. cerevisiae* and *S. paradoxus*. We generated and sequenced 20 F1 haploid progeny from a cross between *S. cerevisiae* and *S. paradoxus* using the method developed by Bozdag, *et al*. (2021). Phenotyping of these interspecific hybrids show a wide range of growth rates at different temperatures and fitness defects in mating-type specification.

We evolved 320 independent populations of haploid and homozygous diploid hybrids for over 1,000 generations in rich glucose media. We find substantial fitness gains in the evolved populations and we are currently sequencing the genomes of these strains to identify the underlying beneficial mutations. We hypothesize that these mutations compensate for genetic incompatibilities in hybrid protein complexes.


ABSTRACTS
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Molecular Biology Applications in Fungi

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Fungi are everywhere and play vital roles in most ecosystems. They are a diverse group of organisms with an estimated range of species from 1.5 to 5.1 million. Fungi are integral to the functioning of ecosystems, agriculture, human health, biotechnology and environmental remediation. Researching fungi is not only relevant but also essential for understanding their biological roles and, importantly, to apply what we know about them to the benefit of the industry. In this presentation we highlight the significance of studying molecular biology of fungi and we provide a general panorama of essential tools such as DNA & RNA extraction, gene expression analysis, cell biology assays, mutation detection and genome editing. Through a fungi-centered perspective, this presentation underscores how these innovative technologies contribute to a more comprehensive understanding with wide-ranging implications.


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ABSTRACTS
FLASH TALKS

Yeasts from open agave fermentation show geographic population structure wi sparse conservation of introgressions

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The budding yeast S. *cerevisiae* is frequently isolated from open fermentations of cooked agave juice used to produce distilled spirits. Due to the limited number of genomes available from this environment, the genetic structure, ecology, and origin of agave-related S. *cerevisiae* have been overlooked. Here, we report the genomic sequences of 209 genomes from S. *cerevisiae* associated with open fermentations from over 60 sites representing all the producing regions in Mexico. Phylogenomic analyses group more than 90% of the sequenced isolates in an extended version of the known Mexican Agave clade which is within the domesticated clade of the species, but it is differentiated because it has a high diversity and a high number of introgressed genes from the sister species S. *paradoxus.* Strains from the Mexican Agave clade show population structure correlated with geography, for instance, isolates from the northeast form an isolated clade while stains from the northwest are fully homozygous. Introgressed genomic regions show sparse conservation and differential evolution patterns that suggest a complex history within different subclades. Meanwhile, isolates from Tequila form a cluster with signs of admixture between the Mexican Agave clade and an undefined population and therefore show an intermediate number of introgressions. Surprisingly, we found only three Wild/Pre-domestication isolates and two more isolates show admixture between them and the Mexican Agave clade despite the fact that fermentation occurs spontaneously in open tanks. Finally, a few isolates of commercial origin were identified. This evidence suggests the influence of geography and human practices on the overall structure and it provides insight into the structure of populations exposed to a megadiverse semi-industrial environment. This study not only sheds light on the genomic consequences of interactions between wild and domesticated microbes but also provides a resource to conserve the agave fermentation micro-ecosystem which has great cultural and economic value.

LOX1 and PLP1 lead a transcriptional reprogramming essential for injury-induced conidiophore development in *Trichoderma atroviride*

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Fungi use oxidized lipids, also known as oxylipins, as signaling molecules to induce asexual development. However, physiological and molecular mechanisms triggered by oxidized lipids that lead to asexual development have yet to be understood, especially in the context of mechanical stress. To better understand the role of oxylipins in fungi, we utilized genetic techniques, mass spectrometry, and phenotypic analysis to decipher the function of two genes: a patatin-like phospholipase (*plp1*) and a unique lipoxygenase (*lox1*), which produce oxidized PUFA derivatives in *Trichoderma atroviride* when mechanical damage occurs. Our findings indicate that damaged signaling and sensing components regulate the expression of *plp1* and *lox1*. Additionally, we observed that mutations in these genes affected the emergence of aerial hyphae, blocking injury-induced conidiation. Our functional loss analysis also showed that both genes are essential for producing the wound oxylipin 13-HODE and the transcriptional reprogramming required for conidiation. According to the transcriptomic analysis, *T. atroviride* requires LOX1 and PLP1 to induce at early stages of the response transcription factors involved in asexual development, such as a BrlA homolog, Hox2, and Azf1, and later activate lipid metabolism and structural proteins involved in aerial mycelium emergence. These results highlight the cooperative function of *lox1* and *plp1* in regulating molecular and physiological processes in damaged-sensitized cells, which leads to reproductive aerial mycelium development and survival through asexual reproduction.

Keywords: oxylipins, injury-induced conidiation, transcriptome

CFEM proteins in *Neofusicoccum parvum* and their role in the pathogenicity process

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Cysteine-Rich Secretory Proteins (CRISPs) are recognized as fungal effectors by the plant immune system. Among the CRISPs, there are fungi-exclusive CFEM proteins (Common in Fungal Extracellular Membrane) that contribute to pathogenesis. This is a current research topic in the field of plant-pathogen interactions. *Neofusicoccum parvum*, a filamentous fungus and a member of the Botryosphaeriaceae family, is considered an extremely aggressive endophytic pathogen capable of causing stem cankers, necrosis, branch wilts, dieback, and other diseases in a wide range of hosts, including woody species of agricultural and forestry importance. In this study, we identified twelve genes encoding CFEM domain-containing proteins from the genome of *N. parvum* (UCR-NP2). Subsequently, we performed predictive analyses of their biochemical and molecular properties using bioinformatics approaches. The analysis of CFEM-domain proteins allowed us to classify them into three groups: 1) effector proteins, 2) Glycosylphosphatidylinositol-anchored proteins, and 3) G protein-coupled receptors (GPCR) proteins. Furthermore, RT-qPCR analysis showed significant differences in mRNA expression levels for three CFEM proteins belonging to the group of GPI- anchored proteins and effector proteins. The results showed that CFEM genes are transcriptionally regulated under certain conditions and suggest a specific contribution of certain CFEM proteins in the pathogenesis process. Our findings contribute to a better understanding of the functional roles of CFEM domain- containing proteins in the infection process of *N. parvum*.

Keywords: CFEM domain, *Neofusicoccum parvum,* effector, GPI-anchored proteins.

This work belongs to the area of fungus-host interactions.

Effects of human Tau protein expression on the yeast mitochondrial physiology.

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Area: Biochemistry

Tau is a microtubule associated protein which is expressed in the mammals peripheral and central nervous system. Tau abnormal aggregation in neurons is a hallmark of some neurodegenerative known as tauopathies. Growing evidence shows that there is a link between aggregation of Tau protein and mitochondrial dysfunction, which appears to be an early event in the development of neurodegenerative diseases. However, it remains unclear whether Tau expression affects directly or indirectly the mitochondrial function. In this work, we found that when expressed in the yeast *Saccharomyces cerevisiae*, a portion of the 2N4R isoform of Tau is located into mitochondrial sub-compartments. Mitochondrial- located Tau appeared to generate mitochondrial stress and to activate the retrograde yeast response. Tau also affected mitochondrial dynamics and degradation, inducing fragmentation of the mitochondrial network and increasing mitophagy upon nitrogen starvation and in stationary phase. We also observed that Tau impaired the mitochondrial respiration when glucose or lactate were used as carbon sources. Our results demonstrate that some effects of Tau on human mitochondrial physiology are recapitulated in yeast, which makes this species a useful model for the study of the molecular mechanisms present in neurodegenerative diseases.

Keywords: Tau, mitochondria, yeast

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The vacuolar proteases of the yeast multi-resistant to antifungal *Candida auris* in autophagy conditions

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Abstract:

Candida auris is a multidrug-resistant pathogen, which has been linked to healthcare-associated infections, being capable of causing fungemia mainly in immunocompromised patients with a fatality rate of 67%. Moreover, molecular studies have allowed to identify *C. auris* based on its genetic information and geographic distribution in five different clades. In México, the first case of invasive candidiasis due to *C. auris* on a Monterrey hospital was made public in 2020. That same year, different epidemiological organizations issued an epidemiological alert. On the other hand, the vacuolar proteolytic system of yeasts plays a role in different physiological functions, including their virulence, morphogenesis, maintenance of homeostasis, and turnover of senescent or non-functional organelles and proteins. Interestingly, all these processes have been associated to autophagy. This mechanism gives the cell the ability to survive on different microenvironments and stress inducers. This process is mediated by autophagy related genes (*ATG*s) involved in the formation of the autophagosome, which is degraded by vacuolar proteases for the subsequent reuse of monomeric and oligomeric structures. Studying the vacuolar proteases of *C. auris* will allow to suggest the role of these enzymes in autophagy and other related processes. In this work, a search for the most likely putative vacuolar proteases genes in the *C. auris* genomes of two different strains, one from Spain (*C. auris* 49, Clade III) and the other from Mexico (*C. auris* 201498, Clade IV), was performed. We also performed measurements of 5 specific activities of putative proteases from enzyme extracts for *C. auris* strains as well as analysis of the expression of genes encoding CauPrA (aspartyl protease), CauPrB (serine protease), CauCpY (carboxypeptidase), CauApe1 (aminopeptidase) and CauDap2 (dipeptidyl- aminopeptidase) enzymes and the *atg8* gene by RT-qPCR under different conditions of rapamycin-mediated autophagy induction and nutritional stress. We found an increase for all five activities tested, as well as a differential increase in the expression of protease coding genes in the carbon source-deficient media and in the rapamycin-added medium in which both *C. auris* strains were cultured. In these same media we also found an increase in the expression of the *ATG8* gene, suggesting the role of *C. auris* proteases in the adaptation to nutritional stress caused by an autophagy event, under the conditions evaluated.

The volatile 6-pentyl-2H-pyran-2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root meristem via an ethylene-mediated phosphate (Pi) deficiency response.

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Abstract

Plant growth-promoting fungi are integral components of the root microbiome that help the host resist biotic and abiotic stress while improving nutrient acquisition. *Trichoderma atroviride* is a common inhabitant of the rhizosphere, which establishes a perdurable symbiosis with plants through the emission of volatiles, diffusible compounds, and successful root colonization. The 6-pentyl-2H-pyran-2-one (6-PP) is the principal volatile biosynthesized by *T. atroviride*, and it has been reported to promote plant growth and regulate root architecture. Its biological activity modulates the expression of several auxin-responsive genes to control lateral root formation. However, high concentrations of 6-PP suppress root tip growth and imply the function of the ETHYLENE INSENSITIVE2 (EIN2) protein, a key component in the ethylene signaling pathway [1]. Our results reveal that loss-of-function mutation of EIN2 in *Arabidopsis* confers complete insensitivity to the 6-PP effect, which is mainly attributed to the misregulation of PIN-mediated polar auxin transport and the stem cell niche (SCN) maintenance. Besides, we report that 6-PP induces the expression of phosphate (Pi) transporter genes (*ARABIDOPSIS THALIANA PHOSPHATE TRANSPORTER 1*, *ATPT1* and *AtPT2*) in roots, markedly induced by Pi starvation. However, these responses differ from those of the *ein2* mutant, showing less Pi transporters induction at root tips. According to the functional category enrichment analysis of differentially expressed genes, 6-PP induces a strong immune response in the seedlings, switching on processes such as response to organonitrogen compound and chitin in the *ein2* mutant background, whereas in the wild-type Col-0 seedlings these genes are repressed. Interestingly, genes encoding elements of the Pi starvation response increased their expression level in response to 6-PP in WT seedlings relative to *ein2* mutant plants, indicating that inhibition of root meristematic activity involves possible crosstalk between ethylene signaling and Pi deficiency response.

1] Garnica-Vergara *et al*. (2016). The volatile 6-pentyl-2H-pyran-2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root morphogenesis via auxin signaling and ETHYLENE INSENSITIVE 2 functioning. New Phytologist. 209; 1496-1512.

Pseudogenization-driven gene loss shapes genome evolution in Hanseniaspora

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Gene family contractions and gene loss have played significant roles in the evolution of fungal genomes. Among the mechanisms driving gene loss, pseudogenization stands out as a major contributor; high mutation rates during this process lead to rapid genome reduction. Fungi from the *Hanseniaspora* genus are a great model to understand gene loss since some spices are characterized by the smallest genomes among the budding yeasts. Furthermore, *Hanseniaspora* can be separated into two lineages, a slow evolution lineage and a fast evolution lineage with reduced genomes. In this study, we isolated and sequenced 16 *Hanseniaspora* isolates from different geographic regions across Mexico. Among them, ten were identified as *H. lachancei*, two as *H. pseudoguilliermondii*, two as *H. guilliermondii*, and one as *H. opuntiae*. Comparative analysis of these genomes revealed the dynamic nature of gene family sizes and compositions, with metabolic, DNA repair, and cell cycle control functions being the most affected. Interestingly, the distribution of pseudogenes does not correlate with genome size or gene count, but instead shows lineage specificity, indicating that distinct remodeling processes operate during the evolution of each organism's genome. Overall, we observed a wide range of pseudogene integrity, suggesting that each lineage may exhibit varying degrees of partial activity. This hypothesis will be further tested by phenotyping the isolates under a variety of growing conditions. Our findings highlight the importance of pseudogenization-driven gene loss as a mechanism that shapes genome evolution in autochthonous *Hanseniaspora* populations, providing insights into their adaptation strategies.

Candida glabrata secretome molecules from stationary-phase induce a quiescence-like state in growing cells.

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Candida glabrata is an opportunistic fungal pathogen that causes infections in immunocompromised patients. Yeast cells can be classified into proliferative and nonproliferative states. The proliferative state is referred to as the logarithmic phase (LP) where growth and mitotic cell division occurs, while the non-proliferative state is characterized by cessation in cell growth and is commonly known as the stationary phase (SP). In cultures that have reached SP, cells attain a distinct quiescent state distinguished by increased cell density, resistance to different stresses, and an extended lifespan. *C. glabrata* oxidative stress response (OSR) depends on the metabolic state since SP cells are more resistant to oxidative stress compared to LP cells. It has been proposed that *C. glabrata* cells increase their resistance to oxidative stress through secreted molecules that activate signal transduction pathways that modulate the OSR. In this study, we show that BG14 (parental strain) LP cells increase their resistance to oxidative stress when exposed to a cell-free conditioned medium (CM) from SP cells or water, indicating that nutrient starvation induces resistance to oxidative stress. However, the presence of signaling molecules are required since CM from *aro8*Δ *aro9*Δ *aro10*Δ or *msn2*Δ *msn4*Δ, exhibited a reduced resistance to H_2O_2. We have identified in *C. glabrata* secretome the alkene 1-dodecene (C12) and the aromatic alcohols (AAs) tyrosol and tryptophol that increase LP cells resistance to H_2O_2 but signaling through different pathways. RNA-seq data confirm the induction of OSR genes in LP cells exposed to CM, C12 and AAs. Interestingly, the transcriptional landscape of LP cells exposed to CM or C12 shows the activation of quiescent-like state genes. Together, these data show that *C. glabrata* CM molecules modulates a response involved in the transition to quiescence-like state along with an increase in resistance to stress including oxidative stress.

Molecular and sensitive specific detection of *Candida glabrata*

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There are five *Candida* species responsible for up to 90% of candidemias reported worldwide including *Candida glabrata*, an emergent and opportunistic fungal pathogen that is usually listed as the second most common species responsible for invasive candidiasis in humans. Recently, the WHO included *C. glabrata* as the leading species in its first ever published high priority group of the fungal priority pathogen list. *C. glabrata* has an innate low susceptibility to fluconazole, for this reason, an opportune and sensitive detection method is crucial, but current detection methods are time consuming and have a limited sensitivity. In this work, we developed a novel method for *C. glabrata* detection based on a computerized, unbiased method for oligonucleotide design using the most frequent repeats present in the genome, to amplify them by end point PCR. These oligonucleotides can specifically amplify highly repetitive regions of *C. glabrata´*s genome, generating a distinguishable and easily visualized ladder pattern only when *C.glabrata´*s genomic DNA is present, but not when other related *C. glabrata* species' DNA is present, indicating that these oligonucleotides display high specificity. In addition, these oligonucleotides also have high sensitivity for detection of *C. glabrata*, as a minimal amount of genomic DNA is required, compared to other methods.

Evolution of *Candida glabrata* throughout the course of an infection

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Abstract

Candida glabrata is an emergent and opportunistic fungal pathogen that colonizes and survives in different niches within its host. The objective of this work is to determine the phenotypic and genotypic changes that occur in sequential clinical isolates of *C. glabrata* throughout the course of an infection. The clinical isolates come from the same patient (P7), from blood and urinary tract cultures.

We found phenotypic variation among these isolates regarding sensitivity to thermal and oxidative stress and susceptibility to two classes of antifungals. Only isolate P7-3 (from urine culture) is highly resistant to caspofungin, while the other isolates are sensitive. However, this isolate P7-3, is sensitive to fluconazole (FLC) and isolates P7-1, P7-2, P7- 4, and P7-5 are resistant. The sequence of the *PDR1* gene, which encodes for the Pdr1 transcription factor, shows that the five P7 isolates are identical to each other throughout the protein sequence except for the C-terminus of the last isolate (P7-5) which contains a mutation, G1099C, in the putative Pdr1 transactivation domain. In addition, all the isolates contain 4 polymorphisms with respect to the *PDR1* gene of the reference strains CBS138 and BG14 (S76P, V91I, L98S and T144P). We found that the *CDR1* gene, which codes for the major drug efflux pump of *C. glabrata*, is highly overexpressed in the resistant isolates P7-2 and P7-5. In addition, the resistance to FLC of the resistant isolates P7-1, P7-2 and P7-5 depends on the Pdr1 transcription factor. Taken together, these data show phenotypic and genotypic variation between isolates from different niches within the patient, suggesting that there is microevolution during an infection and may result in a better adaptation of *C. glabrata*, depending on the anatomical site in which it is found.

Trichoderma brevicompactum 2IG2102 has features of biological control agent and produces secondary metabolites that contribute to its antagonism towards fungal phytopathogens

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The novel strain *Trichoderma brevicompactum* 2IG2102 was isolated from soil associated with banana plants growing on the coast of Oaxaca, Mexico. Optical microscopy and analysis of 3 phylogenetic markers confirmed the identified species belonging to the clade 6, a clade whose species had not been reported and characterized in Mexico. *T. brevicompactum* 2IG2102 inhibits the mycelial growth of 2 phytopathogenic *Fusarium* strains and accelerates the germination of tomato and cucumber seeds. The species of the *Trichoderma* clade 6 produce trichotecenes, a group of terpenes that repress the translation in cytoplasm and mitochondria. Identification of the *tri5* gene encoding the enzyme that produces trichodiene -the universal precursor of trichothecenes- was the first approach to get an insight into the trichothecene biosynthesis in *T. brevicompactum* 2IG2102, which was later confirmed by detecting both its transcript by RT-PCR and the trichodiene by SPME-GC-MS. A trichothecene-enriched extract was able to inhibit the mycelial growth of *Fusarium* spp., and the analysis of metabolic profiles by HPLC/MS-MS revealed the composition of trichothecenes and other metabolites with potential biological activities. An additional role of trichothecenes as modulators of chromatin structure has been recently proposed. In this context, we demonstrated that non-lethal concentrations of the trichothecene-enriched extract were able to impair the growth of yeast deletion mutants affected by histone acetylation and deacetylation.
This is the first report of characterization of a species from the *Trichoderma* clade 6 in Mexico that displays biological control traits and the capacity to produce toxic compounds that might be incorporated in organic pesticides or herbicides.

Keywords: Biological control, phylogeny, phytopathogens, trichothecenes, *Trichoderma*

The unexplored apical and subapical organization of the endoplasmic reticulum in growing hyphae of *Neurospora crassa*

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Abstract:

The cytoplasmic order of the endoplasmic reticulum (ER) in relation to polarized protein secretion in most of the multinucleated cells of filamentous fungi is still unknown. The ER consists of an interconnected membranous network that, in general, forms a series of flattened cisternae (cER), and tubule-like structures (tER). In fungal hyphae there is an extensive network of ER membranes that emerge from the nuclear envelope into the cytoplasm and constitute what is known as the peripheral ER (pER). The filamentous fungus *Neurospora crassa* is a well-established model organism to study polarized growth. We have recently observed in living hyphae of *N. crassa* by confocal laser scanning microscopy (CLSM) the ER-shaping protein YOP-1 tagged with GFP localized primarily at apical and near-apical regions. Remarkably, those regions coincide with the regions, where other ER markers, as for example the ER chaperone BiP, are not as abundant. Due to the multinucleated nature of *N. crassa* hyphae, we evaluated the organization of YOP-1-GFP in a cell co-expressing histone H1 tagged with RFP. We determined hyphal elongation rates (HER) and length of the nuclear exclusion zone (NEZ) in the YOP-1-GFP/H1-RFP strain. HER were similar for the WT and for the YOP-1-GFP/H1-RFP strains. The length of the NEZ (14.1 ± 2.2 µm) corresponded to the region of higher fluorescence intensity of YOP-1-GFP (~10-12 µm). In contrast, the region of higher fluorescence intensity of BiP-RFP and NCA-1-RFP (~12 to 60 µm) corresponded to hyphal regions II and III, where a higher number of nuclei were found. Moreover, YOP-1-GFP revealed also pleomorphic structures just below the tip; some of them looked like condensed patches, which were also observed in a WT strain stained with ER-Tracker Blue-White DPX. Those subapical ER patches are very dynamic, move along the hyphal growth axis, and seem to be connected to the SPK. Three-D reconstruction of CLSM Z-stacks, revealed connections of the pER patches with other pER membranes that extended to the tip. In addition, it could be observed that some pER membranes (YOP-1-GFP) were surrounding the SPK core, where mCherry-YPT-1 was used as marker of microvesicles. Finally, we confirmed the presence of smooth tER at the apex and rough cER at the subapex of cryo-fixed mature *N. crassa* hyphae by transmission electron microscopy (TEM). Therefore, our results suggest that YOP-1 positive ER membranes at the hyphal apex correspond to interconnected tER, whereas the BiP positive ER membranes at the hyphal subapex correspond to cER. Moreover, in the region between the apex and the subapex, both ER types are most probably interconnected, forming distinct arrangements during hyphal growth.

Key words: endoplasmic reticulum, tubules, cisternae, filamentous fungi

Transcriptomic profile of *Colletotrichum lindemuthianum* pathotypes

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Keywords: Pathogen, Pathotypes, CAZymes, CWDEs

Fungi are the main organisms capable of degrading plant cell walls (PCW), such is the case of *Colletotrichum lindemuthianum*, a pathogenic filamentous fungus of the common bean *Phaseolus vulgaris*. This species of fungus displays a hemibiotrophic nutrition/infection strategy, where it secretes an arsenal of plant cell wall degrading enzymes (CWDEs), some of which are also important virulence factors. In addition, it is well established that *C. lindemuthianum* presents pathotypes with diversity of virulence against differential bean varieties. Molecular analysis by AFLP of several isolates collected in two geographic regions of Mexico, where different bean varieties are cultivated under different conditions, showed high genetic diversity and four genetic groups were detected. Considering the diversity of bean varieties that the species infects, among *C. lindemuthianum* pathotypes there may be differential gene expression adjusted for differences in PCW, as an important component of the host immune system. The objective of this work was to evaluate the global transcriptomic profile, focusing mainly on the comparison of CWDEs among four pathotypes of *C. lindemuthianum* grown on different carbon sources. Total RNA was purified from mycelia grown for 48h in Mathur minimal media supplemented with fresh ground *P. vulgaris* green beans or glucose as the unique carbon source. Construction of mRNA libraries was performed with TruSeq stranded mRNA system (Illumina Inc.) and sequencing on an Illumina NovaSeq6000 platform. Trinity and Trinotate software were used for transcriptome assembly and annotation, respectively, using the previously sequenced genome as reference. The size of the four transcriptomes from the glucose treatment ranged from 28.04 to 28.94 Mb, whereas for the four transcriptomes from the green bean treatment it ranged from 27.26 to 31.55 Mb. The BUSCO prediction of full length and single copy orthologous genes for the glucose treatment ranged from 307 to 402, while for the green bean treatment it ranged from 203 to 284. For the functional annotation, the GO, UniProt, Pfam, CAZy, EggNOG and KEGG databases were used, with blastp and blastx (NCBI) algorithms. In pathotypes grown on glucose, annotations ranged from 15,885 to 17,655 genes, whereas when grown in green beans, annotations ranged from 14,001 to 15,481 genes. In all the compared databases, two pathotypes grown on green beans present a greater number of genes, including those that code for CAZymes and CWDEs. All pathotypes cultured on glucose had the lowest number of CAZYmes genes and transcripts. There is differential global expression among four pathotypes of *C. lindemuthianum*, which varies with the carbon source.

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Blood serum stimulates the virulence through the enhancement of the mitochondrial oxidative metabolism and rhizoferrin production in Mucorales

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Abstract:

Mucormycosis is an opportunistic fungal infection caused by members of the order Mucorales, with *Rhizopus arrhizus*, *Lichtheimia corymbifera*, and *Mucor circinelloides* as the main responsible agents. *M. lusitanicus*, formerly known as *M. circinelloides f. lusitanicus*, is a dimorphic fungus and has been used to study this infection, partly because there are well-established tools available for its genetic manipulation. This work analyzed how blood serum enhances mitochondrial oxidative metabolism in Mucorales and how it affects their virulence. Our results demonstrated that Mucorales spores produced on serum had more mitochondrial activity and content in non-fermentable carbon sources, and were cultured under low oxygen levels, they stimulated hyphal growth more than those produced on yeast-peptone glucose medium alone. Spores from *M. lusitanicus* and clinically relevant mucoralean species produced or germinating in serum had increased respiration rates, reactive oxygen species (ROS) levels, and growth at 36°C. The cell-free supernatants of the culture broth (SS) from spores produced on serum increased toxicity against *Caenorhabditis elegans*, and these spores showed higher virulence after their inoculation in *Galleria mellonella*. Adding non-lethal concentrations of potassium cyanide or N-acetylcysteine to the culture during aerobic and anaerobic growth of Mucorales spores produced on serum lowered SS toxicity, suggesting that mitochondrial metabolism is important for virulence induced by serum. Lack of fermentative metabolism in the alcohol dehydrogenase 1 mutant strain revealed similar phenotypes as well as the presence of serum in the wild-type strain. Meanwhile, the impairment in mitochondrial oxidative metabolism, exhibited in the deletant in the ADP-ribosylation factor like 2-encoding gene, showed low virulence even in the presence of serum. A *M. lusitanicus* strain deleted in the rhizoferrin synthetase-encoding gene is avirulent to larvae in serum presence. Our results suggest that blood serum favorably regulates mitochondrial oxidative metabolism and siderophore rhizoferrin production in all Mucorales species examined, which results in increased virulence.

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Gene co-expression network and the regulation by the RNAi machinery during mycoparasitism in *Trichoderma atroviride*

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Abstract:

Trichoderma atroviride is a filamentous fungus widely used in agriculture, among its applications it stands out as a biological control agent against different phytopathogenic fungi of agricultural importance. The process in which some of the *Trichoderma* strains exert biocontrol is called mycoparasitism [1]. Therefore, different genes that participate in this process have been described by expression analysis and loss of function experiments [1], but one of the challenges is to be able to understand the relationships that genes have with each other during this process. In this work, a weighted gene co-expression network was elaborated from 90 RNA-seq libraries of the wild-type strain of *T. atroviride* and mutants in the RNAi machinery in confrontation with *Alternaria alternata, Rhizoctonia solani* AG2 and *Rhizoctonia solani* AG5, in three different stages of mycoparasitism: before contact, during contact and after contact. We chose these fungi because they cause severe symptoms in plants, but they are also efficiently controlled by the *T. atroviride* wild-type strain. However, knockout mutants of the *dcr*2 and *ago*3 genes are unable to control the growth of any of these species. In this work we show the first network of gene co-expression during mycoparasitism in *Trichoderma*, which gave us information on different modules of genes that are associated with particular biological functions during mycoparasitism in the WT strain and in the *T. atroviride* RNAi mutants, in addition, we identified the hub genes of each of the modules, which gives us information to know how these genes are related and how the RNAi machinery plays an important role in this process.

[1] Dou, K. *et al*. (2022). Functional Genetics of *Trichoderma* Mycoparasitism. Advances in *Trichoderma* Biology for Agricultural Applications. Fungal Biology. Springer, Cham. 39-83.

Characterization of GPI-Proteins ACW-1 and CCG-6, and CBM-52 Protein NCW-3 in *Neurospora crassa* as Possible Anchors for a Surface Display System

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Neurospora crassa is a filamentous fungus that has been used to elucidate eukaryotic molecular mechanisms as a model organism. However, its cell wall proteome remains poorly characterized and the functions available within. This does not only represent a gap in the fundamental knowledge of this fungus, but precludes its application as a biotechnologically promising host. Given its characteristics as simple nutrition requirements, non-pathogenicity, rapid growth and readily available molecular tools, *N. crassa* could be used to develop surface-functionalized, whole-cell biocatalysts through protein display. Protein display endows the surface of a host with innovative and complex catalytic activities by fusing and co-expressing a protein, or enzyme, of interest with a native cell wall protein (CWP) as the anchor. The objective of this work was to characterize three CWP: two glycosylphosphatidylinositol (GPI)-anchored proteins, ACW-1 (anchored cell wall protein 1, NCU08936) and CCG-6 (clock-controlled protein 6, NCU01418), and a carbohydrate binding domain protein, NCW-3 (non-anchored cell wall protein 3, NCU07817) to evaluate them as possible anchors to implement a protein display system on the cell wall of *N. crassa*. Homologs for those CWPs in other fungi were searched and their 3D structure predicted by AlphaFold. ACW-1 had a well-characterized homolog in *S. cerevisiae*, Ecm33p, which is involved in cell wall structure maintenance. It also had a highly ordered structure composed of long and short intercalated β-sheets. CCG-6 had Sed1p in *S. cerevisiae*, involved in the yeast resistance against starvation and extensively used as anchor in yeast protein display. Although not well characterized, CCG-6 homologs were highly conserved. NCW-3 did not have characterized homologs. In experiments involving ACW-1, CCG-6 and NCW-3 knock-out strains exposed to diverse cell wall stresses (Congo Red, CR; Calcofluor White, CFW; osmotic stress, and temperature), *Δacw-1* showed an affected morphology with aberrant polarization but similar vegetative growth to WT in normal conditions, sensitivity to CR, and improved resistance to CFW, osmotic stress and temperature than WT. *Δccg-6* showed a severely affected morphology and inhibited growth in normal conditions, and it was the most sensitive strain to all stresses. *Δncw-3* showed an aberrant morphology and sensitivity to CR, osmotic stress and temperature and overall resistance to CFW. GFP-tagged ACW-1 and NCW-3 localized to the septa and hyphae periphery in mature mycelia, whereas GFP-tagged CCG-6 did not localize to a particular cellular compartment. ACW-1, CCG-6 and NCW-3 might be structural elements of different importance in the maintenance of the cell wall, ACW-1 being the least necessary one while CCG-6 a crucial protein for its correct function. ACW-1 has a non-vital cell wall structural function, so it would be the most suitable anchor, even in the case of a deleterious effect due to the display system. This work was supported by CONACYT-SENER Sustentabilidad Energética, grant 245750.

Trichoderma requires the Arabidopsis DICER-LIKE 3 and ARGONAUTE 9 proteins to modulate the expression of the Nitrile Specifier protein 4 (NSP4) gene

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Plants have developed several types of defenses that allow them to suppress damage caused by pathogens. At a cellular level, the recognition of pathogens triggers a complex defense network in which phytohormones play important roles. In addition, small non-coding RNAs participate in plant defense through RNA- directed DNA Methylation (RdDM). Plants synthesize secondary metabolites called glucosinolates, which are hydrolyzed by myrosinase enzymes, and together with specifier proteins, they synthesize antimicrobial molecules involved in the defense of plants against pathogens and insects. Defense mechanisms are also triggered by beneficial microbes such as growth-promoting fungi (i.e., *Trichoderma* spp.). *Trichoderma* promotes plant growth and confers them protection against foliar pathogens such as *Botrytis cinerea* and *Pseudomonas syringae*.

Here, one 24-nucleotide small RNA from Arabidopsis, which is accumulated in *Arabidopsis* during its interaction with *Trichoderma*, putatively target *NSP4* gene. *NSP4* codes for one of the five nitrile-specifier proteins that participate in the synthesis of simple nitriles in the model plant *Arabidopsis thaliana*. To assess the role of NSP4 from Arabidopsis in systemic disease resistance induced by *T. atroviride,* the *NSP4* insertional mutant line was treated with the fungus, and eighteen-day post-inoculation (dpi) was inoculated with foliar pathogens. We showed that NSP4 modulates the triggering of systemic disease resistance against *Botrytis cinerea* and *Pseudomonas syringae* mediated by *Trichoderma*. The *nsp4* mutant did not respond to *Trichoderma*-induced priming and was affected in the expression of genes related to Salicylic Acid and Jasmonic Acid/Ethylene pathways. To determine which proteins are involve in the modulation of *NSP4*, this gene was assessed in Arabidopsis mutant lines whose wild-type genes code for core proteins involved in the RdDM pathway. We proved that *NSP4* expression is regulated in a dependent manner by sRNA-mediated silencing pathways in the presence of *Trichoderma*. Also, we showed that Arabidopsis DCL3 and AGO9 are mainly associated with the regulation of *NSP4* mediated by *Trichoderma* and they play a negative role in Arabidopsis immunity against *B. cinerea*. Finally, we showed that in the presence of *Trichoderma*, the *NSP4* promoter is methylated, thus repressing its expression, which allows the fungus to better colonize Arabidopsis roots.

Effects of culture time and carbon source on the proteome of *Neurospora crassa* extracellular vesicles

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Abstract:

Neurospora crassa, a widely studied fungal organism in molecular biology and biochemistry, has revealed significant insights into secretory vesicles and their impact on hyphal morphogenesis. However, our understanding of extracellular vesicles (EVs) in *N. crassa*, including their biogenesis and functional significance, remains limited. In this study, we investigated the proteome of *N. crassa* EVs (FGSC #988) and explored how growth time and carbon source affect their composition. We isolated EVs from cultures grown for 16 hours (S16) and 24 hours (S24) in Vogel minimal medium supplemented with sucrose (1.5% w/v) at 30°C and 150 rpm. Additionally, EVs were collected from 16-hour cultures grown under the same conditions but with glucose (1.5% w/v) as the carbon source (G16). EVs were obtained through differential ultracentrifugation. The hydrodynamic diameter (D_h) of EVs was assessed using Dynamic Light Scattering (DLS), and protein analysis was performed using SDS-PAGE and LC-MS/MS. Protein identification was accomplished through MASCOT and ProteoIQ. Our D_h measurements demonstrated significant variations based on the substrate, with G16 EVs displaying a broader D_h range (28 to 712 nm) compared to S16 EVs (D_h range: 20-164 nm), while S16 and S24 EVs exhibited no discernible differences. Proteomic analysis identified 716, 702, and 383 proteins for G16, S16, and S24, respectively. Remarkably, a considerable number of the identified proteins were associated with cell wall synthesis and remodeling in all conditions. The most abundant protein in S16 and G16 was a glycosidase (Q7S222), while an anchored cell wall protein (ACW) 12 dominated in S24. Several proteins, including glucanosyltransferases, ACW proteins (3, 4, 5, 8, and 11), cell wall protein PhiA, and clock-controlled protein-14, were shared among all conditions and implicated in cell wall biogenesis. Moreover, proteins involved in vesicle processes, such as SEC proteins, YOP-1, Rab GTPases, and vacuolar sorting proteins, were exclusively detected in S16 and G16, suggesting that growth time may influence EV biogenesis mechanisms. Overall, our findings highlight the essential role of EVs in transporting proteins necessary for cell growth in *N. crassa*, particularly in processes related to cell wall biogenesis. Furthermore, we underscore the influence of growth time on EV formation, providing valuable insights into this crucial biological phenomenon.

Secreted Rich Cystein Protein 1 (SCP1) from *Trichoderma atroviride* is a new effector candidate involved in plant interaction process.

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The filamentous fungus *Trichoderma atroviride* has been extensively studied due to its antagonistic capacity against phytopathogenic fungi, as well as the symbiotic relationships that the fungus is capable of establishing with plants, promoting hormonal balance and resulting in improved plant growth and stimulation of the plant defense system. Although the involvement of some protein effectors in this fungus – plant interaction has been demonstrated, the process by which *Trichoderma* establishes symbiosis with plants is not yet fully understood. From available transcriptomes of two different *Trichoderma* species in interaction with plants, we selected the scp1 gene as effector candidate, to carry out its functional characterization and contribute to the understanding of the association process between *Trichoderma* and plants. We generated plasmids and constructions to obtain *scp1* null mutant strains as well as *scp1* overexpressing strains. We are carrying out monoconidial cultures to analyze the participation of *scp1* during the interaction with *Arabidopsis thaliana* seedlings, focusing on plant growth, root architecture and the activation of defense-related genes. Moreover, we generated strains that overexpress the SCP1-mCherry chimera protein, in which we were able to confirm that the protein is secreted. Additionally, SCP1-mCherry chimera protein was partial purified from a heterologous system and we successfully identified SCP1-mCherry protein inside *Arabidopsis* root tissue, possibly in the apoplast. The analysis of the predicted structure of SCP1 identified homologous regions to Thuricin CD, a bacteriocin from *Bacillus thuringiensis*, suggesting that SCP1 might work by altering the composition of the root microbiome. The results obtained so far contribute to the identification and partial characterization of SCP1 as a new protein effector, for which root internalization was demonstrated and a novel participation is predicted.

The yapsins of the yeast multi-resistant *Candida auris* in osmotic and cell wall stress conditions

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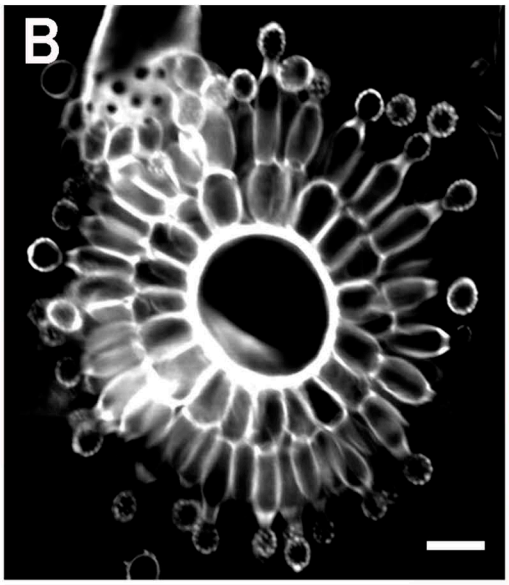
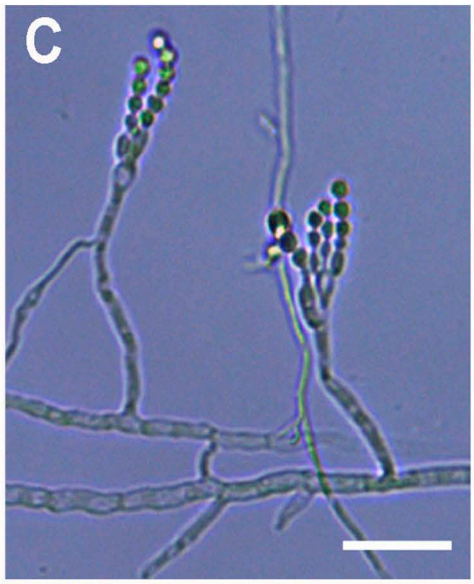
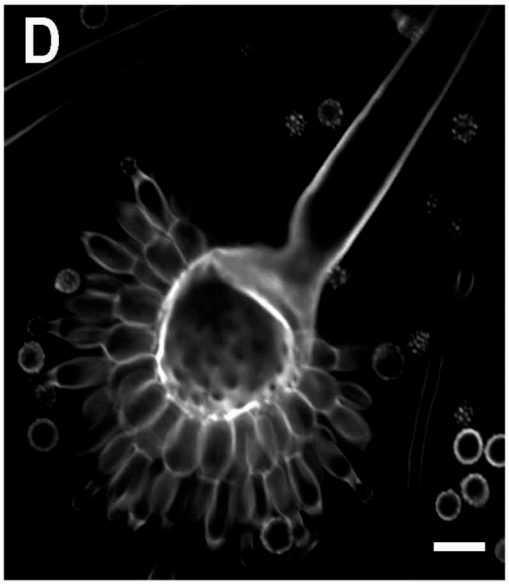
Candida auris is an opportunistic pathogen first identified in 2009 in Japan, since then its reports have been increasing around the world and have been classified into five clades. Its main characteristic is multi-resistance to first-line antifungals (azoles, polyenes, and echinocandins) and its ability to generate outbreaks in the nosocomial environment. Furthermore, *C. auris* is characterized by being halotolerant and growing at 40-42ºC. The *C. auris* yapsins, a family of aspartyl proteases anchored to the cell wall and cell membrane. In other yeast species, these enzymes have been implicated in the stability and function of these ultrastructures. The CauYPS genes coding yapsins were searched and characterized to describe the main characteristics of the CauYps deduced proteins, such as their primary, secondary and tertiary structure. The bioinformatics analysis allowed the identification of seven putative yapsin coding sequences (*CauYPS*1-7) in the genome of *C. auris* B11220 uploaded in GenBank at NCBI. Most of YPS genes of *Candida* species belonged to the CTG clade. The *CauYPS* genes are found on four of the seven chromosomes in the genome. We evaluated the expression of *YPS1* and *YPS7* in the strains *C. auris* 49 and *C. auris* 20-1498 belonging to clades III and IV, respectively in different stress conditions and we found that these genes are expressed depending on the clade of the strain and the type of stressor. In other yeast species, these yapsins play a role in the maintenance of the cell wall, pH and vacuolar homeostasis, in addition to being related to the evasion of the host's immune response. Other enzymes, such as hemolysin, secreted aspartyl proteases (Sap's), DNAses, phospholipases and esterases have been little studied in *C. auris*. The strains of *C. auris* 49 and 20-1498 did not express hemolysin activity in blood agar medium but expressed esterase activity and aspartyl proteases activity. Inhibition by pepstatin and sensitivity to various stressors suggests a possible role of yapsins in wall and membrane function. The phenotypic difference between *C. auris* 49 and 20-1498 strains is most likely due to their belonging to different clades and are a reflection of different genomic structures, which can be verified by genome analysis and gene expression analysis.

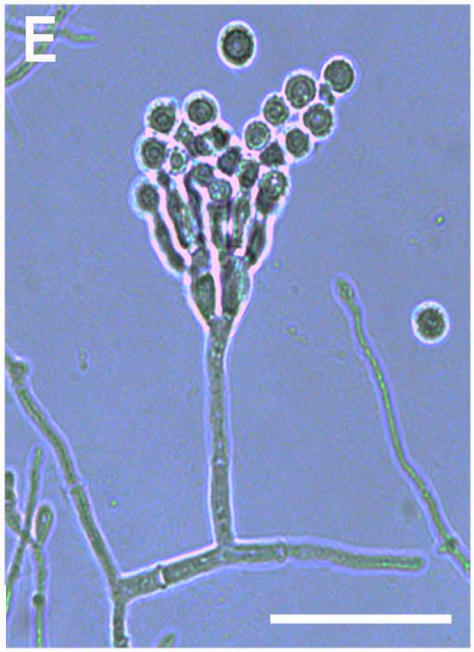
Keywords: *Candida auris,* yapsins, stress.


ABSTRACTS
POSTER SESSIONS









POSTERS

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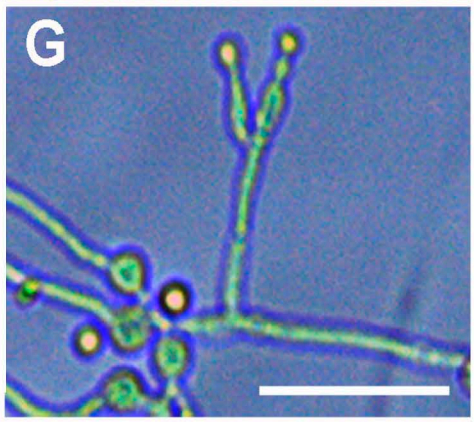

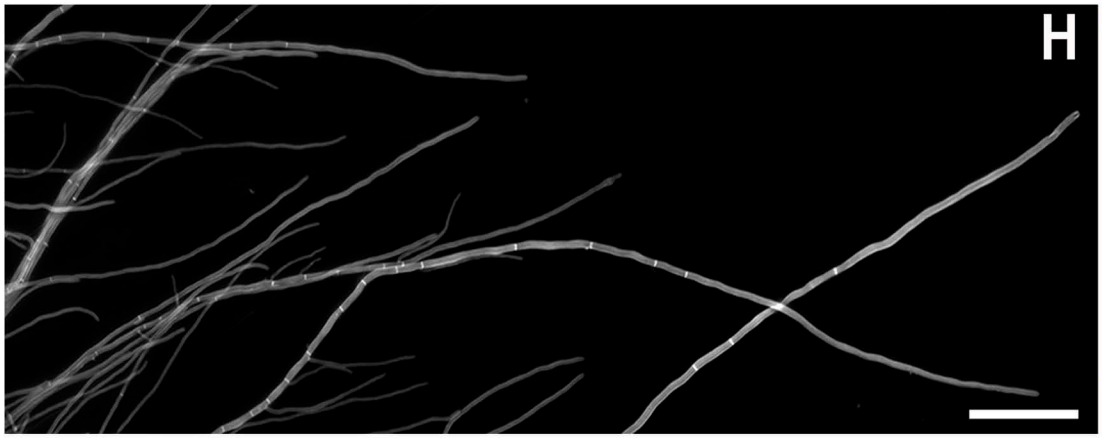

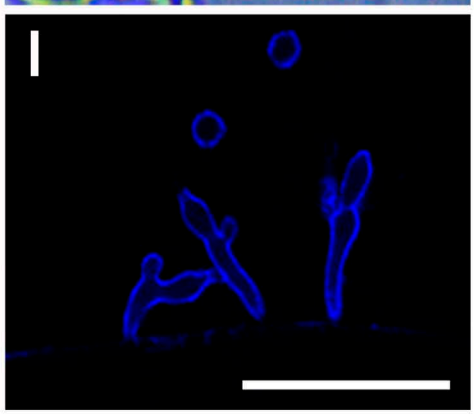

The peroxisome protein translocation machinery is developmentally regulated in the fungus *Podospora anserina*

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Abstract:

Peroxisomes are dynamic and highly versatile organelles that are essential for multiple developmental processes. Peroxisome function depends on the protein constitution of its matrix, which is defined by two conserved protein import pathways. These pathways are driven by the cycling import receptors Pex5 and Pex7, respectively, which recognize in the cytosol the proteins destined to peroxisomes and conduct their import into the organelle. The import process depends on the peroxisome docking/translocation machinery, which is composed of the peroxisome membrane proteins Pex13 and Pex14 and that constitutes the channel for protein translocation across the peroxisome membrane. In the model fungus *Podospora anserina*, peroxisomes are required to induce meiotic development. This process relies on PEX13 but not on PEX14. Additionally, PEX14 is partially dispensable for peroxisome protein import during meiotic development, suggesting a developmental regulation of the translocation machinery Here we show that PEX13 abundance in hyphae is maintained at low levels by the activity of the peroxisome ubiquitin ligase complex and of the ubiquitin-conjugating enzyme PEX4, in conjunction with PEX5, PEX14 and PEX8, which bridges the ubiquitination and the docking/translocation machineries. In addition, we found that the AAA ATPase complex –involved in receptor recycling and pexophagy induction– and PEX3 and PEX19 –implicated in peroxisome membrane formation– are also required to restrain PEX13 levels, and that in their absence PEX13 localizes to mitochondria. Moreover, we found that PEX13 levels increase during sexual development, where its abundance further increased during meiocyte and meiotic-spore differentiation. Our findings show that the peroxisome protein translocation machinery is subject to complex developmental regulation, which modulates PEX13 abundance along meiotic development.

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Identification of molecules with antibacterial activity from different fungal pathogens.

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Pathogenic bacteria, viruses, and fungi cause infectious diseases around the world. The response against infectious diseases has been the design and development of antibiotics, antifungals, and antivirals with different mechanisms of action. These pathogens can survive in the presence of antimicrobials through different mechanisms: mutations in target proteins, increase in expression of efflux pumps, and uptake of genetic material from other organisms, among others. The World Health Organization (WHO) has declared a worldwide emergency in acquired antimicrobial resistance by different pathogenic bacteria, fungi, and viruses. The WHO priority is the research and development for new antibiotics against multi-resistant bacteria, fungi, and viruses. Yeast have shown to secrete metabolites with antibacterial activity. In our laboratory, it has been shown that the secretome of *Candida glabrata* has antibacterial activity. In this work, we are conducting an antibacterial activity screening from different yeast (*Candida glabrata, Candida albicans, Candida parapsilosis, Candida tropicalis, Candida krusei* and *Saccharomyces cerevisiae*). We are including a screen for synergistic effects with various antibiotics with different modes of action.

"*ALT2* and prostaglandin biosynthesis: a new metabolic pathway in the yeast *Saccharomices cerevisiae*"

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Prostaglandins are lipid metabolites that participate in inflammation, cell repair, and act as autocrine and paracrine hormones in mammals. *Candida albicans* has been reported to produce prostaglandin E2 (PGE2) from arachidonic acid, although its function in fungi remains unknown. In 2019, it was discovered that *Saccharomyces cerevisiae* also has the ability to produce PGE2.

In our research, we have uncovered the involvement of the *ALT2* gene encoded enzyme in PGE2 biosynthesis in the yeast *Saccharomyces cerevisiae*. Furthermore, we have found that the absence of this gene reduces cell aggregation during the stationary phase. In mammals, PGE2 plays a role in platelet aggregation. Therefore, by studying the regulation mechanism of cell aggregation in yeast, we aim to gain insights into the role of Alt2 in this process. Moreover, our research aims to lay the groundwork for unraveling the genetic mechanisms involved in PGE2 biosynthesis and its conservation in fungi and mammals. Consequently, our hypothesis suggests that Alt2-interacting proteins and their direct interactors are involved in the biosynthesis of prostaglandin E2 in *Saccharomyces cerevisiae*.

Differential CWDEs secretion capacity among *Colletotrichum lindemuthianum* pathotypes

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Abstract:

The ascomycete *Colletotrichum lindemuthianum* causes anthracnose disease in the bean plant (*Phaseolus vulgaris*). This species exhibits a hemibiotrophic nutrition/infection strategy or lifestyle that includes the secretion of a group of enzymes (CWDEs) that work in a coordinated and synergistic manner to degrade the cell wall of their host. The plant cell wall (PCW) is mainly composed of cellulose, hemicellulose, pectin, and lignin. Knowledge about the fungal enzymes that degrade PCW polysaccharides comes mainly from studies focused on their isolation and characterization due to their importance in multiple biotechnological applications in industrial processes. It is known that some pathogenic fungi show greater hydrolysis than non-pathogenic fungi in cultures with different natural substrates. However, in general these evaluations have been carried out with an individual as a representative of each species. *C. lindemuthianum* presents pathotypes/races with diversity of virulence against bean varieties that also have diversity of resistance. In addition to their virulence diversity, these pathotypes may have differential hydrolytic capacity adjusted for differences in PCW of bean varieties. The objective of this work was to evaluate the secretion of CWDEs in *C. lindemuthianum* pathotypes in culture with glucose, which is an easily accessible carbon source, and water hyacinth (*Eichhornia crassipes*), a hemicellulose-rich natural carbon source. Growth and enzyme secretion of eight pathotypes in culture with Mathur medium supplemented with glucose or water hyacinth were determined. After incubation from 1 to 12, 14 and 16 days, the mycelial growth (dry weight) was determined, and the extracellular medium was recovered for the determination of the enzymatic activity of the CWDEs α-L- arabinofuranosidase (ABF), β- xylosidase (XYLO), endoxylanase (XYL), and cellobiohydrolase (CBH), with fluorogenic and colorimetric substrates. The results showed that for all pathotypes in cultures with glucose, the XYL and ABF activities were basal and almost null in the case of XYLO and CBH. This was consistent with the process of catabolic repression, where genes encoding CWDE are repressed by negative regulators in response to high glucose concentrations. On the other hand, in all the pathotypes in cultures with water hyacinth a high enzymatic secretion was detected. However, a differential behavior was observed in mycelial growth and in the hemicellulolytic secretion capacity among the *C. lindemuthianum* pathotypes. In particular, one pathotype showed better growth and greater secretion of XYL and ABF, compared to the rest of the evaluated pathotypes. The better mycelial growth on a hemicellulose-rich carbon source was consistent with a high hemicellulose degradation and thus a higher assimilation and metabolism of released monosaccharides (mainly xylose and arabinose).

Establishment of growth conditions to increase the production of fungal dyes.

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Fungi are diverse eukaryotic organisms that inhabit in soil or decaying plant matter and could synthesize specialized metabolites with different biological activities that are exploited by many industrial sectors. In this study, various growth conditions were evaluated with the aim of increasing the volume of colored exudates of ten fungal strains. For this purpose, the fungal development was evaluated in varying culture media to know what carbon source they have preferred. In a second experimental stage, the production of colored exudates was evaluated in co-cultures in both solid and liquid media. Each culture medium was inoculated with a 0.5 cm of fungal mycelium disk and incubated at room temperature of 28-30°C for 15 days. For the inoculation in liquid medium, the experiment was divided into two parts: the first one, 0.5 cm of fungal mycelium disk was inoculated directly and for the second one, each 0.5 cm mycelium disk was inoculated in two different tubes and after 15 days, the supernant were interchanged. For the co-culture trials, 45 interactions were performed, in medium at pH 5.5, and then incubated for 15 days at a temperature of 28-30°C. The results showed that a greater volume of colored exudate is produced in the Potato Broth followed by the Malt Extract Broth. For the co-cultures, it was determined that in certain strains there was a growth inhibition when they were interacting. It was also observed that in some strains there were a stimulation in the production of colored exudates in solid and liquid media. Chemical characterization of the exudates and their subsequent comparison will allow us to establish the nature of the exudates and determine their possible application. This study represents an approach to know the growing condition in co-culture to the improve the yield of colored exudates from fungi.

The Role of Subcellular Localization on the Functional Diversification of the Paralogous Proteins Bat1 and Bat2

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Paralogous genes are duplicated genes in the same organism that evolved from either a partial or total duplication of the genome. The study of paralogous genes in the yeast *Saccharomyces cerevisiae*, showed that the retention and functional diversification of duplicated genes played an important role in the acquisition of facultative metabolism.

BAT1 and *BAT2* are paralogous genes that codify for branched-chain aminotransferase enzymes in *S. cerevisiae* (Bat1 and Bat2). Utilization of branched chain amino acids (valine, leucine and isoleucine) as nitrogen source and the synthesis of these amino acids are functions which are exclusively carried out by these two enzymes. Bat1 and Bat2 have a 73.6% of aminoacidic identity and similar kinetic profiles. However, *BAT1* expression is under the control of the transcriptional regulator Gln3, which depends on the quality of the nitrogen source; while BAT2 expression is controlled by Gcn4 that activates the transcription of some genes when amino acid deprivation occurs. This information indicates that *BAT1* and *BAT2* have opposite expression profiles: *BAT1* is preferentially expressed when the cell has a primary nitrogen source (biosynthetic conditions) and *BAT2* expression is mainly achieved when the amino acidic concentration in the medium increases (catabolic conditions).

This transcriptional divergence correlates with studies showing that Bat1 has a biosynthetic profile, whereas Bat2 has a catabolic one. In glucose and ammonia (biosynthetic conditions), a *bat1*Δ mutant shows a reduced growth rate compared to a wild type strain and the *bat2*Δ mutant, while in glucose and VIL (catabolic conditions a *bat2*Δ mutant has a deficient growth rate compared to the wild type strain and the *bat1*Δ mutant. The opposite profiles of the enzymes Bat1 and Bat2 can be explained by the expression regulation of *BAT1* and *BAT2.* Moreover, these enzymes have also diversified on their subcellular localization: Bat1 is a mitochondrial enzyme while Bat2 is cytosolic. We suggest that differential localization of Bat1 and Bat2 could be related to their biological function.

We have re-localized the enzymes to the opposite compartment in presence and absence of its paralogue. In the case of Bat1, the first 18 aa residues of the protein were removed achieving re-localization from mitochondria to the cytosol. Whereas with Bat2, addition of a mitochondrial localization sequence resulted in re-localization to the mitochondria. Then we performed growth rate analysis under different growth conditions to determine the importance of subcellular localization on the function of Bat1 and Bat2 biosynthetic or catabolic role.

Key words: Paralogues Subcellular Localization

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Amyloid beta peptide expression' effect in the physiology of the endoplasmic reticulum of yeast.

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Alzheimer disease (AD) is a progressive neurodegenerative pathology leading to gradual cognitive and behavioral changes and loss of memory. The hallmarks of AD include accumulation and aggregation of b-amyloid (Ab) peptides with extracellular deposition on senile plaques, two major isoforms of Ab peptides associated with AD are Ab40 and Ab42, the first is the most abundant peptide and the latter being more toxic and prone to form oligomers.
Accumulated evidence suggest that aggregation of Ab peptides are necessary but insufficient causes of dementia and that additional factors are required. These factors include several subcellular processes including loss of Endoplasmic Reticulum (ER) homeostasis manifested as chronic ER stress; however, there is still controversy in whether cellular physiological impairments are downstream effects of Ab aggregation and toxicity.
The purpose of this project is to determine the molecular mechanism by which expression of Ab peptides affect the ER homeostasis, or if decline in organelle function is a prerequisite for the establishment of Ab toxicities.
We use yeast as a model organism to fulfill this objective by reason of the yeast models have been successfully used to model some aspects of AD pathology; however, they have not been used extensively and so far, studies lack mechanistic details to explain AD peptide toxicities.
Here, we expressed the Ab40 and Ab42 peptide isoforms with and without tagged forms with signals for ER retention and for the secretory pathway. We found that expression of the Ab40 and Ab42 isoforms with signal for the secretory pathway show an increase of ER stress-reporter and are more sensitive to tunicamycin, induce accumulation of misfolded protein in the ER lumen, in the stationary growth phase.

Biochemistry
Keywords: yeast, amyloid beta, endoplasmic reticulum

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Heterologous expression and characterization of hydrophobins from the polyurethanolytic fungus *Cladosporium tenuissimum* A3.I.1 for their potential application in plastic biodegradation

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The global plastics industry produced 390.7 MTon in 2021,[1] an amount that is continuously increasing due to the high plastic demand and low recycling. Plastic waste is usually burned or dumped in landfills, causing adverse environmental effects. An alternative for plastic waste treatment is biodegradation using microorganisms or their enzymes or proteins. Hydrophobins (Hfbs) are proteins with surface activity able to modify the hydropathy of materials helping the binding of hydrolytic enzymes, hence their degradation. *Aspergillus oryzae* RolA Hfb adsorbed into a polyester polybutylene succinate co-adipate increased the hydrolytic activity of CutL1 cutinase over the polymer.[2] The fungus *C. tenuissimum* A3.I.1, isolated from a decomposing polyurethane (PU) foam collected at a municipal dump site, grew in and degraded around 80% of the PU coating Impranil® and 50% of a polyether PU foam in three weeks.[3] However, its biodegradation mechanism is unknown. This work aimed to determine the expression of Hfb genes in *C. tenuissimum* A3.I.1 during its growth in Impranil®, carry out the heterologous expression of the overexpressed Hfb genes, and characterize their surface activity to have information about its possible participation in the biodegradation of polyesters and PU.

The transcriptome of *C. tenuissimum* A3.I.1 grown in a medium with Impranil® or Dextrose was analyzed. The fungus exclusively expressed four class I Hfb genes in Impranil® (Foldchange 4-5), suggesting that the Hfbs may be involved in Impranil® biodegradation. The Impranil® overexpressed genes were cloned and expressed in *Pichia pastoris* X-33. The Hfbs superficial activity on different materials will be determined by measuring the contact angle to know their capability to change the surface from hydrophobic to hydrophilic this would allow hydrolytic enzymes to bind and attack the plastic surface for the biodegradation process.

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The Mitochondrial Alternative Oxidase in Ustilago maydis Is Not Involved in Response to Oxidative Stress

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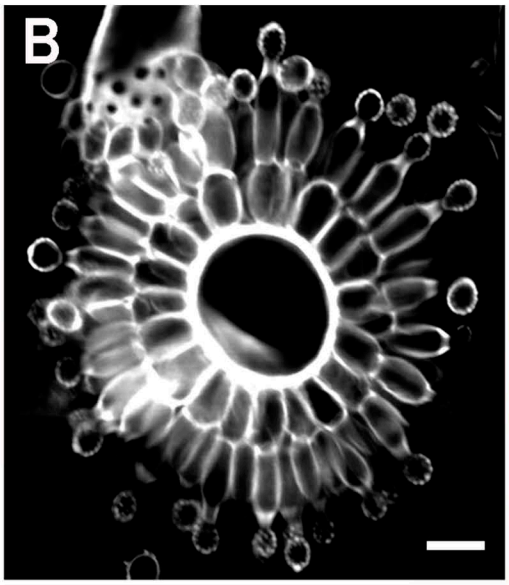
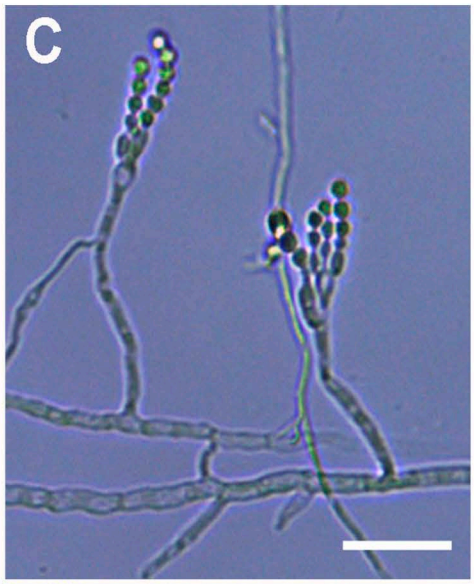
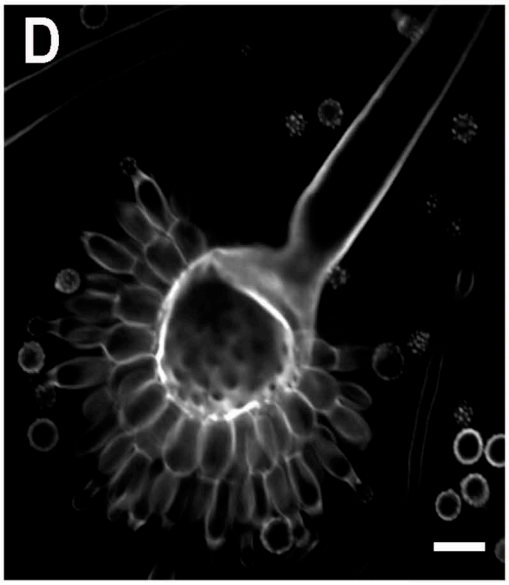
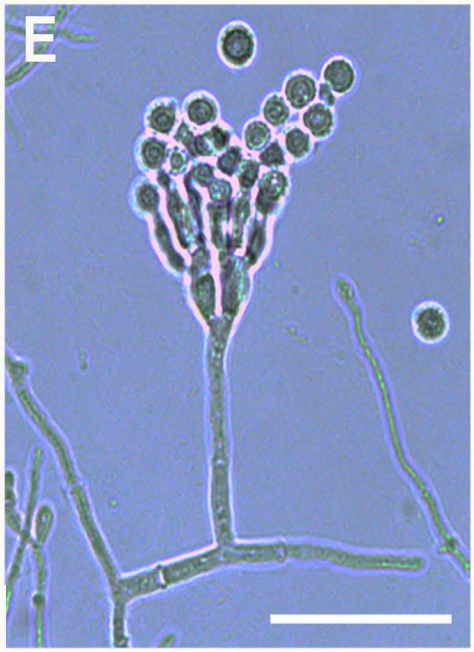
U. maydis emerged as a significant eukaryotic model to study several processes. This yeast is an aerobic microorganism with the classic mitochondrial cytochrome pathway, a cyanide-resistant alternative oxidase (Aox1), and three alternative dehydrogenases. The involvement of Aox1 in different types of stresses and processes, such as desiccation, pH and temperature changes, vegetative and filamentous growth has been discarded, but it hasn't been explored its participation in the response against oxidative stress. In this work we analyzed the participation of Aox1 in the response against oxidative stress. To achieve this goal, four strains with different degrees of *Aox1* expression were used: FB2 wild-type, FB2aox1Δ, FB2aox1-Gfp, and FB2Potef:aox1-Gfp. The oxidative stress was induced by 1 mM paraquat. The replicative capacities, mitochondrial respiratory activity, Aox1 capacity, and the activities of catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase, all antioxidant enzymes, were assayed. Also the GSH/GSSG ratio was determined. The results showed that there was no significant difference when comparing control cells against the mutant strains, suggesting that the Aox1 in *U. maydis*, is not essential to cope with the oxidative stress.









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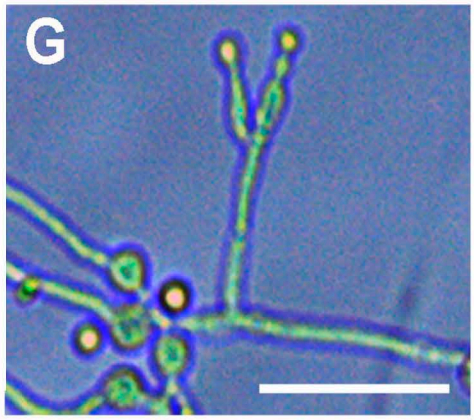

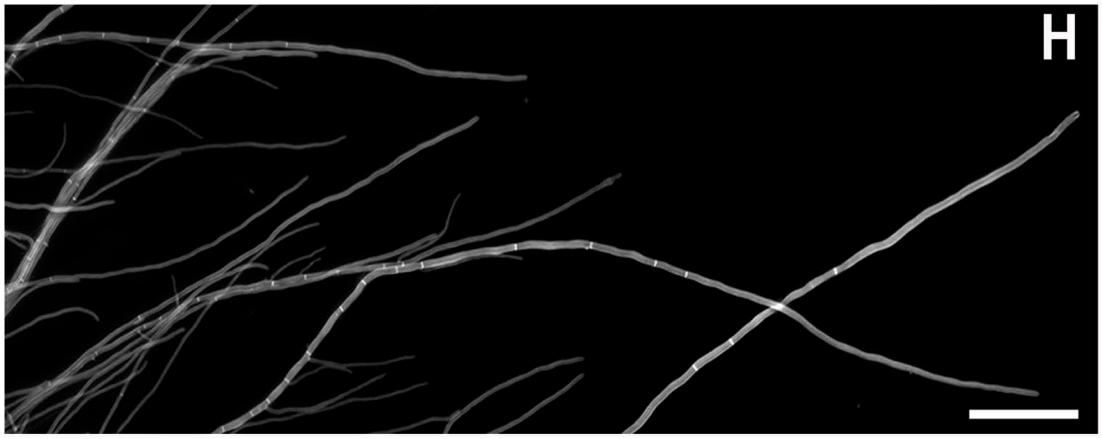

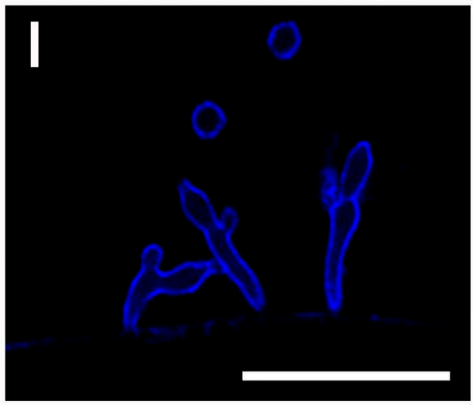

Evaluation of kinetic growth parameters of parental, reconstituted, and hybrid strains of *Pleurotus eryngii.*

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Abstract

Pleurotus eryngii is an edible mushroom with a great interest in large-scale production due to its excellent culinary characteristics and the presence of metabolites with pharmacological activity. Implementing strategies for genetic improvement could help optimize productivity by combination of desirable features from different strains and species and development of hybrid and reconstituted strains, which are obtained by mating compatible neohaplonts. In this study, we evaluated if hybridization and reconstitution of strains affect the vegetative growth of the new strains.

By modeling experimental data of mycelial invasion on malt-agar extract (MAE) and sterile wheat grain (WG), kinetic growth parameters were obtained, i.e., specific growth rate (μ_{max}) and lag phase (λ), for 2 parental strains (FQ and MB), 20 hybrid strains, and 15 reconstituted strains. The parental strains FQ and MB on MAE showed a μ_{max} of 8.21 ± 0.38 mm d^{-1} and 8.53 ± 0.5 mm d^{-1} and λ of 1.54 ± 0.15 days and 1.38 ± 0.13 days, respectively. As reported by Alpuche-Gonzales et al. (2023), the reconstituted strains FQ exhibited a shorter lag phase than the parental strain (between 0.85 and 1.42 days) as well as shown by the reconstituted strain MB6XMB10 with a λ value of 1.15 ± 0.37 days. The hybrid strains expressed statistically similar values of μ_{max} and λ as the parental strains (7.51 - 8.70 mm d^{-1} and 1.39 - 1.61 days). The kinetic parameters obtained on wheat grain for reconstituted and hybrid strains showed no difference to those obtained for parental strains. It is also important to measure now these kinetic growth parameters on the lignocellulosic substrate used for commercial cultivation of P. eryngii (Zou et al., 2019).

Alpuche-González, C., Ornelas-García, B., Leal-Lara, H., Villanueva-Arce, R., De Las Mercedes Gómez Y Gómez, Y., Franco-Hernández, M., Garín-Aguilar, M., & Del Toro, G. V. (2022). Optimization of *Pleurotus eryngii* culture parameters and development of improved strains by mating of compatible neohaplonts. Revista Mexicana de Ingenieria Quimica, 22(1), pp.1-17.

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Mucor circinelloides and *Coprinopsis cinerea has* genes are *bona fide* hyaluronic acid synthases?

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Hyaluronic Acid (HA) is a heteropolysaccharide composed of repeated dimers of N-acetyl glucosamine and D-glucuronic acid. It is one of the principal components of the mammal´s extracellular matrix and is involved in numerous functions like cellular division, immunological response, and tissue repair, just to mention a few. As well, this molecule is synthesized by some bacteria, viruses, and the pathogenic yeast *Cryptococcus neoformans,* the only reported fungi that synthesizes HA so far. In those organisms, the enzyme that synthesizes HA is the Hyaluronic Acid Synthase (HAS). We discovered sequences in several filamentous fungi homologous to *C. neoformans* HAS (Cn-HAS), among them *Mucor circinelloides* (Mc-HAS; 66% identity) and *Coprinopsis cinerea* (Cc-HAS; 82% identity). Even though the biological role of HASs in filamentous fungi is completely unknown, we hypothesize they could be involved in the pathogenic role of *M. circinelloides,* as has been demonstrated for *C. neoformans* HAS and, in the case of *C. cinerea, it* could influence the morphogenesis and development of the fungus. Therefore, we aim to demonstrate that these putative HAS are able to produce HA.

To elucidate their catalytic activity, Mc-HAS and Cc-HAS were heterologously expressed in *Saccharomyces cerevisiae*. By Western blot, we found that both putative HASs are correctly expressed in the heterologous system and located in the cell membrane, with high levels of expression. With respect to Mc-HAS, additionally to the band of the expected weight, a band of the double of molecular weight was observed, therefore it could be possible that it forms dimers. In the next step, we will confirm the activity of the enzymes and if they are able to synthesize HA.

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Pectinase enzymes produced from yeast using citrus waste

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Abstract

Since 1930, pectinases have experienced significant growth in the food industry and represent 20% of the global enzyme market. However, the generation of pectinases often involves the use of non-GRAS microorganisms, making them costly to handle. In response to this challenge, the use of selective and active yeasts in substrates derived from waste for pectinase production is proposed. This study explores the potential of citrus waste as a substrate for the sustainable and economically viable production of pectinases using yeast.

The experimental approach was divided into two stages. In the first part, the pectinolytic activity of *Saccharomyces*, *Kluyveromyces*, *Pichia* strains isolated as epiphytic mycoflora from Italian lemons and agave musts, belonging to the yeast collection of the Laboratorio de Biotecnología Industrial (CBG-IPN), was evaluated using commercial pectin as the substrate. The goal was to select strains with the highest activity. In the second stage, the activity of the selected strains from the previous stage was evaluated using a citrus waste- based medium, and the 3,5-dinitrosalicylic acid (DNS) methodology was employed to detect reducing sugars and quantify exo-pectinolytic activity. The results indicate that some strains can produce pectinases in both substrates. Other studies have also demonstrated the feasibility of using agro-industrial waste such as grape skins and beet pulp for pectinase production. Among the evaluated strains, *Saccharomyces cerevisiae* LCBG-3Y4 exhibited the highest specific enzymatic activity (35.21 U mg^{-1} protein). This study highlights the possibility of producing pectinases sustainably and economically using citrus waste and yeast.

Remotion of Cr (VI) with biomass of water Kefir and SCOBY of Kombucha

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In recent times, the pollution caused by hexavalent chromium has been increasing in soil and water bodies, due to its excessive use in various industries. Specifically, in the state of Guanajuato, this rise in water pollution by chromium can be attributed to mining and the leather industry. Given these circumstances, there is a growing need to explore alternatives for treating effluents containing chromium. Chromium is one of the most toxic heavy metals with a significant negative environmental impact. Hexavalent chromium (Cr VI) is the predominant form of this pollutant, in contrast to its less oxidant, less toxic, and less water-soluble trivalent form (Cr III). Therefore, the development of new technologies, particularly through the implementation of bioremediation, is crucial for effectively treating water contaminated with Cr (VI). Using biomass and/or exopolymer substances derived from microbial consortia, such as the exopolysaccharides found in kombucha and water kefir, could serve as alternatives to complement or even replace conventional chemical methods in removing this metal from aqueous phases. This study focuses on the use of biomass from two consortia, SCOBY (Symbiotic Culture of Bacteria and Yeast) of kombucha and dried grains of water kefir. The adsorption capacity of these biomaterials is assessed in a low-volume and cost-effective system (10 mL), maintaining constant pH, temperature, and rpm under a discontinuous regime of operation for various initial concentrations of Cr (VI), and bioadsorbent masses are tested. The Cr (VI) is determined using the diphenylcarbazide technique, which acts as an indicator of Cr (VI). Pseudo first and second order models are employed to determine the type of adsorption kinetics, while the Langmuir and Freundlich models are used to identify the type of equilibrium isotherm for the adsorption bioprocess. The objective of this research is to evaluate the removal capacity of Cr (VI) from synthetic aqueous solutions using the adsorption of ions on the exopolysaccharides of kombucha and water kefir, aiming to identify the phenomenon. The results of the adsorption experiments demonstrate a removal capacity of 98%. The dry biomass of kombucha exhibits a remarkable ability to remove Cr (VI) in liquid systems, achieving adsorption rates of 98%. Consequently, it holds promise as an alternative method for effectively eliminating this contaminant.

Lacasse from *Trametes sanguineus*: A biocatalyst for modifying quercetin

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White-rot fungi have attracted significant attention due to their ligninolytic complex, which includes oxidative enzymes such as peroxidases, tyrosinase, and laccases. *Trametes* (*Pycnoporus*) sp. is a white-rot fungus classified as a food-grade microorganism that can produce pigments and enzymes, including laccases. Laccases (EC 1.10.3.2) are multicopper oxidases. These enzymes, which are glycoproteins with molecular masses of 50-103 kDa, utilize oxygen to oxidize phenols, polyphenols, aromatic amines, and other non-phenolic compounds. On the other hand, quercetin is one of the most extensively studied flavanols (3, 3′, 4′, 5, 7-pentahydroxyflavone) can be found in natural sources such as red cherries, apples, grapes, blueberries, blackberries, citrus fruits, broccoli, tea, cocoa, onions, and green leafy vegetables. It exhibits several beneficial effects on human health, including senolytic and angioprotective properties, antioxidant activity, inhibition of allergic substances, and potential anti-cancer activity. However, quercetin faces challenges in being considered an effective drug due to its limited absorption, poor aqueous solubility, instability in physiological media, poor permeability, and low bioavailability. Enzymatic modifications have been employed to enhance the stability and solubility of flavonoids.
In this study, a crude enzymatic extract of laccase from *Trametes sanguineus* (TS lac) culture and purified laccase from *Trametes versicolor* (TV lac) (Sigma-Aldrich 38429) were used to modify quercetin. An irregular fractional screening experimental design was implemented, considering the following factors: phosphate buffer (pH 6.5, 100 mM), acetate buffer and 3% ethanol (pH 5.5, 100 mM), 0.4 U/mL, and 0.8 U/mL of laccase, and temperatures of 30 and 35°C. These factors were studied to investigate the effect of the enzyme on the structure of quercetin. The final concentration of quercetin was 10 mM, with a reaction time of 24 hours and agitation at 200 rpm. The modifications in the quercetin structure were detected using UV-Vis spectroscopy (200-400 nm). Additionally, FTIR-AR spectra were obtained for the samples, which exhibited noticeable differences in UV-Vis spectra between quercetin and modified quercetin (9 samples). After analyzing the FTIR spectra, 5 samples were selected for further investigation using ^{1}H and ^{13}C NMR spectra recorded in DMSO-D_6. These samples were as follows: ***1***. TS lac, 0.4 U/mL, acetate buffer, 35°C; ***2***. TS lac, 0.8 U/mL, phosphate buffer, 35°C; ***3***. TS lac, 0.4 U/mL, acetate buffer, 30°C; ***4***. TV lac, 0.8 U/mL, phosphate buffer, 35°C; and ***5***. TV lac, 0.8 U/mL, acetate buffer, 35°C. The most significant changes were observed in sample number two (TS lac, 0.8 U/mL, phosphate buffer, 35°C), which exhibited a greater decrease and disappearance of the OH signals. This indicates partial oxidation of quercetin into its phenolate form, a step preceding complete oxidation. This may represent a possible oxidation mechanism of quercetin via ionic and non-radical pathways. Under these conditions, the crude extract from TS lac partially oxidized quercetin, which will be further studied for its effect on cellular cancer lines.
This research contributes to the advancement of biocatalytic approaches for modifying natural compounds with potential therapeutic applications using an enzyme produced by a robust fungus.

Keywords: Enzymatic modification, white-rot fungi, flavonols

Expression analysis of the polyketide synthase gene *adaA* in a chromium reducer environmental strain and two collection strains of *Aspergillus*.

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INTRODUCTION. The widespread use of chromium in diverse industrial processes has converted it into a serious environmental contaminant. Trivalent [Cr (III)] and hexavalent [Cr (VI)] chromium are the most stable and abundant chromium forms. The Cr (VI) in chromate or dichromate is highly toxic to different life forms. While, Cr (III) is less harmful. Some metabolites and enzymes produced by microorganisms participate in the reduction of Cr (VI) to Cr (III) directly or indirectly. In previous studies in the working group was observed that the environmental strain Ed8 of Aspergillus tubingensis produces the metabolite TAN1612 in the presence of citrate. Additionally, it was observed that the Cr (VI) reduction capacity correlates with the production of this metabolite. The *adaA* gene codes for a polyketide synthetase, which is the enzyme that initiates the synthesis of this metabolite since it uses Acetyl-CoA and Manonyl-CoA for its cyclization.
OBJECTIVE. Analyze the expression of the *adaA* gene in the environmental strain Ed8 of *A. tubingensis* and compare it with the expression of this gene in the collection strains NRRL593 of *A. tubingensis* and FGSCA593 of *A. niger* at concentrations of 0 mM, 10 mM, and 25 mM of citrate and 0, 12, and 24 h of growth.
METHODOLOGY. Cultures with 48 h of growth were transferred to fresh medium, and biomass samples were taken at 0, 12, and 24 h. RNA extraction was performed with trizol, and cDNA was synthesized. The oligos were designed with sequences of the specific *adaA* gene of each strain and the histone gene was used as internal control for the determination of expression using semiquantitative and quantitative RT-PCR. To detect the metabolite and its Cr (VI) reducing capacity, the supernatant of the 24 h medium was analyzed by LC-MS.
RESULTS. In preliminary results using semiquantitative RT-PCR, the *adaA* gene expression in the Ed8 strain increases as time and citrate concentration increases. While in the collection strains, the expression of *adaA i*s low and no effect of citrate is observed. The determination of the expression of the *adaA* using real-time PCR is being carried out. For this, the efficiency of the primers is being determined, and the relative expression will be analyzed using the $2^{-\Delta\Delta Ct}$ method. In the collection strains, the analytical signal of the metabolite was not detected, and a low capacity to reduce Cr (VI) under the same conditions was determined.
CONCLUSION. The Ed8 strain at a citrate concentration of 25 mM and a time of 24 h has higher expression of the *adaA* gene compared to the collection strains, which indicates a direct correlation between the presence of citrate, the analytical signal of the metabolite, the capacity reduction of Cr (VI) and the expression of the *adaA* gene.

A new terpene from *Trichoderma virens* activates JA plant defense pathway and controls growth of *Sclerotium cepivorum*

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Fungi of the genus *Trichoderma* are widely recognized as biocontrol agents in agriculture, as well as beneficial plant symbionts, improving growth and plant defense through the jasmonate and salicylic acid signaling pathways. In particular, *Trichoderma virens* produces a large number of volatile and non-volatile chemical compounds. Among the main metabolites produced by *T. virens* are terpenes, which are reported to be excellent bioactive compounds. In our work group, the *T. virens* strain OETvCyt2 was generated and characterized by the production of five terpene-like compounds. The modified metabolic profile correlates with a greater antagonistic capacity of the OETvCyt2 strain against the phytopathogen *Sclerotium cepivorum* and the upregulation of defense genes in *Arabidopsis thaliana* seedlings. Based on these results, we purified a new unreported terpene, from culture supernatants of the OETvCyt2 strain. It was determined by RT- qPCR that this terpene (15μM) positively regulates jasmonate signaling pathway in *Solanum lycopersicum*, by inducing *PIN2* gene expression. The same terpene dose also delays the growth of *S. cepivorum* by 74%, and at 50μM it inhibits sclerotia germination *in vitro,* until 10 days of incubation. Finally, we tested that fungicide Folicur (150ng/mL) noticeably affects germination. Similar results were obtained using a combination of the terpene (5μM) and Folicur (8ng/mL) after 7 days of incubation. Our results show the possible biotechnological application of the purified terpene in crops, either to improve plant defense or to directly treat some of its pests, representing alternatives to the use of conventional pesticides.

The action of chitosan on the development of soil isolates from the desert of Baja California Norte, Mexico.

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Because chitosan's positive charges of the NH3+ group can interact with the negative charges of cell membranes to inhibit the growth of other microbes, it has been suggested as a biological control agent. In Baja California Norte's, in the desert of the town of San Felipe soil samples were taken from the zone of petrified shells. All samples were maintained in sterile bags until the seeding. The isolates of microorganisms from microbiotas of distinct area of soils were seeded on PDA plates. Because there is no data of the antifungal activity of chitosan on extremophile fungi, the goal of this study is to explore the effect of chitosan on fungus that inhabit the desert of northern Baja California. By directly seeding soil samples on medium potato-dextrose agar (APD) plates, 13 isolates of fungi were obtained and tested with chitosan at doses of 1, 3, and 5 mg/mL. The growth diameter was assessed after 5 and 7 days of incubation at 28 °C. Different halos of inhibitions were seen in various isolates, but one intriguing finding was that some of the isolates displayed chitosanolytic activity, indicating a significant potential for biotech applications for these new isolates.

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Utilization of Ureasa from *Ustilago maydis* of three strains in biocement production

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Ustilago maydis is a phytopathogenic fungus, which belongs to the Basidiomycetes and infects maize plants (*Zea mays*). It is a fungus that goes through two phases, one saprophytic in the form of yeast and another micellar that infects and parasitizes the plant. The saprophytic form is used in research as a model of a phytopathogenic fungus. In recent years, progress has been made in aspects of molecular biology and the metabolic pathways of certain metabolites of the fungus. The biotechnological application of this fungus includes the production of secondary metabolites, enzymes, biosurfactants, and polysaccharides, among others, using the yeast form. *Ustilago maydis* is a urease-positive fungus and has been an identification characteristic for *Ustilago*, urease hydrolyzes urea into ammonium and carbonate, and the pH change that is generated during the reaction causes calcium to precipitate, generating hardness in foundation mixes.

In this work, we use three *Ustilago maydis* yeast strains: FB1, FB2, and AB33. The latter corresponds to a mutant built in the laboratory. The production of urease induced by the presence of ammonium was analyzed in solid medium plates.

Urease activity in a liquid medium was determined by conductometry. The best-producing strain was the AB33 mutant, which was used for the formation of biocement in a mixture of calcium chloride, urea, and sand combined with a culture of the mutant with glucose. Both FB1 and FB2 were analyzed in the same way and the biocement was treated for 12 days. As a preliminary result, it was obtained that both FB2 and AB33 produced rigid biocement.

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Characterization of *Periconia macrospinosa* HAgJ2 isolated from *Agave tequilana* as a biocontrol agent

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Summary:

Periconia macrospinosa is a species of fungus belonging to the dark septate endophytes (DSE). This species has limited information regarding the colonization of roots of nonmycorrhizal plants and its anti-phytopathogenic characteristics. The strain HAgJ2 was isolated from the root tissue of *Agave tequilana*, a crop located in Atotonilco El Alto, Jalisco. The fungus was identified by microscopical observations and the sequencing and analysis of the ITS 2 region. To date, there is no record of the presence of this genus or species in the *A. tequilana* microbiome or in the geographical location. The aim of this study was to identify the potential of *P. macrospinosa* HAgJ7 as a biocontrol agent, considering its possible impact in reducing the use of chemical fertilizers and pesticides, which alter soil composition and contaminate water.

We determined the antifungal and antioomycete activity of *P. macrospinosa* HAgJ2 in different culture media. The antimicrobial properties depended on the culture media and the inoculation method of HAgJ2 and the pathogens. *P. macrospinosa* HAgJ2 completely inhibited or affected the growth of fungi and oomycetes such as *Phytophthora capsici*, *Sclerotium rolfsii*, *Fusarium proliferatum*, *Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Colletotrichum gloesporioides* on PDA plates; these microorganisms are economically important phytopathogens. We have also identified the specific time of growing of *P. macrospinosa* HAgJ2 to produce enough antimicrobial metabolites on PDA plates to show antagonistic activity.

Evaluation of the antifungal activity of silver nitrate against the fungus that causes in Agave salmiana black mold.

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Abstract

The Agave salmiana maguey is a plant from which all its parts can be used to obtain products of commercial value, such as: sap or mead, inflorescence or quiote, inulin, barbecue leaves or fibers, etc., however, it is found threatened by pests and diseases that reduce production. Among the most aggressive is the one known as smallpox or bold, caused by the fungus Bipolaris zeae. Which attacks and dries the leaves; In addition, it has been reported that it is also capable of infecting cereals such as corn, barley, among other crops.

Silver-based antimicrobials may be effective in treating infections due to Ag+ ion toxicity. In this research project we determined the minimum inhibitory concentration of silver nitrate ($AgNO_3$) against the phytopathogen B. zeae. It was observed that the conidia treated with a solution of $AgNO_3$ greater than 100 ppm inhibit their growth by ~40% in PDA medium. In addition, there was a reduction of 86 and 95% in the percentage of germination of conidia, at concentrations of 100 ppm and 200 ppm respectively.

Subsequently, we decided to test the antifungal activity in planta, we analyzed its effectiveness in corn and agave plants. To do this, we added $AgNO_3$ to young maize plants at the already established concentrations, before (preventive treatment) and after (corrective) inoculation with conidia of the fungus. The data obtained suggest that the corrective treatment at 200 ppm works better, since there was no statistically significant difference between the control and the treatments in the preventive treatment. In Agave salmiana plants, infected and treated with a concentration of 200 ppm, a 36% decrease in the radial growth of black spots was observed. These results show that $AgNO_3$ at concentrations of 200 ppm inhibit the growth of this phytopathogenic fungus.

Key words: Silver nitrate, Agave salmiana, bold, Zea mays, Bipolaris zeae.

A comparison study of pellet capacity formation between *Rhizopus stolonifer* and *Pleurotus ostreatus* and the effect of Methotrexate on spore germination.

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Fungi have been used in processes due to their ability to produce enzymes, secondary metabolites, polysaccharides, etc. Some enzymes can be used to degrade compounds as medicines. Methotrexate (MTX) is an inhibitor of Dihydrofolate reductase commonly used in cancer treatments and other diseases. It has demonstrated that long exposure can cause cytotoxic, genotoxicity, mutagenesis, and teratogénesis. Studies have reported medicines degradation using strains of Zygomicetos and Basidiomycetes, fungi belonging to these divisions can form fungal pellets which are dense networks of hyphae or spores that are agglomerate to the formation of a spherical body. These pellets are produced under certain conditions (pH, agitation, inoculum size temperature, etc.) and provide advantages over traditional cultivation.

The objective of this work was to determine the best conditions for pellets production by *Rhizopus stolonifer* and *Pleurotus ostreatus* and the effect of MTX on the spore germination of both strains. A design 3x3 simplified factorial with 3 levels and 3 replicas following the model of Taguchi, in which the variables were: inoculum size, source concentration of carbon, and stirring speed, where was found that the most significant variable is agitation. In addition, a concentration of 100 ppm Methotrexate inhibits 50% of *R. stolonifer* spore germination. Preliminary tests were also performed on the effect of MTX on the growth of strains in a solid medium where MTX did not make structural changes in any of the strains. In addition, a DL 50 was carried out which indicated that a concentration of 100 ppm of methotrexate inhibits 50% of the germination of *R. stolonifer* spores.

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Degradation of chlorpyrifos and lambda-cyhalothrin by rhizospheric fungi from *Typha domingensis* plant in the Turbio River

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The Turbio River, situated in the state of Guanajuato, is one of the most polluted water bodies due to industrial discharges in Mexico. Agricultural activities contribute diffusely to pollution through pesticide runoff and improper handling of their residues. Nevertheless, there are natural wetlands alongside the Turbio River that have the potential to eliminate numerous pollutants due to presence of terrestrial and aquatic vascular plants, microorganisms, and substrate support. Consequently, this study is centered around identifying fungi within the roots of *Typha domingensis* from the Turbio River with the capability to degrade chlorpyrifos and lambda-cyhalothrin. These could serve as alternatives for treating diverse environmental contexts. In this research, eleven filamentous fungi were isolated from both the rhizosphere and the adjacent water of *T. domingensis*. Fungal genotyping was performed by extracting genomic DNA and amplifying and sequencing the ITS 1-4 region. Macroscopic and microscopic characterization, along with sequencing results, confirm the existence of the genera *Penicillium*, *Aspergillus, Talaromyces*, *Diaporthe, Graphium, Trematosphaeria*, and *Pyrenochaetopsis*. *Penicillium* sp. Rz-1a and *Penicillium* sp. Rz-5b, *Talaromyces* sp. Rz-1c, and *Diaporthe* sp. Rz-1b exhibited the highest growth rates in both pesticides-rich and pesticides-free mediums. In the degradation experiments (employing 25 and 50 ppm of chlorpyrifos and lambda-cyhalothrin), the isolates strains demonstrated significant degradation percentages for chlorpyrifos within 14 days. Specifically, *Penicillium* sp. Rz-5b, *Penicillium* sp. Rz-1a, *Talaromyces* sp. Rz-1c and *Diaporthe* sp. Rz-1b achieved rates of 82.55%, 80.82%, 79.30%, and 76.07%, respectively. Similarly, degradation of lambda-cyhalothrin was highest, with *Penicillium* sp. Rz-5b, *Talaromyces* sp. Rz-1c, *Penicillium* sp. Rz-1a, and *Diaporthe* sp. Rz-1b showcasing degradation percentages of 87.30%, 83.84%, 83.67%, and 77.83%, respectively. These findings underscore the rhizospheric fungi´s capacity to degrade pesticides at *T. domingensis,* even within severely polluted environments such as the Turbio River. These isolated strains have the potential to be pivotal components in remediation initiatives, as they can be employed as inoculants in artificial wetland systems. Moreover, the study of their metabolic pathways involved in pesticide degradation holds promise for future biotechnological applications, potentially in the development of bioreactors.

Isolation and selection of thermotolerant filamentous fungi producing amylases obtained from the Tolantongo caves.

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Introduction: The isolation of thermotolerant microorganisms present in natural environments has generated great interest in recent years, due to the extracellular enzymes or secondary metabolites that they can produce, among which are useful enzymes in the degradation of different substrates, as they are the amylases. These enzymes break down starch to dextrin, maltose, or free glucose and are used commercially in many industrial processes, such as food production, textile manufacturing, and detergents.
In the present work, thermotolerant filamentous fungi were isolated from sediment and water samples from the river, tunnel and caves of Tolantongo. The amylolytic, proteolytic, chitinolytic, and chitosanolytic activities in the isolated fungi were evaluated at a semiquantitative level, by measuring hydrolysis halos or mycelium growth on agar plates with the respective substrate.

Methodology: The study was carried out with 35 filamentous fungi isolated from the Tolantogo caves located in the Cardonal municipality, Hidalgo (Mexico), at approximately 1422 m of altitude between the geographical coordinates 20°38’38.3’’N y 98°59’34’’ W.
The water and sediment samples were collected from three zones: Tunnel (I), Thermal caves (II) and the thermal river (III), were placed in sterile tubes, transported in the thermal containers, and stored under refrigeration conditions at 4°C until analysis. Three techniques for the isolation of fungi wee carried out: successive dilution, soil washing, and direct inoculation on plates, the medium employed was PDA added with chloramphenicol and the samples were incubated at 35°C for 7 days. The enzymatic activities (amylolytic, proteolytic, chitinolytic and chitosanolytic) of isolated fungi were evaluated at a semiquantitative level by measuring hydrolysis halos or growth on agar plates with the respective substrate. The fungi that presented the highest enzymatic index of amylolytic activity were selected to measure the production of amylases in solid and liquid fermentation states using the methods modified described by Suresh et al. 2010 and Olagoke, 2014, respectively.

Results: All the isolated fungi exhibited single or combined hydrolytic activities. Five isolates (14%) produce only one of the five tested hydrolases, isolate H12 and isolate H29 (protease), isolate H5 and isolate H16 (amylase) and isolate H33 (halophilic protease). While 40% (14 isolates) showed two activities, being mostly the activities (amylolytic and proteolytic). On the other hand, 11 isolates (32%) showed three activities and 5 isolates (14%) showed 4 activities. It was observed that the fungi isolated from the caves presented a broad enzymatic potential, because in the preliminary screening plate all fungi showed 3 or 4 activities.
The 88% of the isolates presented amylolytic activity (31 isolates), while the other 4 (12%) did not present this activity. Of the 31 amylolytic fungi, 21 belong to the samples from the river (68%), 9 were isolated from caves (29%) and 1 corresponds to the tunnel (3%).

Characterization of *Metarhizium* response to different abiotic factors

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Abiotic factors such as high temperature and UV-A rays induce the formation of reactive oxygen species, which causes cell damage. These factors affect the viability of entomopathogenic fungi, decreasing their efficiency in the biological control of pest insects.

Salicylic acid and Riboflavin have been reported to enhance resistance to these abiotic factors (1, 2). In this work, two strains of *Metarhizium* (*M. robertsii* ES-10 and *M. robertsii* ES-14) isolated from the state of Guanajuato were tested. Conidia from the two strains were obtained on rice supplemented with riboflavin or salicylic acid under light/dark or constant-dark conditions. The survival of conidia subjected to thermal stress at 37° and 40°C and UV-A rays, applying an energy of 9000 $\mu J/m^2$, was evaluated. The results showed that the *M. robertsii* ES-10 conidia are more resistant to thermal stress at 40°C, and the conidia obtained in dark conditions of the *M. robertsii* ES-14 strain are more resistant to UVA light.

The interaction of *M. robertsii* strains with broccoli (*Brassica oleracea* var italica) seedlings and seeds was also tested. Higher water uptake was observed in seedlings in interaction with the conidia of *M. robertsii* ES-10, regardless of the conditions in which the conidia were produced. This effect was also observed with the dark-produced conidia of the *M. robertsii* ES-14 strain. In the interaction experiments carried out in soil for agricultural use, the seedlings treated with *M. robertsii* ES-10 conidia, regardless of the conditions in which they were produced, showed an increase in the length of the stem and leaf-primordia and an increase in the dry weight of the plant.

In conclusion, although some conidiation conditions favor tolerance to high temperatures and ultraviolet light, the genetic background of each strain impacts the responses to the different abiotic conditions and the interaction between *Metarhizium* and broccoli.

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Exploring *Neurospora crassa* PIR proteins as molecular anchors for cell-surface protein display

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Protein display on microorganism's cell surface may lead to innovative biotechnological applications such as vaccines and antibodies, bio-absorbents, or even whole-cell biocatalysts. At the molecular level, a cell-surface protein display system consists in the use of a cell wall resident protein (anchor) to immobilize a functional protein (passenger) that is co-translationally fused to the former. The passenger can be fused either to the N- or C-terminus or within the anchor. When an N-terminal fusion is performed, a signal peptide (SP) is necessary at the N-terminus of the passenger so that the display fusion is efficiently secreted and translocated into the wall. PIR proteins, a family of proteins attached to the wall through an alkali-sensitive ester bond between a specific glutamic acid within the PIR motif and β-1,3-glucans, have been successfully utilized as anchor for protein display in yeasts. However, in filamentous fungi this approach has been poorly applied. Moreover, PIR proteins in filamentous fungi have not been characterized yet. Thus, the aim of this work was to characterize *Neurospora crassa* PIR proteins as anchors to determine the best display configuration using eGFP as passenger. Two PIR proteins are inferred in *N. crassa* genome, PIR-1(NCU04033) and PIR-2 (NCU07569). Because PIR-1 bear an N-terminal Kex2 pro-peptide whose function is unclear, two different fusions were constructed, 1) eGFP inserted just after SP, between amino acid 22 and 23; and 2) eGFP inserted after Kex2 processing site, between amino acid 243-244 (GFP-PIR-1 and KexGFP-PIR-1). As expected, GFP-PIR-1 did not fluoresce, demonstrating the processing of the Kex site. In contrast, KexGFP-PIR-1 fusion accumulated as patches close to the apical dome surrounding the *Spitzenkörper*, and around nuclei in distal hyphae regions. Moreover, because PIR-1 was predicted to be a GPI anchored protein, two additional fusions of GFP at the PIR-1 C-terminus were built: 1) PIR-1-GFP, and 2) a truncated version of PIR-1 (PIR-1ΔGPI-GFP) where GFP was attached 2 amino acids before the ω-site. Unlike PIR-1-GFP, PIR-1ΔGPI-GFP fluorescence was not abundant in the cytosolic, instead, it revealed a fluorescent signal at the apex. These observations suggest that PIR-1 is a *bona fide* GPI protein, whose N-terminus is important for the correct maturation of the protein. PIR-2 was only labeled to the C-terminus. Fluorescence accumulation in the apical dome of GFP-labeled PIR-2 was higher than the other strains. Taking these results together, we can conclude that PIR-1 N-terminus is not suitable for protein display; nevertheless, a fusion in the C-terminus of ω-site truncated PIR-1 could be a potential anchoring fusion for cell surface protein display in *N. crassa,* but PIR-2 is apparently the best protein anchor.This work was supported by SENER-CONACYT Sustentabilidad energética, grant 245750; FODECIJAL-COECYTJAL grant 8186-2019, and DyD-COECYTJAL, grant 9888-2022.

Multiple display of peptides with affinity to Al and Zn on the cell surface of *Neurospora crassa* using the hydrophobin class 1, EAS, as an anchor

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The intrinsic characteristics of fungi have made them a subject for research and development of state-of-the-art technologies. In the biotechnology sector, they have become interesting hosts for the display of proteins. This technique allows biotechnologically interesting proteins and enzymes to be immobilized on fungi cell surface. This is achieved through the co-translational fusion of a resident cell wall protein that functions as an "anchor" and a passenger protein/enzyme that provides new qualities to the host.

Genomic mining of filamentous fungi has revealed a wealth of cell wall proteins that could be used as anchors for protein display. Among these proteins are hydrophobins, which are exclusively produced by filamentous fungi and have biophysically and biotechnologically interesting properties. These proteins are secreted as monomers by hyphae in search of a hydrophilic::hydrophobic interface, where they undergo polymerization to allow the growth of aerial hyphae through a highly ordered rodlet-like layer, which resembles amyloid fibrils.

The ordered arrangement of hydrophobins is very attractive for their application in protein display technologies since, hypothetically, they would allow the display of several proteins at the same time in an ordered manner. Achieving this goal, although challenging, would be a milestone in the advancement of protein display systems on the cell surface of filamentous fungi. Based on previous investigations that identified specific regions of the *Neurospora crassa* hydrophobin EAS (NCU08457) that can be modified without affecting its fold, in this work we aim to fuse Al- and Zn-binding peptides to EAS loop 1 and loop 2 to demonstrate hydrophobins´ functional capacity for multiple protein display. Once we obtain the mutant strain, we will test its ability to adsorb metal ions of aluminum and zinc on the hyphal cell surface. The results obtained will be presented at the conference.

Identification and characterization of a filamentous fungus isolated from plastic bags in an estuary in Mazatlán, Sinaloa.

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Plastics are durable synthetic material created by humans that are widely used in various industries such as construction, housing, food, textile, and medicine, among others. Their excessive use and difficulty in degradation have led to a significant environmental problem due to the accumulation of this material in ecosystems, resulting in the generation of microplastics and nanoplastics that can enter the food chain, causing adverse effects that are still poorly understood in plants, animals, and humans. It is projected that by 2050, 34 billion tons of plastic will be produced annually. Currently, the world produces 300 million tons of plastic waste per year, with Mexico contributing around 3.8 million tons. Despite the challenges in plastic degradation, microorganisms and/or their enzymes that have the capability to degrade these types of polymers have been employed. One of the most studied organisms is the bacterium *Ideonella sakainesis*, which utilizes a system of two enzymes, PHETase and MHETase, to depolymerize PET. Other bacteria such as *Pseudomonas aeruginosa*, *Thermobifida fusca*, and fungi like *Fusarium oxysporum*, *Trichoderma spp.*, and *Fusarium culmorum* have also been studied and characterized. Recently, in our laboratory, a fungus was isolated from an estuary in Mazatlán, Sinaloa, that has the ability to grow on plastic bags. Furthermore, its growth capability was confirmed in a minimal Vogel's medium using high-density PET as the sole carbon source. PET degradation analysis, phylogenetic inference, and other assays are currently in progress.

Evaluation of the Micellar Invasion Efficiency of *Pleurotus ostreatus* on Lignocellulosic Residues in Agar Matrix

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Area: Biotecnología

The production of edible mushrooms is an activity with a high social, economic and environmental impact; recognized as an important agroindustry because mushrooms have the adaptability to grow on a wide variety of agro-industrial waste. *Pleurotus ostreatus* is the second most produced edible mushroom species worldwide due to its content of essential amino acids, such as arginine, glutamine and glutamic acid, as well as vitamins, minerals and high content of proteins, fibers and carbohydrates. Mexico is currently positioned as the leading producer in Latin America with 80% of total production. Considered an alternative to meat consumption due to the number of nutrients that provides, its organoleptic characteristics and its importance as food for human consumption, it is necessary to generate knowledge that helps to standardize the production process of these mushrooms since, quantitatively, there are few studies that guarantee optimal conditions for their development. The objective of the proposal is to evaluate the efficiency with which the *Pleurotus ostreatus* mycelium invades agro- industrial substrates available in the state of Aguascalientes, the test was carried out individually and in combination using a 0.5% agar matrix, they were exposed to temperature, constant humidity (80% relative humidity and 23°C respectively), by measuring the growth radius the necessary information was obtained to determine the growth rate in the vegetative phase. As a result, it was observed that there are significant differences in the growth rate in relation to the substrates used. In conclusion, the proposed culture methodology for *Pleurotus ostreatus* and the quantification of the growth rate are parameters that quantitatively determine the best substrate to use in the production of edible mushrooms.

Keywords: Growth rate, agroindustry, edible fungi, agroindustrial waste, substrates.

Phenanthrene and Anthraquinone biodegradation by *Aspergillus spp.* isolated from contaminated soil in Reynosa, Mexico.

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Polycyclic aromatic hydrocarbons (PAHs) are non-polar, uncharged compounds consisting of benzene rings arranged in angular or linear arrangements. Natural combustion processes and anthropogenic activities cause PAHs to be released as recalcitrant pollutants into the environment, they have also been reported as mutagenicity and carcinogenicity in microorganisms, plants, and animals. These facts have highlighted the need of exploring bioremediation techniques, specifically, the use of fungi as potential bioremediation agents (Mycoremediation) have stood out because of their intra and extracellular enzymatic activities. In this study, fungal strains were isolated from contaminated soil samples collected from "La Escondida" lagoon in Reynosa, Mexico to evaluate their ability to degrade phenanthrene (PHE) and anthraquinone (AQ). Tolerance tests and growth in medium with PHE and AQ as sole carbon source was conducted, as well as qualitative detection of the three main extracellular enzymes involved in lignin degradation: Laccase (Lac), Lignine Peroxidase (LiP), and Manganese Peroxidase (MnP). Three promising fungi isolates (H7, H35 and H36) were identified by ITS sequencing as *A. niger, A. terreus, and A. ochraceopetaliformis*. Their PHE and AQ biodegradation potential was evaluated through High-Performance Liquid Chromatography (HPLC) and the resulting metabolites were identified with Gas Chromatography-Mass Spectrometry (GC-MS). *Aspergillus niger* isolate was positive for LiP and MnP after 21 days of culture, and degraded PHE (11.6%) and AQ (22.8%) from an initial concentration of 250 mg/mL.

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ANÁLISIS DEL POTENCIAL DE DEGRADACIÓN DE NAPROXENO Y AMPICILINA DE CEPAS DE HONGOS AISLADAS EN REYNOSA, MÉXICO.

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Pharmaceuticals are chemical substances of natural or synthetic origin that interact with biological systems to induce changes in physiological or biochemical functions. They are widely utilized for the treatment of various diseases. Nowadays, there are over 3,000 pharmaceuticals available in the market, with 20% employed for human use and 80% for animal use. However, pharmaceuticals also have environmental risks as they can be detected as residues in aquatic environments, such as groundwater, surface water, and soil. Currently, there is a lack of rigorous regulations governing the management and disposal of pharmaceuticals used in household settings. Moreover, their complex elimination is influenced by various factors, including molecular structure, physicochemical properties, biotic and abiotic factors, contributing to environmental contamination.

Conversely, bioremediation, utilizing microorganisms, offers a potential solution for the elimination of various xenobiotics, including pharmaceuticals. Filamentous fungi have demonstrated the capacity to degrade a wide range of pharmaceuticals through diverse metabolic pathways, utilizing them as alternative energy sources.

Therefore, the objective of this study was to identify and evaluate the potential of filamentous fungi in the degradation of commonly used pharmaceuticals, such as naproxen and ampicillin. Initially, the isolated fungi were identified using ITS sequencing markers, followed by assessing their tolerance to different concentrations and exposure times of naproxen and ampicillin. Additionally, the degradation capacity was determined, and the degradation metabolites were identified using Ultra-High-Performance Liquid Chromatography with Mass Detector. Fungi isolated from soils in the city of Reynosa, Tamaulipas, were identified as belonging to the genera Aspergillus, Penicillium, and Trichoderma. Furthermore, the results revealed that these identified fungi exhibited the ability to tolerate high concentrations (100 mg/mL) of naproxen and ampicillin. Significantly, the fungi also demonstrated their capability to degrade both pharmaceuticals at concentrations of 50 mg/L, thereby identifying the respective degradation metabolites. These findings highlight the potential of fungi as promising organisms for the remediation of pharmaceutical-contaminated sites.

Degradation analyses were performed at a concentration of 50 mg/L for both naproxen and ampicillin, wherein the fungi exhibited the capacity to degrade these pharmaceuticals. The identification of metabolites and quantification of drug degradation were carried out using UPLC-MS techniques.

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Diversity of secondary metabolites produced by *Beauveria bassiana* grown on different nutrients.

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Beauveria bassiana is an entomopathogenic fungus widely used in the control of various insect pest, and part of its entomopathogenic activity is derived from the production of secondary metabolites. It is well known that entomopathogenic fungi produce a wide range of secondary metabolites that can exhibit antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer activities, among others, and these can be used in many different industries. Fungal growth and secondary metabolite production can be influenced by various abiotic factors such as temperature, pH, or nutrients in the environment where they develop. The aim of this work was to evaluate the growth and differences in the metabolic profiles of the *Beauveria bassiana* GHA strain, subjected to different culture media. For this purpose, the GHA strain was cultured in PDA, SDA, SNA, YEPDA, MEA, PDH, OATS, RICE, and CHEERIOS media, at 28 °C for 21 days. Fungal growth was measured by milligrams of biomass produced, and differences in metabolic profiles were evaluated through a GC-MS analysis. Results indicated significant differences in biomass production among media. The highest fungal growth was observed in SDA and OATS media; otherwise, the fungus did not grow in PDA, MEA, and SNA. The GC-MS analysis detected 156 metabolites, including 54 methanol-extracted metabolites (34,62%), 65 ethyl acetate-extracted metabolites (41.67%), and 37 hexane-extracted metabolites (23.72%). These metabolites can be classified into amines, alcohols, fatty acids, terpenes, carboxylic acids, hydrocarbons, and amino acids. In conclusion, metabolite and biomass production were different among media and the presence of the different nutrients available exerts a strong influence in *B. bassiana* development.

"Caracterización morfológica de levaduras nativas aisladas de bebidas alcohólicas tradicionales y su potencial para fermentar azucares"

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Alcoholic beverages and fermented foods have been consumed for more than 9,000 years or so; being used for its conservation increasing its useful life. For this reason, they must first go through a microbiological alteration, degrading carbohydrates in order to obtain anaerobic energy and different metabolites; of which one of the most used at an industrial level is ethanol. Within these traditional fermented drinks are a large number of microorganisms, mostly yeasts and lactic acid bacteria. The main objective of the work is to isolate and morphologically characterize different autochthonous yeasts from traditional fermented beverages such as: Pulque, Tascalate, Tepache, Pozol, Calpis, Tejuino, and Wine.

To do this, samples were collected from different areas of the Mexican Republic; (Teotihuacán) State of Mexico, (Mezquital Valley) Hidalgo, and (Cuauhtémoc, Miguel Hidalgo) CDMX. Some drinks such as Tejuino, Tascalate, and Pozol were left to ferment for 5 days at a temperature of 28°C without depression to proliferate microorganisms. Subsequently, the samples were seeded by cross-streaking in SDA media until growth was observed.

Thirty-two yeasts were isolated and characterized based on their microscopic and colonial morphology. The beverages with the highest number of different isolates were: 6 colonies for Pulque, 5 for tepache, and 3 colonies for Tejuino, each with different characteristics within each beverage. Yeasts that grew in already purified SDA were kept in slant tubes and 70% glycerol.

The isolates were subjected to carbohydrate fermentation tests with the Durham test to identify whether they can produce CO_2 by metabolizing it. The sugars that were tested were glucose and sucrose. The yeasts isolated from the beverages with the highest fermentative capacity were those obtained from Pulque, Tepache, Pozol, and Tejuino, revealing their potential as ethanol producers.

Enhancing PET degradation through tannase overexpression in *trichoderma atroviride*

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Abstract:

The extensive usage and slow degradation of plastics have resulted in an escalating environmental and public health challenge. Plastics gradually break down into microplastics over time, infiltrating the food chain and posing detrimental effects on human health. To address this issue, alternative strategies have been explored to promote plastic reuse, recycling, and accelerated degradation, given their ubiquitous presence in our food, water, and air. Research efforts have focused on investigating microorganisms capable of breaking down or depolymerizing plastics, with filamentous fungi, including *Fusarium*, *Penicillium*, and *Trichoderma sp*., emerging as promising candidates.

In our laboratory, we have made a significant discovery: two strains of *Trichoderma* possess an enzyme structurally similar to *Ideonella sakaiensis* MHETase, a bacterium known for its ability to bioassimilate polyethylene terephthalate (PET). Leveraging the distinct characteristics and structural similarity at the sequence level, as well as employing three-dimensional modeling and docking analyses to explore potential ligand functionality, we successfully overexpressed this gene in *Trichoderma atroviride* using a constitutive promoter-driven expression vector. This transformation has yielded ten transformants that are currently undergoing comprehensive characterization to evaluate their efficacy in PET degradation via in vitro assays.

These promising findings represent a significant advancement in the quest for effective plastic degradation solutions, offering potential applications for sustainable management of this pervasive global contaminant.

Detoxification of tequila vinasse by *T. sanguineus* for water reuse in seedling growth

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Tequila is one of the most important agroindustries in Mexico and it is in continuous growth. According to the Tequila Regulatory Council, approximately 650 million liters of tequila were produced in 2022, representing a 2.4-fold increase in production over the past 5 years. Tequila vinasse, a by-product generated during agave fermentation, is a wastewater characterized by its dark color and composition, which includes water, alcohol, sugars, phenolic compounds, proteins, among others. Its high chemical oxygen demand (COD) and pH levels classify it as hazardous to the soil, plants, and aquatic organisms, requiring the development of treatment strategies or proper disposal methods. Although agave vinasse is specific to Mexico, it shares the same challenges in terms of its management and disposal as vinasse derived from other sources. Current policies have prioritized strategies to address the adverse effects resulting from anthropogenic activities and promote waste recycling. In Mexico, the circular economy law enacted in 2021, promotes the valorization of agro-industrial wastes as a means to optimize resource utilization and minimize waste generation. In this study, different concentrations of tequila vinasse (100%, 70%, and 50%) were subjected to a 10-day treatment with *Trametes sanguineus*. Significant reductions in color, phenolic content, and COD were observed across all conditions, with approximately 70% decolorization, 60% reduction in phenolic content and 30% reduction in COD. Subsequently, the potential of the treated vinasse for seedling growth was assessed. *Trametes sanguineus* was removed from the vinasse, and the resulting supernatant was applied directly to lettuce and tomato seeds. Seedling growth assays were conducted, revealing that the treated vinasse supported the growth of these plants, with root-related features comparable to the control. Considering that seedling production is an essential part of horticultural crop production, treated vinasse at a concentration of 50% could be used in the production of tomato and lettuce seedlings.

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Potential Degradation of LDPE Using Enzymatic Extract From *Trametes sanguinea*

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Introduction. In 2021, plastic production reached 390.7 million metric tons, with 14.4% corresponding to low-density polyethylene (LDPE)[1]. This increase has led to a significant pollution problem due to inefficient recycling methods[2]. New alternatives are required to reduce this contamination problem such as enzymes and microorganisms capable of degrading plastics[3]. In this work, the fungus *T. sanguinea,* found in an oil-contaminated region in the port of Veracruz[4], was been considered. This fungus possesses a laccase[5] with a potential capacity for LDPE degradation, as this type of enzyme has been previously associated with plastic biodegradation[6]. The objective of this study was to investigate LDPE degradation by *T. sanguinea* and optimize the conditions for this process.

Methodology. The enzymatic extract of *T. sanguinea* was obtained using a culture media comprising 2L of 50% water-diluted tequila vinasses at pH 5. The culture media contained 10% v/v of biomass, and the extraction process lasted for 10 days. The analysis of optimal conditions for LDPE degradation using the enzymatic extract of *T. sanguinea* was performed by incubating it at pH 4.5 and three different temperatures (30°C, 40°C, and 50°C) for 96 hours, followed by enzymatic activity measurement using ABTS as a substrate[7]. The evaluation of LDPE degradation by the enzymatic extract of *T. sanguinea* was based on the difference in weight of LDPE, which was incubated for 96h at 30°C and 220 rpm with the extract at pH 4.5. The piece of plastic was pretreated with a exposure of ultraviolet light (UV) during 168 hours.

Results. From the analysis of optimal conditions for enzyme activity, it was concluded that pH 4.5 and 30°C were the best conditions for preserving the enzymatic activity of laccase in the enzymatic extract of *T. sanguinea*. Under this condition 98% of the activity was observed after 96 h. These conditions of temperature and pH were used for the evaluation of LDPE degradation. After 96h, a 20% reduction in LDPE weight was observed, indicating an interaction and potential biodegradation of the plastic by the enzymatic extract of *T. sanguinea*. **Acknowledgments.** This project is financially supported by the FORDECYT- PRONACES/1727997/2020 fund.

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Biotecnología

Isolation and characterization of yeasts from a wastewater valorization plant

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The use of microorganisms such as microalgae and yeasts is handy in wastewater treatment, as it allows the removal of micronutrients and the accumulation of macromolecules of biotechnological interest. In addition, biomass is obtained as a by-product that can be used in the production of bioethanol and biofuels of interest in different sectors (Nicula et al., 2023; Ko et al., 2020; Spagnuolo et al., 2019).

In this study, the isolation and identification of yeasts from “Atzintli", a pilot plant for the valorization of wastewater and microalgae of the Instituto de Ingeniería, UNAM, is carried out within the project of the interdisciplinary research group: Intensification of processes to obtain biocompounds from wastewater, whose main objective is to increase the methodologies to isolate biocomposites from residual water, under the concept of circular economy. Previously in this project, Romero (2019) isolated two yeasts from local wastewater, one of them identified as *Candida utilis*, which has a high ethanol and biomass production rate that is able to compete with *S. cerevisiae*.

In a first stage, by means of classical microbiology techniques with rich and selective culture media for fungi, six yeasts with different morphological characteristics have been isolated, among those some stand out for their high duplication rate, and two others for the production of pigments with potential biotechnological applications. Once the identification at genus and, if possible, species level by sequencing of ribosomal genes and ITS1 and ITS2 spacers has been completed, we will study the population dynamics of these yeasts in 14-day co-culture with microalgae in wastewater.

In parallel, we have characterized the growth dynamics of some yeasts isolated from these wastewater to identify the conditions (carbon and nitrogen sources, micronutrients) that allow the proliferation of the yeasts individually to determine those that favor better yield for the subsequent production of biomass and bioethanol.

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Does CuRDX, a putative cupredoxin, localize to the cell wall of Neurospora crassa?

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Cell wall proteins are essential for the structure and function of fungal cells. They are involved in a variety of important processes, such as stress resistance, nutrient uptake, and signaling. The study of cell wall proteins is therefore of great interest to fungal biologists as it can help to understand how fungi works and how they interact with their environment. Previously, in our work group, an *in-silico* analysis of the cell wall proteome of filamentous fungi was carried out. This analysis identified a putative cupredoxin-like protein (NCU04496, CuRDX) in *Neurospora crassa*, which was found to potentially resides to the cell wall and, based on plant´s homologs, it is hypothesized it could have functions related to ligninolysis. In the same study, two plasmids were built: *Pccg1::curdx::v5::gfp (CuRDX-53)* and *Pccg1::ps::gfp::v5::curdx (CuRDX-54)*, where CuRDX is N- and C-terminally bound to *GFP*. Using a knockout strain, it was observed that CuRDX plays an important role in morphology and branching, thus strengthening the hypothesis that CuRDX is present in the wall of *N. crassa*. The aim of this work is to verify the presence of CuRDX in the wall of *N. crassa*, using molecular biology, microscopy and biochemical tools. Results obtained will be formally presented during this congress.

Generation of a FRET system for *Saccharomyces cerevisiae* with the potential to detect the formation of oligomers of the hyaluronic acid synthase, *CPS1*, of *Cryptococcus neoformans*

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Hyaluronic acid synthases (HAS) are the enzymes responsible for the synthesis of hyaluronic acid (HA), a polysaccharide involved in essential physiological processes such as cell migration, adhesion and differentiation, angiogenesis, tissue homeostasis, proteoglycan assembly and phagocytosis. The molecular weight of HA directly influences their biological function, which in turn depends on the organism that produces it (vertebrates, viruses, bacteria and fungi) due to the adopted oligomerization state of HAS, which can be monomeric, homo- and/or heteromeric. In vertebrates, where HAS are better characterized, depending on its oligomerization state, they have different location, activity and processivity. Understanding the synthesis mechanism of HA is important for the biotechnological production of HA since it has wide application and market demand in pharma, medical, cosmetic, among other sectors.

In fungi, *Cryptococcus neoformans* synthesize HA through CPS1 (*cps1* gene, CNAG_04320), the first and only fungal HAS confirmed so far. However, the molecular processes CPS1 uses to synthesize and translocate HA are not known. CPS1 has the potential to form homo-oligomers responsible for regulating the synthesis of HA. This oligomerization can be elucidated through the development of a FRET (*Fluorescence Resonance Energy Transfer*) system. This project aimed to design and construct a series of plasmids used to evaluate whether CPS1 from *C. neoformans* (Cn-CPS1) forms oligomers or not through a heterologous FRET system in *Saccharomyces cerevisiae.*

This system consists of two molecular assemblies built by Gibson assembly. The constructions were made fusing *Cn-cps1* gene, extracted from *C. neoformans var. grubii* H99 genomic DNA, to genes encoding the fluorescent proteins *mTq2* or *mNG.,* as the FRET pair. Between these fluorophores, *v5* or *6xhis* epitopes were added to allow the possibility of immunodetection and localization. The assemblies were subcloned into pYES2 derivatives, MpYES2-7 and MpYES3, which led to plasmids *MpYES2-7::Cn-cps1::v5::mTq2* and *MpYES3::Cn-cps1::6xhis::mNG*. Plasmid construction was confirmed by restriction analysis and sequencing.

The generation of the plasmid system *MpYES2-7::Cn-cps1::v5::mTq2* and *MpYES3::Cn-cps1::6xhis::mNG* will allow to demonstrate, through FRET, if protein-protein interactions occurs between CPS1 monomers, with meaningful implications for *C. neoformans* CPS1 activity and processivity regulation. This work is being funded by CONAHCYT-Ciencia de Frontera, grant 2019-552259.

Stoichiometric balance in *Pleorotus dijamor* on agave substrate (*Agave* sp.)

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The mushroom species widely consumed in the world are *Agaricus bisporus*, *Lentinula edodes* and *Pleorotus ostreatus*. The genus *Pleorotus* is a member of the Basidiomycota division, consists of about 40 species. Some of them, are appreciated as food for their nutritional value, contain proteins, essential amino acids, bioactive compounds, antioxidants, and unsaturated fats. On the other hand, the ecological function in the degradation of organic matter in forests and recycling compound of plant residues such as leaves, stems and trunks. In according to *Pleorotus* genus ability to degrade cellulose, agro-industrial wastes from corn, rice, wheat, paper, sawdust, coffee pulp and other materials have been proposed as substrate for their growth, and thus, the cultivation The objective of this search was to establish the elemental stoichiometric balance in production of *P. dijamor* on agave residues under laboratory condition. Residues from the tequila industry were collected and fermented to produced mushroom. The project focuses on the transformation of the raw material into consumable material without considering the complexity of the process related to its metabolism, the biological efficiency, the production rate, the biodegradation rate and the analysis of the elemental matter balance and its relationship with stoichiometric equation through the elemental balance methodology for the elementals of the C. H, O, N to observe if there are difference in its elemental composition between the different crops. The results show the elemental stoichiometric balance between of C, H, O and N of three harvest under laboratory conditions. For the determination of C-mol of mushroom, the weight 1 g C-mol was calculated for the corresponding elemental composition in % w/w for C, H, O and N and salts for different results in the elemental composition of the mushroom C-mol: $X = CH_{\alpha}O_{\beta}N_{\gamma}$. Finally, the stoichiometric equation was represented as follows:

$$\phi_1 S + \phi_i S_i \rightarrow \phi_2 X + \phi_j P_j ; i = 1, 2, \dots, M;\ j = 1, 2, \dots, N$$

Currently, the experimentation is in the stage of generating algorithm of the elemental equation. These findings allow us to know the coefficients of the main elements involved in the accumulation of fungal biomass and the capacity of the fungus to degrade the substrate.

A new species of *Talaromyces* produces secreted antifungal metabolites

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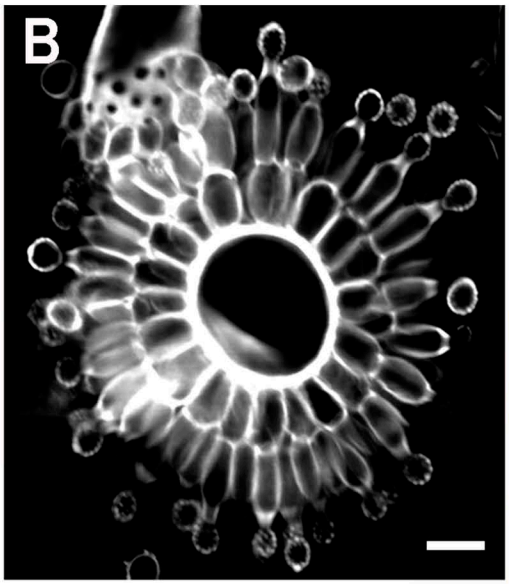
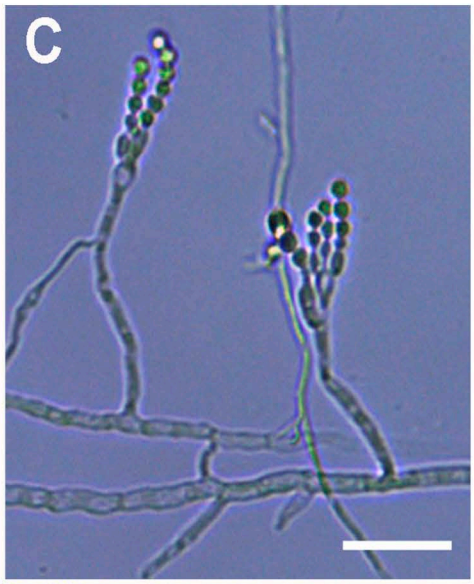
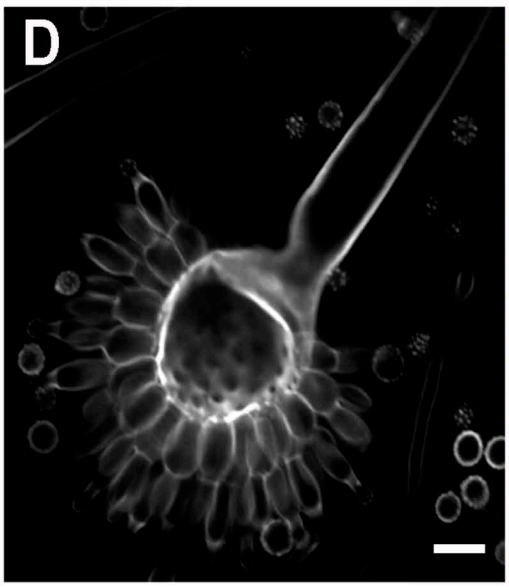
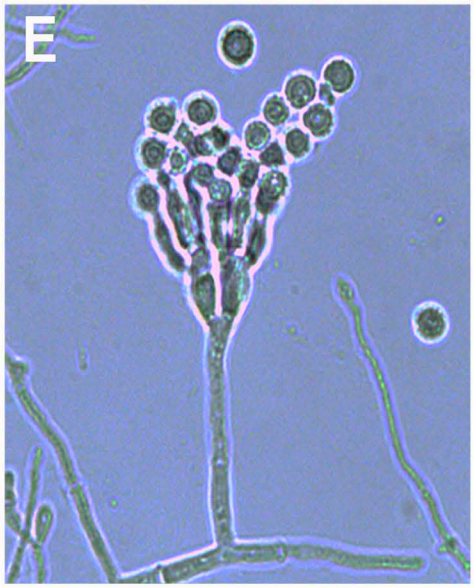
The is a current interest in microorganisms to be implemented as environment-friendly biocontrol agents. In our group, we are focused on searching for antagonists of *Fusarium oxysporum* f.sp. *cubense* (*Foc*), the causal agent of banana wilt. Three new strains were isolated form pseudostems of banana plants, all of them repressed the mycelial growth of our model strain *Foc* M5. Such antagonists were identified a a new *Talaromyces* species by phylogenetic inference of 3 informative *loci* as well as the morphological analysis. By performing different confrontation tests, we demonstrate that the antagonism does not require direct contact between fungi, therefore, diffusible molecules are involved in the repression of *Foc* M5 growth. An ethyl acetate extract form liquid cultures from the 3 *Talaromyces* strains also showed the inhibitory effect. The metabolomes of these extract were determined by HPLC/MS-MS. Metabolites from different biosynthetic origin might have antifungal activity, but specifically, the metabolic network of polyesters is composed by metabolites that were previously reported as inhibitors of the cell wall formation, and they might have a prominent role in the repression of mycelial growth of M5. In accordance with the inhibition of cell wall formation, the ethyl acetate extract also promoted hyphal swelling of *Foc* M5. This work suggests a new species of *Talaromyces* that can be implemented as a biological control agent against *Foc.*

Key words: Antifungal activity, biological control, *Fusarium*, metabolome, *Talaromyces*









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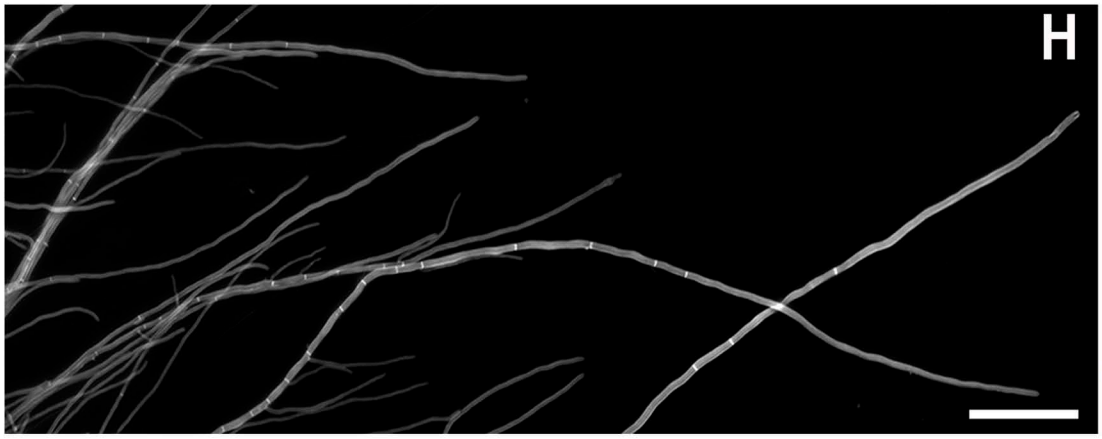

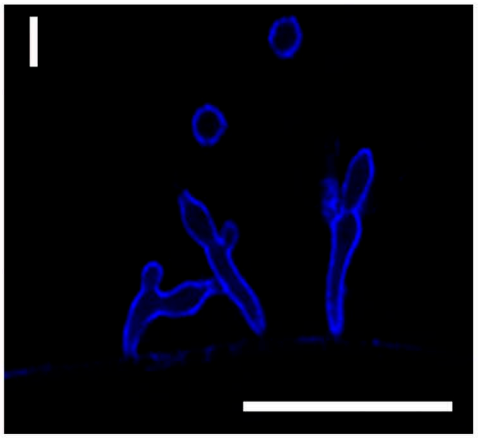

Unraveling the functional role of fungi isolated from human milk

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Abstract:

Breast milk serves as the primary source of nourishment for newborns, providing essential nutritional, microbiological, immunological, and energy content. While bacteria have been extensively studied as a significant component of breast milk microbiota, the role of fungi in this context remains largely unexplored, often regarded as contaminants rather than native microbiota. Consequently, their niche within the gut microbiota of newborns is currently unknown. To address this knowledge gap, we employed interdisciplinary approaches to isolate various strains of fungi, which were subsequently characterized phenotypically. Among the isolated organisms, a novel *Candida* strain exhibiting biochemical inconsistencies, the ability to grow at 37°C, the absence of hemolytic activity, and an accelerated growth rate in human milk lipids was identified. We have performed complete sequencing of this *Candida* strain and are currently studying its metabolic and pathogenic properties to shed light on its significance within the breast milk microbiota. This research aims to enhance our understanding of the functional role of fungi in breast milk, thereby contributing to a more comprehensive understanding of the complex microbial ecosystem supporting newborn health.

High fertility of yeast hybrids associated with agave fermentations

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Hybridization is the result of the mating of two diverged organisms. Although this process can bring advantages, such as outcompeting the parental species under certain conditions, it also has important consequences at the genomic level. For example, it leads to gene loss, rearrangements, loss of heterozygosity (LOH), and aneuploidies. Remarkably, hybridization is associated with sterility due to these genomic changes and genomic incompatibilities overall. Yeast from the genus *Saccharomyces* can generate hybrids and produce spores, but its viability is around 1%. The production of traditional agave spirits in Mexico provides a unique environment for microorganisms. It is mostly a handmade process where open fermentation occurs, and specific practices vary from place to place. In this system, in addition to *S. cerevisiae*, the most usual yeast in fermentations, we commonly isolate hybrids of this species with its sister species *S. paradoxus.* Unlike *S. cerevisiae*, *S. paradoxus* is often associated with natural environments, specifically with bark from trees of the *Quercus* genus. Surprisingly, most of the isolated hybrids were able to sporulate efficiently and had a spore viability between 14 and 38%, much higher than previously reported viabilities in this type of hybrid. The *S. cerevisiae* population from agave fermentations has a significant number of introgressions from *S. paradoxus*, which could explain their high fertility. Additionally, to further understand the biology and evolution of the hybrids in agave fermentations, we phenotypically characterized the hybrids in a variety of environmental conditions. Overall, our research aims to explain whether hybridization is favored due to a gain in fertility and to determine phenotypic characteristics that allow them to outgrow the parental species in this type of fermentation. This will help us understand how yeast hybrids can overcome the sterility barrier that is expected when two species interbreed.

Genetic modification of *Candida maltosa*, a nonpathogenic CTG species

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Candida maltosa is closely related to important pathogenic *Candida* species, especially *C. tropicalis,* but to our knowledge it has never been isolated from humans. For this reason, it is an ideal organism to understand the genetic underpinnings of the pathogenicity of *Candida* species through comparative studies. The aim of this research was to generate a better assembly of the *C. maltosa* genome and to develop genetic engineering tools that will allow us to study this species at a molecular level. To this end, we used short and log-read sequencing to build a polished genomic assembly composed of 14 Mbp, 58 contigs and 5,518 genes. Using the Saccharomycetes BUSCO database, genome completeness was estimated to be close to 96%. This assembly represents a substantial improvement from the currently available sequence that is composed of thousands of contigs. To be able to edit the genome of *C. maltosa* we generated a set of triple auxotrophic strains so that gene deletions can be performed similarly to what has been routinely done in pathogenic *Candida* species. We used the FLIP-SAT system optimized for *C. tropicalis* to tandemly delete *LEU2, HIS1 and ARG4*. The triple auxotrophic strains allow deletion of the two alleles of given gene and its subsequent reintegration for complementation assays. As a proof of concept, we generated knockouts of genes associated to biofilm formation in other *Candida* species, *EFG1* and *ROB1*. Characherization of these miutants showed that both have a role in biofilm formation and filamentous growth. The auxotrophic mutants and the new genome assembly are a key step to start using *C. maltosa* for comparative and evolutionary studies.

A landscape of evolution in the Fungal Tree of Life through syntenic blocks

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Area: Genómica Funcional y Comparativa

Abstract

Fungi is one of the largest groups in nature, and as a result, they play a crucial role in nutrient recycling and have significant ecological impacts.
Thanks to technological and computational tools, our understanding of fungi's roles in food production, pharmaceuticals, and ecological processes has been enhanced.
To appreciate the evolutionary landscape, it is necessary to understand Genome doubling events, such as whole-genome duplication (WGD), as they contribute to fungal diversity and rapid adaptation.
Synteny analysis has proven valuable in studying genomic organization and revealing conservation and plasticity in fungal genomes.
In this context, we utilized computational and bioinformatic tools to conduct a large-scale comparative genomics analysis of the Fungi Tree of Life, aiming to gain a comprehensive understanding of fungal evolution and synteny blocks.

Keywords
synteny, Fungi, evolution, genomic comparative, genome duplication, computational biology, bioinformatic

Search for hyaluronic acid synthases in Fungi: Do filamentous fungi synthesize hyaluronic acid?

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Hyaluronic acid (HA) is a biologically relevant polysaccharide composed of N-acetyl- D-glucosamine and D-glucuronic acid. It is found in vertebrates, some bacteria, viruses, and the pathogenic yeast *Cryptococcus neoformans*, the only fungus reported to synthesize HA. This polysaccharide plays essential biological roles; it is involved in cell proliferation, differentiation, and tissue repair, among others. The enzyme that performs the synthesis of HA is the Hyaluronic Acid Synthase (HAS), which is a transmembrane protein belonging to the processive glycosyltransferase family 2 (GT2).

In the Kingdom Fungi, the universe of HASs has not been thoroughly explored. In this work, we designed a strategy to find putative HASs in the fungal proteome. As a query, we used reference sequences that were previously reported to be *bona fide* HASs. Moreover, we systematically identified essential residues related to the HA synthesis and also all those regions highly conserved through the sequences. Using these results, we created an HMM profile in order to detect new hypothetical HASs. Retrieved sequences were manually curated to get more accurate predictions. Additionally, a search was conducted for the enzymes that are involved in the biosynthesis of the HA precursors (UDP-glucose 6-dehydrogenase, UTP-glucose-1- phosphate uridylyltransferase and UDP N-acetylglucosamine pyrophosphorylase) in fungus, using HMM profiles. The orthology of the results obtained was reviewed through a phylogenetic analysis and their putative activities were also predicted with ttianhao/CLEAN.

We found 69 putative sequences that have the characteristics of classical HASs. To support this result, we also found that fungi species that encode such proteins have all the machinery to synthesize the HA precursors. Additionally, six new motifs were found conserved in all the putative fungal HASs sequences (*i.e.*, GGVXT, WEXL, RTAXY, LXD, FXXRW, WXXXP), which can be used to design new targets to elucidate their role in the biosynthesis of HA in fungi. We inferred the secondary and tertiary structures of reference HASs (bacteria, vertebrate, and virus) and putative fungal HASs. In contrast to animal and bacterial HASs, a 3 alpha-helices transmembranal pore was found to be characteristic of fungal HASs. This structural characteristic could be involved in the processivity of the enzyme, related to the molecular weight of the HA synthesized and/or to their biological function in Fungi. This work is being supported by CONAHCYT-Ciencia de Frontera 2019, grant 552259.

UstilagoNet: a database of gene regulatory and co-expression interactions in *Ustilago maydis*

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This database contains information related to expression data, co-expression interactions, and interactions between transcription factors (TFs) and target genes in *Ustilago maydis*. UtilagoNet consists of 168 samples of gene expression data that have been processes and normalized, belonging to 19 series, which correspond to the GPL3681 platform in the NCBI.
Additionally, it contains the co-expression relation between 5’766 predicted open reading frames (ORFs), coverage of 85.23% of *U. maydis* genome. Finally, it contains 23’932 regulatory interactions between 219 TFs and 2’849 target genes. The gene regulatory networks were inferred by two approaches: first, previously described networks were used as a template, and the interactions were inferred through comparative genomics, secondly, the interactions were inferred using machine learning algorithms by utilizing the expression data from the 168 samples. The database allows querying for each gene, and it returns basic gene information, average gene expression in each condition, co-expressed genes, regulating TFs, and, if applicable, gene targets. Additionally, it allows consultation of various genes that returns regulatory circuits and co-expression modules. Finally, it allows for the independent download of each complete dataset.

Universidad Autónoma del Estado de Morelos

Centro de Investigaciones en Biotecnología

Whole deep genome analyses of an undescribed strain of *Trichoderma*, isolated from a Milpa in Morelos, Mexico

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Abstract

Fungi are often classified using various species concepts that use different criteria. This hampers comparative studies among individuals or populations of the Fungal kingdom. Nowadays, genomics provides powerful tools to reunite the diverse species concepts and integrate their taxonomy into a more unbiased manner. In this work, we ensembled *de novo* the complete genome sequence of a fungal strain (BMH-0061) isolated from a “milpa” through long reads using the Oxford Nanopore MinION technology. With these data, we adapted the specific-species concept applying the phylofenetic concept of species (which has been recently used for prokaryotes and microbial eukaryotes). We evaluated the hypotheses of Genomic coherence, phylogenetics, discriminatory diagnostic characters as well as genetic elements that contain biological transition signatures among phylogenetically close taxons. After these deep analyses of the whole genome sequence, we concluded that this strain belongs to a new undescribed *Trichoderma* species and defined its taxonomical limits. We named this new species as *Trichoderma tlahuicanensis* after the native ethnicity that inhabited the place from which the strain was isolated, the Tlahuicas. Also, an exploratory study was conducted to infer its biological and ecological characteristics. Morphological studies are under way as well as the evaluation as a mycoparasite, according to what we found in the genome.

Genomic analysis of new bacterial species from extreme environments

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The search for new bacteria species from extreme environments has become of great interest due to their various biotechnological applications, such as the creation of biopolymers, biodegradation, enzyme production, to mention a few. The bacteria, defined as ML-Red is an extremophile, isolated from Mono Lake, California from an alkaline and hypersaline environment. Its genomic DNA and 16SrRNA was sequenced and used to search for its closest genome and genus. The results show a close relation with *Nathrohydrobacter* and *Rhodobaca* however it clearly presents another separate branch that can lead to the possibility of a new genus. Based on its physiology analysis, its optimal conditions to grow are the following: pH 9.5, salinity of 40/60 g/L, grows in dark and light and does not grow anaerobically. During lab cultivation variants arose from the original isolate, such as the ML-White, whose gDNA was also sequenced. It shows interesting genomic differences with the ML-Red. This last one presents a larger genome (3.47 Mbp) in 3 separate contigs that contains two large plasmids, while the ML-White has a shortened genome and did not contain one of the large plasmids that harbors the photosynthetic reaction center genes amongst other ones. Further genomic sequencing, using Illumina and Nanopore sequencing and growth analysis was performed to investigate the mechanism and the origin of these molecular changes.

Key words: DNA sequencing, extremophile bacteria, genomics.

The role of the transcription factor Gln3 in fluconazole resistance in *Candida glabrata*

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Candida glabrata is a pathogenic yeast that can cause candidemia in immunocompromised individuals. These yeasts often exhibit innate resistance to azoles, the primary main drugs used to combat this disease. Moreover, this fungus can thrive in various stressful and nutrient-depleted growth conditions. Our research group has demonstrated Gln3 to be the main transcription governing nitrogen assimilation in *C. glabrata*. We also published in 2021, that there was an increased fluconazole resistance in strains lacking Gln3. This resistance was accompanied by an increased expression of major fluconazole detoxification pumps and their regulatory transcription factor. These findings Gln3's potential involvement in fluconazole resistance in *C. glabrata*.

To investigate Gln3 role in fluconazole resistance, this project involved generating mutants in the *gln3*Δ genetic background for *CDR1*, *CDR2* and *PDR1* genes. Subsequently, we assessed their fluconazole resistance in a minimal medium supplemented with ammonia as a nitrogen source. Additionally, we evaluated the expression of selected antifungal resistance-associated genes responsible for maintaining the integrity of the cell membrane, including *ZAP1*. We observed a reduced fluconazole susceptibility at 8 and 16 µg/mL in *cdr1*Δ and *pdr1*Δ mutants lacking Gln3 compared to individual mutants in these genes. When analyzing the expression of membrane stability-associated genes like *MAR1*, *SSA3*, *RTC3* and *GAC1* in the *gln3*Δ strain, higher expression levels were observed compared to the parental strain. Regarding *ZAP1*, we noted increased expression levels in the *gln3*Δ strain versus the parental strain when cells were grown without antifungals. However, this difference in expression was not observed in the presence of fluconazole. We continue to investigate the relationship between the Gln3 transcription factor and genes governing membrane stability in *C. glabrata*. This involves generating mutant strains (both single and double) in the *gln3*Δ genetic background and assessing fluconazole resistance, alongside measuring cell permeability in these mutants.

Prediction of Biosynthetic Gene Clusters in Mexican Isolated Fungi from Strawberry Fields

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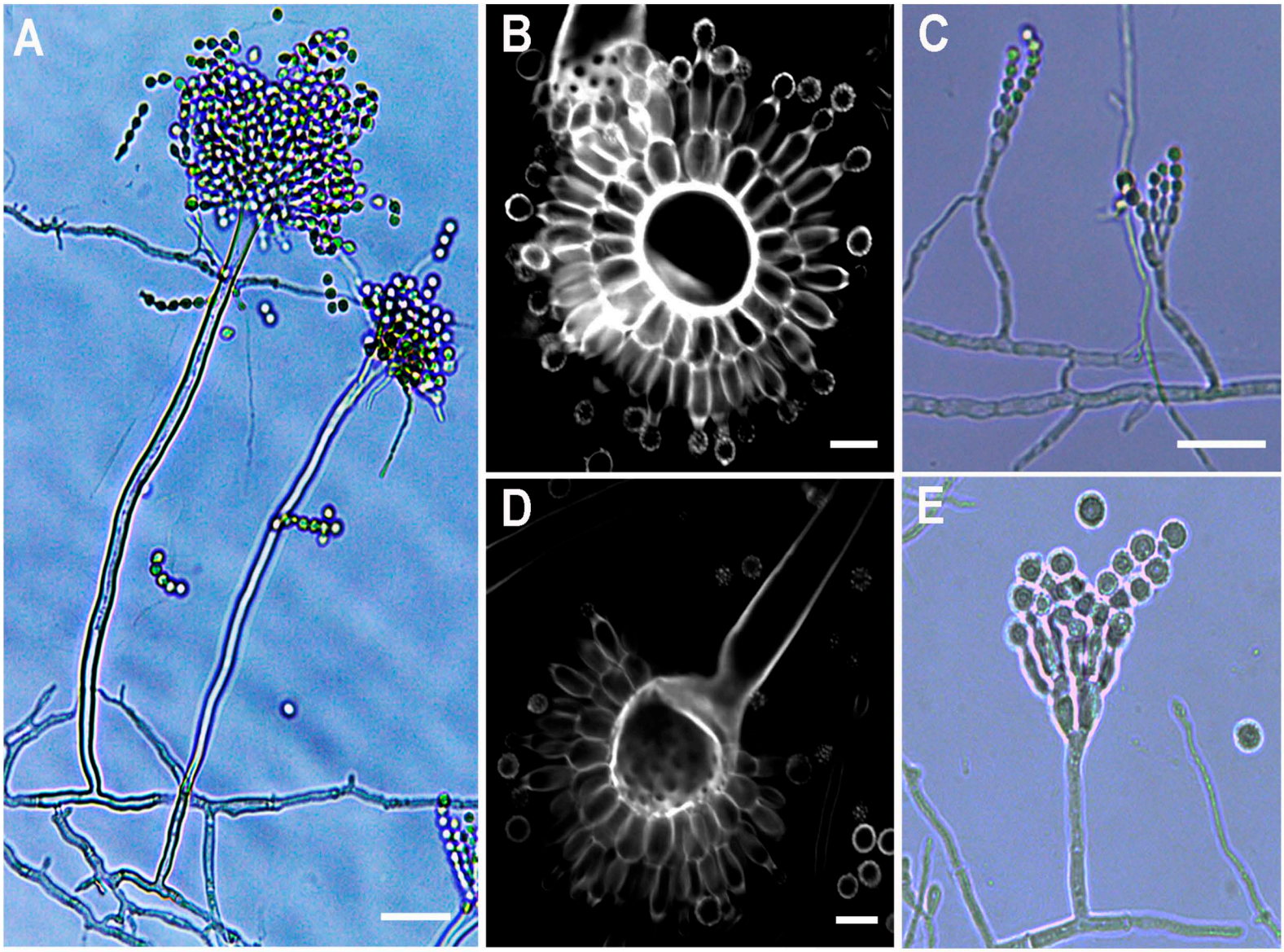
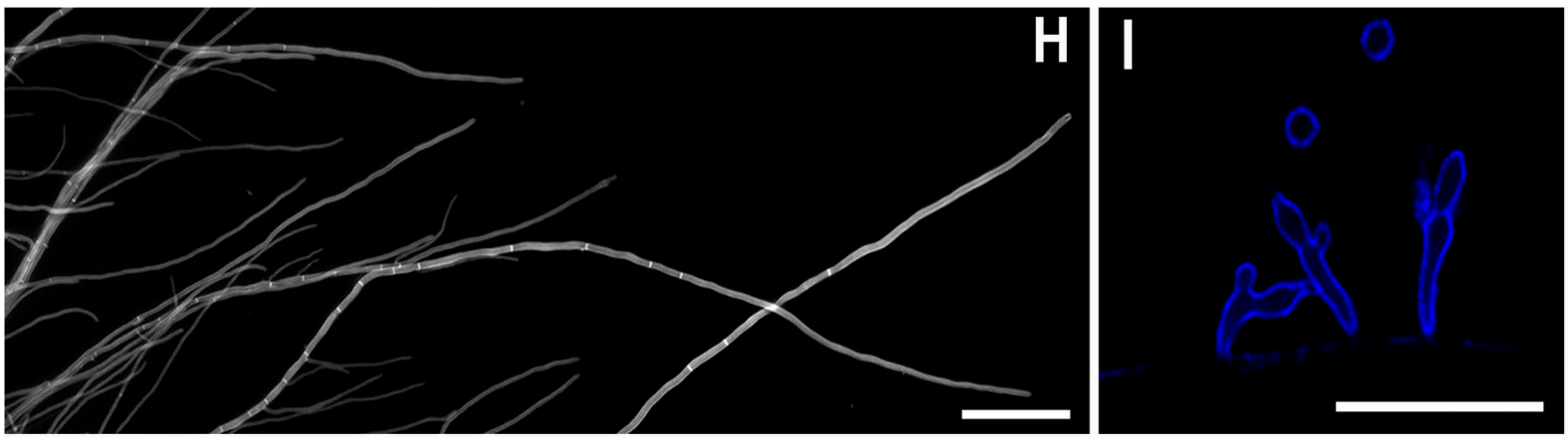
Biosynthetic gene clusters synthesize natural products such as antibiotics and other specialized metabolites. In this study, two fungi were isolated from Mexican strawberry fields. Genomes were phylogenetically organized using the ITS marker, and it was determined that they belong to the genus *Fusarium*, closely related to the species *oxysporum* and *chlamydosporum*. These microorganisms underwent genome sequencing using Illumina, complemented with nanopore technology. The assembled genome was used for the prediction of biosynthetic gene clusters. The availability of public genomes of *Fusarium* enables comparative genomic studies, allowing for a deeper understanding of the diversity and function of the metabolites produced by these fungi.



POSTERS

FUNGI DEVELOPMENT



Intracellular organization, cell wall integrity and endocytosis are dependent of Actin Cytoskeleton in *Metarhizium brunneum*.

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M. brunneum is an entomopathogenic fungus used for the biological control of pests in agriculture. This fungus also can form other beneficial interactions with plants as an endophyte. While interacting with either insects or plants, the fungus must defy different barriers and toxic compounds, which trigger events of cell differentiation and organelle transport to achieve homeostasis. In fungi, cell polarity is an essential process for proper growth and morphogenesis. The actin cytoskeleton is required for normal apical growth and maintaining cell shape and organelle organization (peroxisomes, lipid droplets, vesicles, etc.). To describe the role of the actin cytoskeleton in the intracellular organization of *M. brunneum*, we determined the concentration that inhibited mycelial growth rate by 50% (LC50) of Latrunculin B (Lat B) (anti-actin drug). It was observed that the minimum inhibitory concentration of 1.5 µg/mL caused aberrant growth with apical branching and increased hyphal thickness. In addition, the dynamics of peroxisomes and lipid droplets were affected in presence of Lat B, with an abnormal distribution and decrease in the displacement speed. The cell wall stained with calcofluor white was altered, with irregular thickening, forming some deposits in spots close to the membrane, which caused an increase in the sensitivity to Congo red compared to the WT. The Apical Vesicle Crescent (AVC) stained with FM4-64 was localized in the apical dome moving in the growth direction, but under Lat B treatment this structure was not visible. On the other hand, it was shown that hyphae treated with Lat B decrease their endocytic activation compared to WT.

Nanoscopic dynamics of endocytic patches during hyphal growth of *Neurospora crassa*

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The actin cytoskeleton in filamentous fungi is a key component in both exocytosis and endocytosis, processes that determine hyphal morphogenesis. So-called “actin patches” are sites of endocytosis and are composed of F-actin filaments linked by a variety of actin-binding proteins, such as fimbrin, coronin, and the Arp2/3 complex. The precise size, as well as the origin and transport mechanisms of endophytic vesicles remain a topic of interest; their study has been limited by the resolution capacity of confocal and evanescent field microscopy (TIRF) techniques. In this work we report single particle tracking and velocimetry of the endocytic machinery from TIRF microscopy images processed by a novel super-resolution algorithm. The actin patches are sufficiently dispersed in the subapical and basal regions of the hypha, allowing their individual analysis. However, in the subapical ring they are highly concentrated, making it difficult to observe individual patches. Fluorescence nanoscopy offers the opportunity to describe changes in the size, shape, and direction of motility of individual structures. Our work provides new evidence for the directional transport of vesicles along the hyphae observed through nanoscopic images of live cells in a model filamentous fungus, and provides a detailed mesoscopic description of the origin and fate of actin patches, thus contributing to the understanding of its role in the formation of hyphae.

Role of the endoplasmic reticulum-shaping proteins YOP1 and RTN1 in *Podospora anserina* mitochondrial dynamics

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YOP1 and RTN1 proteins, belonging to the YOP1/DP1 and reticulon families, respectively, participate in shaping and regulating the dynamics of the endoplasmic reticulum (ER), by promoting membrane curvature. We have shown that the development of the filamentous fungus *Podospora anserina* implicates a precise regulation of ER dynamics, which involves the activity of the reticulon and YOP1 family proteins. During hyphal growth, the ER is characterized by pleomorphic and highly dynamic peripheral ER subdomains, which are associated to the polarized growing apical hyphal region. These domains are enriched for YOP2 and RTN1, and their formation depends on YOP1. During sexual development, RTN1 also defines apical ER subdomains along ascus (meiocyte) differentiation and growth, but it is redistributed to the perinuclear cell region during the subsequent meiotic progression. This protein is necessary for sexual development, a process that also relies on proper mitochondrial dynamics. Here we show that the dynamics of the ER and mitochondria during development are closely interrelated. We show that there is a close association of mitochondria with the ER subdomains defined by RTN1, and we analyzed the impact of eliminating the reticulon and YOP1 family proteins in mitochondrial dynamics during both the vegetative and sexual phases of the fungus. Our findings show that, despite their close association to the ER, mitochondria dynamics is only moderately affected upon loss of the ER membrane curvature proteins, suggesting further mechanisms implicated in sustaining mitochondria-ER interactions and crosstalk.

Organelle dynamics in absence of kinesin-3 motor proteins in *Podospora anserina*.

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Ascomycetes possess two kinesin-3 motor proteins, KIN2 and KIN3. Kinesin-3 motors have been proposed as global regulators of intracellular trafficking during polarized growth of fungal hyphae and in neuronal axons. *KIN2* orthologues of *Neurospora crassa* and *Aspergillus nidulans* (*NKIN2* and *UncA*, respectively) are required for peroxisome and early endosome transport, but their involvement in mitochondrial dynamics remains a matter of debate, and their role in endoplasmic reticulum (ER) motility remains unknown. In the basidiomycete *Ustilago maydis*, KIN2 (named Kin3 in this organism) mediates peroxisome and ER, but not mitochondrial, movement, through an endosomal mediated co-transport, known as hitchhiking. In *A. nidulans*, peroxisome hitchhiking requires PxdA as specific adaptor, which is not needed for mitochondrial displacement, implying that mitochondria do not share this mechanism. However, in *N. crassa* it has been suggested that KIN2 is required for mitochondrial binding to microtubules. Here we studied the role of *Podospora anserina* class 3 kinesins on peroxisomes, mitochondria, and ER dynamics during hyphal polarized growth. We deleted *KIN2* and *KIN3* and found that *KIN2*, but not *KIN3*, is required for optimal mycelial growth and proper hyphal ramification. We found that the genetic elimination of KIN2 reduces peroxisome abundance and motility and disturbs apical ER dynamics. In addition, we discovered that loss of KIN2 altered the distribution of mitochondria, without impeding their motility. Finally, we found that KIN3 absence did not notably affect the dynamics of these organelles, and that the *ΔkinΔkin3* double deletion recapitulated all *Δkin2* defects. Our research revealed a major role for the kinesin-3 motor KIN2 in the regulation of *P. anserina* organelle dynamics. This research was supported by grants CONACYT-DFG 277869 and PAPIIT-DGAPA, UNAM IN227823.

Role of the small GTPase MIRO1 in *Podospora anserina* organelle dynamics

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Cell differentiation and development rely on precise spatiotemporal regulation of organelle dynamics. Sexual development of the filamentous fungus *Podospora anserina* depends on a dynamic regulation of the formation and dynamics of multiple organelles, including mitochondria, peroxisomes and the endoplasmic reticulum (ER). This process requires the activity of distinct organelle membrane fission and remodeling factors, and involves a differential organelle intracellular distribution at specific developmental stages. Cytoskeleton-based organelle transport is crucial to define organelle localization in eukaryotic cells. In metazoans, the Mitochondrial Rho GTPase MIRO provides a key motor protein adaptor for mitochondrial transport. In addition, this protein facilitates mitochondria-ER interactions, and regulates peroxisome fission and transport. In *P. anserina*, we have shown that MIRO1 is dispensable for mitochondrial or peroxisome transport in vegetative hyphae, but is relevant for ascospore germination, as the growth of the germinative mycelium is affected upon MIRO1 loss. Here, to further understand the contribution of MIRO1 to *P. anserina* organelle dynamics, we studied the genetic interactions between *MIRO1* and genes involved in ER remodeling (*YOP1*), mitochondria-ER interactions (*MDM10*), and peroxisome and mitochondrial fission (*DNM1*). Moreover, we analyzed, mitochondrial, peroxisome and ER dynamics along ascospore germination and found that peroxisome arrangement and motility are affected in absence of MIRO1. Our findings show that MIRO1 plays an important role in the regulation of peroxisome dynamics during ascospore germination. This research was supported by grant CONACYT-DFG 277869 and PAPIIT-DGAPA, UNAM IN227823.

El papel del complejo de unión entre retículo endoplásmico y mitocondria (ERMES) en la regulación de la dinámica del retículo endoplásmico en *Podospora anserina*

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En la célula, diferentes organelos interactúan entre sí mediante sitios de contacto. En los hongos, la unión entre el retículo endoplásmico y la mitocondria está mediada por un complejo de proteínas denominado ERMES, que está formado por las proteínas asociadas a la membrana mitocondrial MDM10, MDM12 y MDM34 y la proteína del retículo endoplásmico MMM1. Adicionalmente, MDM10, MDM34 y MDM12 forman sitios de contacto con los peroxisomas. El complejo ERMES tiene funciones de transporte de fosfolípidos y calcio, así como un papel fundamental en la fisión mitocondrial. Asimismo, MDM10 se asocia a los complejos SAM y TOM implicados en biogénesis mitocondrial. En el hongo filamentoso Podospora anserina, el desarrollo sexual involucra una regulación precisa de la formación y la dinámica de las mitocondrias, los peroxisomas y el retículo endoplásmico. Hemos demostrado que MMM1 es un gen esencial en este hongo. Asimismo, mediante una mutación termosensible en el gen MDM10 ($mdm10^{L240P}$), hemos observado que MDM10 es necesaria para la morfología mitocondrial, el posicionamiento de los septos y el desarrollo sexual normal. En este trabajo, demostramos que la mutación mdm10L240P también genera defectos en la morfología del retículo endoplásmico, caracterizados por un incremento en los dominios apicales del retículo endoplásmico periférico, y que este defecto se suprime al eliminar a YOP1, proteína que controla la curvatura de la membrana del retículo endoplásmico. Asimismo, demostramos que la eliminación genética de RTN1 –una segunda proteína de curvatura del retículo endoplásmico– o, más notablemente, de YOP1, exacerban las alteraciones negativas producidas en el desarrollo del hongo por $mdm10^{L240P}$. Nuestros resultados indican que el complejo ERMES es necesario para la correcta distribución y dinámica del retículo endoplásmico.
Este proyecto fue financiado por los donativos CONACYT-DFG 277869 y PAPIIT-DGAPA, UNAM IN227823.

Mycotoxin-producing fungi presence in medicinal and aromatic plants; Eucalyptus (*E. melliodora* L.), horsetail (*E. hyemale*), and skunkweed (*P. alliacea* L.)

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Herbs with medicinal properties are considered one of the oldest sciences, being the first medicines empirically used to cure diseases in emerging and developing countries such as Asia, Africa, and Latin America. Worldwide, it is estimated that about 80% of the population uses traditional herbal medicine for primary health care, and in Mexico is an integral part of alternative medicine, and is closely linked to the culture and traditions of the population, its use is based mainly on empirical experience, in addition to its accessibility of acquisition and low cost. However, they are often exposed to contamination by several microorganisms responsible for the decrease in commercial quality, such contamination can begin in the field during the entire vegetative cycle of the crop and continue during the harvesting process, drying, and storage conditions, depending largely on various environmental conditions, such as temperature and moisture content, which are the main aspects that modulate the growth of fungi and the production of mycotoxins. The most important mycotoxin-producing fungi are those belonging to the genera *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium*. They represent a potential risk to human health through the contaminated food consumption. Aflatoxins (AF), produced by *Aspergillus* and *Penicillium* species, have mutagenic, teratogenic, and immunosuppressive activities, causing acute and chronic effects in humans. Based on the above, this research aimed to search for the presence of mycotoxin-producing fungi in medicinal and aromatic herbs. The isolation of fungi was carried out using. Method for enumeration of fungi and yeasts in foods. Fungal genera were identified by dichotomous keys and multiplex polymerase chain reaction (PCR). Of the total samples of medicinal and aromatic herbs analyzed, 50.4 NOM-111-SSA1-1994, Goods and Services % showed no contamination, while 49.6% showed fungal contamination, mainly of the genus *Aspergillus* (60%) and the genus *Penicillium* (33.33%).

It is important to emphasize the need to know the mycological quality of medicinal and aromatic herbs used to cure any health condition and the need to implement good manufacturing, handling, and storage practices.

Role of Auxins Produced by *Trichoderma atroviride* in Its Growth and Development

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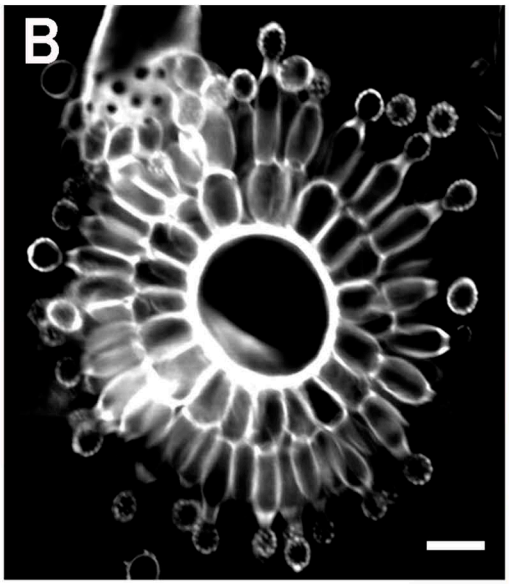
Numerous species of fungi, including those that do not directly interact with plants, produce, and secrete auxins. This raises the possibility that these hormones play an endogenous role in these organisms. However, the role of indole-3-acetic acid (IAA) in fungi has not been thoroughly investigated, and as a result, there are no reports available on the subject.

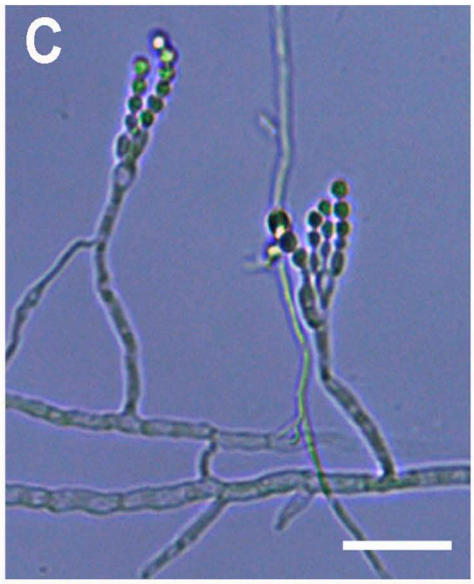
With the purpose of elucidating the endogenous role of IAA in *T. atroviride*, we generated mutants lacking the *ald* gene, which encodes the enzyme aldehyde dehydrogenase, a key enzyme in the final step of the indole-3-pyruvate (IPA) pathway. To achieve this, we assessed the involvement of IAA in growth, light- and injury-induced conidiation. In addition, we evaluated the impact of IAA on the response to cellular stress, including oxidative and osmotic stress.

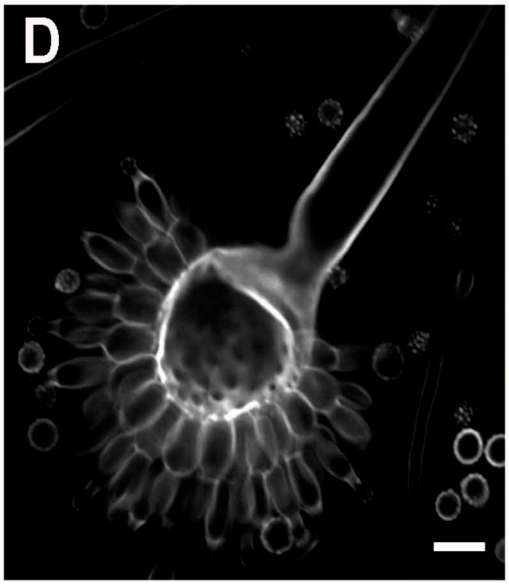
Our results indicated that IAA could be acting as a regulator with a role that can be either positive or negative, depending on the stimulus. In the context of growth under constant light conditions, its action appears to be negative, as mutant strains exhibited accelerated colonial growth compared to the wild-type strain. In contrast, IAA appears to exert a negative regulation on mechanical injury-induced asexual reproduction.

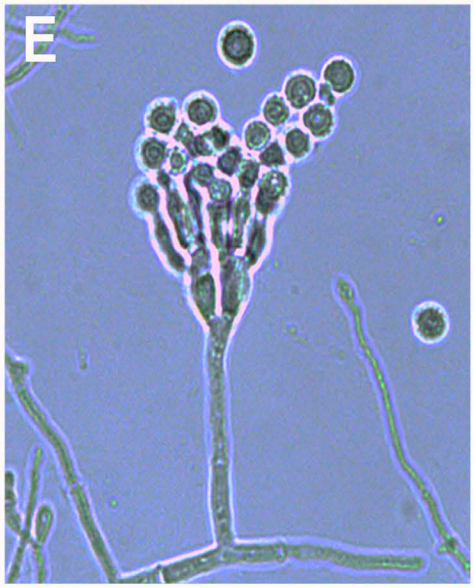
Our results also suggest that IAA could play a repressive role in response to osmotic and oxidative stress, mediated by the MAPK Tmk3 pathway, as mutants displayed greater tolerance under these stress conditions.

Overall, these findings suggest that IAA plays an essential role in regulating various responses and may be interacting with the MAPK Tmk3 signaling pathway to control asexual reproduction in response to these stimuli.









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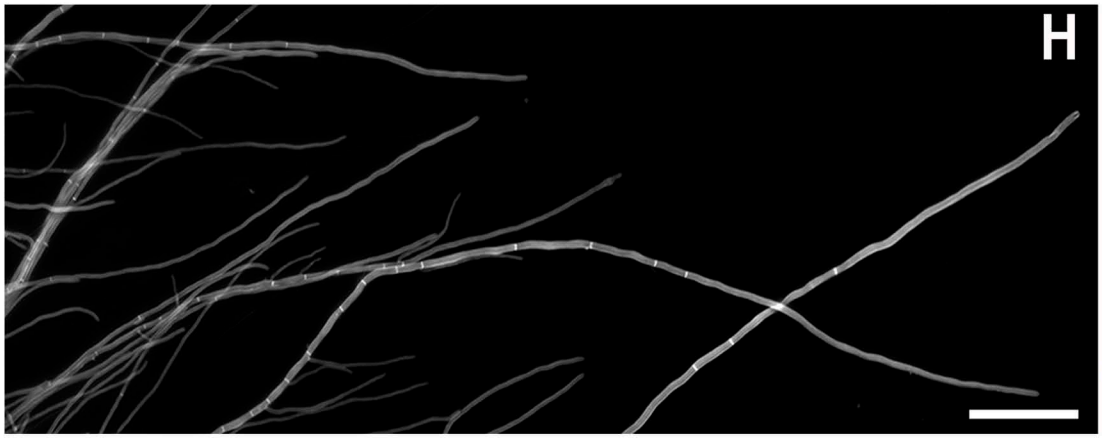
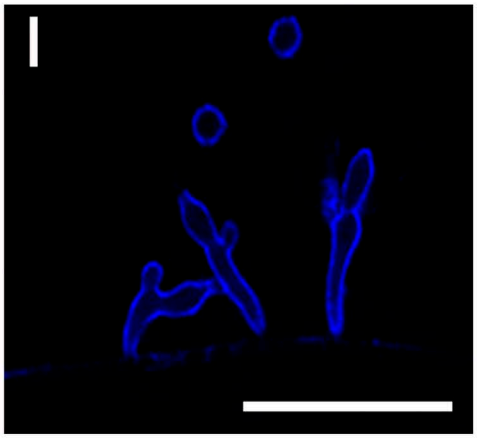
FUNGI-HOST INTERACTION





Immunomodulatory effect of an NSAID-antifungal therapy for the treatment of eumycetoma in mice

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Eumycetoma is a chronic fungal disease characterized by the appearance of granulomas, protuberances on the skin and deep tissues, which causes inflammation leading to an increase in volume and deformation of the affected area, thus causing the destruction of the surrounding tissues. These grains are the result of multiple traumatic inoculations of etiological agents. Eumycetoma is mainly caused by *Madurella mycetomatis* and it predominantly affects the extremities of young adult males. The treatment commonly includes the use of azoles and surgery to remove the grains, although this has proven to be an ineffective and expensive therapy in which constant recurrences are reported. It has been suggested that inflammation plays a key role on the effectiveness of the therapy, therefore, in this work we used a murine model of *M. mycetomatis* eumycetoma to evaluate the effect of a combined diclofenac-amphotericin B therapy on the secreted cytokines to the bloodstream. Four groups of immunocompetent BALB/c mice with eumycetoma were treated for 28 days as follows: the control group was administered saline solution, the second group was treated with diclofenac, the third group received amphotericin B and the last group received combined diclofenac-amphotericin B therapy. After treatment was completed, sacrifice was performed by cervical dislocation and blood was recovered by cardiac puncture using heparinized syringes to subsequently obtain plasma and perform quantification of the following cytokines IL-1β, IL-6, IL-17A, IL-22, IL-25, IL-33, sCD40L, IL-4, IL-10, IL-17F, IL-21, IL-23, IL-31, IFN-γ, and TNF-α using a multiplex system. Our data showed that after the 4-week period of treatment, a Th2 cytokine profile predominates over a Th1 response. And the residual Th17 response is limited to the anti-inflammatory IL-22. These results positively correlate with the reduction in size and number of fungal grains at the same period. Interestingly, these results were only observed on the group treated with the combination of diclofenac-amphotericin B, unlike those groups receiving monotherapy or saline as control. Together, our results suggest that a combined diclofenac-amphotericin B therapy might be more effective than monotherapies for the treatment of eumycetoma caused by *M. mycetomatis*.

Identification of strawberry pathogens and their biological control

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Fungal diseases in strawberries generate growth reduction, leading to the death of plants and causing significant economic losses. Mexico is one of the five main producers and exporters of strawberries worldwide. Recently, the strawberry crop in the Bajio - Mexico has shown an increasing incidence of diseases, with reports of the presence of *Neopestaloptiosis rosae*, an important emerging pathogen in the region. In an effort to provide solutions to this problem, we contacted a small strawberry producer in Guanajuato State and we collected plants with disease symptoms to analyze the fungi present in the crown and roots. We isolated two species that were identified as *Fusarium spp.* and *Macrophomina spp*, through ITS analysis; both genera of fungi are reported as important strawberry pathogens. Among the organisms reported as biological control agents for fungal diseases are those belonging to *Trichoderma* genus. These filamentous fungi are capable of associating with plants, promoting better growth and improving plant defense, and displaying antagonistic activity against several phytopathogenic fungi. In our laboratory fungal collection, we have *Trichoderma* strains with high mycoparasitic activity. We carried out direct confrontations of these *Trichoderma* strains against the two putative pathogens and a strain of *N. rosae*. We determined that two *Trichoderma harzianum* strains can antagonize all three phytopathogenic fungi *in vitro*. Currently, we are growing strawberries in a greenhouse using soil from agricultural fields. We have inoculated the three phytopathogens and we are going to determine the degree of protection conferred by the strain that has shown the best *in vitro* antagonistic capacity. With the results obtained, we hope to be able to propose viable biological treatments that help to control diseases in strawberries in our region.

Role of two secreted proteins in development and pathogenesis of *Fusarium* sp. associated with the ambrosia beetle *Xylosandrus morigerus.*

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The secreted proteins have an important role in the pathogenesis of phytopathogenic fungi. Among of them are enzymes such as proteases, necessary for nutrient breakdown and acquisition, and protection from host´s immune system. In addition, small proteins, generally cysteine-enriched (SSCP-small secreted cysteine-rich proteins) unique for fungi exist in the secretome, several of which have been demonstrated to play a role in pathogenesis.
Serine proteases (SPs) are among the most abundant secreted proteins in phytopathogenic fungi; the S10 family (SP10), known as serine carboxypeptidases, are reported as abundant in plant-associated fungi but scarcely functionally studied in plant pathogens. Among SSCP are cerato-platanins (CPs), which are suggested with role in cell wall remodeling and relevant in the pathogenesis in some fungal species, and have been demonstrated as elicitors of the plant immune response.
Fusarium sp. INECOL-BM-04, isolated from the ambrosia beetle *Xylosandrus morigerus* is pathogenic to forest and agricultural species. In order to get insight of the molecular mechanism that drive the virulence of *Fusarium* sp. we characterized replacement mutants in a SP10 and a CP. In comparison to WT *Fusarium* sp. ΔSP10_6-5 does not present affectation in mycelia morphology, but there is a reduction of the colony diameter in different culture media, reduction in conidia production, and the pathogenic process in *Populus nigra* is affected. While *Fusarium* sp. ΔCP1-4 showed dense white aerial mycelia and absence of mycelia and culture pigmentation grown in PDA and bigger colony in MM. Interestingly, this strain is more tolerant to cell wall stress.
These results suggest that the secreted SP10 and CP have role in development and pathogenesis *Fusarium* sp. INECOL_BM-04 associated with the ambrosia beetle *X. morigerus*, denoting the relevance of the secretome in the life cycle and style of this fungus.

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Small RNAs during mycoparasitism in *Trichoderma atroviride*

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Abstract:

Small RNAs are short fragments ranging from 20 to 30 nucleotides that can perform post transcriptional gene silencing (PTGS) in eukaryotic species (1). In *Trichoderma atroviride* they are involved in growth, asexual development, and hyphal regeneration (2, 3). In this work, we obtained small RNA-Seq libraries of *T. atroviride* WT strain and a mutant strain of a dicer2 gene (Δ*dcr2*), which is defective in mycoparasitism. The WT and Δ*dcr2* strains were confronted against *Alternaria alternata* in three different times: before *T. atroviride* contacted the fungal prey, at the physical contact with the prey and when *T. atroviride* overgrew the prey. The small RNA size distribution showed peaks of 21 and 22 nucleotides in the WT strain, however those peaks were lost in Δ*dcr2* strain. We identified thirty microRNA-like RNAs (milRNAs) in the WT libraries, but they were lost in the Δ*dcr2* strain. We also identified small interfering RNAs (siRNAs), on average we detected 2,100 and 789 siRNAs in control and mycoparasitism conditions in the WT strain libraries. However, most small RNA clusters were lost in the *Δdcr2* mutant, remaining only 36 and 99 siRNAs in Δdcr2 strain. We searched targets for the differentially expressed milRNAs and siRNAs under mycoparasitic conditions and that were lost in *Δdcr2* strain. The targets were involved in transport, protein regulation and transcription regulation.

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Analysis of the influence of genes encoding for proteins with nitronate monooxygenase activity on different *Metarhizium* lifestyles

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Nitroalkanes are hydrocarbon derivatives from fuels, chemical industry wastes, tobacco smoke, pesticides, pharmaceuticals, etc. Some organisms, including plants and fungi, also produce nitroalkanes in the form of toxins as a defense mechanism (1).

Metarhizium brunneum contains a family of six genes in its genome that code for proteins with nitronate monoxygenase activity. These genes catalyzing the oxidation of nitroalkanes to their corresponding carbonyl and nitro compounds to protect the host from the toxic effects of the agent and, in the case of some organisms, use them as a source of nitrogen and carbon (1). These proteins are characterized by a higher affinity to nitroalkane 2-nitropropane, and their biological activity in vivo is still not well described.

In this work, the construction of the null mutant of the *NMO3* gene by homologous recombination and the effect of the deletion on the virulence of the fungus on *Plutella xylostella* insect and during the interaction of the fungus with sorghum (*Sorghum vulgare*) plants was carried out. Additionally, the ability of *M. brunneum* strain CARO19 and the null mutants of the *Nmo1(Δnmo1-B2), Nmo2 (Δnmo2),* and *Nmo5 (Δnmo5)* genes, to grow in the presence of 2-nitropropane, 1-nitropropane and nitroethane at different concentrations was evaluated.

(1) Cervantes *et al* (2020). *Applied Microbiology and Biotechnology*. DOI.org/10.1007/s00253-020-10450-0.

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The role of *Trichoderma atroviride* small RNA 2 in plant immunity

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The arms race between plants and pathogens has gained significant relevance due to the growing worldwide demand for food. Therefore, research aimed at improving crop plant resistance to biotic and abiotic stress, promoting growth, and quality of plant products is booming. In the rhizosphere, plants regularly interact with a wide variety of microorganisms such as bacteria, oomycetes, and fungi. However, a cross-kingdom interaction can be deleterious or beneficial to plants when there is contact with pathogens or beneficial fungi from the *Trichoderma* genus. These filamentous soil fungi can achieve a mutualistic relationship with plants and deliver multiple benefits by which they can be used as a bio-stimulant and bio-fertilizer. During the interaction, *Trichoderma* may initially be recognized by the plant as a pathogen, hence triggering the plant's defense mechanisms. However, this first immune response can be suppressed by *T. atroviride* through a cross-kingdom silencing mechanism that has been actively described to improve the local, and systemic defense response of the host plants. These biocontrol fungi trigger a physiological phenomenon called priming and enable plants to respond more rapidly and robustly to a virulent interaction.

In this project, we aim to characterize the role of small RNA 2 (sRNA2) of the beneficial microorganism *Trichoderma atroviride* and its potential target genes in the model plant Arabidopsis thaliana. Such gene targets code for proteins involved in the synthesis of defense compounds, redox signaling, and other processes that contribute to the plant's ability to resist pathogens. And to understand how this mutualistic relationship enhances the pathogen defense mechanisms of the plant. Co-infiltration data performed on the interaction of srna2 and its target in a crop plant will be presented.

Keywords: Trichoderma, Silencing mechanism, Cross-kingdom interaction, Plant Immune system, plant.

SCP3 as an effector candidate from *Trichoderma atroviride*

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One of the main problems in agriculture is related to diseases caused by phytopathogenic fungi. Biological control offers represents an alternative for its treatment. Fungi belonging to the *Trichoderma* genus are among the most successful biological control agents in agriculture, due to their antagonistic properties towards pathogens. Additionally, *Trichoderma* is able to beneficially associate with plants, and there is increasing evidence that this association is established through effector proteins, whose function modifies the physiology of the plant, enabling the biological interaction. To contribute to the knowledge of factors involved in the associations that *Trichoderma* establishes with plants and other microorganisms, we searched for proteins with effector characteristics in the *Trichoderma atroviride* genome. The SCP3 protein was selected since bioinformatic analysis indicated the presence of a secretion signal peptide as well as a nuclear localization sequence. Moreover, the available transcriptomic data show *scp3* expression during the interaction of *T. atroviride* with *Arabidopsis thaliana*. The main objective of this work is to determine the ability of the SCP3 protein to internalize in the tissue of the hosts reported for *T. atroviride*, whether they are plants or fungi. Currently, we are purifying SCP3 tagged with the mCherry fluorescent protein, from a heterologous system and we will analyze the localization of this chimeric protein in *A. thaliana* root cells and some fungal pathogen strains.

Exploring the interaction between obligate and facultative mangrove marine fungi isolated from Pichilingue, La Paz, Baja California Sur

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Mangrove forests are a unique plant community that hosts rich assemblages of species in the coastal intertidal zone of tropical and subtropical latitudes. The mangrove forests are facing natural and anthropogenic disturbance effects like climate change, invasive species, and unsustainable management. Consequently, the mangrove degradation and loss negatively impact mangrove productivity and ecosystem services provided. Few studies on the physiology of microscopic fungi from mangroves have been published. A group of microscopic fungi called “manglicolous fungi” inhabit this ecotone. These microorganisms may play a role in the nutrient cycling in these habitats. During the performance of their complex ecological functional activities, several of them can produce unique natural products with biological activity as primary metabolites, secondary metabolites, antibiotics, enzymes, alcohols, organic acids, and biopolymers. Invasive fungal species with tolerance to saline environments may be a threat to the mangrove health. The activity of those introduced nonnative fungal species could cause an alteration of the community structure manifested by a decrease in abundance values or. This study explores the interaction between three fungal species *in vitro.* The fungal strains of the obligate marine fungus *Lulworthia* sp and the facultative marine fungi *Trichoderma* sp. and *Fusarium* sp. were collected from mangrove area adjacent to the Unidad Académica Pichilingue UABCS. The fungi were obtained in culture using the standard protocols for isolation and identification. Voucher strains were deposited at the MGV culture collection, Instituto de Biología, UNAM. A known spore concentration of *Trichoderma* sp. and *Fusarium* sp. was *in vitro* tested on the strain of *Lulworthia* sp. The effect of the facultative marine fungi on the development of the marine obligate fungus was shown to be antagonistic. As such, *Lulworthia* sp. is especially susceptible to the presence and number of spores of the *Trichoderma* and *Fusarium* sp. The finding highlights the need to study the fungal community structure all year around.

Functional analysis of a predicted lysozyme encoded by the *scp2* gene from *Trichoderma atroviride*

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Nowadays, the increasing response of plant pathogenic microorganisms to current agrochemical pesticides has made it necessary to develop more efficient alternatives focused on reducing environmental and health repercussions, without increasing the associated production and usage costs. Antimicrobial peptides which have shown an antagonistic activity against phytopatogenic bacteria and fungi has been identified in various microorganisms. These molecules are usually cationic with low molecular weight (2-20 kDa), and usually exhibit an amphipathic alpha-helix structure. Among the studied organisms, the fungi belonging to the *Trichoderma* genus stands out as they produce metabolites with antimicrobial potential. *Trichoderma atroviride* is also recognized as a beneficial plant symbiont. The antimicrobial activity is likely related to the fungus´s ability to compete with other microorganisms present in the rhizosphere, contributing to the establishment of an association with the plants. To explore the antimicrobial potential of *T. atroviride*, our work group carried out an analysis of its genome to identify genes whose biological products have the potential to act as antimicrobial agents. The *scp2* gene was selected as a promising lysozyme candidate. In this study, our goal is to determine the antimicrobial activity of SCP2 against phytopatogenic and beneficial bacteria such as *Xanthomonas campestris, Agrobacterium tumefaciens, Rhizobium etli* and *Paraburkholderia caledonica*. We are working on purifying the protein from a heterologous system. Our results will contribute to a better understanding of the association process between *T. atroviride* and plants, as well as the potential use of SCP2 protein in controlling some phytopathogenic bacteria.

A Potential Role of Effector Proteins during *Trichoderma atroviride* and Plant Growth – Promoting Bacteria Interactions

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Trichoderma spp. are soil borne fungi that establish beneficial interactions with different plants, and are capable of mycoparasite several fungal phytopathogens, such as *Fusarium* spp. Such interactions result in positive effects to the plants, like improved plant growth and development, and enhanced resistance against pathogens. *Trichoderma* uses different strategies to establish communication during its interactions with other organisms, such as the use of effector proteins, like Epl1, to modulate plant physiology and defense responses to colonize plant roots, or to attack its fungal prey. In the soil, these fungi are not alone, and can establish different types of interactions with other organisms present, like plant growth – promoting bacteria (PGPBs), which can lead to a synergistic or to an antagonistic relationship among them, thus affecting their overall benefits on the plant or its fungal prey, and possibly, the role of effector proteins. The aim of this work was to determine the expression of *T. atroviride* genes coding for effector proteins during the interaction with different PGPBs, *Arabidopsis thaliana* plants or the phytopathogen *Fusarium brachygibbosum*, to elucidate their role in the establishment of these interactions; and to determine if the combined interaction enhances the beneficial effects of *T. atroviride* and PGPB on the plant and against the pathogen. Our results showed that, during the interaction with *F. brachygibbosum* and the PGPBs, the effector coding genes *epl1, tatrx2* and *tacfem1* increased their expression, especially during the consortia with the bacteria. During the interaction of *T. atroviride* with the plant and the PGPBs, the genes *epl1* and *tatrx2* increased their expression, mainly with the consortium formed with *Pseudomonas fluorescens* UM270, with *Bacillus velezensis* AF12 or with *B. halotolerans* AF23. We also observed that the consortium of *T. atroviride* with *Rouxiella badensis* SER3 is better at inhibiting the pathogen growth, but the consortium of *T. atroviride* with *P. fluorescens* UM270 is better at promoting *Arabidopsis* growth. Our results show that the efficacy of biocontrol agents such as *Trichoderma spp.* could be improved in co-culture with different strains of other beneficial microorganisms, and effector proteins have a potential role in establishing these interactions, not only with plants and pathogens, but also with other beneficial microorganisms such as bacteria.

Identification of *Lasidioplodia theobromae* peptides putative implicated in pathogenesis

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Lasidioplodia theobromae belongs to Botryosphariaceae family and is a critical pathogen of several woody plant species including those of agroeconomic importance. The symptoms provoke by *L. theobromae* results in cankers, dieback, and fruit and root rots. Currently, a vast number of pathogenicity factors in this fungus have been described including secondary metabolites, hydrolytic enzymes and phytotoxins. However, it remains to be elucidated other pathogenicity factors such as peptides. Peptides in some fungi have importance in pathogenicity, nevertheless the information in this area is scarce in *L. theobromae*. In this work through bioinformatic analysis we identified a peptide collection with putative effectors properties. Then we established in vitro pathosystems using different forestry host to analysis the transcripts level of the different peptides in order to infer their related with pathogenicity. This is a first approach to highlight the role *L. theobromae* peptides during the infection process.

Área:

- Interacciones hongo-huésped

Antimicrobial effect of SCP1 from *Trichoderma atroviride* over beneficial and pathogenic rhizosphere bacteria

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Trichoderma atroviride is a beneficial plant symbiont fungus. The plant-*Trichoderma* interaction is modulated by a wide diversity of molecules, including effector proteins. Some of these effectors have already been functionally characterized, highlighting their participation in the modification of plant physiology to facilitate the interaction process. However, little has been described about whether effectors in *Trichoderma* have the ability to modify the rhizosphere microbiome. Previous observations in our work group demonstrated that *scp1* gene expression is induced during co-culture with *Arabidopsis thaliana* and partially purified chimeric protein SCP1 fused to the fluorescent protein mCherry is able to internalize into root tissue. The structural analysis showed that SCP1 has high similarity to Thuricin CD, a bacteriocin from *Bacillus thuringiensis*. These results suggest that SCP1 could not only have an effect on the interaction with the plant, but also by manipulating the root microbiome. In this work, our goal is to determine the antimicrobial effect of the SCP1 protein on beneficial and phytopathogenic soil bacteria, as well as analyze its protective effect on *A. thaliana*, during a bacterial infection. Preliminarily, we observed that the total proteins, obtained from a liquid culture of a *T. atroviride* strain that overexpress the chimera SCP1-mCherry, showed antimicrobial activity against *Xanthomonas campestris*, *Agrobacterium tumefasciens* and *Escherichia coli;* while such effect was not observed when proteins obtained from a wild type strain were used. Currently, we are in process to obtain pure SCP1 protein from a heterologous system, in order to confirm our previous results and test the SPC1 activity against *Paraburkholderia caledonica* and *Rhizobium etli,* and finally to analyze the impact of SCP1 on the severity of the infection generated by *X. campestris* in *A. thaliana* seedlings.

The photorespiratory enzyme glutamate:glyoxylate aminotransferase 1 (GGAT1) plays a key role in the regulation of plant defense responses induced by *Trichoderma* in Arabidopsis

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The mutualistic fungi *Trichoderma* spp. colonize the roots of a broad spectrum of plants, promote plant growth and induce the systemic resistance against foliar pathogens. However, the underlying mechanisms by which these beneficial microorganisms prime plant immunity is not well understood. In a previous work (González-López *et al*., 2021), we found that *Arabidopsis thaliana* Col-0 wild-type plants secrete an array of proteins with enzymatic functions during their interaction with *Trichoderma atroviride*, including the glutamate:glyoxylate aminotransferase 1 (GGAT1), which catalyzes the conversion of glyoxylate to glycine (Gly) during photorespiration. In this work, we found that Arabidopsis plants bearing a mutant allele of *GGAT1* (*ggat1-2*) exhibits compromised expression of the Salicylic Acid-responsive gene *PR-1a* and increased expression of the Jasmonic Acid- and Ethylene-responsive gene *PDF1.2*, which correlated with an enhanced susceptibility to the bacterial hemi-biotrophic pathogen *Pseudomonas syringae* pv. *tomato* DC3000, and enhanced resistance to the necrotrophic fungus *Botrytis cinerea*. We hypothesized that the absence of *GGAT1* in Arabidopsis, causes an accumulation of glyoxylate in the cell, which elicits plant defense against *B. cinerea*. In accordance with this, exogenous application of glyoxylate to Col-0 plant roots, triggered the systemic resistance against *B. cinerea* and induced the expression of *PDF1.2* and *PR-1a*. The photorespiratory-associated amino acid serine (Ser) applied in plant roots failed to rescue the *ggat1-2* susceptibility to *B. cinerea*. In addition to glyoxylate, Ser and Gly caused increased expression of *PR-1a* in Col-0 plants and enhanced accumulation of hydrogen peroxide in leaves when applied exogenously in roots. In addition, Ser, but not Gly, conferred resistance to *B. cinerea* and provoked increased expression of *PDF1.2* in wild-type plants. We further found that overexpression of the *EDA9* (EMBRYO SAC DEVELOPMENT ARREST9) gene in Arabidopsis, that encodes a 3-phophoglycerate dehydrogenase involved in Ser biosynthesis, enhanced plant resistance against *B. cinerea*, indicating that Ser positively modulates plant resistance to *B. cinerea*. Finally, we found that both *EDA9* and *GGAT1* were up-regulated in *Arabidopsis* during its interaction with *T. atroviride*, suggesting that the beneficial fungus modulates the levels of plant resistance-related amino acids in their host. Taken together, our findings suggest a potential connection between photorespiration and plant immune response triggered by *Trichoderma*.

Key words: *Trichoderma*, Arabidopsis, *Botrytis cinerea*, GGAT1, photorespiration.

Abundancia de especies de *Candida* presentes en mujeres adultas jóvenes con sobrepeso y que realizan diferente actividad física

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Introducción. La obesidad es una enfermedad crónica multifactorial, que incrementa el riesgo de padecer enfermedades crónicas no transmisibles, reducción de calidad de vida y muerte prematura. La OMS ha declarado la obesidad como epidemia mundial del siglo XXI. México ocupa el segundo lugar a nivel mundial en prevalencia de población adulta con obesidad. El humano alberga al menos 100 billones de microorganismos, los cuales constituyen la Microbiota, (bacterias, virus, hongos y parásitos). La fracción fúngica de nuestra Microbiota es denominada Micobiota, siendo *Candida* uno de los géneros constituyentes principales. El humano porta distintas especies comensales del género, dentro de estas se encuentra a *Candida albicans* comúnmente la más aislada. No obstante, podemos encontrar otras especies como; *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata* (*Torulopsis*), *Candida guilliermondii*, *Candida krusei* y *Candida kefyr*. C. albicans habita con frecuencia en la mucosa oral, vaginal y gastrointestinal de individuos sanos como un comensal inofensivo. Un equilibrio inter e intra especies que habitan nuestro organismo es relevante para mantener la salud en el humano. Una disbiosis en las comunidades comensales de individuos sanos puede variar la diversidad y la densidad fúngica. Esta disbiosis o variación puede contribuir al sobrepeso y obesidad.
Metodología. Se realizó la toma de muestras orales y fecales en mujeres adultas jóvenes (18 a 35 años) que practican rugby o soccer y tienen sobrepeso, la identificación de especies de *Candida* se realizó por medio de métodos tradicionales, la muestra oral se obtuvo, recolectando la saliva en ayunas en PBS, para la recolección de las SS se les solicito la primera evacuación de la mañana, las muestras se sembraron en chromoagar para su primer aislamiento, posteriormente identificadas mediante morfología microscópica, tubo germinal y auxonograma. Se tomaron medidas antropométricas y peso de cada joven, se tomó en cuenta los criterios de inclusión y exclusión, además del consentimiento informado, se aplicó un cuestionario de alimentación y antecedentes generales.
Resultados y discusión. Las especies identificadas en mujeres con sobrepeso que practican rugby son muy distintas de las que practican soccer. Las mujeres pertenecientes al grupo de rugby mostraron menor diversidad de especies y menor UFC, siendo *Candida albicans* la más frecuente seguida de *C. Spp*, *C. glabatra*; se encontró también el caso de mujeres con sobrepeso negativas al crecimiento de *Candida* en sus heces fecales, al contrario, las mujeres de soccer con sobrepeso y normopeso que presentaron una mayor variedad de especies y mayor número UFC tanto en muestras fecales como orales, siendo *C. albicans* la más frecuente, seguida de *C. glabatra* y *C. sp*.
Conclusiones. La obesidad, el sobrepeso, la actividad física, así como la alimentación pueden ser factores determinantes en la variedad y número de especies presentes de *Candida.*

Palabras clave: obesidad, sobrepeso, micobiota, actividad física, *Candida sp*.

Influence of bacterial endosymbionts on the pathogenic capacity of *Metarhizium*

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Metarhizium is a genus of entomopathogenic fungi distributed worldwide. This property has enabled its use as an active ingredient in producing biopesticides to control agricultural pests (1). Recent studies have shown that this fungus can form mutualistic associations with plants (2). In addition, we have demonstrated that two *Metarhizium* strains carry bacteria that can be transmitted from one generation to the next. From these isolates, we perform their characterization, as well as from some associated bacteria. We identified the fungal species as *M. robertsii* and *M. pinghaense*, both associated with the genera *Bacillus* and *Agrobacterium* bacteria.

On the other hand, we investigated whether these bacteria affect the growth of the fungus. For this purpose, we generated cured strains of both fungal species. We then performed germination, radial growth, and conidiation tests under different growth conditions. In general, the bacterial endosymbionts delayed the germination of the conidia. However, they did not affect the radial growth of the fungus.

Moreover, the rate of conidiation was different in the two *Metarhizium* species. Bacterial endosymbionts in *M. robertsii* significantly increased conidiation when the fungus was cultivated under dark conditions in a minimal medium, but we found no significant difference when it was cultivated in a photoperiod or in rich medium. The opposite occurred in *M. pinghaense*, where bacterial endosymbionts' presence reduced the conidiation rate under the same growth conditions. We then examined the virulence of the fungal strains with a bioassay using *Galleria mellonella* as a model. In this analysis, we found that the presence of bacterial endosymbionts in *Metarhizium* reduced the insect's virulence level.

The results of this project show that *Metarhizium* can form a symbiotic relationship with multiple bacterial species, unlike other fungal models where it is restricted to a single species. In addition, these bacteria may affect the growth of the two *Metarhizium* species tested differently, possibly due to the difference in bacterial load between each species. However, in both cases, the presence of these bacterial endosymbionts significantly reduced the virulence of *Metarhizium* against *G. mellonella*, although the possibility that other bacterial associations or combinations of these with *Metarhizium* have an enhancing effect is not excluded.

(1) San Aw, K. M., & Mun Hue, S. (2017). *Journal of Fungi.* https://doi.org/10.3390/jof3020030
(2) Sasan, R., & Bidochka, M. (2012). *American Journal of Botany.* https://doi.org/10.3732/ajb.1100136

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Keywords: *Metarhizium*, biological control, endosymbionts.

Peptidases involved putatively in the pathogenicity of INECOL_BM-04 and INECOL_BM-06 *Fusarium* sp. strains isolated from the ambrosia beetle *Xylosandrus morigerus*

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The study of fungi associated with ambrosia beetles has recently gained interest because they cause emerging diseases in woody species. *Xylosandrus morigerus* is an ambrosia beetle with a wide distribution in Mexico, and recently we isolated from the beetle two strains named INECOL_BM-04 and INECOL_BM-06 with pathogenic potential in woody plants. The molecular mechanisms produced by hemibiotrophic and necrotrophic fungi such as species of the genus *Fusarium* are necessary in the infection process. However, virulence factors, such as peptidases, of fungi associated with ambrosia beetles are still unknown. The objective of this study was to identify, using bioinformatics and experimental approaches, the peptidases involved putatively in the pathogenicity of INECOL_BM-04 and INECOL_BM-06 strains. The proteome analysis of both strains revealed 27 and 26 peptidases respectively and we group them in the 4 families: S8, S10, S53 and M28. The percent identity between the sequences of both strains were above 90% and a signal peptide was predicted in 81% of the sequences. Experimental approaches using polyacrylamide-SDS gels and activity assays revealed different electrophoretic patterns between the two strains. Differences were also observed in the cellular and extracellular contents generated in the different growth media (PDB and PDB supplemented with 2% casein). Additionally, using electrospray ionization mass spectrometry (ESI-MS/MS), we were able to identified three peptidases from the M24, A1, and S10 families, the last one also inferred by bioinformatics analyses.

These results represent the first approach to studying peptidases and their potential involvement in the pathogenesis of *Fusarium* sp. associated with ambrosia beetles. Future research should focus on the functional characterization of the identified peptidases.

Keywords: S8, S10, molecular virulence factor, proteases

Area: fungus-host interactions.

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Identification and characterization of *Neofusicoccum parvum* peptides potentially involved in its pathogenesis

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The short open reading frames encoded peptides (SEPs) are molecules of up to 100 amino acids involved in various cellular processes including mechanisms of pathogenesis in fungi. *Nefosucoccum parvum* belongs to the Botryosphaeria family that groups important broad-range phytopathogens, and is responsible for significant damage in woody plants including agronomical and forestall species. Until now, the SEPs produced by *N. parvum* related to its growth and pathogenesis are unknown. Here, we established both bioinformatic and experimental approaches to elucidate pathogenesis-related SEPs. First, using the public *N. parvum* UCR-NP2 genome and several bioinformatics tools, were inferred 290 SEPs that were characterized in silico. Then, methodological strategies were improved for the extraction and enrichment of SEPs from *N. parvum* mycelium. Isolated SEPs were identificated through electrospray ionization mass spectrometry (ESI-MS/MS). In summary, the ESI-MS/MS analysis allowed us to identify a total of 45 SEPs between the first 36 and 72 h of *N. parvum* growth, of which 20 have no known function, 12 are related to genetic information processing, 8 with metabolism and growth and 5 were predicted as effectors. To our knowledge, this is the first study that identify SEPs produced by *N. parvum* and their potential role in pathogenesis of this economically relevant fungus.

Keywords: *Neofusicoccum parvum*, peptides, phytopathogen

This work belongs to the area of fungus-host interactions.

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Mass Spectrometry detection of Molecules related to the interaction between mycotoxin-producing fungi and commercial coffee beans.

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SUMMARY

Mycotoxin-producing fungi in packaged coffee beans are more common than we imagine. *Aspergillus* s.p., *Penicillium* s.p., and *Fusarium* s.p. are the main producers of these specialized metabolites.

Mycotoxins are chemically stable molecules and persist during food processing, resisting changes in pH and temperature. The most frequent are Ochratoxins; being Ochratoxin A (OTA) one of the most toxic. They are highly toxic to humans and other animals and are linked to kidney disease, cancer, aleukia, liver disease, hemorrhagic syndromes, and immunological and neurological disorders.

In this study, we analyzed different commercially packaged coffee beans, with three levels of roasting (light, medium, and strong), from which we isolated fungi that were identified by macroscopic and microscopic phenotype, as well as by molecular biology (PCR-ITS).

With the analytical platforms developed in the Laboratory of Biochemical and Instrumental Analysis (LABI), we evaluated with Mass Spectrometry (MS) the production of mycotoxins at different moments of interaction between the fungus and the grain, as well as the production of other molecules that possibly are involved in the interaction.

Effect of combined NSAID-antifungal therapy on the reduction of eumycetic grains

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Eumycetoma is a chronic granulomatous disease caused by fungi that enter the host through traumatic inoculation. The clinical manifestations consist on the formation of fungal grains surrounded by an inflammatory infiltrate affecting the skin, subcutaneous tissue and sometimes bone. These grains form channels from which more grains are excreted, in addition to seropurulent fluid causing an increase in volume and deformation of the affected region. Although there is a great etiological variety, the most common causative agent is *Madurella mycetomatis*. The treatment of eumycetoma is lengthy and includes the administration of antifungals (usually azoles) for prolonged periods, followed by surgery for the removal of the grain and a period of post-surgical antifungals. However, this scheme is usually unsatisfactory, with a high relapse rate and in extreme cases leads to complications such as limb deformity or amputation. One of the factors that could serve as protection to the grain from the action of antifungals is the inflammatory mechanism itself, which could play a role in pathogenesis by creating a barrier that prevents the antifungals from reaching the eumycetic grains. Therefore, in this work we used a mouse model of *M. mycetomatis* eumycetoma to evaluate the effect of a combined diclofenac-amphotericin B therapy. Four groups of immunocompetent BALB/c mice were inoculated intraperitoneally with 120 mg wet weight of fungal suspension and allowed to develop eumycetoma for 50 days. At the end of this time, the treatment was administered for 4 weeks. The control group was administered physiological saline, another group was treated with diclofenac, the third group was administered amphotericin B and the last group received combined diclofenac-amphotericin B therapy. At the end of the treatment mice were sacrificed, and the tissues of the peritoneal cavity were recovered and processed by traditional histological technique. The tissue slides were stained either with hematoxylin-eosin to observe the inflammatory infiltrate; or with PAS stain to observe the fungal structures or with Masson's stain to observe the collagen capsule. The results obtained suggest that *M. mycetomatis* grains are reduced both, in number and size using the combined therapy compared to the untreated control and the monotherapy groups. Also, combination therapy led to a reduction of fungal structures and misstructured eumycetic grains. Taken together, the results suggest that diclofenac-amphotericin B combination therapy may be an effective alternative for the treatment of *M. mycetomatis* eumycetoma.

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Dissecting AGO-sRNA binding affinities between kingdoms of life through RNA-Protein docking analyses

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Introduction: small RNAs (sRNAs) are a class of short non-coding RNAs, with sizes < 200 nucleotides, which modulates the expression of target genes at transcriptional, posttranscriptional, and translational levels, in a process called RNA interference (RNAi). The sRNAs interact with Argonaute (AGO) proteins to perform target regulation. The thermodynamic affinity of sRNAs to a specific AGO define the mechanism by which the sRNA will modulate target expression. Cross-kingdom RNAi (ck-RNAi) refers to the inter-organism communication based on the exchange of sRNAs between different biological kingdoms. The ck-sRNA-AGO affinity profiles remains a poorly understood phenomenon. Using *Trichoderma atroviride-Arabidopsis thaliana* mutualistic interaction sRNA- and mRNA-seq libraries, we previously identified potential ck-sRNAs and their putative targets, as well as their possible biological outcomes. To explore more in deep about how ck-sRNAs would have affinities with their potential AGO proteins, we performed an RNA-Protein (RNP) docking analysis using the different domains of distinct kingdoms AGOs with a set of miRNAs from *A. thaliana* (ath-miR390a) and *Homo sapiens* (hsa-miR20a) with known sRNA-AGO selection and our ck-sRNAs candidates.

Results: We found that sRNA binding affinities profiles for AGO domains were identical in all studied AGOs, with Piwi being the most stable and AGO Linkers (L1 and L2) resulted the least stable. Additionally, we found that regardless of their origin, all AGOs can perform stable binding to every tested miRNA, with miRNA 5p arms having slightly better affinity. Also, we discovered that the sRNA-binding stabilities of the PAZ, MID, L1, and Non characterized domains vary depending on the AGO.

Conclusions: According to our findings, AGOs exhibit a conserved sRNA binding affinity order among their domains. However, certain AGOs have a different binding affinity context than others, being a potential factor in their capacity for cross-kingdom sRNA loading.

Keywords: small RNAs, Cross-kingdom RNAi, AGO, RNP-docking, miRNA, tRH, Trichoderma.

Identification of interactor proteins of FkCER1 and FkCER2, two Cerato-platanin proteins from ambrosial fungus *Fusarium kuroshium*.

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The ambrosial fungus *Fusarium kuroshium*, is the causal agent of the Fusarium dieback, a disease that produces serial damages in ornamental and wildland trees species, as well as in those with agronomic interest. This fungus is native from Asia, but in 2015 was identified in Tijuana, Mexico. Despite its infectious potential, little is known about the molecular mechanisms involved in pathogenesis. Therefore, using transcriptomic data, we identified two cerato-plantanin proteins of *F. kuroshium*; FkCER1 and FkCER2. Cerato-platanin proteins are cysteine-rich proteins unique to fungi and are proposed to be involved in development as well as in host virulence response. In this work, we intend to identify those proteins from both plant and fungus interacting with FkCER1 and FkCER2. Consequently, we expressed FkCER1 in a heterologous system, and the purified protein was used to perform interaction experiments in *Citrus cinensis* and *Fusarium solani* using immobilized metal chelate affinity chromatography, electrophoresis 2D and ESI-MS/MS spectrometry. Finally in this work, we will infer possible plant protein targets, and fungal proteins involved in defense response.

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Establishment of *Candida glabrata* biofilm on epithelial cells

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The invasive infections by *Candida* spp. has increased around the world, at a rate of 700,000 cases per year. Recently, non-albicans Candida, such as *C. glabrata, C. tropicalis* among others, have been increasingly identified in cases of candidosis. *C. glabrata* is a haploid, asexual yeast, whose cell wall is a complex structure composed of two layers. *C. glabrata* expresses different factors of virulence, for example, the formation of biofilm on biotic or abiotic substrates. This biofilm contributes to the establishment and persistence of the infections. Currently, the formation of biofilm on epithelial cells it has been little explored. Accordingly, the aim of this study was stablished the *C. glabrata* biofilm on epithelial cells. The formation of a confluent monolayer of epithelial A549 cells was standardized by testing different concentrations and incubation times. The cell concentration 4×10^5 cells/mL in 24 well-plates with incubation for 48 h at 37°C with 5% CO_2 were the conditions to get a confluent monolayer. Afterwards, different concentrations of yeasts as inoculum (5×10^4, 1×10^5, 2×10^5, and 1×10^6 blastoconidia/mL), and different times of interaction (4, 12 and 24 h) were tested for the establishment of biofilm on the confluent cell monolayer. The inoculation of 2×10^5 blastoconidia/mL after 4 h of adherence was the yeasts concentration with better biofilm formation at 24 h. In addition, the biomass quantification confirmed this finding. In conclusion, the *C. glabrata* biofilm was established on epithelial cells *in vitro* conditions.

Proteinase A is involved in the cell death of *Ustilago maydis*

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To determine the importance of the *Ustilago maydis* PEP4 gene, which encodes the acid vacuolar proteinase A, in cell death, we propose using *U. maydis* as a eukaryotic model organism to study the molecular mechanisms underlying autophagy, apoptosis, and necrosis pathways. To assess the significance of the *U. maydis* pep4 gene, orthologous to human Cathepsin D, in cell death, we compared the ability of the wild-type and Δpep4 strains to accumulate autophagic bodies within vacuoles when exposed to carbon stress conditions.

Additionally, we evaluated the resistance to stress conditions and cell death between the wild-type and Δpep4 strains. Wild-type and Δpep4 cells were incubated in complete medium (MC) until they reached the stationary phase. Subsequently, the cells were incubated in minimal medium to induce necrosis, and then processed for multiphoton microscopy. Our observations revealed that Δpep4 cells exhibited greater resistance to H_2O_2 compared to the wild-type cells. This suggests that proteinase A is indeed involved in programmed cell death in *U. maydis.*

Interaction networks between yeasts from agave fermentations

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The production of traditional agave spirits such as mezcal, depends on open agave fermentations in which many yeast and bacteria species interact forming a complex microbial community. These interactions often depend on the production of compounds that can either enhance or inhibit the growth of specific species, thereby altering ecological succession and population dynamics. Among the mechanisms of antagonistic interactions some yeasts have the ability to produce “killer” toxins. These secreted yeast toxins inhibit the growth of sensitive strains of the same or related species, but not of other toxin secreting yeasts. These interactions can influence the quality of the final product, as documented in wine fermentations, but little is known about the interaction networks between microorganisms in agave fermentations. This study aims to identify the interactions among yeasts from over 90 agave fermentation tanks from all agave spirit producing regions throughout Mexico. Taxonomic identifications were performed using ITS amplicon sequencing, and co-occurrence analyses were used to identify the potential interactions. After detecting positive and negative relationships, pairwise co-cultivation experiments will be conducted to corroborate the observed interaction patterns. Finally, we will perform killer assays with isolated strains of specific species to determine which are expressing killer toxins and which are sensitive to them. Our work will help to understand the yeast interaction network of agave fermentations which most probably impacts the fermentation outcome. The knowledge generated will also have the potential to guide strategies for the optimization of fermentation processes, such as the assembly of mixed starter cultures.

Characterization of the response of *candida glabrata* clinical isolates to immune cells

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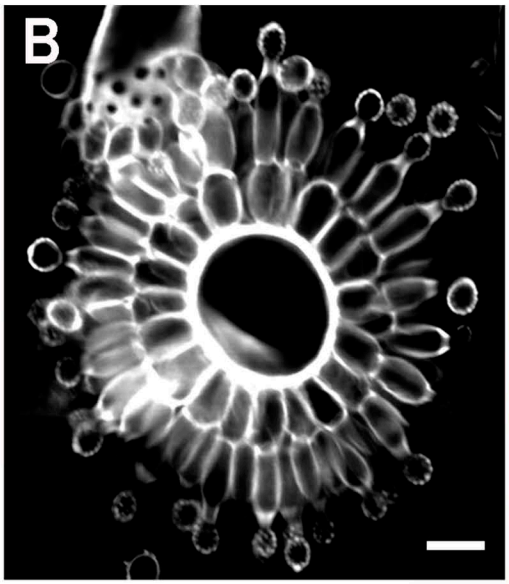
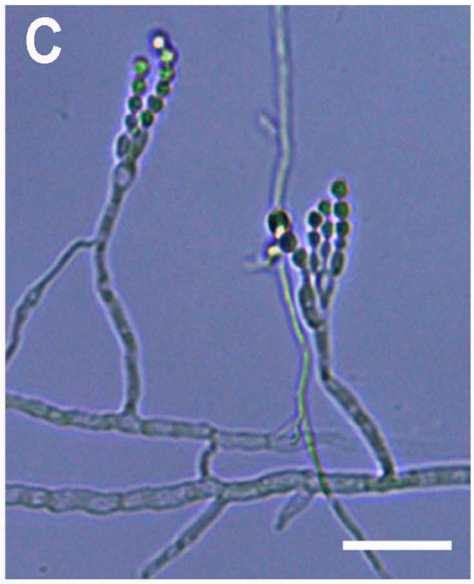
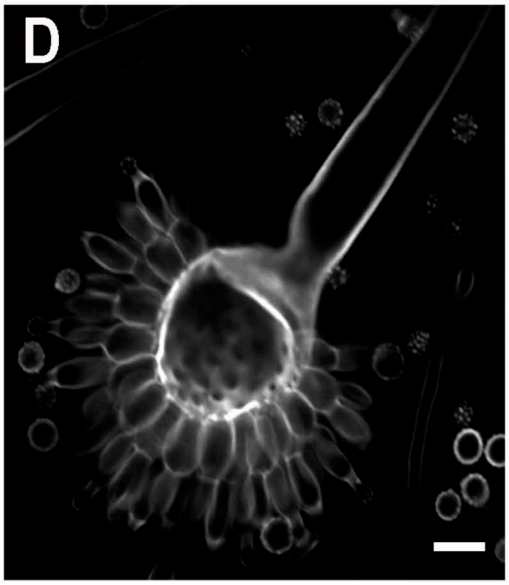
Infections by fungal pathogens are increasing due to a rise in an immunocompromised population. Several species of *Candida*, such as *Candida glabrata,* have increased their prevalence dramatically as a cause of bloodstream infections in part due to their high intrinsic and acquired resistance to antifungals. In addition, *C. glabrata* is characterized by a relatively low susceptibility to neutrophils, effectively evading the immune response. *C. glabrata* can induce a low-grade inflammatory response by silently transiting within the host, leading to a delay in neutrophil activation and a successful establishment and persistence in the host.

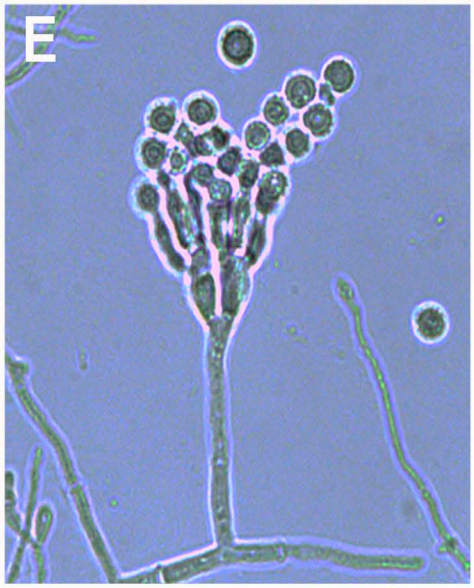
To gain insight into the mechanisms used by *C. glabrata* to partially resist neutrophil attack, in this work we aimed to characterize the microevolution of *C. glabrata* clinical isolates and a standard laboratory strain during phagocytosis by neutrophils *in vitro*, by repeatedly exposing yeast cells to neutrophils, and phenotypically characterizing the surviving *C. glabrata* cells with respect to the response to oxidative and thermal stresses as well as the susceptibility to antifungals and the stability of the phenotypes of the surviving evolved cells. Data of the characterization of cells obtained from experiments in which we performed three consecutive exposures of *C. glabrata* to neutrophils will be presented.

Keywords: *C. glabrata*, neutrophils, antifungals, oxidative stress, microevolution, immune response.









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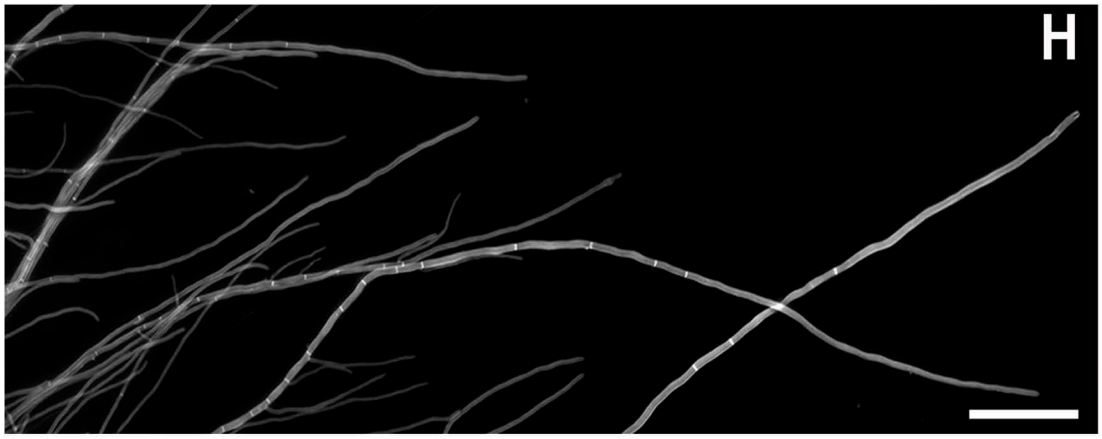

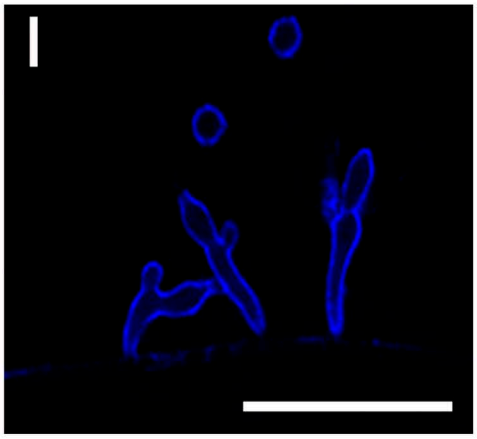

"Neo-functionalization in *Saccharomyces cerevisiae*: A Novel Nrg1-Rtg3 chimeric transcriptional modulator is essential to maintain mitochondrial DNA integrity"

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Abstract

In *Saccharomyces cerevisiae* the transcriptional repressor Nrg1 (Negative Regulator of Glucose-repressed genes) and the b/Zip transcription factor Rtg3 (Retrograde regulation) mediate glucose repression and mitochondria to nucleus signaling, respectively. Here we show a novel function for these two proteins, in which alanine promotes the formation of a chimeric Nrg1/Rtg3 regulator that represses the *ALT2* gene (encoding an alanine transaminase paralogue of unknown function) expression. A *NRG1/NRG2* paralogous pair, resulting from a post-wide genome, small scale duplication event, is extant in the *Saccharomyces* genus. Neo-functionalization of only one paralogue resulted in Nrg1, able to interact with Rtg3. Either *nrg1*Δ or *rtg3*Δ single mutant strains are unable to utilize ethanol and show a typical petite (small) phenotype on glucose. Neither of the WT genes complemented the petite phenotype, suggesting irreversible mitochondrial DNA damage in these mutants. Neither *nrg1*Δ nor *rtg3*Δ mutant strains express genes encoded by any of five polycistronic units transcribed from mitochondrial DNA *in S. cerevisiae*. This, and the direct measure of the mitochondrial DNA gene complement confirms that irreversible damage of the mitochondrial DNA occurred in both mutant strains and is consistent with an essential role of the chimeric Nrg1/Rtg3 regulator in mitochondrial DNA maintenance.

***Key words*:** Chimeric regulator, Mitochondrial DNA integrity, Neo-functionalization and *Saccharomyces cerevisiae.*

Identification and characterization of long noncoding RNAs in *Candida glabrata*

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Candida glabrata is an opportunistic pathogenic fungus that presents higher resistance to treatments than *Candida albicans*. Yeasts of the genus *Candida* have been reported to exhibit various mechanisms of resistance to treatments. The role of lncRNAs in candidiasis is of interest because it has been reported that these non-coding RNAs can have a regulatory role in the immune response in other diseases caused by pathogens such as *Candida albicans* or *Aspergillus fumigatus*. These transcripts can be expressed under certain conditions, and they can act on the genome, transcription factors, histone modifying complexes and protein binding factors, among others.

When a pathogen colonizes its host, it requires a refined machinery to acquire nutrients such as carbon and nitrogen, among others. Previous studies from our laboratory on nitrogen catabolism in *C. glabrata* indicated that the preferential use of available nitrogen sources depends on a widely studied mechanism known, in *Saccharomyces cerevisiae,* as Nitrogen Catabolite Repression (NCR), for which a key protein is Gln3. Therefore, from a transcriptomic analysis obtained from a *gln3*Δ strain grown in minimal medium with ammonia as a nitrogen source, a list of possible lncRNAs for *C. glabrata* was obtained.

In this study, using a *gln3*Δ mutant and a wild-type strain, we generated mutants by double homologous recombination for some of the lncRNAs identified in *C. glabrata* to elucidate whether they are involved in the regulation of the expression of their neighboring genes. So far, we have found that there is no difference in the doubling times of the mutant strains obtained; there is an ability to hydrolyze starch for the *gln3*Δ mutant which is lost in the *gln3*Δ *lnc10587*Δ strain and finally, we observed a decrease in the expression of the genes surrounding *lnc383*, when this lncRNA is deleted, compared to the parental strain BG14.

KEYWORDS: lncRNA, *C. glabrata*, *GLN3*.

Characterization of Stress-Response Pathways in the fungal pathogen *Candida glabrata*

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All cells are exposed to environmental changes. Microorganisms respond to environmental signals by regulating their gene expression. **E**nvironmental **s**tress **r**esponse (ESR) has been studied in *Saccharomyces cerevisiae,* which is closely related phylogenetically to *Candida glabrata*, an opportunistic fungal pathogen that causes invasive candidiasis. The ESR is in part regulated by the transcriptional factors Msn2 and Msn4. These proteins recognize the STRE sequence in promoters of genes that encode proteins with functions related to the oxidative stress response, nutritional stress, or heat shock, however; in *C. glabrata* it is unknown how these proteins act coordinately during the ESR. The purpose of this work is to evaluate the ESR in *C. glabrata* by the characterization of the transcriptional factors Msn2 and Msn4 and their regulatory pathways.

"Importance of subcellular localization of proteins that are part of the Nrg1/Rtg3 transcriptional chimeric complex in *Saccharomyces cerevisiae*"

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In *Saccharomyces cerevisiae*, the transcriptional repressor Nrg1 (Negative Regulator of Glucose-repressed genes) and the transcripcional factor Rtg3 (ReTroGrade regulation), mediate glucose repression and mitochondrial to nucleus signaling. In our laboratory, we demonstrated a novel function for both proteins, which is the formation of a chimeric Nrg1/Rtg3 regulator. Nrg1 and Rtg3 interaction is favored by the presence of alanine. In addition, this chimeric regulator represses the expression of *ALT2* gene (encoding an alanine transaminase paralogue of unknow function). Additionally, we found that the nrg1Δ and rtg3Δ single mutants strains are unable to utilize ethanol as a sole carbon source and show a typical petite (small) phenotype in glucose. Most interesting was the finding that complementing the mutants with the WT genes did not allow wild type recovery indicating that lack of either Nrg1 or Rtg3 result in irreversible damage to respiratory metabolism and to the mitochondrial DNA stability.

Therefore, it can be concluded that the presence of the Nrg1/Rtg3 chimeric regulator is of great importance for the maintenance of mitochondrial DNA in *S.cerevisiae*, which has given rise to several lines of novel research projects. In the present work we are interested in to localizing the subcellular location of the Nrg1 and Rtg3 proteins using fluorescent markers as well as the formation of Nrg1/Rtg3 chimeric complex using BIFC (Biomolecular Fluorescent Complementation Assay). The principal objective of this research is to understand the mechanisms of interaction between both proteins and to verify the subcellular compartments that are involved both in the function of the proteins individually and in the formation of the complex to obtain more information about their composition and the importance of their organization in their function. We will also evaluate the correlation of the formation of the Nrg1/Rtg3 complex with the presence of alanine *in vivo* and to determinate when this regulator is required in the life cycle of *S.cerevisiae.*

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Participation of the orthologous genes *NRG1* and *RTG3* in the yeast *Kluyveromyces lactis* respiration.

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The transcriptional regulator proteins Nrg1 and Rtg3, in the yeast *Saccharomyces cerevisiae*, form a novel and recently described hybrid complex, involved in mtDNA maintenance. If one of the coding genes of these proteins is eliminated, the respiratory function in the cell is compromised, the culture renders fermentative only and displays the “Petite” phenotype. In a more profound study, mtDNA is disrupted and it is clear that complementing each of the single mutant strains with the corresponding wild type gene, does not restore the parental phenotype; these data suggest the relevance of the Nrg1-Rtg3 complex for the respiratory metabolism in *S. cerevisiae*.

The petite-negative yeast *Kluyveromyces lactis*, that presents a predominantly respiratory metabolism, also posses orthologous genes to *NRG1* and *RTG3* in its genome. Although It is plausible that the products of these genes, are involved in respiration and mtDNA maintenance, their function in *K. lactis*, has not been documented.

In this work, we are interested in describing the function of the Nrg1 (KlNrg1) and Rtg3 (KlRtg3) proteins in *K. lactis* respiratory metabolism, through the use of the directed gene mutation technique and the observation of the mutant strains phenotype over different carbon sources.

We are also interested in determining whether KlNrg1 and KlRtg3 are forming a complex; this objective will be accomplished by a co-immunoprecipitation assay.

This series of experiments will shed light over the evolution of hybrid transcriptional regulators in divergent lineages as is the case of *Saccharomyces* and *Kluyvemormyces*.

"The Hap2-3-5-Gln3 Hybrid Transcriptional Complex: Identification of its Organization and the Gene Circuit Under Its Control"

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Abstract: The *S. cerevisiae* transcriptional response to different physiological conditions depends on a repertoire of transcriptional modulators that decipher regulatory information through their specific binding to cognate sequences and their capacity to recruit the components necessary for the formation of a given transcriptional complex. These regulators contain two domains: the DNA-binding domain and the activation domain, which can be found in a single polypeptide or in different polypeptides. In yeast, both the DNA-binding and activation domains are present in a single polypeptide. However, The HAP complex, is constituted by four polypeptides: Hap2, Hap3, Hap5 which constitute the DNA-binding domain and Hap4 which corresponds to the activation domain. In 1989 it was proposed that yeast could form hybrid transcriptional modulators by creating complexes as Hap2-3-5 (DNA binding domain) and X (a foreign activation domain which is not Hap4)[1]. This new regulator Hap2-3-5-X would elicit a unique response different from that generated by the Hap2-3-5-4 complex. In 2011, our research group discovered the first hybrid modulator consisting of the Hap complex binding domain and a foreign factor substituting Hap4: Hap2-3-5-Gln3, which had a novel transcriptional role absent in Happ2-3-5-4 and in Gln3 transcriptional modulators when these work independently[2]. The aim of this study is to determine the organization, target gene network, physiological role and chromatin interaction of the hybrid transcriptional modulator Hap-2-3-5-Gln3, with the pertinent promoters comparing its properties with those of the native regulators that form it (HAP complex and Gln3). The methodology employed includes techniques such as: Next Generation Sequencing (RNA-seq), Coimmunoprecipitation (CoIP), Real-time PCR (qPCR) and Nucleosome Scanning Assay (NuSA). So far, we have worked on the analysis of the interaction of the proteins that form the complex in the presence of repressive (glutamine) and non-repressive (proline) nitrogen sources and so far the finding obtained has been that as expected, in proline as sole nitrogen source (non-repressive nitrogen source) the formation of the Hap-Gln3 complex is not observed, while when yeast is grown on a repressive nitrogen source such as glutamine, Hap-Gln3 complex is formed, surprisingly mediating *GDH1* expression on repressive nitrogen sources. The physiological role of hybrid transcriptional regulators will be discussed.

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Characterization of *Candida glabrata* strains derived from azole-induced microevolution.

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Candida glabrata is an opportunistic pathogenic yeast that produces infections in immunocompromised patients, and in severe cases, systemic infections. The management of systemic infections is of great concern because of the increased prevalence of Candida worldwide and the rapidly acquired and innate resistance to azole and other first-line antifungals of *C. glabrata*.

C. glabrata has developed a series of strategies to develop fluconazole resistance (FLC^R). The main mechanisms of azole resistance are orchestrated by *PDR1* transcription factor mainly through increased expression of efflux pumps which transport antifungals outside the cell. Loss of mitochondrial function is also associated with the surge of FLC^R strains during infections and *in vitro* microevolution experiments. In addition, epigenetic modifications such as histone methylation marks H3K36 generated by Set2 and H3K4 catalyzed by Set1 are related to gene expression changes linked to FLC^R.

In this work we performed a preliminary characterization of FLC^R mechanisms of *C. glabrata* strains derived from a previous *in vitro* microevolution experiment in the presence of fluconazole. We isolated a series of isolates from three strains with FLC^R phenotype. Different outcomes of stability of FLC^R phenotype were observed since a group of isolates acquired a mitochondrial dysfunction phenotype (Gly-) and other isolates present FLC^R and respiration competence (Gly+). Reversibility of FLC^R phenotype was observed after growth in the absence of selective pressure for some colonies of all strain lineages tested. The occurrence of unstable FLC^R phenotypes suggests the presence of unstable epigenetic adaptations in these evolved strains. To explore the role of epigenetic modifications (methylations of lysines 4 and 36 of H3) on the surge of microevolved FLC^R strains, we will analyze the role of histone H3 methylations produced by Set1 and Set2 in the rapid FLC^R emergence and whether these changes are linked to mitochondrial dysfunctions. We will evaluate the presence of mitochondrial DNA and respiratory capacity, in these FLC^R strains to explore the connection between mitochondrial function, histone methylation and fluconazole resistance.

The understanding of mechanisms which mediate the emergence of transitory or permanent FLC^R in *C. glabrata* allows the use of integrated treatments that avoid the surge of antifungal resistance during persistent infections. This underscores the importance of developing alternative drugs which target genes required for the onset of resistance.

Differential expression of cell wall proteins associated with *Candida albicans* biofilms inhibition by phenolic compounds from oregano (*Lippia greaveolens* Kunth) stem.

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Infections associated with *Candida albicans* biofilms and consequent antifungal resistance are on the rise with the increased use of indwelling medical devices (1). It is necessary to develop new therapeutic strategies due to the high morbidity and mortality and the massive economic expense associated with this type of infection. The essential oil extracted from leaves of the Mexican oregano (*Lippia graveolens*) is used in the manufacture of cosmetics and drugs. Usually, after the essential oil extraction from oregano leaves, the stems are no longer of commercial value. However, these remnants contain flavonoids and phenolic acids (3) with antifungal potential. In this study, we evaluated the effect on the inhibition of *C. albicans* biofilm formation of an ethanolic extract from the stem of Mexican oregano (*Lippia graveolens* Kunth) and its associated molecular mechanisms. Ten biofilm-forming strains of *C. albicans* were included. Assays to determine the Minimum Inhibitory Concentration (MIC), Minimum Lethal Concentration (MLC), and the effect on the inhibition of biofilms were carried out in microtiter plates using RPMI medium and the ethanolic extract of the stem of Mexican oregano at different concentrations. Plates were incubated at 37 ^{o}C for 24 h, and the biofilm formation was measured in a spectrophotometer at 450 nm. After, the Cell Wall Proteins (CWPs) of *C. albicans* were extracted and analyzed on SDS-PAGE gels and sequenced using LC-ESI-HDMSE. Finally, the expression of some selected CWPs was validated by RT-qPCR. The MIC and MLC to the oregano stem extract determined for the ten isolates of C. *albicans* in planktonic cells were 16 mg/mL and 32 mg/mL, respectively. In the biofilm inhibition tests of the ten *C. albicans* isolates with the oregano stem extracts we observed that the MIC and MIC/10 inhibition biofilms formation was between 70 to 90%. A total of 30 CWPs were identified, some of which were differentially expressed, such as flocculin, *SSA4*, *HSP70*, *RTB1*, *TSA1*, and *ALS3*, whose expression was validated by RT-qPCR.

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Effect of teotihuacanin on the expression of ABC-type transporters in the fungal pathogen *Candida glabrata*.

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The incidence of invasive *Candida* infections in severe immunosuppressed patients has increased significantly in the last 30 years, and *C. glabrata* accounts for more than 25% of invasive clinical cases worldwide. The use of antifungal drugs as prophylactics has led to an increase in resistance, particularly to fluconazole (FLC). Resistance to azoles is mediated by the transcription factor Pdr1, which in turn induces the expression of ATP-binding cassette (ABC) transporter encoding genes. Research is now focused on finding inhibitors or modulators that block the extrusion activity of these transporters, known as efflux pump inhibitors (EPI). These compounds can be found as secondary metabolites from medicinal plants. For example, teotihuacanin (TH), a diterpene isolated from *Salvia amarissima*, showed a synergistic effect with vinblastine, increasing its sensitivity to the MCF-7 cancer cell line. Resistance to vinblastine is also mediated by ABC transporters, which opens the possibility of using TH and other phytochemicals as fungal EPI. The purpose of this study is to determine whether TH and FLC increase the susceptibility of *C. glabrata* to FLC by inhibiting the activity of ABC transporters. We will screen for antifungal activity of TH in FLC-sensitive and FLC-resistant strains; evaluate whether the presence of TH affects the expression of ABC transporters; and determine the cell localization of ABC transporters in the presence of TH. By comprehensively investigating the role between ABC transporters and TH in FLC-resistance in *C. glabrata*, this research could provide valuable insights into the ongoing global crisis of antifungal resistance. The data will focus on the antifungal activity of TH in FLC-sensitive and resistant strains.

The chimeric regulator Nrg1-Rtg3-alanine maintains the expression of mitochondrial DNA in *S. cerevisiae.*

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Abstract:

Transcription factors are direct regulators of gene expression. Traditionally, these factors are modulated by their nuclear concentration and activated by phosphorylation or ligand binding, leading to different signal-transduction pathways. Nrg1 works by negatively regulating genes encoding gluconeogenesis enzymes, those implied in the Krebs cycle, and those that metabolize carbon sources other than glucose. Rtg3 and Rtg1 form a transcription complex that activates genes of the retrograde signaling pathway between the mitochondrion and the nucleus as a response to mitochondrial dysfunction, resulting in the induction of antioxidant defenses and stress resistance. In previous work, the Nrg1-Rtg3 chimeric complex was found while studying the paralogous genes *ALT1* and *ALT2*. Northern Blot analysis showed that alanine induced *ALT1* expression and repressed that of *ALT2*. In both *nrg1Δ* or *rtg3Δ* mutant strains, *ALT1* expression is not repressed in the presence of alanine, indicating that alanine acts as a co-regulator of Nrg1 and Rtg3. These data suggest that interaction between Nrg1 and Rtg3 leads to transcriptional *ALT2* repression. In this study, we measured the expression of mitochondrial-encoded genes by real-time PCR in a wild-type, *rtg3Δ* and *nrg1Δ* strains, which were grown on glucose-GABA or glucose-GABA-alanine. Since mitochondrial genes are expressed as polycistronic transcripts, we determine the expression of cytochrome oxidase subunits II and III (*COX2, COX3*), apocytochrome b (*COB1*), and two ATP synthase subunits (*ATP6, ATP9*). To compare the expression to nuclear-encoded genes, we analyzed the expression of cytochrome c oxidase subunits VI and VIII (*COX8, COX6*). In addition, we predicted the tridimensional structure of Nrg1, Rtg3, and the DNA promoter with the I-TASSER server, and after that, we used the HDOCK server to predict the interaction between them with or without alanine. This analysis was made to determine if the alanine interacts directly by increasing the stability of the Nrg1-Rtg3-DNA complex. This leads to the conclusion that, in the presence of alanine, Nrg1 and Rtg3 form a chimeric regulator complex that maintains the expression of mitochondrial DNA; however, a molecular dynamic simulation is needed to further analyze the stability and equilibrium of the chimeric complex.

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Analysis of the change in the expression of virulence and resistance genes in *Candida albicans* exposed to electric current and UV radiation.

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Introduction: *Candida albicans* is one of the main causes of nosocomial infections, mainly affecting immunocompromised patients, critically ill patients, hospitalized in intensive care units, as well as children and neonates who develop fungemias and other infections due to endogenous contamination or through biomedical devices by biofilms (main mechanism of virulence) that confers protection against external agents thanks to the formation of layers that causes inadequate penetration of antifungals, in addition the adhesion produced allows them to resist physical and chemical disinfection methods.

High intensity ultraviolet light such as UVC is applied in healthcare centers as a technique for control and prevention of nosocomial infections since it is highly efficient in preventing microbial growth, however, this efficiency varies according to the surface on which it is worked, different wavelength must be considered for water, air and surfaces, in addition, UV rays must directly affect the pathogen.

It is known that the generation of electrochemical potentials is effective in eradicating microbial pathogens, it has been proposed as a sterilization method by physical alteration by producing nanopores in biological materials (such as cell suspensions) causing the permeability of cell membranes.

This phenomenon (electroporation) has been used in medicine and biotechnology, such as electrochemotherapy, extraction of various compounds and microbial inactivation in food preservation. It is known that the electrical approach controls bacterial adhesion using electrical interactions between bacterial cells and conductive surfaces through electron transfer. Among the various methods to control biofilms, the approach that uses an electric current can be used to separate adhered bacteria or prevent bacterial adhesion.

Objective: To analyze changes in inhibition, morphology and resistance to antifungals in *Candida albicans* when exposed to electric current and UV radiation.

Materials and methods: Prospective study. Different generations of a *Candida albicans* ATCC 10231 strain will be exposed in aqueous medium to sublethal treatments with UV light (254nm) and electric current (50V, 100µA) in different combinations for 5 minutes for subsequent analysis of inhibition percentage (MTT), morphology (SEM), biofilm production (microplate quantification), susceptibility to antifungals (MIC) and gene expression (RT-qPCR).

Results: Preliminary results are expected by September 2023.

Enrichment of a set of genes specifically regulated and related to the overproduction of β-glucans in the BMA2 strain of *Ustilago maydis*.

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β-glucans are glucose homopolymers linked by β-glycosidic bonds, which can be produced by bacteria, fungi, plants and algae. β-glucans have multiple properties, including anticancer, antioxidant, and immunostimulatory activity; as well as physicochemical properties that can be used by industries to use them as a vehicle for drugs, gelling material, additives, etc. β-glucans are structural components, which are regularly produced by cells. The BMA2 mutant strain of *Ustilago maydis* has an overproduction of β-glucans, the yield is competitive with those available on the market, and the regulatory mechanisms responsible for the increase in production seek to be explained in detail, *U. maydis* used as a model organism to investigate biotechnological and biological processes. With the analysis of a microarray of the BMA2 strain and the wild type, the enrichment of a set of specifically up- and down-regulated genes significantly related to the β-glucan biosynthesis pathway. It was determined that various mRNAs that encode enzymes related to carbohydrate metabolism (CAZymes) are upregulated, in addition to the possible increase in β-glucan levels since glycosyl hydrolases are downregulated in the BMA2 strain. The glycosyl hydrolases are involved in the hydrolysis, synthesis or modification of carbohydrates and glycoconjugates. Likewise, members of the Rho GTPases family involved in signaling act as molecular switches and exert upregulation; therefore, the overproduction of β-glucans may be a response to cellular stress and the possible lack of expression of glycosyl hydrolases. We explore the β-glucan biosynthesis regulation and molecular dynamics to improve the production systems we have up to now and improve the knowledge of the mechanisms of adaptation to stress in fungi.

Abf1 is involved in different cellular processes and may form dimers with itself and other proteins.

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Abstract

In the human fungal pathogen *Candida glabrata*, Abf1 or ARS binding factor 1, is a DNA binding protein involved subtelomeric silencing of several *EPA* genes, which encode adhesins that constitute some of the virulence factors of this pathogenic yeast. We previously determined that *Cg*Abf1 is essential for viability and in this work, we sought to determine the function of *Cg*Abf1 in *C. glabrata*. We designed a "shut off" system for *CgABF1* depletion using the *MET3* promoter, which is repressed by the addition of methionine and cysteine and we generated an over-expressing system in which *CgABF1* is driven by the *MT1* promoter, inducible by adding copper to the media. We evaluated the viability in both systems and observed that under both conditions, depletion, and over-expression of *Cg*Abf1, there is a dramatic loss of viability starting at early time points of depletion and overexpression. These data correlate with the abnormal distribution of nuclei in the cells depleted or with overexpression of *Cg*Abf1. The related, non-pathogenic yeast *Saccharomyces cerevisiae* contains the orthologous gene *ScABF1* encoding the *Sc*Abf1. Both proteins share homology throughout the protein, and the identity is strongest at the bipartite DNA binding domains and the CS2 domain involved in silencing (90.24% and 65.22% and 86.67% identity, respectively). We constructed a predicted three-dimensional model of Abf1 which reveals disordered regions that are usually involved in DNA binding processes. In addition, we determined the activity of the *MT-1* promoter required to overexpress Abf1 without loss of viability, by adjusting the $CuSO_4$ concentration. Data from experiments a) to determine whether Abf1 forms homodimers and/or interacts with other silencing proteins, using the Bimolecular Fluorescence Complementation system (BiFC), b) activity of the *ABF1* promoter and c) heterologous complementation of *abf1*Δ strain with *Sc*Abf1 will be presented.

Molecular study of the pH response of T9 environmental strain of *Trichoderma harzianum*

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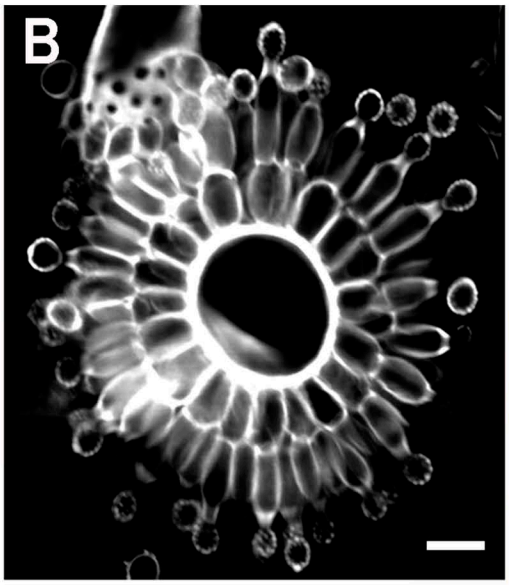
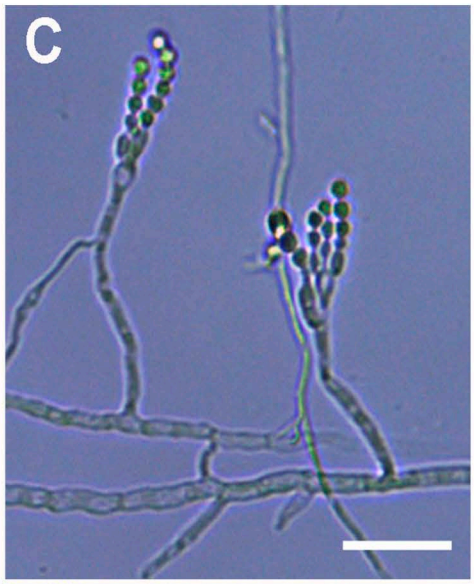
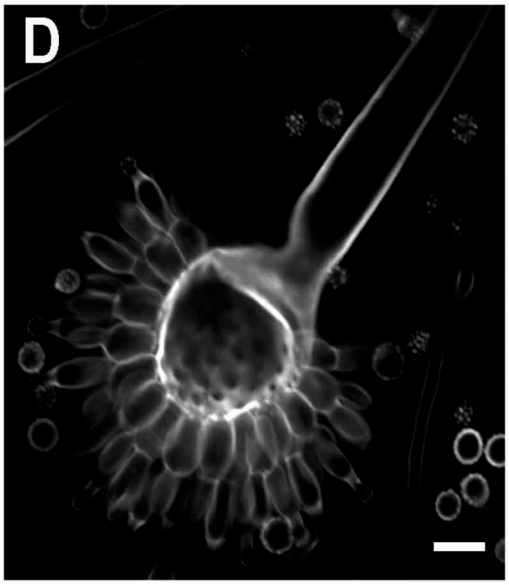
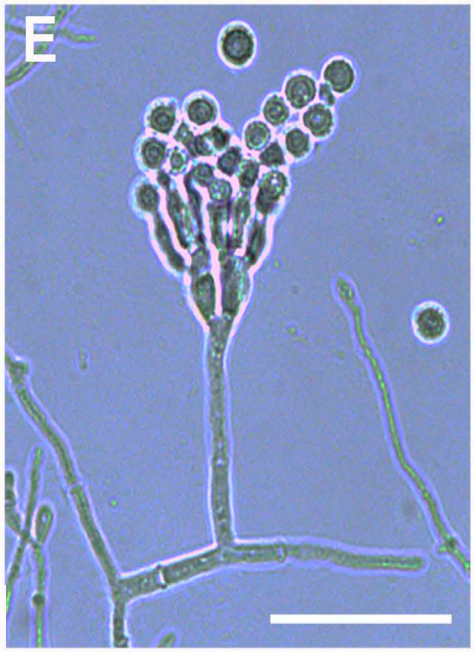
Soil pH is one of the most important abiotic factors for plants since it determines the bioavailability of nutrients. It also affects the survival of rhizosphere microorganisms, which in turn, must activate regulatory mechanisms that coordinate the expression and activity of resistance genes to overcome pH-stressing condition. *Trichoderma* genus fungi are common habitants of rhizosphere, recognized as biocontrol agents in agriculture, due to its mycoparasitic activity. As beneficial symbiont, *Trichoderma* is able to improve plant growth and defense. In fungi, the molecular mechanism involved in response to alkaline pH is controlled by the zinc-finger transcription factor PacC. In *Trichoderma*, PacC is relevant for the mycoparasitic process, regulating the production of cell-wall degrading enzymes and secondary metabolites. Previously, we isolated the alkaline tolerant *Trichoderma harzianum* T9 strain from arid soil in Nuevo León state, which is able to growth *in vitro* at pH 11. Additionally, the T9 strain showed better growth promotion in *Sorghum bicolor* seedlings, in alkaline agricultural soil. In this work, we analyze the *pacC* gene sequence from T9 strain and two reference strains and compare its response to acidic and alkaline culture conditions, as well as during the mycoparasitic interaction against phytopathogen fungi. By sequencing, we did not find any differences in the *pacC* coding and regulatory sequences. However, in T9 strain, we observed higher *pacC* expression at early exposure to alkaline growth conditions. Moreover, under alkaline conditions, T9 strain had the best antagonistic activity against phytopathogens. We are currently determining whether sodium-extrusion family (ENA) transporters could be involved in the ability of the T9 strain to tolerate alkaline conditions.









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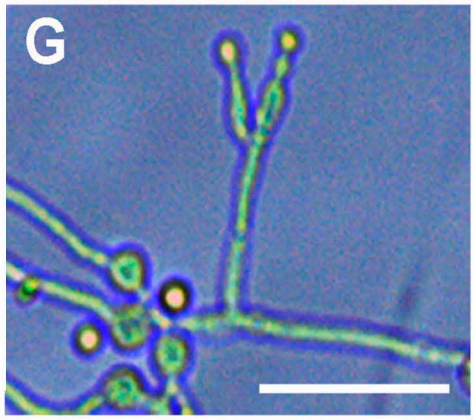

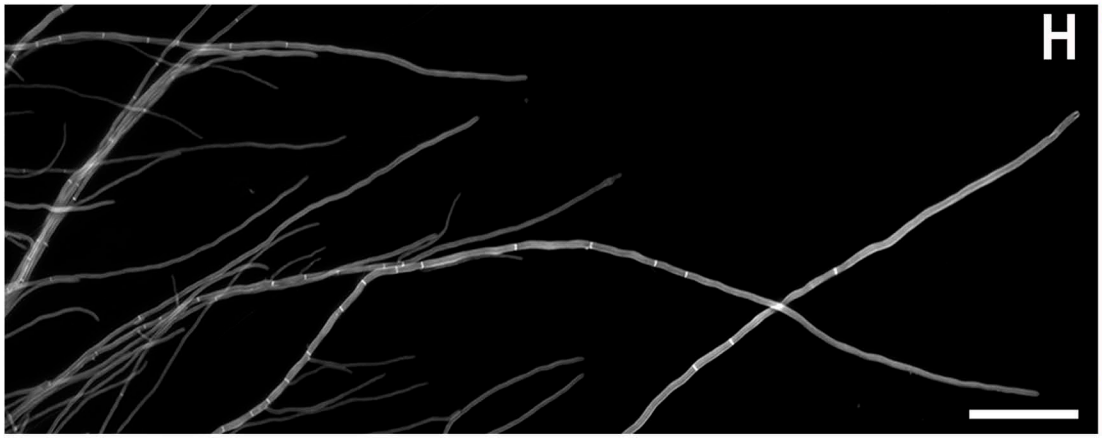

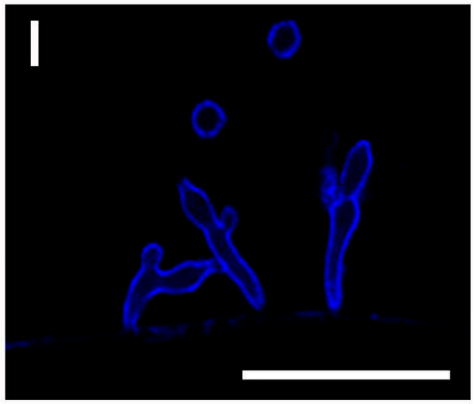

Determinación de Ocratoxina A en café soluble

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Coffee is a widely consumed beverage by the population, it has become a daily and social activity, its desirability is linked to its aroma, flavor and its positive or negative effects for the consumer. Within the different presentations of coffee, soluble coffee continues to be one of the most consumed, due to the fact that it can be easily obtained, at a lower price and is easy to prepare. Coffee contamination can occur in different production stages, from harvesting, drying, or bean storage. During these stages there are several factors that can alter the coffee quality. In this sense, pollution is mainly caused by an excess of humidity, which is provoked by a bad handling in its production, or during the harvest by direct contact of beans with contaminated soils. Some filamentous fungi produce secondary metabolites of low molecular weight known as mycotoxins. Ochratoxin A (OTA), the most relevant and prevalent form of the Ochratoxin group, mycotoxins can cause negative effects on consumer health. OTA is associated with kidney and immune system dysfunction. Therefore, this work aimed to register for the mycotoxin-producing fungi presence in soluble coffee. The presence of fungi in this type of coffee was determined using the NOM-111-SSA1-1994 "Method for the enumeration of molds and yeasts", the determinations were carried out in duplicate. Fungi identification was through dichotomous keys; of the analyzed samples of soluble coffee, 40% did not present fungi contamination, while 60% had the presence of fungi of the *Aspergillus* genus. Of these, 89% were *A. fumigatus* and 11% *A. niger.*

OTA presence in coffee was quantified using the ELISA assay, giving a range of 0 to 87 µg/kg. The detection and control of contamination by filamentous fungi is the responsibility of coffee growers and manufacturers, who must implement quality control measures to guarantee that the final product is free of contaminants and safe for the consumer.

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Evaluation of 4*H*-chromenes in P-glycoprotein (P-gp) of *Candida* spp: phylogeny, modelling, molecular docking and enzymatic activity

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The worldwide incidence of fungal infections caused by yeasts belonging to the genus *Candida* has been increasing in recent years, in the recent pandemic, cases of fungal co-infection were reported in patients who had the SARS-CoV-2 virus. Although there are multiple reasons for this increase, one of the main risk factors is an immunosuppressed system of the patient and the existence of resistance mechanisms to a variety of traditional antifungal drugs. One of the most important mechanisms are the efflux pumps (ATP-binding cassette ABC transporters superfamily), which are known to interfere and prevent drug entry into the fungal cell. For this reason, with the purpose of proposing new compounds different from those already known that can act on the P-gp of these species, in this work an-*in silico* analysis of such enzymes and the evaluation of derivatives of 4*H*-chromenes was carried out. Initially, a multiple alignment of the amino acid sequences of the P-gp of *Candida* spp., was carried out, finding domains conserved with the P-gp of *H. sapiens*. Phylogenetic studies demonstrate the close relationship that exists between some of the P-pg of *Candida* spp., and *H. sapiens*. Likewise, 3D models of these enzymes and their respective mutants were generated and evaluated, evidencing a structural similarity above 60% with that of *H. sapiens*. On the other hand, once these models were obtained, the molecular docking study was carried out, in which it was reflected that the compounds analyzed in this study (4*H*- chromenes) recognized amino acid residues of the active site (binding domain a ATP) of the P-gp of *Candida* spp., and that the binding energy values obtained were better than that obtained in the reference compound (Liniquidar). Finally, with the purpose of knowing the participation of one of the amino acid residues of the active site of such enzymes, the interactions between the mutated proteins and the 4*H*-chromenes were carried out, where it was possible to show that the amino acid mutation Lys by Asp affected the interaction and the binding energy values, inferring that this residue plays an important role in the mechanism of action carried out by 4*H*-chromenes and the P-gp of *Candida* spp., as well as the inhibition of growth in *Candida* spp., and the enzymatic activity of the P-gp of *Candida* spp., in conclusion, these analyzes allowed us to predict the importance of these yeast P-gp as a proposed inhibition for 4*H*-chromenes in therapy against fungal infections caused by these species.

Soil, rhizosphere and phyllosphere meta-taxonomics for the evaluation of the cessation of the use of glyphosate in orange cultivation in northern Veracruz

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Citrus fruits are the second crop group that most uses glyphosate in Mexico. To evaluate the impact of glyphosate on crops, studies have been carried out at the soil and rhizosphere levels. There are studies where effects are detected both in the microbiota and biodiversity, water, and soil by the use of this herbicide, while other research affirms that there is no conclusive associated risk with specific exposure. Glyphosate may have an effect on the composition and dynamics of bacterial and fungal microbiomes in the soil, and consequently, on the rhizosphere and endospheric communities of the crops to which it is applied. The objective of this study is to analyze the dynamics of the microbiota of soils, rhizosphere and phyllosphere of orange trees in conventional and organic cultivation, as well as of orchards in transition, all in approximately similar soils, through meta-taxonomic characterization. The first stage of the analysis showed that rhizospheric microbiome of oranges in agroecological transition is slightly different from that of conventionally grown oranges that include glyphosate application but, in terms of biological diversity, no significant differences were observed between them. The microbiome of the surrounding soil of the studied oranges exhibited distinct differences in the biological diversity they contained. While fungal communities in conventionally grown soil showed low phylogenetic diversity, those in agroecological transitioning soil were more phylogenetically diverse. These results indicate that meta-taxonomic strategies are useful for monitoring the transition from conventional to organic orange growing practices. However, it is necessary to continue exploring metataxonomic strategies that facilitate the monitoring of crops in agroecological transition. Therefore, we are currently working on a meta-taxonomic monitoring approach to characterize the communities of the orange tree phyllosphere subjected to sublethal doses of glyphosate. We seek to eliminate the background noise caused by the heterogeneity of the soils found in the orange orchards on hills, which are the most predominant in northern Veracruz. Integrating the results obtained from the rhizosphere and soil analysis with those of the phyllosphere (which will be available by the date of the congress) will give a more complete view of the impact of the environmental application of glyphosate in orange tree associated microbial communities.

Circular economy in a brewery: The use of Brewer's Spent Grain for the growth of *Pleurotus ostreatus* and *Pleurotus djamor*.

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Key words: *Pleurotus*, Brewer's Spent Grain, Circular economy

Introduction

México is the fourth country worldwide in the production of beer. In 2021 the production was around 13,470 million liters,[1] during this process there is the obtention of different residues such as Spent hop, Spent yeast and Brewer's Spent Grain (BSG), the last one being the highest in quantity. Approximately for each liter of beer produced there is the generation of 200 grams of BSG which can end up in dumps; this is a problem because this by-product can attract different problems such as plague of cockroaches, buzzards, rats and microorganisms.[2] Combined with, there is a recent problem in different cities of México that are reporting the appearance of clandestine dumps near to populated zones, provoking diseases in people. The chemical composition of BSG is mainly lignocellulolytic material and has a humidity near 70%; these characteristics make the residue ideal for the cultivation of different organisms, such as fungi. *Pleurotus* genre has the capacity to grow in this kind of by-product and has the advantage of being edible.[3] By this way there is also the opportunity of promoting the circular economy in a process in which the residues can be used as a new raw material for the obtention of food. By this way it is possible to achieve at least 4 of the Sustainable Development Goals which are the number 2, 9, 11 and 12. The objective of this work was to use the BSG as a substrate for the culture of the fungus *Pleurotus ostreatus* and *Pleurotus djamor* promoting the circular economy in a brewery.

Material and methods

The process starts with the recollection of BSG from a brewery; then it is sterilized, mycelia is inoculated, and the mix is put in plastic vessels. The colonization process takes place in a period between 2 and 3 weeks without any incidence of light, after that, holes are made in the walls of the vessel and moved into a chamber where humidity is maintained between 70-80 %. After 1-2 weeks fruitful bodies are mature and collected.

Results and discussion

The time for growth of *Pleurotus ostreatus* and *Pleurotus djamor* were of 23 ± 2 days for a first fructification with biological efficiency of 30.91 ± 5.51 and 21.02 ± 2.90, a yield of 20.59 ± 4.37 and 13.16 ± 2.08 respectively. When compared with other sources of alimentation sources such as broccoli need a period of time of 60 days and meat a period of at least 2 years for cows.

Figure 1: *Pleurotus ostreatus* and *Pleurotus djamor* growth successfully in BSG

Conclusions

BSG can be used as substrate for the culture of edible fungus of the *Pleurotus* genre. This application makes possible the usage of a residue as a raw material for the production of high value products, promoting also the circular economy in a brewery, showing good results in a short period of time while compared to other food sources.

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Using Yeast to Study Human Gene Variants Associated with Mitochondrial Disorders

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Mitochondrial diseases (MD) are highly heterogeneous disorders with multiple organ manifestations, caused by mutations in either mitochondrial or nuclear DNA. More than 1,500 nuclear genes are involved in mitochondrial structure and function; defects in approximately 27% of them have been associated with MD. Whole genome and exome sequencing has allowed the identification of new gene variants in patients with mitochondrial disorders, however, not all variants are pathogenic. *Saccharomyces cerevisiae* is a suitable model organism to analyze the impact of such gene variants on metabolism.

Around 40% of the human genes involved in mitochondrial structure and function have an ortholog in the baker's yeast *Saccharomyces cerevisiae.* Since this yeast can grow with a dysfunctional mitochondrial metabolism and even in the absence of mitochondria, it has been used as a powerful tool to validate and understand the molecular mechanisms of pathogenic gene variants associated with MD. This project aims to implement *S. cerevisiae* as a tool for the systematic analysis of human pathogenic gene variants by evaluating the impact of complete knock-out and single nucleotide substitution mutants in mitochondrial function and structure.

In this work, we examined the human gene *TUFM* and its *S. cerevisiae* homolog, *TUF1*, as proof of concept. *TUFM* encodes for the Elongation Factor EF-Tu, involved in the synthesis of mitochondrial proteins; in 2019 a new single nucleotide variant in this gene was reported in a patient with MD. The effects of the single nucleotide variant and the complete knock-out in *S. cerevisiae* metabolism were assessed by contrasting their growth in fermentable and non-fermentable carbon sources, and by measuring the membrane potential with a fluorescent dye as an indicative of the mitochondrial function. Additionally, the analysis of homologous and heterologous complementation of the mutants was conducted. Our findings show that the knock-out mutant exhibits a modest growth delay in comparison to the wild-type strain and the single nucleotide substitution mutant under fermentative conditions, however under non-fermentative conditions the growth of the knock-out mutant was impaired. The selection of other genes and further evaluation are underway.

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Microevolution of *Candia glabrata* cells in the presence of antifungals

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Opportunistic infections pose significant health risks for individuals with weakened immune systems, often exhibiting resistance to standard treatments. *Candida glabrata* (*C. glabrata*) is an opportunistic pathogenic fungus in humans known for its phenotypic plasticity, enabling adaptation in diverse environments. In Mexico *C. glabrata* is the second most common Candida species causing candidiasis, a condition resulting from microbial dysbiosis induced by a weakened immune system or prolonged antibiotic therapy. Some *C. glabrata* strains have shown resistance to fluconazole (FLC), one of the primary treatments for candidiasis, through mechanisms such as overexpression of efflux pumps and mitochondrial DNA mutations. Additionally, recent research has suggested a link in histone methylation patterns, particularly H3K36 methylation, and alterations in gene expression leading to FLC resistance in *C. glabrata*. The aim of this study is to determine the contribution of epigenetic factors to FLC resistance in evolved mutants of *C. glabrata* that were chronically exposed to FLC *in vitro*. We have observed that there are at least two types of FLC resistant (FLC^R) evolved mutants: a) FLC^R mutants with dysfunctional mitochondria (Gly-) and b) FLC^R mutants capable of respiration (Gly+). We propose that modifications in histone methylation patterns, particularly H3K36 methylation, may play a role in modulating FLC resistance in Gly- and Gly+ mutants. We are analyzing the stability of FLC^R and FLC susceptibility (FLC^S) phenotypes in Gly- and Gly+ evolved mutants, determining the relative amount of mitochondrial DNA in the Gly- and Gly+ mutants, and generating *set2*Δ mutant strains to assess susceptibility to FLC. Data from these experiments will be presented. By comprehensively investigating the interplay between histone methylation, mitochondrial function, and FLC resistance in *C. glabrata*, this research could provide valuable insights into the underlying mechanisms.

"EVALUATION OF THE ANTIFUNGAL EFFECT OF THE EXTRACT OF *Pleurotus ostreatus* ON *Aspergillus parasiticus*"

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A. parasiticus has the capacity to produce secondary metabolites known as aflatoxins, highly carcinogenic, teratogenic and mutagenic compounds, causing great damage both to health and economics. Today, the search for inhibitors of the development of *A. parasiticus* and the production of aflatoxins continues. On the other hand, it has been previously reported that the interaction between plant extracts with microorganisms can modify the normal development of *Aspergillus* fungi. The objective was to evaluate the interaction of the extract of *Pleurotus ostreatus* with *A. parasiticus.*

The crude extract prepared from *Pleurotus ostreatus (P. ostreatus)* and ethanol was partially characterized, finding a total polyphenol concentration of 3.06 µg ac. Gallic/mg of extract, were detected by means of colorimetric tests, tannins, terpenoids and saponins. In the trials on the biomass production of *Aspergillus parasiticus*, the effect of the *P. ostreatus* extract, a decrease in the biomass produced was found as a function of the control used, which was dimethyl sulfoxide (DMSO) at 1% solution in which dissolved the extract used. The percentages of biomass decrease were 64.39, 55.62 and 48.56% for the concentrations of (100, 90 and 80 mg of *P. ostreatus* extract/mL of DMSO, respectively. Microscopies of the extract interacting with the *A. parasiticus* strain were also performed, observing that the spores treated with the extract presented morphological modifications, which may imply damage to the *A. parasiticus* cell wall. Likewise, tests of antifungal activity were carried out with the technique of inhibition halos and poisoning of the medium, finding favorable results with the concentration of 100 mg of extract/mL of 1% DMSO, inhibiting the *Aspergillus parasiticus* strain.

Evaluation of internal region spacer of ribosomal DNA sequence for molecular identification of filamentous fungi in the Colección Nacional de Cepas Microbianas y Cultivos Celulares (CINVESTAV-Mexico).

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The Colección Nacional de Cepas Microbianas y Cultivos Celulares (CDBB) (establish in 1977) is a Microbial Biological Resource Center specialized in the conservation, identification and provide reference strains and isolates of biotechnological application, composed of 2000 cultures, including algae, bacteria, filamentous fungi (640 strains) and yeasts.

The CDBB provides annually an average of 30 filamentous fungi and yeasts, in addition to offering the identification service, at the request of the users of the collection, such as researchers from public institutions and industries.

Currently, the sequencing of universal gene fragments is a very useful and reliable tool, due to its easy amplification and rapid taxonomic discrimination, considering the extensive public databases of these sequences. The objective of this work is present the taxonomic category in which greater discernment resolves the internal region spacer region of DNA ribosomal (ITS), for filamentous fungi group.

In 2012, CDBB implemented the characterization by molecular biology with the ITS regions. The sample was the authentication of 130 internal filamentous fungi from CDBB and 70 services of external identification.

The method characterized 48 genera, out of 200 strains, resulting in 2 corrections: *Acremonium*, deposited as *Fusarium*, and *Pyricularia* deposited as *Cercospora*.

We found 114 species distinguished with the ITS fragment, although it was complemented with the morphology and geographical distribution, there were three genera that suggest amplifications with more gene fragments to have a more robust certainty (*Aspergillus*, *Fusarium* and *Penicillium*) due to the diversification of these genera and the new species described.

The other genes that we expect to increase the robustness of the identification are ß-tubulin and calmodulin, in addition to an extensive database in the 18s, D1/D2 LSU, COI and actin gene fragments. The ITS region determined 100% of the strains in the genus, and 94% at the species level.

This fragment has a high efficacy at the species level, and is totally accurate at the genus level, for immediate identifications or authentications. In addition to found cryptic species, which can only be genetically discerned as *Histoplasma* and *Trichophyton.* Only three genera (*Aspergillus*, *Fusarium* and *Penicillium*), with greater diversity, bring us closer to the species group that belongs.

Fusarium metallothioneins: a study in *Fusarium kuroshium*

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Metallothioneins (MTs) are a family of ubiquitous and very diverse proteins with high affinity to Zn^{2+}, Cu^{+} and other metals. MTs are characterized for their relatively short sequences with distinct with cysteine-rich motifs: CXC, CXXC, CC, CCC and others, where X is any amino acid. The MTs have been studied mainly in mammals, where they participate in metal homeostasis, heavy metal tolerance and protection against oxidative stress. Recently, a role for MTs in the virulence of fungal pathogens has been proposed. Ambrosia fungi are symbionts of ambrosia beetles, which are cultivated by the latter as their sole food source. Some species of ambrosia fungi are phytopathogenic to plants and are a threat to forests, landscapes, and agriculture. In this study we identified two MT-like proteins in *Fusarium kuroshium,* an ambrosia fungus and causal agent of Fusarium dieback. However, their properties and roles are poorly understood. The aim of this work was to provide an initial view of the phylogenetic relationships of one *Fusarium kuroshium* metallothionein, and their orthologs in the *Fusarium* and other close genera. Furthermore, we are trying to find out signature motifs of the identified protein sequences. The possible connections between *Fusarium* MTs and pathogenicity are discussed.

Keywords: cysteine-rich, metallothioneins, *Fusarium*.

STUDY OF HUMAN GUT MYCOBIOTA IN DYSBIOSIS AND THEIR METABOLIC PROFILE

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Introduction.

The gut microbiota is one of the most studied environments with critical functions. Complex relations are established between the microbiota and its host and the microorganism forming the microbiome. Interactions can be symbiotic relationships of type commensal, mutualism and others. In the end, these relationships are mediated by chemical molecules. Since fungi are far less abundant than bacteria in the human gut (0.1% or 10^5-10^6 cells per gram of feces), their effects on microbiota and host health are not characterized. Additionally, fungi are heterotrophs and can produce an arsenal of exoenzymes and specialized molecules with diverse structures that give them a range of possible functions. In this work, we studied filamentous fungi isolated from fecal samples of hospitalized patients from Mexico City, to understand whether these microorganisms could grow in human physiological conditions and to describe their metabolic profiles.

Methodology.

Fungal strains from 39 human fecal samples were isolated using agar BHI and antibiotics. The molecular identification of each strain was performed using ITS, B-tubulin, and elongation factor 1-alpha genes. Then, metabolomic analyses using LC-MS-MS/MS data from their organic extracts were done using an *in-house* database of fungal metabolites (>500 compounds) and the GNPS plataform.

Results.

We obtained six filamentous fungi belonging to *Penicillium* and *Paecylomices* genera. Two of these fungi were able to grow at 37 °C and bile salt media (sodium taurocholate) suggesting these species could survive in the human gut. The metabolomic analysis of these strains reveales the presence of different classes of molecules, including carboxylic acids, fatty acyls, glycerophospholipids, indoles and derivatives, and others. Additionally, we identified several small molecules with antimicrobial properties.

Acknowledgments.

This work was partially supported by DGAPA-PAPIIT UNAM IA206823 (ARR), and by DGAPA-PAPIIT UNAM IN203923 (MF).

Design of a novel multiplex qPCR system to detect pathogenic Mucoralean fungi from clinical human samples

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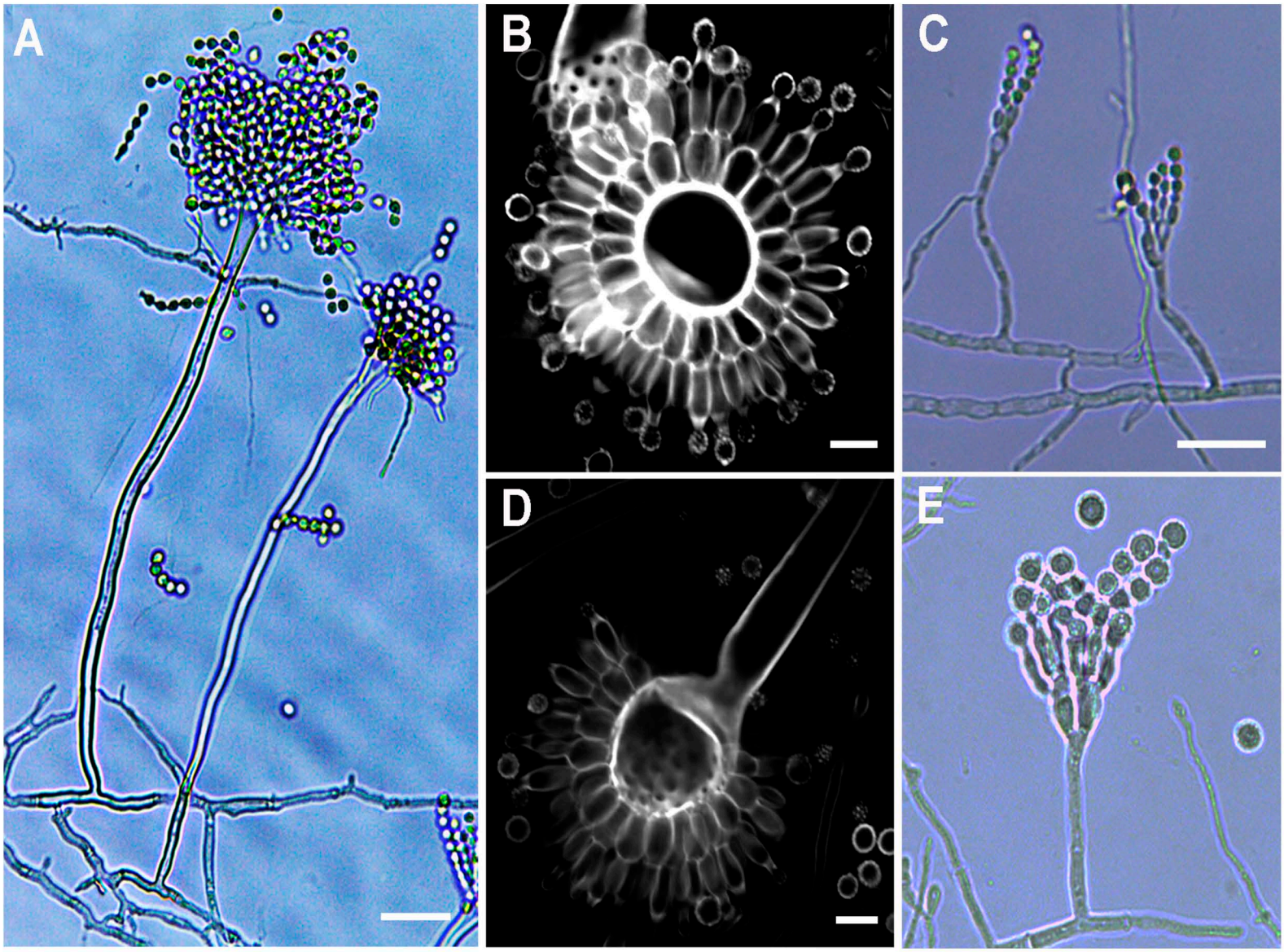
Abstract

Mycoses are a serious public health issue worldwide. Particularly, some species of the early diverging Mucorales fungi can lead to a rare but life-threatening infection called Mucormycosis mainly in immunocompromised patients. The most prevalent pathogenic Mucorales species is *Rhizopus arrhizus.* Moreover, some species of *Lichtheimia* and *Mucor* have clinical importance as well. It has been proposed that the high mortality rate in this fungal infection could be associated with delayed treatment as a result of an inopportune diagnosis or misidentification. Several technical difficulties have been reported to obstruct the microscopy identification or culture isolation of clinically relevant Mucorales fungi. In this work, we present the design of a novel non-invasive molecular method based on multiplex qPCR to simultaneously detect fungal DNA from three relevant pathogenic species of Mucoralean fungi (*Lichtheimia corymbifera, Mucor lusitanicus, and R. arrhizus*). Firstly, by employing bioinformatics, we identified *tfc-1* homologues for the three *Mucorales species;* this gene encodes a subunit of the RNA polymerase III transcription initiation factor complex. Remarkably, this housekeeping gene has been validated previously as a normalizer gene for qPCR analysis in *M. lusitanicus*. Afterwards, we designed hydrolysis probes with specific fluorophores and oligonucleotides for each *Mucorales* species; the human Ribonuclease P was also included in this system as an internal endogenous control. The results obtained in this work provide evidence of a novel, sensitive, and specific molecular system for the detection of pathogenic Mucorales DNA.



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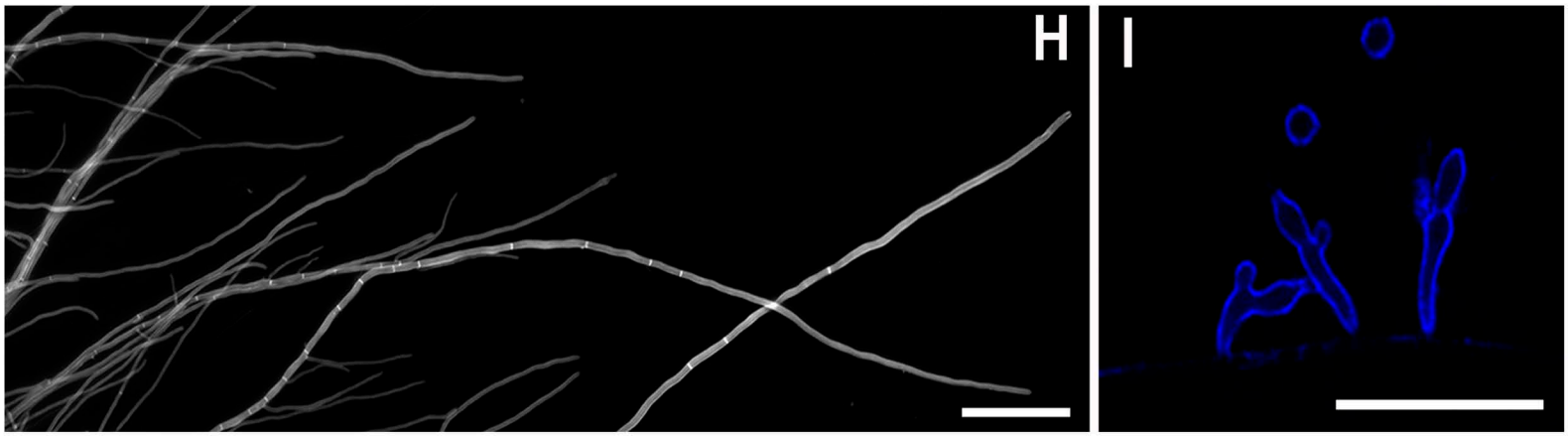
SIGNAL TRANSDUCTION



The role of phosphatidylinositols in cell growth and differentiation in the fungus *Aspergillus nidulans*.

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Abstract

Our laboratory focuses on investigating the role of reactive oxygen species (ROS) in the physiology and cellular differentiation of *A. nidulans.* In a recent study, we examined the changes in the fungal phosphoproteome in response to H_2O_2. We discovered significant modifications in the phosphorylation of the enzymes Stt4 and Mss4, which are crucial for phosphoinositide synthesis (1). These enzymes, along with the phosphatase TepA, are involved in the synthesis and degradation of phosphoinositides, which play important roles in various cellular processes (2).

In humans, the homolog of TepA is a protein that acts as a tumor suppressor, with multiple functions in malignant tumors, including the regulation of cell motility and directionality(3). These findings led us to investigate the function of phosphoinositide-synthesizing enzymes TepA and Stt4. As a first step, tried to generate mutants lacking the Stt4 and TepA enzymes.

ΔtepA mutants exhibit reduced conidiophore production at 3 days of growth, indicating a delayed but not impaired development, while no differences were found during sexual development. Sensitivity to different types of stress was assessed using H_2O_2 and menadione as oxidative stress agents, calcofluor and congo red for cell wall stress, and the response to nutrient deficiency was also evaluated. However, no differences were observed under any of these conditions compared to the wild-type strain.

With regards to *ΔStt4* mutants, only heterocaryotic transformants were obtained, indicating that stt4 is an essential gene in *A. nidulans*. *ΔStt4* mutants terminal phenotype corresponds to conidia that are form short and irregular germ tubes, filled with numerous vacuoles, before dying. Our results suggest that phosphatase TepA plays a minor role in *A. nidulans* physiology, while Stt4 role in phosphoinositide synthesis essential.. However, many questions remain unanswered, which motivates us to continue with further investigations.

AcknowledgmentsThis research was funded by grant PAPIIT-UNAM IN215622.

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Conservation of the Pal/Rim pathway in Ustilaginomycetes

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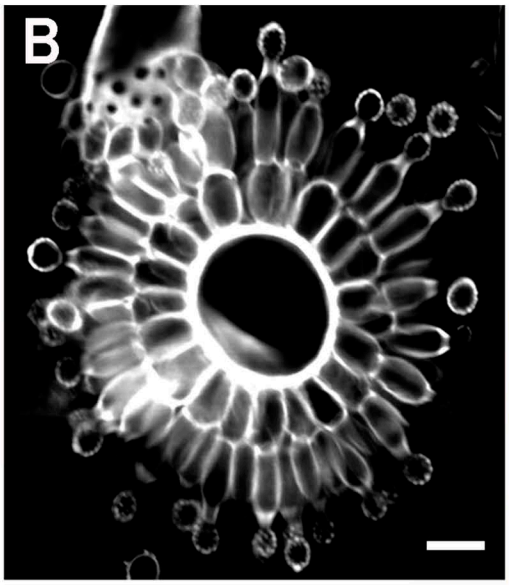
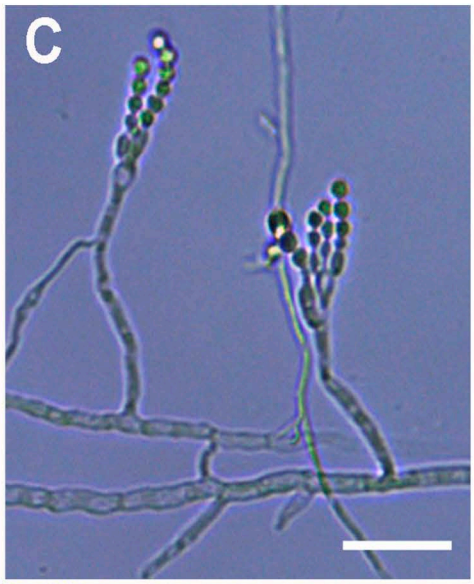
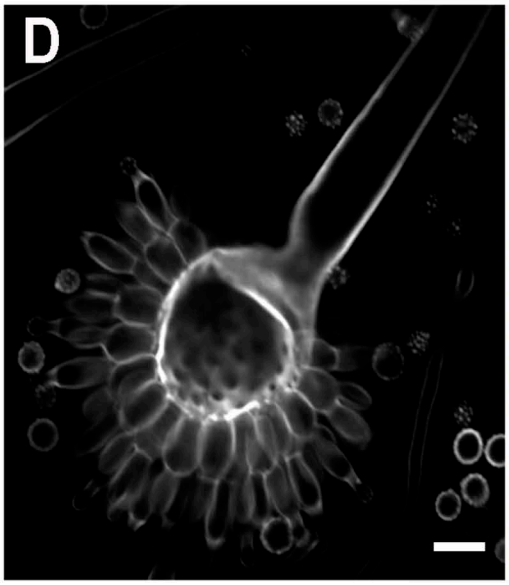
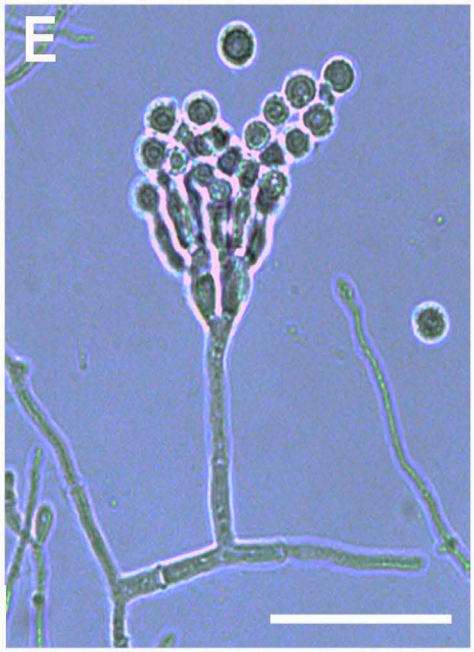
The ability of fungi to effectively sense and internalize signals related to extracellular changing environments is essential for their survival. This adaptability is particularly important for fungal pathogens of humans and plants that must sense and respond to drastic environmental changes when colonizing the hosts. One of the most important physicochemical factors affecting fungal growth and development is pH. Most fungal species possess mechanisms such as the Pal/Rim pathway for external pH adaptation. Using a comparative genomic approach, in this work we explored the conservation of the whole Pal/Rim pathway in the best sequenced and annotated Ustilaginomycetes. Our data reveal that the Rim/Pal pathway is conserved in Ustilaginomycetes, some of them considered important models for studying the fungal plant interaction. This study contributes to understanding the molecular mechanisms of these fungi for responding to extracellular stresses.









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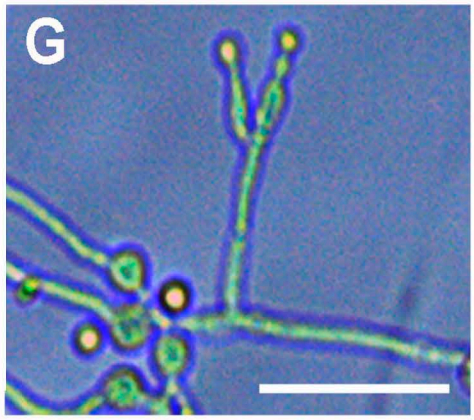
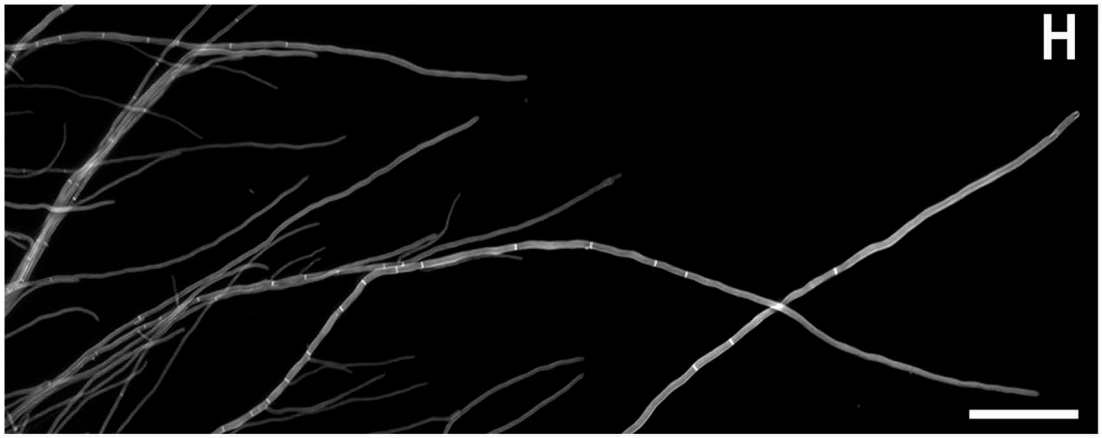
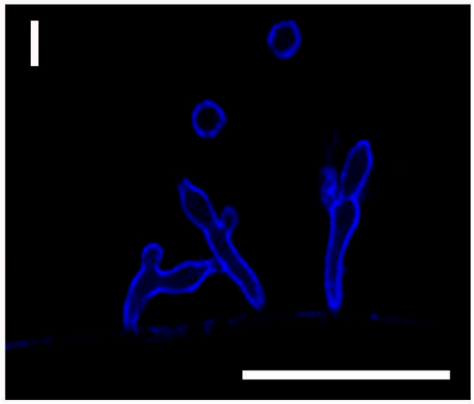
STRESS







Functional role of the metalloprotease Ermp1 from *Schizosaccharomyces pombe* in the stress response in endoplasmic reticulum

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Abstract:

Ermp1 is a transmembrane metalloprotease from the endoplasmic reticulum (ER) of the fission yeast *Schizosaccharomyces pombe*. Although its function has not been demonstrated, there is evidence indicating that Ermp1 is related to the maturation of ovarian follicles in rats[1] and the upregulation of the unfolded protein response (UPR) in humans[2]. Because UPR is conserved in humans and yeast[3], Ermp1 could play an important role in the adaptive response to reticular stress. To know the cellular function of Ermp1 and determine a model of ER stress in *S. pombe*, an *in silico* analysis of protein- protein interactions was performed. The analysis included the prediction of a network of 37 proteins that interact with Ermp1, using the BioGRID database and DIOPT Ortholog Finder. Relevant functional annotations such as calcium transport, vesicular trafficking and lipid metabolism were identified, which are processes regulated by UPR during stress[4]. Furthermore, analysis of the Ermp1 transmembrane region with the MIB server revealed that it could transport calcium due to its subcellular location. Since Ermp1 is a protease, PROSPER server evaluated 6 target proteins to predict proteolytic cleavage sites. Molecular docking was also performed using the ClusPro and BioLuminate tools. The protein-protein docking results showed interactions between the Ermp1 catalytic pocket and the proteolytic cleavage consensus sequences. This suggests that Ermp1 would be related to protein degradation, a process regulated by UPR to decrease protein load in ER[4]. On the other hand, an ER stress model of the wild strain of *S. pombe* was standardized with Thapsigargin, Tunicamycin and DTT comparing different concentrations with respect to time. Cellular growth was observed to decrease after 8 hours of incubation. Viability was not significantly affected by Thapsigargin treatment during the 24 hours of incubation, unlike cells treated with Tunicamycin and DTT, where the viability decreased by up to 50%. Therefore, the logarithmic phase of yeast growth is the ideal time to assess the adaptive response to ER stress since the cells do not compromise their viability. In conclusion, this study suggests that Ermp1 is overexpressed during the stress adaptation phase in ER, due to its relationship with processes highly regulated by UPR, such as calcium maintenance and proteolysis. This hypothesis would be supported by the quantification of gene expression.

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Production of mannosylerythritol lipids as an antimicrobial agent, secreted by strain FMA2, genotype NRG1 of *Ustilago maydis.*

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There is evidence that microorganisms usually produce various substances in stress situations produced by both physical and chemical stimuli (Aréchiga-Carvajal & Ruiz-Herrera, 2005; Hewald et al., 2006). In the case of *Ustilago maydis*, it has been described that it produces two types of extracellular compounds, one is ustilagineous acid and the second is the production of large amounts of surfactant compounds such as mannosylerythritol lipids (MEL) under conditions lacking nitrogen (Aréchiga-Carvajal & Ruiz-Herrera, 2005; Becker et al., 2021; Cortes-Sánchez et al., 2011; Hewald et al., 2005). These glycolipids can be used as biosurfactants, as well as being a biodegradable option to produce detergents and pharmaceuticals (Becker et al., 2021).

Objective

Produce and test the efficiency of mannosylerythritol lipids with antimicrobial capacity against pathogenic bacteria.

Methodology

The *U. maydis* strain will be reactivated in Complete Medium at 28 °C for 24 hours under constant agitation. YEPS Medium pH 6 will be inoculated with the FMA2 strain at 1x105 c/ml; incubate for 96 h at 28 °C with shaking. a 1:1 solution will be made with ethyl acetate, later, the ethyl acetate will be evaporated at 60°C and the product obtained will be suspended in methanol. The antimicrobial capacity test will be performed using the Kirby Bauer method.

Results

The inhibitory activity of the extracts obtained against *E. coli* and *M. Morganii* was measured and compared with the inhibitory activity of methanol. The extracts obtained showed inhibitory capacity against *E. coli* and *M. Morganii* from 48h.

Conclusions

Glycolipids play an important role in the development of therapeutic agents against various diseases, based on their anticancer and anti-inflammatory properties, as well as their antimicrobial capacity, which includes antibacterial, antifungal, and antiviral properties. Interestingly, it was observed that mannosylerythritol, derived from ustilaginous acid, had a positive effect on the pathogenic strains tested, achieving a visible inhibition halo in all repetitions.

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Trx3 (thioredoxin), Prx1 (peroxiredoxin), and glutathione (GSH) participate in the mitochondrial thiol redox balance in *Candida glabrata*

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Candida glabrata is a pathogenic yeast recently classified within the group of high-priority fungal pathogens by the WHO. The high resistance to different stress is central in the virulence attributes of *C. glabrata*, which allows it to survive and replicate within phagocytic cells. In the case of oxidative stress, C. *glabrata* uses a multitude of antioxidant mechanisms that respond to different types of reactive oxygen species under specific conditions. From our previous work, we know that glutathione is an abundant and essential molecule in *C. glabrata*. Superoxide dismutases, Sod1 and Sod2, inactivate the superoxide ion, while different peroxidases inactivate H_2O_2: a single catalase, Cta1, confers resistance to high concentrations of H_2O_2 in acute exposure, while peroxiredoxin Tsa2 and sulfiredoxin Srx1, protect against chronic exposure. The peroxiredoxin/sulfiredoxin system uses thioredoxin as reductants, which are small proteins that restore sulfhydryl groups from the sulfenic form. Recently, we have characterized the role of thioredoxins, Trx2 and Trx3, and thioredoxin reductases, Trr1 and Trr2, in response to different types of stress. Trx2, Trr1, and Trr2 are the main cytosolic reducers of H_2O_2-scavenging enzymes, while Trx3 is a mitochondrial thioredoxin. Interestingly, the null mutant (*trx3*Δ) does not have a phenotype of sensitivity to several types of stress (oxidative stress, heat shock, or xenobiotics). Trx3 is the only mitochondrial thioredoxin and there is no thioredoxin reductase that localizes to mitochondria, however, there are studies that points to an essential function of glutathione in mitochondria. Therefore, we made a *gsh2*Δ *trx3*Δ double mutant to address the role of Trx3 and GSH in mitochondria. The *gsh2*Δ *trx3*Δ mutant did not grow in non-fermentable carbon sources (glycerol or ethanol), while the single mutants did. This result suggests that Trx3 participates cooperatively in mitochondria to maintain the redox balance. Furthermore, peroxiredoxin (Prx1) is localized in the mitochondrial matrix and reduces H_2O_2 and we believe that Prx1 could be the target of Trx3. We used a **bi**molecular **f**luorescence **c**omplementation (BIFC) assay and observed that Prx1 and Trx3 physically interact. In order to identify which reductase acts on Trx3, we generated null mutants in *GLR1* (mitochondrial and cytosolic forms of glutathione reductase) and *GRX2*, which encode for a glutaredoxin; and constructed double and triple mutants in combination with *trx3*Δ and *prx1*Δ. We analyze the phenotypes of sensitivity to different stressors and the growth on non-fermentable carbon sources. Currently, we are evaluating the redox status of Trx3 and Prx1 to determine which reductase enzyme is acting on these proteins.

OxrA protein as part of the antioxidant response and its possible role on mitochondrial dynamics in *Aspergillus nidulans*

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Abstract

Aerobic organisms generate reactive oxygen species (ROS) as product of their metabolism. ROS are molecules derived from O_2, mainly through its partial reduction, which can have both damaging and signaling roles (1) in cellular processes, such as gene regulation, signal transduction and cell differentiation (2). A global phosphoproteomic analysis carried out in our laboratory to identify putative proteins and pathways involved in H_2O_2 signaling (3). H_2O_2 induced the dephosphorylation of the protein AN3004 (Oxidant resistant or OxrA), which contains a TLDc domain at its C-terminus, conserved in all eukaryotes (4). Notably, in mouse the TLDc domain is enough for this function (5). Although the molecular mechanism of Oxr1 function is not yet understood, this protein has been localized in mitochondria.
From fungi to animals, the deletion or reduced expression of Oxr1 results in sensitivity to oxidative be required for the expression of several antioxidant genes (6). This and the fact that H_2O_2 induces mitochondrial division in *A. nidulans* has led us to determine if OxrA is part of the antioxidant response and if it plays a role in mitochondrial dynamics in this fungus. Our results indicate that the lack of OxrA results in oxidative stress sensitivity and that *ΔoxrA* mutants show a delay in asexual development, which is enhanced in the presence of H_2O_2.

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Quorum sensing in *Candida glabrata*

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During a *Candida glabrata* infection, cells are internalized by phagocytic cells and are subject to the presence of reactive oxygen species (ROS, which damage all biomolecules) playing a central role in the elimination of the pathogen. In immunocompromised patients, *C. glabrata* can overcome oxidative stress, proliferate within the macrophage, and thus persist in the host. *Candida glabrata* has a well define machinery to respond to oxidative stress: transcriptional factors that detects and respond to ROS and antioxidant systems to maintain the redox balance within the cell. We have observed that *C. glabrata* is more resistant to ROS in stationary phase cells (SP) than logarithmic phase cells (LP). Furthermore, SP supernatant protects LP cells against ROS. These data suggest that the SP supernatant contains molecules that induce the oxidative stress response or contains secreted antioxidants that diminished ROS damage. Analysis of *C. glabrata* secretome and transcriptional regulation of *CTA1* (catalase 1) identified the enhancer molecule, 1-dodecene (C12). To have a genome-wide analysis, we sequenced the transcriptome of *C. glabrata* cells from SP, LP, LP incubated with SP supernatant, and LP with C12. Differential gene expression analysis shows repression, mainly from transcription and translation activity, ribosome biogenesis, and DNA synthesis and repair, and correlates with the cessation of cell duplication when LP cells were incubated with SP supernatant. Up-regulation of genes encoding proteins involved in energy metabolism, oxidative phosphorylation, non-fermentable carbon utilization, and proteins involved in autophagy, fatty acid metabolism, peroxisome formation, antioxidant enzymes, and some amino acid biosynthetic pathways. Genes encoding for the MAPK and phosphatidylinositol signaling pathways were also upregulated in SP cells and LP cells incubated with SP supernatant or C12 .These results suggest that the secreted molecule C12 present in the SP supernatant have the potential to arrest the cell cycle, induce autophagy and energy metabolism based on cell respiration. We propose that this molecule could signaling by the MAPK and phosphatidylinositol pathways. Quorum sensing molecules have been reported in *Saccharomyces cerevisiae* (2-phenylethanol, tyrosol, and tryptophol) and *Candida albicans* (farnesol) and this work is the first report of a quorum sensing molecule in *C. glabrata*.

Tolerance to Cu, Cr and Pb of *Trichoderma asperellum* and *Trichoderma longibrachiatum*

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In the soil there are bacteria and fungi that promote plant growth and are biocontrol agents of pathogens. Therefore, the injury caused by heavy metals in microorganisms is relevant for the crop's productivity. The fungi from Trichoderma genus have all these functions in the crops, however their tolerance mechanisms to heavy metals are poorly understood and this knowledge could be useful for the integration of Trichoderma in the crops and soil management. Previously, we reported that the isolates To of *T. asperellum* and *T. longibrachiatum* promoted the growth of onion plants and are potential agents of biocontrol. The isolate To of *T. asperellum* was tolerant to Cu-based fungicide and reduced the injury caused by Cu in onion plants. *T. longibrachiatum* also reduced damage caused by infection with *Sclerotium cepivorum* and saline stress in onion plants. Based on above, in this study we compared the tolerance to Cu, Cr and Pb of three isolates of *T. asperellum* and *T. longibrachiatum*. The four isolates of Trichoderma were grown in liquid culture medium with 100 mg L^{-1} of Cu, Cr, and Pb and it was evaluated the production of biomass and conidia and calculated the tolerance index (TI). The heavy metals content was determined by EDX-coupled Environmental Scanning Electron Microscopy. In the presence of heavy metals Cu, Cr, and Pb, the mycelium pigmentation and morphology depended on the isolate of Trichoderma and the heavy metal. However, in *T. longibrachiatum* it was most conserved the mycelium color and the hyphal grew forming aggregates with the exposure to Cu, Cr, and Pb. *T. longibrachiatum* was most tolerant to the three heavy metals than the isolates of *T. asperellum*. In *T. longibrachiatum*, the TI was 100 % with Cu and Cr, and with Pb was 81.5 %. While in *T. asperellum*, the TI values for Cu were from 88.3 to 94.9%, for Cr were 81.9 to 69.4 % and with Pb were from 59.9 to 73 %. In isolates of *T. asperellum*, the Pb was the metal most accumulated, followed of Cu and Cr. In contrast, in *T. longibrachiatum* the Cu was the metal most accumulated followed by Pb and Cr. In conclusion, we propose that *T. longibrachiatum* could be used in the integrated management of onion crop, due its potential as agent of biocontrol, promotor of plant growth, and tolerance to the heavy metals Cu, Cr, and Pb.

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