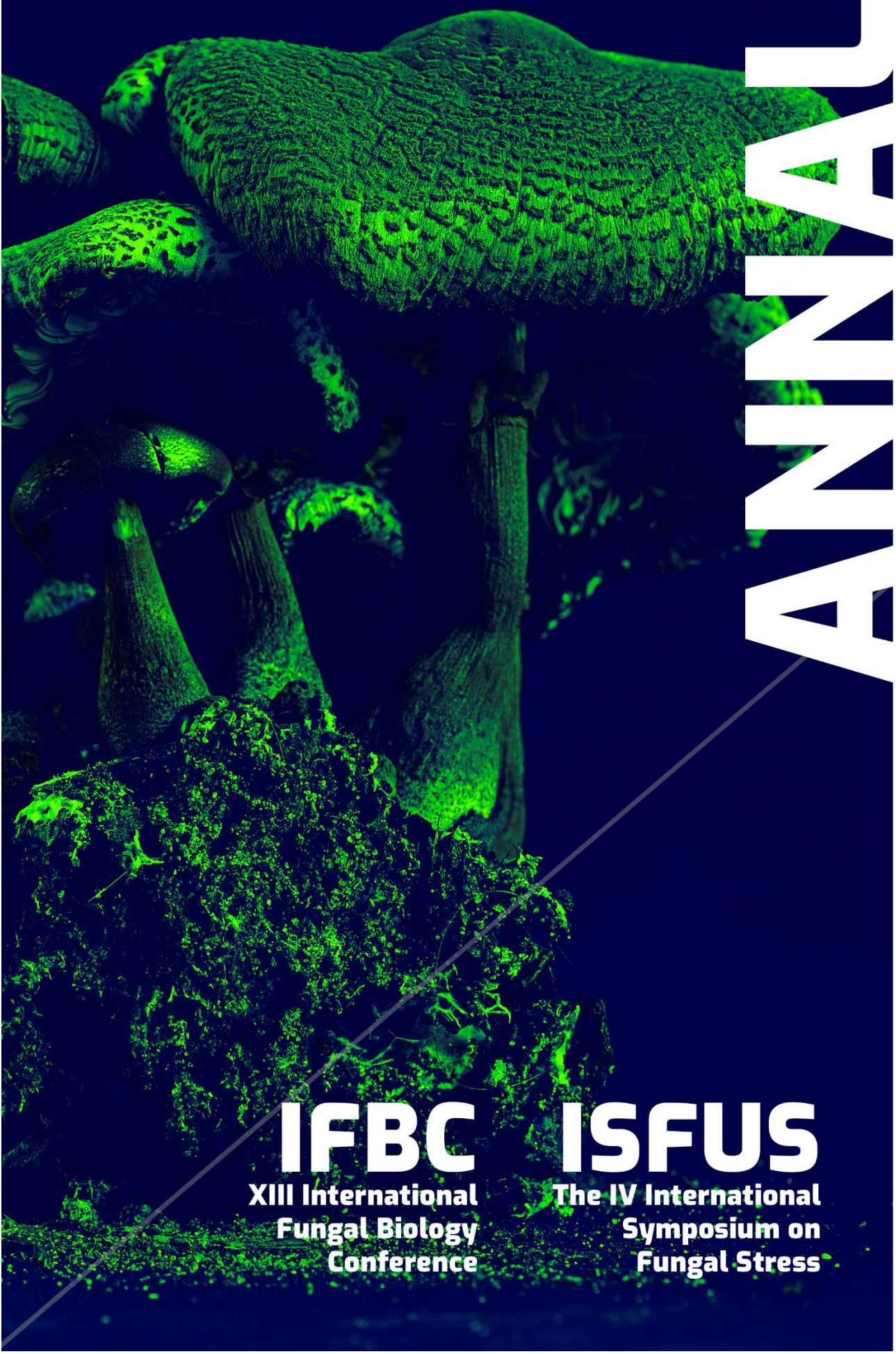
HOTEL NACIONAL INN
SEPTEMBER 25 - 29, 2022

ANNALS



IFBC ISFUS

XIII International Fungal Biology Conference

The IV International Symposium on Fungal Stress

FAPESP CAPES

FUNDING

FAPESP

CAPES

HOSTS

UNIVERSIDADE BRASIL

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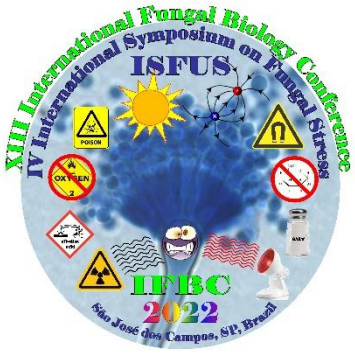

Annals of the

XIII International Fungal Biology Conference (IFBC)

IV International Symposium on Fungal Stress (ISFUS)

São José dos Campos (SP)
Hotel Nacional Inn, 2022

Dados Internacionais de Catalogação na Publicação (CIP)

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1. Biologia, ciências da vida

Hotel Nacional Inn CDD - 370

Ficha catalográfica elaborada por **Even3** – Sistema de Gestão de Eventos

ABOUT

Fungi are essential for life on our planet and play central roles in processes like industry, agriculture and medicine. Yet few people are aware of the importance and impact that these organisms have for human life and the environment. Fungi are our food and fermentate the bread we eat, and the wine and beer we drink. Fungi are producers of medical drugs, industrial enzymes and recombinant proteins, and among the leading producers of bioethanol and biodiesel. Fungi are bioremediators and bug killers and balance ecosystems as major recyclers of organic matter. Fungi can cause major diseases in humans, animals, and plants. Fungi can do all of this, in part because of their high resilience, being able to inhabit hostile environments, such as the interior of a mammalian organism, and to deal with many types of stress, including oxidative stress, high temperature, UV radiation, dryness, nutrient deprivation, osmotic stress, and the presence of toxic compounds. Such ability to respond to, survive in, and transform the environment, lead us to try to understand the biochemical and molecular mechanisms by which fungi are so plastic in their ability to adapt to stress. For beneficial fungi, resistance to stress is what we seek. To damage fungi, their resistance to the stresses must be overcome. Therefore, knowing the mechanisms of stress adaptation in fungi can broaden the possibilities of intervention to modulate their ability to adapt to specific environments in the interest of society.

This meeting featured talks from scientists from several countries who work on all these topics at the frontier of the research in fungi, including ecology and environment, health, sustainability, agriculture, industry, technology, molecular and cellular biology, biochemistry and industrial processes. This event is a unique opportunity to discuss with the most renowned scientists in these fields.

Based on the great success of the first (Rangel et al. 2015), second (Alder-Rangel et al. 2018), and third ISFUS (Alder-Rangel et al. 2020, Rangel and

Alder-Rangel, 2020), the IV ISFUS was a joint meeting, together with the XIII International Fungal Biology Conference (IFBC) at the Hotel Nacional Inn in São José dos Campos, SP, Brazil. This increased the visibility of both meetings and attracted participants from all over the world.

The **International Fungal Biology Conference** started in 1965 in Bristol, UK and since then has been held in Provo (Utah, USA 1973), Gwatt (Switzerland 1980), Stirling (Scotland 1987), Helen (Georgia, USA 1991), Konstanz (Germany 1996), Groningen (The Netherlands 1999), Guanajuato (Mexico, 2009; https://funguscongress.ucr.edu), Nancy (France 2006), Ensenada (Mexico 2009), Karlsruhe (Germany 2013; http://www.iab.kit.edu/conference/index.php), Incheon Songdo, (South Korea 2017), and now in São José dos Campos (Brazil, 2021). This Conference series is focused on exciting new fields at the frontiers of Fungal Cell Biology. Both ISFUS and IFBC are designed to provide a great environment to promote scientific and social interactions. Participants are expected attend every talk; therefore, no concurrent sessions are held. Poster sessions are always very lively at these meetings and reach a similar audience as oral presentations in other meetings.

The joint ISFUS-IFBC meeting brought together complementary and exciting frontier fields of fungal biology that attracted many young researchers and students in this field, from all over the world.

TOPICS

IV ISFUS

1. Stress mechanisms and responses in fungi
2. Fungal photobiology, clock regulation, and stress
3. Fungal stress in industry
4. Fungal biology in extreme environments
5. Ionizing radiation, ultraviolet radiation, heat, and other stresses in fungal biology
6. Fungal stress in agriculture
7. Stress in fungal pathogenesis
8. Stress in populations, fungal communities, and symbiotic interactions

XIII IFBC

1. **Morphogenes:** Includes polar growth, regulation of branching, asexual and sexual reproduction and germination in both yeast and filamentous fungi

2. **Organellar dynamics:** Includes secretion, vesicle trafficking, nuclear dynamics, vacuolar morphogenesis, mitochondrial interactions, cellular heterogeneity

3. **Cytoskeletal function and dynamics:** Includes aspects of cytoskeleton and growth, cell wall synthesis and septation mechanisms, nuclear division, trafficking, etc.

4. **Cell biology of interactions and communication:** Includes fungal-fungal interactions, fungal-bacterial, fungal-plant, fungal-animal focused on cell biological aspects.

REFERENCES ABOUT ISFUS

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VENUE

São José dos Campos is part of the Vale do Paraíba region, is known as the Capital of the Brazilian Aviation and is among the most populous citiesi n the State of São Paulo.

Although highly industrialized, the landscape of the municipality is very green: more than that, it is classified as an area of environmental protection. It is also full of tourist and cultural attractions.
São José dos Campos, São Paulo, is a center of science and advanced technology research and manufacturing. In addition to making aircraft and satellites, the city is home to a modern industrial complex specializing in companies in the automotive, aerospace, electronics, and telecommunications

Hotel Nacional Inn

The Nacional Inn in São José dos Campos has a extensive structure to hold events. The Centro Nacional Inn de Convenções, has the capacity to receive about 700 people in rooms of various sizes and formats.

The Nacional Inn São José dos Campos is located on Avenida Deputado Benedito Matarazzo: next to Shopping Center Vale, close to Embraer, the Airport, and General Motors. It is 15 minutes from bus station and 30 minutes from City Park, one of the two most visited in São José dos Campos. In a 10 to 15 minute walk it is possible to get to the Santos Dumont and Vicentina Aranha Parks.

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bms British Mycological Society promoting fungal science

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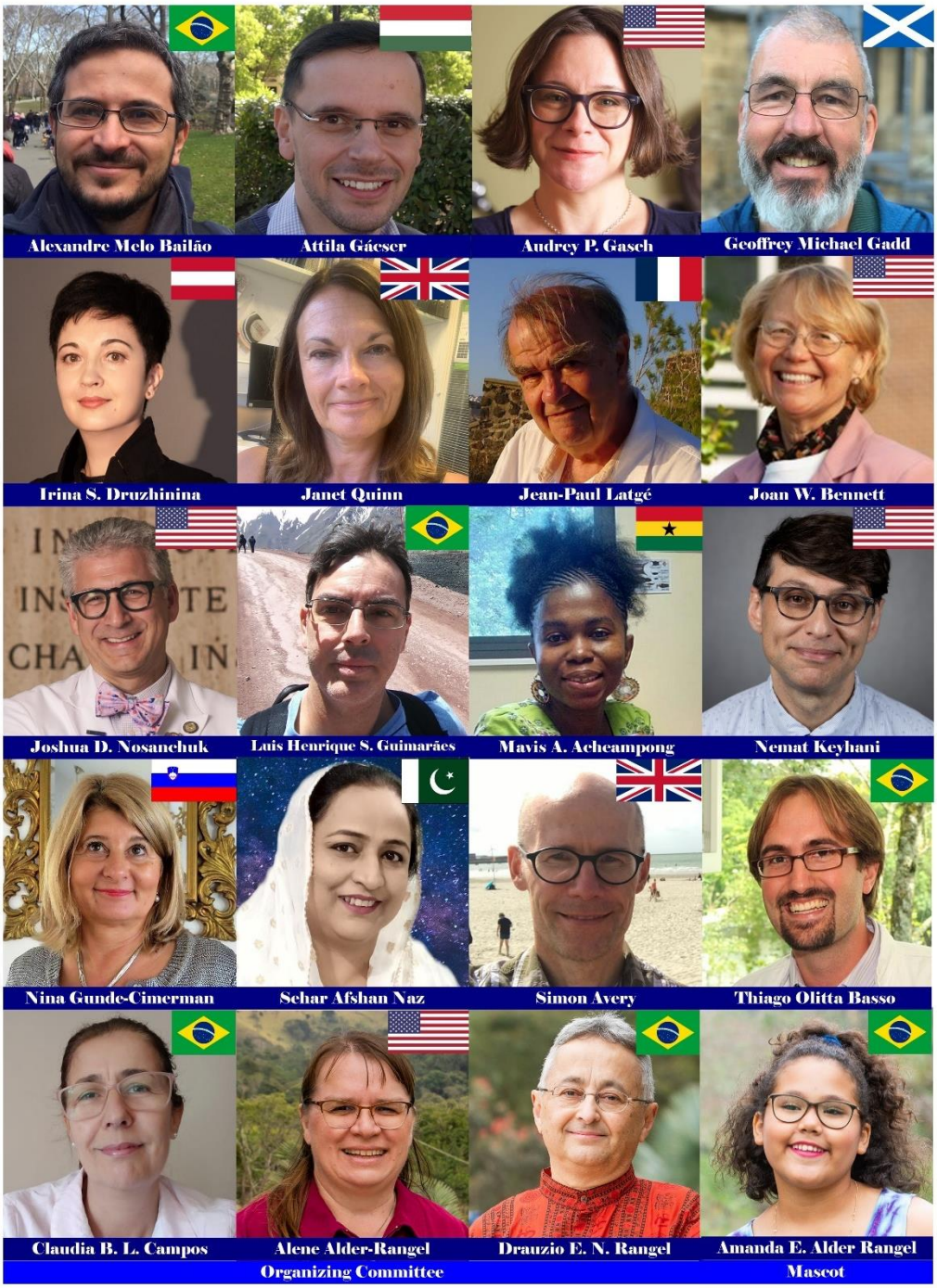
Meet the Speakers

IV International Symposium on Fungal



Meet the Speakers

XIII International Fungal Biology Conference

Group Picture

Speakers of the IV International Symposium on Fungal Stress (ISFUS) & XIII International Fungal Biology Conference (IFBC) in 2022 held in São José dos Campos, SP, Brazil. Front row from left to right: Alene Alder-Rangel, Amanda E. A. Rangel, Drauzio E. N. Rangel. Second row from left to right: Raquel Nascimento (DFG Brazil), Audrey P. Gasch (USA), Nina Gunde-Cimerman (Slovenia), Laila P. Partida-Martínez (Mexico), Xiaorong Lin (USA), Iris Eisermann (UK), Joan W. Bennett (USA), Martine Bassilana (France), Janet Quinn (UK), Fulvia Verde (USA). Third row from left to right: Mavis A. Acheampong (Ghana), Meritxell Riquelme (Spain), Robert Arkowitz (USA), Alfredo H. Herrera Estrella (Mexico), Rosa Reyna Mouriño Pérez (Mexico), Nancy P. Keller (USA), Attila Gácser (Hungary). Fourth row from left to right: Cristina (DFG), Simon Avery (UK), Geoffrey M. Gadd (UK), Ulrich Terpitz (Germany), Jesús Aguirre (Mexico), Jean-Paul Latgé (France), Joshua D. Nosanchuk (USA), Joseph Heitman (USA). Not in the picture: Alexandre Melo Bailão (Brazil), Claudia B. L. Campos (Brazil), Gerhard Braus (Germany), Irina S. Druzhinina (Austria), Nemat Keyhani (USA), Sehar Afshan Naz (Pakistan), Thiago Olitta Basso (Brazil).

Schedule

Time	Sunday	Monday	Tuesday	Wednesday	Thursday	
	September 25	September 26	September 27	September 28	September 29	
09:00 - 09:30		Opening Ceremony	Nancy Keller	Joseph Heitman	Meritxell Riquelme	IFBC
09:30 - 10:00	Meeting with all speakers for lunch and walk in the Vicentina Aranha Park, São José dos Campos. Participants are welcome.	Elsevier Student Awards	Iris Eisermann	Martine Bassilana	Michael Feldbrügge	
10:00 - 10:30		Coffee Break				
10:30 - 11:00		Alfredo H. Herrera Estrella	Xiaorong Lin	Gerhard Braus	Robert Arkowitz	
11:00 - 11:30		Laila P. Partida-Martínez	Fulvia Verde	Rosa Reyna Mouriño Pérez	Jesus Aguirre	
11:30 - 12:00		Ulrich Terpitz	Student Presentation	Student Presentation	Student Presentation	
12:00 - 14:00	Lunch Break					
14:00 - 14:30	Meeting with speakers for collaboration - Hotel Nacional Inn	Joan W. Bennett	Sehar Afshan Naz	Irina S. Druzhinina	Luis Henrique S. Guimarães	ISFUS
14:30 - 15:00		Nina Gunde-Cimerman	Janet Quinn	Audrey P. Gasch	Thiago Olitta Basso	
15:00 - 16:30		Coffee Break and Poster Section				
16:30 – 17:00	Closed meeting with speakers about publication of the Fungal Biology Special Issue.	Geoffrey Michael Gadd	Jean-Paul Latgé	Drauzio E.N. Rangel	Mavis Agyeiwaa Acheampong	
17:00 - 17:30		Research in Germany Session	Joshua D. Nosanchuk	Simon Avery	Alexandre Melo Bailão	
17:30 - 18:00			Attila Gácser	Nemat Keyhani	Claudia B. L. Campos	
18:00	Social Events				Poster Awards	

Organizing Committee

Drauzio E. N. Rangel *– Universidade Tecnológica Federal do Paraná, Dois Vizinhos, PR, Brazil*

Jesus Aguirre *– Universidad Nacional Autónoma de México, Mexico City, DF, Mexico (Chair – IFBC)*

Alene Alder-Rangel *– Alder's English Services, São José dos Campos, SP, Brazil*

Claudia B. L. Campos *– Universidade Federal de São Paulo, São José dos Campos, SP, Brazil*

Schedule

Monday – 26 September 2022

9:00 | Opening ceremony
9:30 | Elsevier student awards
10:00 | Coffee Break

IFBC – Session 1

Cell biology of fungal interactions

Chair: Iris Eisermann

10:30 | Alfredo H. Herrera Estrella

Center for Research and Advanced Studies of the National Polythecnic Institute, Irapuato, Mexico

Trichoderma from predator to prey: the role of fine-tuned regulation of gene expression

11:00 | Laila P. Partida-Martínez

Center for Research and Advanced Studies of the National Polythecnic Institute, Irapuato, Mexico

Fungal holobionts: hidden relationships with ecological consequences

11:30 | Ulrich Terpitz – Julius Maximilian

University, Würzburg, Germany

Green-light sensing in fungi – what do we know so far about fungal rhodopsins?

12:00 | Lunch Break

ISFUS – Session 1

Fungal biology in the environment

Chair: Joshua D. Nosanchuk

14:00 | Joan W. Bennett

Rutgers University, New Brunswick, NJ, USA

Fungal volatiles have physiological consequences

14:30 | Nina Gunde-Cimerman

University of Ljubljana, Ljubljana, Slovenia

Stable hybrids in the populations of black, extremely halotolerant yeast Hortaea werneckii – a new fungal adaptive strategy to extreme environments?

15:00 | Coffee Break and Poster Section

16:30 |Geoffrey Michael Gadd

University of Dundee, Dundee, Scotland, UK

Fungi in metal-rich environments: survival and substrate transformations

17:00 | Research in Germany

Fellowships, Exchange, and Collaboration Programmes

Tuesday – 27 September 2022

IFCB – Session 2

Fungal morphogenesis and polar growth

Chair: Meritxell Riquelme

9:00 | Nancy Keller

University of Wisconsin, Madison, WI, USA

Paracrine signaling guides fungal developmental switches

9:30 | Iris Eisermann

The Sainsbury Laboratory, Norwich, England, UK

Investigating the turgor-driven, septin-dependent infection mechanisms by the rice blast fungus Magnaporthe oryzae

10:00 | Coffee Break

10:30 | Xiaorong Lin

University of Georgia, Athens, GA, USA

Cryptococcal morphogenesis, pathogenicity, and vaccination

11:00 | Fulvia Verde

University of Miami Miller School of Medicine, Miami, FL, USA

Control of cell morphogenesis and chronological lifespan in the fission yeast Schizosaccharomyces pombe

11:30 | Student Presentation

12:00 | Lunch Break

ISFUS – Session 2

Stress in fungal pathogenesis

Chair: Geoffrey Michael Gadd

14:00 | Sehar Afshan Naz

Federal Urdu University of Arts, Science and Technology, Gulshan Iqbal, Karachi, Pakistan

Detection of the antifungal potential of a compound produced by Pseudomonas aeruginosa against Candida species

14:30 | Janet Quinn

Newcastle University, Tyne and Wear, England, UK

Responses of pathogenic fungi to stresses encountered in the host and polymicrobial environments.

15:00 | Coffee Break and Poster Section

16:30 | Jean-Paul Latgé

Institute Pasteur, Paris, France

Aspergillus fumigatus cell wall: past and future

17:00 | Joshua D. Nosanchuk

Albert Einstein College of Medicine, Bronx, NY, USA

Complex modifications of extracellular vesicles in response to cellular stress

17:30 | Attila Gácser

University of Szeged, Szeged, Hungary

The effect of acquired antifungal drug resistance on stress tolerance and virulence of Candida parapsilosis and Candida auris

Wednesday – 28 September 2022

IFBC – Session 3

Cytoskeletal dynamics and growth patterns

Chair: Laila P. Partida-Martínez

9:00 | Joseph Heitman

Duke University Medical Center, Durham, NC, USA

Fungal unisexual and pseudosexual reproduction

9:30 Martine Bassilana

University Côte d'Azur, Nice, France

Building-up tissue invasive filamentous cells – Candida albicans's approach to staying polarized

10:00 | Coffee Break

10:30 | Gerhard Braus

Georg August University Göttingen, Göttingen, Germany

Cellular assembly of the native fungal COP9 signalosome, which is required for fungal stress response and specific protein degradation

11:00 | Rosa Reyna Mouriño Pérez

Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, BC, Mexico

How are MTOCs organized in the filamentous fungus Neurospora crassa?

11:30 | Student Presentation

12:00 | Lunch Break

ISFUS – Session 3

Stress mechanisms and responses in fungi

Chair: Mavis Agyeiwaa Acheampong

14:00 | Irina S. Druzhinina

Royal Botanic Gardens, Kew, London, England, UK

The role of fungal surface-active proteins in stress response and fitness

14:30 | Audrey P. Gasch

University of Wisconsin-Madison, Madison, WI, USA

Functional genomics of the budding yeast stress response uncovers new relationships between stress tolerance and growth rate

15:00 | Coffee Break and Poster Section

16:30 | Drauzio Eduardo Naretto Rangel

Universidade Tecnológica Federal do Paraná, Dois Vizinhos, PR, Brazil

Priming responsiveness of Metarhizium robertsii to magnetic and electric field

17:00 | Simon Avery

University of Nottingham, England, UK

Stressful and stress-free approaches for fungal control

17:30 | Nemat Keyhani

University of Florida, Gainesville, FL, USA

Fungal stress and symbiosis: pH pathways in the laurel wilt pathogen

Thursday – 29 September 2022

IFBC – Session 4

Subcellular heterogeneity and organelle dynamics and interactions

Chair: Gerhard Braus

9:00 Meritxell Riquelme

Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, BC, Mexico

The spectacular hyphae of Neurospora crassa: an excellent model system to test the subcellular responses to environmental stresses

9:30 Michael Feldbrügge

Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Transport of mRNAs in fungi

10:00 Coffee Break

11:00 Robert Arkowitz

University Côte d'Azur, Nice, France

Candida albicans morphogenesis at different temporal and spatial scales

11:30 Jesus Aguirre

Universidad Nacional Autónoma de México, Mexico City, DF, Mexico.

Mitochondrial dynamics and development in Aspergillus nidulans

11:30 Student Presentation

12:00 Lunch Break

ISFUS – Session 4

Fungal stress in industry; Fungal stress in agriculture, medicine; ultraviolet radiation, heat, and other stresses in fungal biology

Chair: Audrey P. Gasch

14:00 Luis Henrique Souza Guimarães
Universidade de São Paulo, Ribeirão Preto, SP, Brazil
Fungal stress tolerance – a story told by the enzymes

14:30 Thiago Olitta Basso
Universidade de São Paulo, São Paulo, SP, Brazil.
Physiological and multi-omics investigation of the stress induced by contaminating bacteria in the context of yeast industrial biotechnology

15:00 Coffee Break and Poster Section

16:30 Mavis Agyeiwaa Acheampong
University of Ghana, Accra, Ghana
UV sensitivity of Beauveria bassiana and Metarhizium anisopliae isolates under investigation as potential biological control agents in South African citrus orchards

17:00 Alexandre Melo Bailão
Universidade Federal de Goiás, Goiânia, GO, Brazil.
Histoplasma response to host-imposed copper poisoning

17:30 Claudia B. L. Campos
Universidade Federal de São Paulo, São José dos Campos, SP, Brazil
Lipid metabolism in calcineurin-induced stress response in Paracoccidioides sp.

18:00 Poster Awards

Research in Germany

would like to invite the participants of the
IV. International Symposium on Fungal Stress - ISFUS & International Fungal Biology Conference – IFBC
to the

Research in Germany – Fellowships, Exchange and Collaboration Programs

to be held on Monday, September 26th, from 5:00 pm to 6:30 pm in São José dos Campos - Brazil

Representatives of the German Research Foundation (DFG) and the German Academic Exchange Service (DAAD) will provide an insight into the German research landscape, including information on funding schemes, collaboration opportunities and exclusive information on grant application strategies. Moreover, scientists working in the field of Molecular and Organismic Biology Research in Germany will give exclusive insights into their experiences in the German research landscape. Afterwards, attendees will get the chance to ask questions and seek individual in-depth advice. The event will be held in English, is free of charge.

Programme:

17:00	**Introduction to the German Research Landscape** Dr. Christina Peters, director of DFG Office Latin America
17:05	**Testimonial** Michael Feldbrugge - Heinrich Heine University Düsseldorf, Germany
17:20	**Funding Opportunities for International Collaboration** Dr. Christina Peters, DFG
17:45	**How to get your research funded by the DFG – Grant Application Strategies** Dr. Catherine Kistner, DFG department of Life Sciences 1: Molecular and Organismic Biology (**online**)
17:55	**Study and Research in Germany: DAAD Funding Programmes** Francine Camelim, DAAD São Paulo
18:00	**Q&A**
18:20	**Final Considerations**

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DAAD Deutscher Akademischer Austauschdienst German Academic Exchange Service

Moments

Participants at IFBC/ISFUS in the auditorium and at the coffee break

Speakers and participants holding hands and sharing happy moments at the opening ceremony

Poster session at IFBC/ISFUS and the stand of the initiative "Research in Germany", the DFG Office Latin America and DAAD Brazil

End of the IFBC/ISFUS meeting with most of the participants and speakers

Participants and speakers of the IFBC/ISFUS on the boat during the excursion to the beach in São Sebastião, São Paulo, Brazil.

ABSTRACTS

MORPHOGENES

IFBC

ANTIFUNGAL ACTIVITY OF GOLD NANOPARTICLES ASSOCIATED WITH RESVERATROL AGAINST CANDIDA ALBICANS

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The development of new antifungal compounds and carrier systems is of great importance due to the restricted arsenal of antifungals, cytotoxicity, limitations of administration routes and emergence of resistant strains. Among the drug carrier systems, inorganic nanoparticles have received considerable attention, especially when associated with potential antifungal compounds. Then, we studied the antifungal effects of gold nanoparticles associated with the polyphenol resveratrol (AuNpRSV) against Candida albicans. Initially, we evaluated the susceptibility of planktonic cells of two C. albicans strains, a reference (SC 5314) and a clinical isolate (Ca60), to AuNpRSV by the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays. AuNpRSV MIC and MFC values were, respectively, 2.46 and 4.92 µg/mL for both strains. The MFC/MIC ratio was 2.00, indicating fungicidal activity of AuNpRSV. The time-kill curves revealed that AuNpRSV reduced the viability of C. albicans SC 5314 and Ca60 at the 8-hour time point. There was no fungal growth for up to 48 hours after AuNpRSV treatment. In the evaluation against C. albicans biofilm, AuNpRSV decreased the biofilm viability of both strains at 1x MIC, with a marked reduction at 5x MIC. In addition, AuNpRSV reduced the filamentation of C. albicans SC5314 and Ca60 at 5x MIC. Altogether, our data demonstrate that AuNpRSV exhibit fungicidal activity against C. albicans by significantly reducing fungal viability up to 8 hours. In addition, AuNpRSV also act against C. albicans virulence factors by decreasing biofilm formation and filamentation.

POSTER PRESENTATION

BUILDING-UP TISSUE INVASIVE FILAMENTOUS CELLS – CANDIDA ALBICANS'S APPROACH TO STAYING POLARIZED

Miguel A. Basante-Bedoya Rocio Garcia-Rodas (mbasante10@gmail.com)
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Our group has been studying the roles of small GTPases and lipids during hyphal morphogenesis in the human fungal pathogen Candida albicans (Bassilana et al. 2020). Using different approaches, such as optogenetics and synthetic physical interactions, together with live-cell microscopy and threedimensional electron microscopy, we determined the importance and dynamics of membrane traffic components during this process, as well as the organization of the C. albicans secretory pathway. Furthermore, using PDMS microfabrication approaches, we probed the ability of filamentous C. albicans cells to penetrate into and grow in substrates of different stiffness (Puerner et al. 2020), and determined how cell growth and morphology are altered during this invasive process. I will discuss how C. albicans responds to external signal to generate invasive filamentous cells, with particular focus on lipids. More specifically the role of phospholipids, including phosphatidylinositol-4-phosphate [PI(4)P] (Garcia-Rodas et al. 2022), and lipid transporters, such as lipid flippases, will be discussed.

References:

Bassilana M., Puerner C., Arkowitz R.A. External signal-mediated polarized growth in fungi. Curr Opin Cell Bio . 2020;62:150-158.

Puerner C., Kukhaleishvili N., Thomson D., Schaub S., Noblin X., Seminara A., Bassilana M., Arkowitz R.A.. Mechanical force-induced morphology changes in a human fungal pathogen. BMC Biol. 2020;18(1):122.

Garcia-Rodas R., Labbaoui H., Orange F., Solis N., Zaragoza O., Filler S.G., Bassilana M., Arkowitz R.A. Plasma Membrane Phosphatidylinositol-4Phosphate Is Not Necessary for Candida albicans Viability yet Is Key for Cell Wall Integrity and Systemic Infection. mBio. 2022;13(1):e0387321.

Acknowledments: Our work is supported by the CNRS, ANR (ANR-11-LABX0028-01, ANR-16-CE13-0010-01, and ANR-19-CE13-0004-01), and EU H2020 (MSCA-ITN-2015-675407) grants.

POSTER PRESENTATION

CANDIDA ALBICANS MORPHOGENESIS AT DIFFERENT TEMPORAL AND SPATIAL SCALES

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We have a long-standing interest in how polarized growth is initiated and maintained during fungal development. In particular, we have been focusing on the yeast to hyphal transition and subsequent filamentous growth, in which growth is restricted to the apex in the human fungal pathogen, Candida albicans. We have been investigating the reorganization of different organelles during such growth processes including initial germ tube formation, subsequent filamentous growth and filament branch formation. In addition, recently we have been examining the physical characteristics of the cytoplasm in these different growth states. We have used loss of- and gain of- function mutants to probe the link between filament morphology and growth rate [1]. To investigate changes in the physical characteristics of the cytoplasm, i.e. cytoplasmic crowding and/or viscosity, we have been using a micro-rheology probe [2]. Based upon growth rate, morphology and the distribution of a range of cellular reporters, our results indicate that C. albicans hyphal branching and growth of the main hyphal filament are distinct developmental states.

1) C. Puerner et al. A myosin light chain is critical for fungal growth robustness in Candida albicans. mBio. 2021. 12: e0252821.

2) M. Delarue et al. mTORC1 controls phase separation and the biophysical properties of the cytoplasm by tuning crowding. Cell. 2018. 174: 338.

ORAL PRESENTATION

CRYPTOCOCCAL MORPHOGENESIS, PATHOGENICITY, AND VACCINATION

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The goals of our research are to gain insights into fungal biology and to use our knowledge to develop novel approaches for the therapy and prevention of fungal diseases. Limited options and adverse effects of current antifungals and the lack of vaccines underscore our challenges to prevent and treat fungal infections. To increase antifungal efficacy, we have developed DectiSomes, liposomes packaged with antifungals that are coated with host dectin receptors, to delivery antifungals specifically to the pathogens. We have shown that, relative to untargeted liposomal drug, DectiSomes bind stronger to various fungal pathogens and show much improved efficacy in mouse models of pulmonary aspergillosis and invasive candidiasis. DectiSomes have the potential to usher in a new antifungal drug treatment paradigm. To develop anti-cryptococcal vaccines, we took advantage of a master regulator Znf2 that controls cryptococcus yeast-to-hypha transition. Cryptococcus cells overexpressing ZNF2 elicit protective immune responses in animals, and protect animals against a subsequent lethal challenge either in the live or heatinactivated form. The antigens recognized by these protected animals reside within the capsule of cryptococcal cells. We are in the process of identifying the protective antigens.

ORAL PRESENTATION

INVESTIGATING THE TURGOR-DRIVEN, SEPTIN-DEPENDENT INFECTION MECHANISMS BY THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE

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Magnaporthe oryzae is a global threat to crop cultivation. The blast fungus forms a specialised dome-shaped, pressure-generating infection structure called an appressorium to enter the plant. Appressorium development depends on the Pmk1 MAP kinase signalling pathway. By performing a quantitative phosphoproteomic approach, we set out to identify direct downstream targets of the Pmk1 MAPK to understand the complex morphogenetic changes during plant infection. We identified 71 putative direct downstream targets of Pmk1 during appressorium development by using discovery phosphoproteomics followed by Parallel Reaction Monitoring (PRM). Putative targets include proteins related to cytoskeleton remodelling, vesicle trafficking, and cell cycle control. One of the targets is Vts1 and both yeast-two-hybrid and coimmunoprecipitation confirmed the association with Pmk1 in early stage appressoria. The phosphorylation of Vts1 is Pmk1 dependent and targeted mutation proved that Vts1 is necessary for proper appressorium development. Within the appressorium a turgor pressure of up to 8MPa is generated and enables the fungus to develop a rigid penetration peg to physically rupture the rice cuticle. We show that the turgor-sensing histidine-aspartate kinase, Sln1, enables the appressorium to sense when a critical turgor threshold has been reached and thereby facilitates host penetration. In parallel, Sln1 interacts with the protein kinase C cell-integrity pathway as a regulator of cAMP-dependent signalling by protein kinase A. Pkc1 phosphorylates the NADPH oxidase regulator NoxR and, collectively, these signalling pathways modulate appressorium turgor and trigger the generation of invasive force to cause blast disease. Turgor is applied as an enormous invasive force and cytoskeletal proteins called septins play a major role during appressorium-mediated plant infection. These GTP-binding proteins function by rigidifying the cortex of cells, scaffolding F-actin and localizing proteins, such as polarity determinants at the plasma membrane. M. oryzae possesses six septins, four of which (the core septins) form a hetero-oligomeric ring structure at the base of the appressorium during infection. We have used a combination of approaches to define the

septin interactome and its function during appressorial penetration by caring out an Ultra-High-Throughput Yeast Two Hybrid analysis coupled with an in vivo immunoprecipitation tandem mass spectrometry (IP-MS-MS) approach using GFP-tagged septins. For each M. oryzae septin we have identified a wide range of interaction partners during appressorium development, including polarity determinants, cytoskeletal components, and a range of regulatory proteins.
Interestingly, we observed that one of the non-core septins Sep7 interacts with Sep3, Sep4, Sep5, and Sep6 specifically during the early stages of appressorium formation, 4h after conidial germination, forming a plasma membrane-associated complex. All our approaches contribute substantially towards the understanding of the complex molecular mechanisms underlying the appressorium-mediated infection of plants.

ORAL PRESENTATION

PARACRINE SIGNALING GUIDES FUNGAL DEVELOPMENTAL SWITCHES

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Oxylipins, or oxygenated lipids, are universal signaling molecules across all kingdoms of life. In the filamentous fungal pathogen Aspergillus fumigatus, oxylipins – both fungal and host derived – mediate developmental switches in development such as hyphal branching and spore production. In vertebrate hosts, oxylipins activate either pro- and anti-inflammatory pathways that can exacerbate or resolve microbial disease. The secreted A. fumigatus oxylipin 5,8-diHODE induces hyperbranching via activation of the fungal transcription factor ZfpA where overexpression and deletion ZfpA mutants yield hyper- and hypobranching phenotypes respectively (1). Aspergillus oxylipins bear structural similarity to vertebrate oxylipins that are perceived by the receptor G2A. Here we explore virulence attributes of ZfpA deletion and overexpression mutants in the zebrafish model of invasive aspergillosis and address the hypothesis that the vertebrate oxylipin receptor G2A may recognize 5,8-diHODE and play a role in host response to A. fumigatus infections.

1. Fungal oxylipins direct programmed developmental switches in filamentous fungi. Niu M, et al. Nat Commun. 2020 Oct 14;11(1):5158.

ORAL PRESENTATION

ABSTRACTS

ORGANELLAR DYNAMICS
IFBC

ENDOSOMAL MRNA TRANSPORT DURING HYPHAL GROWTH

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In eukaryotic cells, proteins are targeted to their final subcellular locations with precise timing. A key underlying mechanism is the active transport of mRNAs and their local translation. These posttranscriptional events are intimately linked to membrane trafficking. A prominent example is the long-distance endosomal transport of mRNAs and their localised translation. Here, we describe current highlights on the fundamental mechanism of the underlying transport process as well as on biological functions. Although progress has been made in identifying various components of the endosomal mRNA transport machinery, a mechanistic understanding of how RNA-binding proteins are connected to endosomes is still lacking. I will discuss how the key RNA-binding protein Rrm4 from the plant pathogenic fungus Ustilago maydis is attached to endosomes using a novel MademoiseLLE (MLLE) domain platform consisting of three individual MLLE domains that function with a defined hierachy i.e. one major domain and flanked by two accessory domains. Thereby, transport of important cargo mRNAs is orchestratetd precisely during hpyhal growth. In addition, we observed that endosomal mRNA transport seems to be be particularly important for mRNAs encoding mitochondrial proteins that mediate efficient organelle import and regulating subcellular mitochondrial activity. These findings disclose a novel intimate link between endosomes and mitochondria, and adds an inspiring new level of complexity to trafficking and organelle biology.

ORAL PRESENTATION

PHYSICAL PROPERTIES OF THE CYTOPLASM AND CELL WALL IN THE HUMAN FUNGAL PATHOGEN CANDIDA ALBICANS DURING MORPHOGENESIS AND ANTIFUNGAL DRUG STRESS

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The human fungal pathogen Candida albicans causes superficial, as well as systemic infections in immunocompromised individuals. This fungal pathogen can undergo distinct morphological transitions that contribute to virulence. Antifungal drug resistance and tolerance during fungal infections has been increasing. While a number of studies have investigated the reorganization of cellular components during the C. albicans yeast to hyphal transition [1], much less is known about the biophysical characteristics of the cytoplasm and the cell wall in live cells and their response to stresses including antifungal drugs. We have used a combination of a micro-rheological probe [2], as well as applied a sub-resolution microscopy approach [3], to investigate changes in cytoplasmic crowding/viscosity and cell wall thickness, respectively, during growth and response to antifungal drugs in this fungal pathogen. Our results reveal that the physical characteristics of the cytoplasm and the cell wall are altered during these processes.

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To be judged for the Journal of Fungi Award.

POSTER PRESENTATION

ROS IN MITOCHONDRIAL DYNAMICS AND DEVELOPMENT IN ASPERGILLUS NIDULANS

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Our group has contributed to establish the role of reactive oxygen species (ROS) as ubiquitous signals that regulate different aspects of development and cell physiology. The study of the roles of the SakA stress MAPK pathway in ROS signaling, in the fungus Aspergillus nidulans, led us to uncover different roles for H2O2 in the regulation of mitochondrial division. Indeed, H2O2 induces an extensive mitochondrial division that depends on the dynamin-like protein DnmA (Drp1 in animal cells) and its receptor FisA. Although the lack of mitochondrial division has minor effects on respiration, it drastically affects polar growth and development, and results in increased levels of mitochondrial ROS. Moreover, H2O2 induces a generalized mitochondrial constriction response, previous to actual division, that involves a gradual depolarization of mitochondria, the participation of Ca2+, and requires a close interaction between mitochondria and the endoplasmic reticulum. Our results support a view of mitochondrial division as the end result of a cascade of signaling events that can be initiated in vivo by H2O2.

Our work was supported by grants CONACYT-DFG 277869 and PAPIIT-UNAM IN200719, IV200519 and IN215622.

THE MICROBODIES OR PEROXISOMES CYTODIFFERENTIATION: INFLUENCE OF THE CARBON SOURCE

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Microbodies are distributed in the different structures of Sclerotina fructigena: hypha, conidiophores, macroconidia, and sclerotia. In microconidia microbodies are apparently not visible in electron microscopy. They have a unique membrane with a matrix that can be finely granular or transparent to the electrons according to their age and their localization in the hyphae. The carbon source could also modify the physiology, kinetic growth, structures, and cytology of the hyphae and directly morphology, and the numbers and texture of microbodies. Lipids, endomembranous system, mitochondria and microbodies, nuclei are often associated (Najim and Turian 1979, Pezet et Pont 1995).

Occasionally we found pseudo-crystalline inclusions more or less electron dense in these organelles, calling also Woroning bodies near the septa (Najim and Turian 1979).

These microbodies are qualified as glyoxysomes or peroxisomes depending of the enzymatic activity related to the physiological conditions (nature of carbon source) (Najim et al.1979).

Studying the cellular differentiation of vegetative hyphae and different structures of the cycle, using transfer medium or stress condition, microbodies are observed with different matrix texture, electron density, and dimensions and sometimes with crystalline inclusions inside Sclerotinia fructigena (Monilia fructigena).

Cytochemical methods in transmission electron microscopy were used to study the structure and functionality of microbodies.

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POSTER PRESENTATION

THE SPECTACULAR HYPHAE OF NEUROSPORA CRASSA: AN EXCELLENT MODEL SYSTEM TO TEST THE SUBCELLULAR RESPONSES TO ENVIRONMENTAL STRESSES

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The shape of the hyphae is dictated by their cell walls and the way their cell wall is assembled during tip growth, a process that requires the polarized secretion of cell wall-building enzymes. We have used the fast-growing fungus Neurospora crassa to identify and analyze by high-resolution live fluorescence imaging key players of the secretory processes leading to localized delivery of vesicles at sites of polarized tip growth. At the hyphal apex, chitin synthases are contained in microvesicles (chitosomes), which concentrate at the core of the Spitzenkörper (SPK), while ß-1,3-glucan synthases are contained in macrovesicles, which occupy the outer layer of the SPK. The position of the SPK defines the place of vesicle fusion and exocytosis, determining both hyphal morphogenesis and growth directionality. We have previously found that the exocyst multimeric complex is crucial for SPK organization and hyphal growth. The exocyst subunits SEC-3, SEC-5, SEC-6, SEC-8, and SEC-15 were previously found localized at the plasma membrane of the cells' apices, while EXO-70 and EXO-84 occupied the frontal outer layer of the SPK, occupied by macrovesicles. The localization of SEC-10 had remained so far elusive. Here, we show that a N. crassa strain expressing an N-terminal GFP-tagged version of SEC-10 at its native locus was fully viable, whereas a strain expressing a Cterminal tagged version displayed severe hyphal growth and polarity defects, with hyphae lacking a noticeable SPK at their apices, indicative of exocyst dysfunction. A sec-10 knockout mutant was only viable in a heterokaryotic state, confirming that this subunit is essential to maintain hyphal morphogenesis. Mass spectrometry analysis revealed fewer exocyst subunits interacting with SEC-10-GFP than with GFP-SEC-10. The most abundant co-precipitate in the GFP-SEC-10 sample, and absent in the SEC-10-GFP sample, was MYO-5, which has been shown in N. crassa to be involved in maintaining apical organization and SPK integrity in N. crassa. Altogether, our data suggest that an unobstructed SEC-10 C-terminus is indispensable for exocyst function and assembly.

ORAL PRESENTATION

ABSTRACTS

CYTOSKELETAL FUNCTION AND DYNAMICS
IFBC

HOW MTOCS ARE ORGANIZED IN THE FILAMENTOUS FUNGUS NEUROSPORA CRASSA?

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?-Tubulin ring complexes (?-TuRC) mediate nucleation and anchoring of microtubules (MTs) to microtubule organizing centers (MTOCs). In fungi, the spindle pole body (SPB) is the functional equivalent of the centrosome, which is the main MTOC, and in addition, non-centrosomal MTOCs (ncMTOCs) contribute to MT formation in some fungi. Such ncMTOCs were characterized in Schizosaccharomyces pombe and in Aspergillus nidulans. In A. nidulans they are anchored at septa (sMTOC) and share components of the outer and the inner plaque of the SPB. Here we show that the Neurospora crassa SPB is embedded in the nuclear envelope, with the ?-TuRC targeting proteins PCP1Pcp1/PcpA located at the inner face and APS-2Mto1/ApsB located at the outer face of the SPB. PCP-1 is a specific component of nuclear MTOCs while APS-2 is also present in the septal pore. Although ?-tubulin was only detected at the nucleus, spontaneous MT nucleation took place in the apical and subapical cytoplasm during recovery from benomyl-induced MT depolymerization experiments. MT dynamics were monitored with the MT plus end tracking protein MTB-3 and revealed MT polymerization from septa. Septal MT polymerization was dependent on the septal proteins SPA-10Spa10 and SPA18Mto2/Spa18 (i.e., the APS-2/SPA-18 protein complex). We conclude that in N. crassa the SPB is the only MT nucleator site, but the septal pore aids to MT network arrangement trough the anchorage of the MT plus-ends.

ORAL PRESENTATION

MICROTUBULE DEPOLYMERIZATION IN THE INTRACELLULAR ORGANIZATION OF METARHIZIUM BRUNNEUM

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Metarhizium brunneum is an entomopathogenic fungus used for the biological control of pests in agriculture. Additionally, the Metarhizium genus is associated with the rhizosphere of plants, enhancing their growth. 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, where the microtubular cytoskeleton plays an important role in the intracellular transport of vesicles and organelles. To describe the role of the microtubular cytoskeleton in the intracellular organization of M. brunneum, we performed depolymerization assays with the anti-microtubules drug benomyl (BML) to assess which organelles' transport was affected. Peroxisomes labeled with the protein KAT-GFP, were observed as bright fluorescent spots evenly distributed along the hypha. Peroxisomes moved in both anterograde and retrograde fashion at different speeds. Exposure to BML, caused peroxisomes to stop moving and their dynamics were different to the WT. Lipid droplets stained with BODIPY were observed as fluorescent particles localized throughout the hypha with slow anterograde and retrograde motions. In cells treated with BML, lipid droplets remained static and started to accumulate throughout the hypha. The Spitzenkörper (Spk) labeled with FM4-64 was localized in the apical dome moving in the growth direction, and when treated with BML the SPK was visibly smaller. Mitochondria were accumulated at the subapex; their dynamics and positioning were independent of microtubules. Cell wall stained with calcofluor white was thicker in the hyphal tip when compared to the subapical and basal regions. Nevertheless, when exposed to BML, the cell wall was thinner in the apical dome. We conclude that microtubules are involved in the transport of chitosomes, peroxisomes, and lipid droplets, as well as in mitochondria position.

POSTER AND ORAL PRESENTATION

FUNGAL HOLOBIONTS: HIDDEN RELATIONSHIPS WITH ECOLOGICAL CONSEQUENCES

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Rhizopus microsporus is an early-diverging fungal species that belongs to the phylum Mucoromycota, and it is important in ecology, agriculture, food production and public health.

Our research has revealed that members of Rhizopus often establish symbioses with gram-negative beta-proteobacteria from the genus Mycetohabitans (Burkholderia sensu lato). This vertically transmitted bacterial symbiont is responsible for the production of toxins that are crucial for the pathogenicity of R. microsporus (1,2,3). Additionally, the endofungal bacteria are essential for the asexual reproduction (4) of their host and also positively affect its sexual cycle, being necessary for abundant zygospore production (5). After more than a decade since the discovery of this unique bacterial-fungal symbiosis, we have now identified new partners: the narnaviruses (6).

In this seminar, I will present some of our most recent results on the role that these bacterial and viral symbionts play in fungal reproduction; how these symbioses affect fungal metabolism, as well as recently described novel Mexican symbiotic Rhizopus strains (7) and how they can help expand our understanding of microbial symbioses and their role in fungal biology and evolution.

ORAL PRESENTATION

GREEN-LIGHT SENSING IN FUNGI – WHAT IS KNOWN SO FAR ABOUT FUNGAL RHODOPSINS?

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For fungi, photoreceptors are of superior importance as light controls many substantial processes in the fungal life cycle such as reproduction and pathogenicity. Among the arsenal of different fungal light sensors reacting to a broad plethora of wavelengths, fungal rhodopsins are responsible for the perception of green light. Fungal rhodopsin are representatives of type I (microbial) rhodopsins and as such consist of seven transmembrane helices that form an interior pocket around the chromophore all-trans retinal. The retinal is covalently bound to the protein via a protonated Schiff-base and undergoes conformational changes upon light-activation that provide protein function. Fungal rhodopsins act either as proton pumps or as sensory proteins, however, detailed knowledge about their physiological function and biological role is still marginal. Considering our recent analyses of different fungal rhodopsins from the ascomycetes Fusarium fujikuroi and Aureobasidium pullulans and the basidiomycete Ustilago maydis, we will summarize the to-date available information about fungal rhodopsins. We will highlight the role of these green light sensors in the biology of fungi while taking into account the occurrence of rhodopsins in dependence of the inhabited niche, especially regarding the green-light-enriched phyllosphere. Besides that, we will compare the biophysical characteristics of different rhodopsins and report on the augmentation of proton-pump activity induced by the plant auxin indol acetic acid observed in certain fungal rhodopsins.

ORAL PRESENTATION

INHIBITION OF IN VITRO GROWTH OF FUSARIUM SOLANI BY RHIZOBACTERIA

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Fusariosis is one of the main diseases that affect soybean, caused by saprophytic fungi belonging to the Fusarium spp complex. They are pathogenic microorganisms that are difficult control, currently the control is done through the use of resistant cultivars and specific fungicides, however, the rapid emergence of resistant isolates to the fungicidal molecules on the market is remarkable (Filho et al., 2020). Therefore, it is necessary to develop sustainable control measures that can be more effective and durable. The objective of this work was to evaluate in vitro the antagonistic effect of three strains of rhizobacteria on the growth of Fusarium solani. The strains belong to the Scheffer Company's collection of microorganisms, obtained from a work of isolation of the rhizosphere and morphologically characterized. The isolated of the pathogen F. solani was kindly provided by Embrapa-Cenargen, the tests were carried out at Scheffer's Laboratory of Biological Resources in SapezalMT. Were performed in vitro tests, concomitantly, being divided into five treatments, namely: T1 – F. solani Only, as a negative control; T2 – F. solani x azostrobin and difenoconazole fungicide, as a positive control; T3 – F. solani x LSB-034; T4 – F. solani x LSB-105; e T5 – F. solani x LSB-106, the experimental design was completely randomized (DIC) with four repetitions. The antagonista effect was tested in three diferente culture media, potatodextrose agar (PDA), nutriente-agar (NA), and a 50% w/v mixture of both (PDA/NA), using the double cultivation technique, where colonies were paired equidistantly. The plates were incubated at 25ºC for 14 days and measurements of F. solani fungus growth were every three days. The percentagem of inhibition of mycelial growth (IMG) was calculated based on the mean diameter of the negative control (DC) and on the mean diameter of each antagonistic collision (DT), at the end, the data were submitted to the Tukey test at 5%. The strain LSB-034 showed not inhibition of F. solani in any of the culture media tested, on the other hand, the strain LSB-105 showed the highest PIC, inhibiting, respectively, 71,62 and 44,26% the fungal growth on BDA and NA culture media. The strains LSB-105 and LSB-106 inhibited, respectively, 76,02 and 74,80% of the pathogen in PDA/NA médium, presenting a result equivalente to the Chemical fungicide, which inhibited, regardless of the culture médium, 83,05% of the mycelial growth of the pathogen. Therefore, presenting itself as a relevant potential for the control of fusariosis (Duré et al., 2018).

POSTER PRESENTATION

MICROCONIDIOGENESIS-MICROCONIDIA ULTRASTRUCTURE AND CYTOCHEMISTRY

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The transformation of polarity of vegetative hyphae into conidiophore could be controlled by environmental factors that influence the differentiation in one or both types of conidia or in sclerotia. The polarity of the vegetative hyphae (spitzenkörper) is disrupted, and different mechanisms and distributions of vesicles (microvesicles and macrovesicles) and reorganization of cytoskeleton (microtubules, microfilaments) will occur in the apices.

Monilia fructigena (Najim et Turian ; a 1979) produces two type of spores, macroconidia that are blastic centripetally type produced successively forming a long chain of citric shape spores; microconidia that are enterogenic type produced successively by phyalides (Cole 1981). M. fructigena produce also sclerotia, at the end of the biological cycle or when the environmental conditions changes: hydric stress, low temperature, oxygen pression, etc.

The first steps of the differentiation are similar during vegetative growth where the hyphae maintain a polarity in apices (Najim et Turian; b 1979).

The typical differentiation changes for vegetative hyphae when light, oxygen, and water as determinant factors change inducing macroconidiogenesis or microconidiogenesis. One of these factors could mark stress conditions and could determine the differentiation.

In this paper, we have tried to explain the biology and physiology of the production and differentiation of microconidia, the cytology and the ultrastuctural aspect of the development. During the ontogeny of microconidia, the cell wall plays an important role that we have tried to understand by also using different cytochemistry methods.

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POSTER PRESENTATION

PRODUCTION AND CHARACTERIZATION OF THE ITACONIC ACIDDERIVED COMPOUNDS 2-HYDROXYPARACONIC AND ITATARTARIC ACID

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Itaconic acid is used in many application fields such as the production of paper, paints and fibers. In 2004, this organic acid has been classified as one of the top 12 value-added platform chemicals derived from biomass. There is also a strong interest in this molecule in the medical and pharmaceutical sectors, both as an anti-bacterial compound and as an immunoregulator for the treatment of autoimmune diseases and viral infections including SARS-CoV-2. While itaconic acid has been studied extensively over the years, not much is known about its two derivatives 2 hydroxyparaconic (2-HP) and itatartaric acid (ITT), although their structural characteristics could also open up several applications. Besides being potential building blocks of industrial polymers, these compounds may also play a role in the immune response of higher eukaryotes due to their metabolic linkage to itaconic acid, a molecule that exerts immunomodulatory effects in macrophages. Evidently, the full spectrum of applications of these novel acids may only be explored when the substances will be available in high quantity and purity.

In this study, we report the production of these two itaconic acid-derived compounds via fermentation of metabolically engineered strains of Ustilago cynodontis. Following production, these compounds were also purified and characterized.

Ustilago cynodontis was found to be a promising host for the production of 2-HP and ITT. Since both molecules are metabolically linked to itaconic acid, a previously engineered itaconate overproducing Ustilago cynodontis strain served as the basis for strain development. By overexpressing the itaconateoxidizing P450 monooxygenase in this strain, itaconate was converted to its lactone 2-HP. The second product ITT is most likely the result of the lactonization of 2-HP, partly catalyzed by a putative 2 HP lactonase. Supernatants from Ustilago cynodontis fermentations on glycerol containing a mixture of 2-HP and ITT were found to inhibit the Pseudomonas putida KT2440 metabolism of glucose and acetate, and to interfere with the itaconate degradation of this strain. The fact that these compounds inhibit the itaconate

degradation suggests an evolutionary arms race between fungi and itaconatedegrading pathogenic microorganisms such as P. aeruginosa. Both 2-HP and ITT were recovered from the fermentation supernatant following a protocol adapted from Guevarra and Tabuchi 1990. 2-HP was recovered first through a process of concentration, lactonization of ITT and extraction with ethyl acetate. Subsequently, ITT could be obtained in the form of its sodium salt by saponification of the purified 2-HP. Finally, several analytical methods were used to characterize the resulting products and their structures were confirmed by NMR. This work provides a promising foundation for obtaining 2 HP as well as ITT in high purity. This will allow to unravel the full spectrum of potential applications of these novel compounds.

POSTER PRESENTATION

REGULATION OF EXTRACELLULAR VESICLE RELEASE MODULATES FLUCONAZOLE RESISTANCE IN CRYPTOCOCCUS NEOFORMANS

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The increasing rates of fluconazole resistance in the human pathogen Cryptococcus neoformans pose many clinical challenges in meningitis management (Iyer et al., 2021). The involvement of extracellular vesicles (EV) in fungal physiology is an increasingly important research field (Rizzo et al., 2021a). However, the genetics regulating EV release and their role in drug resistance mechanisms remain largely unknown. We used a screening approach to address the EV production in a collection of transcription factors (TF) mutants and identified four TF regulating EV release. These mutants also manifested altered fluconazole (FLC) susceptibility. The transcriptomic analysis of the knockouts revealed a downregulation of genes related to membrane homeostasis, and also genes coding for the most abundant protein markers previously found in cryptococcal EVs (Rizzo et al., 2021b). The phenotype of FLC resistance in EV-deficient mutants was not exclusively associated with conventional resistance mechanisms, such as the upregulation of the azole target gene ERG11 nor the drug efflux pumps AFR1-3, suggesting additional regulation mechanisms. To address if EV release could play a role in driving FLC resistance, we analyzed EV production in the presence of the drug. When fungal cells were submitted to sub-inhibitory concentrations of FLC, the EV production was reduced compared to untreated cells. Moreover, an extensive analysis of spontaneous FLC-resistant colonies obtained in vitro showed a

significant decrease in EV production associated with the disomy of chromosomes 1 and 4. When these strains were submitted to subsequential passages in drug-free media, they lost their resistance and aneuploidy phenotypes and restored EV production to the original levels. FLC-resistant clinical isolates also showed a diminishment in EV production. After submitting these isolates to subsequent passages in drug-free media, we identified one strain that reverted FLC susceptibility also restored EV production. Our findings provide evidence of an association between EV production and FLC resistance in C. neoformans, suggesting a new cellular mechanism regulating resilience to a major azole in this fungal pathogen.

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POSTER PRESENTATION

ABSTRACTS

CELL BIOLOGY OF INTERACTIONS AND COMMUNICATION

IFBC

TRANSCRIPTION FACTOR XPP1 INTERACTS WITH SREBP SAH2 TO REGULATE PRIMARY AND SECONDARY METABOLISM IN TRICHODERMA REESEI

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The fungal secondary metabolome holds enormous potential for compounds that can be used for therapeutic and biotechnological applications. However, most of those secondary metabolites are not produced under standard laboratory conditions, impeding the discovery of new natural products.

Previous results showed that the deletion of transcription factor Xpp1 leads to impaired growth but increases the expression of sorbicillinoids and other low molecular weight compounds in Trichoderma reesei. These observations combined with data from an RNA-Seq analysis lead to the conclusion that Xpp1 activates the primary metabolism, and represses the secondary metabolism [1]. Interestingly, the dimerization domain of Xpp1 is highly similar to the one of sterol regulatory element binding protein (SREBP) Sah2. Sah2-homologues in Neurospora crassa (Sah2), Aspergillus fumigatus (SrbA), and Schizosaccharomyces pombe (Sre1) play a critical role in the regulation of the primary and secondary metabolism [2-4].

In this work, we elucidated the interaction of fluorescence-tagged Xpp1 and Sah2 using confocal microscopy. The results show different intracellular distribution of Xpp1 and Sah2 depending on the metabolized carbon source. Further, the localization is also depending on the presence of the other protein partner, strongly suggesting a direct interaction of Xpp1 and Sah2.

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POSTER AND ORAL PRESENTATION

TRICHODERMA FROM PREDATOR TO PREY: THE ROLE OF FINE-TUNED REGULATION OF GENE EXPRESSION

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As cosmopolitan organisms, fungi share a niche with multiple organisms where they are exposed to different competitors and predators. In this talk I will describe the molecular events taking place during mycoparasitism and in the interaction of Trichoderma with insects. I will first describe how Trichoderma atroviride responds to attack by a fungivorous insect. In this regard, filamentous fungi constitute a rich food source that ensures survival and reproduction of their predators and are therefore continuously exposed to mechanical damage. Nevertheless, our understanding of how fungi respond to wounding and predators is scarce. Fungi like plants and animals respond to injury recognizing Damage- and Microbe-Associated Molecular Patterns (DAMPs/MAMPs) that activate Ca2+ and Mitogen-Activated Protein Kinase dependent signaling for the activation of defense mechanisms. Using a transcriptional approach, we studied the capacity of the filamentous fungus T. atroviride to activate specific responses to injury and attack by different arthropods. Attack by Drosophila melanogaster inhibited the transcriptional activation of genes required for hyphal regeneration, and the fungal innate immune and chemical defense responses.

Regarding mycoparasitism, T. atroviride is considered a necrotrophic mycoparasite and has developed the ability to kill other fungi and obtain nutrients from them. Here I will describe the transcriptional response of Trichoderma to the presence of different fungal hosts and the role of small RNA (sRNAs) in mycoparasitism. One of the challenges is to be able to understand the relationships that genes responding during confrontation with other fungi have with each other. We elaborated a gene co-expression network from 90 RNA-seq libraries of the wild-type strain of T. atroviride and mutants of the RNAi machinery in confrontation with three phytopathogenic fungi. We used to Alternaria alternata, Rhizoctonia solani AG2, and Rhizoctonia solani AG5, because they cause severe symptoms in plants, but they are also efficiently controlled by the T. atroviride wild-type strain. However, knockout mutants in sRNAi machinery are unable to control them. This network gave us information

on different gene modules associated to 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 relate and how the RNAi machinery plays an important role in this process.

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ORAL PRESENTATION

A COMPARATIVE STUDY OF TRICHOTHECIUM ROSEUM STRESS RESPONSES

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Trichothecium roseum is a mycoparasitic fungi widely distributed around the world. It is easily found in plant substrates during their decomposition stage and can generate a variety of mycotoxins and secondary metabolites that help it to invade the host. The stress tolerance of Trichothecium roseum (ARSEF 1212) was compared with Metarhizium robertsii (ARSEF 2575) and Metarhizium acridum (ARSEF 324). The three fungi were tested to osmotic stress; oxidative stress; UV radiation; the mutagenic 4-nitroquinoline-1-oxide (4-NQO); Congo red; and heat. Conidia were produced on potato dextrose agar medium and the suspensions were exposed to the different stress conditions. T. roseum (ARSEF 1212) was the most tolerant to osmotic stress, 4-nitroquinoline-1-oxide (4-NQO), and congo red and the least tolerant to heat. M. acridum (ARSEF 324) was the most tolerant to UV radiation and heat and the least tolerant to osmotic and oxidative stress, congo red, and 4-nitroquinoline-1-oxide (4-NQO). Whereas, M. robertsii (ARSEF 2575) presented a pattern of average tolerance when compared to the two other fungi, but it proved to be the most tolerant to oxidative stress and the least to UV radiation.

POSTER PRESENTATION

A LITTLE KEY WILL OPEN A LARGE DOOR: THE ROLE OF FUNGAL SMALL SURFACE-ACTIVE PROTEINS IN FUNGAL REPRODUCTION AND FITNESS

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Fungal biology is impeded by the irregular life cycles, immortality, and versatile reproductive behavior. Fungal bodies develop inside a substrate and have exceptional metabolic and ecological plasticity, which hinders species delimitation. Only some unique fungal traits can shed light on evolutionary forces that shape the environmental adaptations of these taxa. Higher filamentous fungi produce amphiphilic and highly surface-active proteins called hydrophobins (HFBs), which coat spores and mediate environmental interactions. HFBs are unique, non-toxic small fungal proteins with outstandingly high surface activity. They modulate surface properties by selfassembling in interfaces and have a multitude of applications for drug delivery, biosensors, and cosmetics. To explore the applied potential of HFBs, we investigated their functions in the naturally HFB-enriched Trichoderma spp. fungi (Ascomycota) (Cai et al., 2021). We show that HFBs unexpectedly specifically accumulate inside aerial hyphae, where they associate with lipidenriched organelles and putatively line up the vacuolar structures, which contribute to the structure and longevity of aerial mycelium. The maximum of HFB synthesis and secretion happens before the sporulation, providing a massive extracellular matrix suitable for the protective coating of rapidly produced spores. Furthermore, HFBs are involved in the water sensing mechanism of spores and thus are linked to the dormancy/germination switch. Our results reveal that HFBs have a broad range of intracellular functions and are essential for fungal development and fitness. We also exploited a library of HFB-deficient mutants for two cryptic species of mycoparasitic and saprotrophic fungi from the genus Trichoderma (Hypocreales) and estimated fungal development, reproductive potential, and stress resistance. HFB4 and HFB10 were found to be relevant for Trichoderma fitness because they could impact the spore-mediated dispersal processes and control other fitness traits (Cai et al., 2020). An analysis in silico revealed purifying selection for all cases except for HFB4 from T. harzianum, which evolved under strong positive selection pressure. Interestingly, the deletion of the hfb4 gene in T. harzianum considerably increased its fitness-related traits. Conversely, the deletion of hfb4 in T. guizhouense led to the characteristic phenotypes associated with relatively low fitness. The net contribution of the hfb4 gene to fitness was found to result from evolutionary tradeoffs between

individual traits. Our analysis of HFBdependent fitness traits has provided an evolutionary snapshot of the selective pressures and speciation process in closely related fungal species.

In this talk I will summarize how the case ecological genetic study on the function of particular genes – hfb –can be used for a scaled up investigation of fungal fitness based on many species and using the whole genomes.

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ORAL PRESENTATION

ANALYSIS OF THE PROTEOLYTIC ACTIVITY OF CANDIDA BIOFILMS AND EXPRESSION OF SECRETORY ASPARTYL PROTEASE GENES

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Fungi of the Candida genus can form biofilms in the oral cavity and causing infections, called candidiasis. Currently, the treatment of candidiasis has limitations due to the emergence of strains resistant to available antifungals. A promising alternative is the search for therapeutic agents targeting Candida virulence factors, such as the production of secretory aspartyl protease (Saps) enzymes. It has been suggested that, when in biofilms, Candida cells increase secretion of these enzymes and proteolytic activity, making them more pathogenic to host tissues (Rapala-Kozik et. al 2018) However, information on the role of different Candida species and Saps types in the proteolytic activity of biofilms is lacking. This study performed a temporal analysis of the proteolytic activity of biofilms of C. albicans, C. tropicalis, C. glabrata and C. krusei, and verified the expression of secretory aspartyl protease genes (SAP1 to SAP10) of C. albicans during the formation of biofilms. The proteolytic activity of monospecies biofilms of clinical strains of C. albicans (70), C. tropicalis (11), C. glabrata (64) and C. krusei (62) were analyzed after 1h30, 4 and 24h. Also, aliquots of the biofilm were seeded on Sabouraud agar for counting viable cells by calculating Colony Forming Units (CFU/mL). In the period of 4 h of biofilm formation, analysis of the expression of genes from SAP1 to SAP10 of C. albicans was performed. The results were analyzed by ANOVA and Tukey's test, considering a significance level of 5%. It was found that all Candida strains were able to form biofilms, with an increase in the number of viable cells as a function of the time of biofilm formation. After 24 h, the biofilms showed CFU/mL (Log10) of 9.12 for C. albicans, 8.89 for C. glabrata, 8.75 for C. tropicalis and 7.30 for C. krusei. C. albicans had the proteolytic activity higher than the other species at all times of biofilm formation, with a statistically significant difference between them. C. albicans presented an OD value of approximately 0.09 at 1h30, 0.3 at 4h and 0.25 at 24h. As for the results of gene expression, an

increase in the expression of genes SAP1, SAP2, SAP8, SAP9 and SAP10 was found. But the genes SAP4, SAP5 and SAP6 were under-expressed. It was concluded that C. albicans showed higher proteolytic activity in biofilms compared to C. tropicalis, C. glabrata and C. krusei, with an enzyme peak occurring at 4 h. In gene expression, C. albicans increased the expression of SAP1, SAP2, SAP8, SAP9 and SAP10, suggesting that these genes are related to increased proteolytic activity in biofilms and may be possible therapeutic targets for oral candidiasis.

POSTER PRESENTATION

CELLULAR ASSEMBLY OF THE NATIVE FUNGAL COP9 SIGNALOSOME, WHICH IS REQUIRED FOR FUNGAL STRESS RESPONSE AND SPECIFIC PROTEIN DEGRADATION

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The conserved eight-subunit COP9 signalosome (CSN) of the filamentous fungus Aspergillus nidulans assembles through a seven-subunit pre-CSN, which is activated by the integration of the catalytic CsnE/Cs5 deneddylase subunit. The fungal CSN is required for coordinated protein degradation as prerequisite for fungal stress response, development and coordinated secondary metabolism. The assembly of the native fungal pre-CSN was dissected in vivo by combined genetic and biochemical approaches. Strains with functional GFP-CSN fusion subunits were constructed with combinations of deletion mutations in different genes for CSN subunits. The different resulting CSN-subcomplexes were characterized by GFP-affinity purifications and mass spectrometry. Two distinct heterotrimeric CSN subcomplexes could be identified as pre-CSN assembly intermediates. The CsnA-C-H (Csn1-3-8) and CsnD-F-G (Csn4-6-7) trimers can be formed in parallel and independently of CsnB (Csn2). CsnB (Csn2) connects the heterotrimers into a heptamer and is prerequisite for the association of CsnE to the pre-CSN. Surveillance mechanisms control accurate CSN subunit amounts and correct cellular localization for sequential assembly, because losses of CSN subunits change the abundance and location of remaining CSN subunits.

ORAL PRESENTATION

CONTROL OF CELL MORPHOGENESIS AND CHRONOLOGICAL LIFESPAN IN THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE

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The conserved NDR kinase plays a key role in the control of cell morphology and cell proliferation in organisms ranging from yeast and filamentous fungi to human cells. We have previously discovered that fission yeast NDR kinase Orb6 spatially regulates the activity of Cdc42 GTPase, a key morphology control factor, to promote cell shape emergence (Das et al, 2009, 2012 2015). Orb6 also inhibits the degradation of specific mRNAs, thereby promoting polarized cell growth (Nuñez et al., 2016). We find that Orb6 kinase activity is downregulated by a variety of stimuli, such as nutritional deprivation or osmotic stress, suggesting that the Orb6 kinase pathway mediates cellular responses to environmental stress (Chen et al., 2019). Using genomic-scale and proteomic approaches we have identified novel targets of Orb6 kinase and discovered a role for Orb6 kinase in promoting cell adaptation and chronological lifespan. We find that by phosphorylating substrates on specific intrinsically disordered domains, Orb6 kinase promotes vegetative, oscillatory, Cdc42 dynamics and inhibits the emergence of an alternative, “exploratory” pattern of Cdc42 activation, which is normally observed during stress. We propose that an important role of NDR kinase in eukaryotic cells is to enable alternative physiological states, from active cell growth to cell quiescence, to promote cell resilience in the face of stress.

Keywords: cell morphogenesis, cell polarization, Cdc42 GTPase, cell stress, chronological lifespan, Processing bodies, stress granules, phase separation.

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ORAL PRESENTATION

FROM RED QUEEN HYPOTHESIS TO FUNCTIONAL REVERSE CHEMICAL ECOLOGY: RELATIONSHIPS BETWEEN DETOXIFICATION ENZYMES OF WHITE-ROT FUNGI AND THEIR CHEMICAL ENVIRONMENT

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Within their biotope, organisms are permanently in biotic and abiotic interactions. The dialogue between organisms and their environment is notably chemical and involves the production of a large number of molecules of diverse nature. Thus, organisms and in particular plants have acquired during their evolution the ability to synthesize specialized metabolites that partly govern their exchanges and interactions with their environment. The diversity of these metabolites could then result from a long evolution and in particular from coevolutionary mechanisms (Ehrlich and Raven, 1964). This hypothesis suggests that herbivores impose a very strong selection pressure on plants, forcing them to develop increasingly diversified phytochemical defense systems. In this context of "evolutionary arms race" formalized by the "Red Queen" hypothesis, herbivores have developed and are developing detoxification systems that allow them to resist the toxic molecules produced by the specialized metabolism of plants.

A three-phase detoxification mechanism (oxidation, conjugation, secretion) is found in many plant degrading organisms and in particular in fungi that degrade wood. Within forest ecosystems, the degradation of dead wood is indeed carried out by complex microbial communities whose major actors are the socalled decay fungi. Wood in general is composed mainly of recalcitrant polymers (lignocellulose) and more or less toxic metabolites not covalently bound to polymers called extractibles. The so-called white rot fungi possess the ability to degrade and mineralize all organic components of wood and have been widely studied for this property. Phylogenomic analyses suggest that the evolution of these fungi is linked to the presence of gene families encoding enzymes for the decomposition of polymers present in wood such as peroxidases but also enzymes involved in import and detoxification pathways such as cytochrome P450 monooxygenases and glutathione transferases (GSTs).

Since several years, we have developed a " Functional Reverse Chemical Ecology" approach using GSTs from wood-degrading fungi. A high-throughput method based on the measurement of the thermostability of the studied enzymes allowed to quantify the interactions between six GSTs from the white

rot fungus Trametes versicolor and extracts from different wood species from temperate and tropical forests (Perrot et al., 2018; 2020). Quantification of the interactions of these 6 proteins with more than 300 extracts showed a positive correlation between the antifungal properties of these extracts and their interactions with these GSTs of fungal origin (Perrot et al., 2018). This approach, coupled with a targeted fractionation approach, has made it possible to identify molecules of interest within complex mixtures but also to better understand and model the natural sustainability of tropical tree species (Perrot et al., 2018; Barbier et al., 2020). Overall, these data strongly support the hypothesis that herbivore detoxification systems and in particular GSTs can be effectively used as targets in "Reverse Chemical Ecology" approaches. This " Functional Reverse Chemical Ecology" should allow to characterize (i) the influence of a "chemical ecosystem" on the structural and functional evolution of multigenic families involved in detoxification within organisms and inversely, from the same experimental data, (ii) functionally the specialized metabolites from this "chemical ecosystem"

POSTER PRESENTATION

FUNCTIONAL GENOMICS OF THE BUDDING YEAST STRESS RESPONSE UNCOVERS NEW RELATIONSHIPS BETWEEN STRESS TOLERANCE AND GROWTH RATE

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Stress tolerance and rapid growth are often competing interests in cells. Upon severe environmental stress, many organisms activate defense systems concurrent with growth arrest. How stress tolerance and growth rate are coordinately regulated remains poorly understood. Here we use culture-based and single-cell approaches to dissect the role of stress-activated transcription in modulating these responses in budding yeast, Saccharomyces cerevisiae. In response to acute stress, S. cerevisiae cells mount a common gene expression program called the environmental stress response (ESR) comprised of ~300 induced (iESR) transcripts involved in stress defense and ~600 reduced (rESR) mRNAs encoding ribosomal proteins (RPs) and ribosome biogenesis factors (RiBi) important for division. Here I will discuss recent advanced in our understanding of the function of this conserved fungal program, and how stress defense relates to changes in cellular growth rate. We used single-cell sequencing of yeast responding to salt stress, single-cell microscopy to follow transcription-factor localization dynamics and growth rate during stress, and other studies investigating transcription changes and reorganization of ribosome translation during stress. Counterintuitively, our results show that it is the repressor of growth-promoting genes that is correlates with a faster stress acclimation and growth recovery after salt stress. This model fits past work from our lab that suggests that transient transcriptional repression of growthpromoting genes during stress acclimation serves to release translational capacity that can be directed toward induced transcripts supporting stress defense.

ORAL PRESENTATION

FUNGAL AGGRESSORS UNDER STRESS - HOW PRE-CONTACT HOST SENSING PUTS THE MYCOPARASITE TRICHODERMA ATROVIRIDE UNDER STRESS

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Several species of the genus Trichoderma are potent plant protection agents due to their ability to antagonize a wide range of fungal phytopathogens by mycoparasitism. The perception of signals from the host fungus and appropriate morphogenetic responses are essential for successful mycoparasitism. We show that conidial germlings and microcolonies of the strong mycoparasite Trichoderma atroviride preferentially respond to self-signaling cues and compounds secreted by plant roots when tested in chemotropic assays to study their chemosensing capacity, while the perception of potential host fungi has no major relevance at this early developmental stage. Host fungus recognition develops later in mature hyphae of fully differentiated mycelium in which detection of host fungus-derived compounds even overrides sensing of simple nutrients. Investigation of the early interaction stages between T. atroviride and living host fungi revealed in addition that the mycoparasite experiences significant polarity stress when approaching its fungal host. The pre-contact sensing phase was characterized by repeated switching between positive and negative chemotropism in the mycoparasites' hyphae probably triggered by host-derived substances. Accordingly, several low molecular weight substances released by both interaction partners could be detected in the interaction zone substantiating a chemical cross-talk between Trichoderma and the host fungus. Signal perception is integrated via intracellular signaling pathways such as mitogen-activated protein kinase (MAPK) cascades. Deletion of the MAPKs Tmk1 and Tmk3 affected T. atroviride's tip polarization, chemotropic growth, and contact-induced morphogenesis so severely that the establishment of mycoparasitism was highly inefficient to impossible.

POSTER PRESENTATION

FUNGAL STRESS AND SYMBIOSIS: PH PATHWAYS IN THE LAUREL WILT PATHOGEN

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Harringtonia (prev. Raffaelea) lauricola is a filamentous (Ascomycete: Ophiostomataceae) fungus that forms obligate mutualisms with ambrosia beetles in the genus Xyleborus, and is also the causative agent of laurel wilt, a devastating disease that affects members of the Lauraceae family, including the important agricultural crop, avocado. Vectored by its beetle symbiont within specialized fungal transport organs termed mycangia, this fungus can infect the vascular system of healthy trees, leading to wilting and tree death in a matter of weeks or months. Despite its potentially damaging economic impact, little is known regarding H. lauricola physiology or the factors leading to either beetle symbioses or plant pathogenic interactions. We have shown that R. lauricola grows at an optimal pH range from 5-7, with limited growth at higher pH (pH>8), that can, however, be rescued under specific conditions. In order to further examine, pH stress, we have characterized the H. lauricola PacC transcription factor which forms part of the Pal/Rim pH sensing and response pathway in many fungi via characterization of the phenotype of a ?HlPacC mutant. Aspects of mycangial colonization have been examined including the dynamics of initial colonization, and fungal growth and proliferation within the mycangia. In addition, we have characterized the phenotype of a targeted gene knockout mutant of the HlmetR bZip transcription factor that is a positive regulator involved in the uptake and utilization of sulfur sources, and which has been shown to be important for plant pathogenicity. These mutants showed alterations in pH stress and mycangial colonization implicating these pathways as contributing to the mutualistic association of the fungus with its beetle partner. Microscopic methods were also used to capture the transitions the fungus undergoes while colonizing the mycangia. These data are the first steps towards identifying genetic and molecular determinants of fungal-insect symbionts as a new model system for understanding the diversity of fungal genetics and development.

ORAL PRESENTATION

IRREVERSIBLE CELL MEMBRANE DAMAGE OF CANDIDA ALBICANS CAUSED BY 1,3,4-OXADIAZOLE DERIVATIVE

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Severe invasive fungal infections (IFIs) are becoming increasingly frequent in critically ill patients worldwide in recent years, mainly in ICU patients (Almeida et al., 2019). The genus Candida is the most common agent of IFIs, accounting for 80% of fungal infections of the circulatory bloodstream. The limited therapeutic arsenal for the treatment of IFIs associate with the increased antifungal resistance has intensified the search for new effective antifungal agents. The 1, 3, 4 - oxadiazolic derivative (LMM6) is a promising candidate with a broad spectrum of action (Faria et al, 2021). In this sense, LMM6 was tested against C. albicans. Different approaches in vitro were utilized for analyzed the mode of LMM6 action. The cell membrane integrity was evaluated by propidium iodide uptake by yeast cells. The intracellular ROS levels in C. albicans cells was analyzed using the fluorescent dye 2,7-dichlorofluorescin diacetate. The apoptosis assay was realized by FITC Annexin V/Dead Cell Apoptosis Kit. LMM6 affected cell membrane integrity of C. albicans and promotes accumulation of oxygen-reactive species (ROS). These results indicated that LMM6 induces cell death by necrosis through membrane disruption. The fungicidal effect of LMM6 is mediated by accumulation of ROS, leading to irreversible cell membrane damage. In vitro and in vivo assays have shown that LMM6 is a potent antifungal agent that may be useful in future applications.

POSTER PRESENTATION

LASIODIPLODIA SP. ENDOPHYTE AS A SOURCE OF ANTIOXIDANT SUBSTANCES

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Oxidative stress is a cellular condition induced by the unregulated production of reactive oxygen (ROS) and nitrogen (RNS) species, which are reactive molecules generated by biochemical and physiological processes from cellular metabolism under normal or pathological conditions. Antioxidants are compounds that scavenge free radicals and are known to prevent or delay cell damage and health disorders, so they can be of great benefit in improving the quality of life for humans. They can be synthesized by microorganisms to survive hostile situations such as biotic and abiotic stress. Plant associated endophytes have been considered an important and innovative resource of bioactive natural products. Therefore, this study was carried out to evaluate the antioxidant potential of ethyl acetate extracts obtained from optimized culture medium process of Lasiodiplodia sp. endophyte (Ld). Lasiodiplodia is capable of producing high-value bioactive molecules, such as enzymes, secondary metabolites including antimicrobials and antioxidants. Fourteen ethyl acetate extracts from different cultivation time of Lasiodiplodia, in optimized Czapek medium (PO) (Glucose: 15.0g; Sucrose: 24.0g; NaNO3: 0.85g; K2HPO4: 1.0g; MgSO4·7H2O: 0.5g; KCl: 0.5g; FeSO4·7H2O: 0.01g; Yeast extract: 1.0g; Distilled Water: 1000mL), which in preliminary studies showed antimicrobial action against ATCC Staphylococcus aureus and Candida albicans strains, were evaluated against their antioxidant potential. The antioxidant capacity was evaluated according to the DPPH (free radical scavenging effect), FolinCiocalteu (total phenolic) and FRAP (Fe3+ ion reduction capacity) methodology. The results were 14.82 - 43.59% for DPPH; for total phenolic content between 72.68 - 178.23µg Gallic acid equivalent/mg extract; and for the iron reduction capacity 271.67 - 666.67µmol equivalent of ferrous sulfate/g extract. The most promising extracts were POLd-7, POLd-10 and POLd-11, whose responses were, respectively: 35.21; 38.46; 43.59% for the reduction of DPPH radical, total phenolic content (174.73; 143.84; 114.98)µg equivalent of Gallic acid/mg extract and (445.60; 666.67; 471.25)µmol equivalent of ferrous sulfate/g extract, in FRAP evaluation. The extracts mentioned herein showed antimicrobial activity with minimum inhibitory concentration values (MIC) between 50 - 100µg.mL-1 and bacteriostatic effect against S. aureus and MIC values from 25 - 50µg.mL-1 in addition to fungicidal effect against C. albicans. Studies on identifing the substances responsible for the antioxidant and antimicrobial activities of Lasiodiplodia sp. ethyl acetate extracts are being carried out.

POSTER PRESENTATION

RNA-SEQ OF TRICHOPHYTON RUBRUM REVEALS THE REGULATION EXERTED BY HACA IN DIFFERENT BIOLOGICAL PROCESSES

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Trichophyton rubrum is the principal causative agent of dermatophytosis worldwide. These infections are difficult to treat due to fungal strategies to counteract the antifungals used in therapy (Martinez-Rossi et al., 2021). The available antifungal arsenal is still limited, and an aggravating factor is the emergence of resistant fungal strains. Understanding the mechanisms associated with the pathophysiology of these infections is crucial for discovering effective antifungal targets. In this sense, HacA/Hac1, a transcription factor studied in some pathogenic fungi, plays a central regulatory role in response to misfolded proteins (UPR) accumulation in the endoplasmic reticulum (Krishnan and Askew, 2014). However, little is known about its functionality in dermatophytes. To elucidate genes and biological processes regulated by the hacA gene, we performed an RNA-seq using the wild-type and the mutant strain of T. rubrum (hacA) cultivated in the RPMI medium for 24 hours. It revealed 173 differentially expressed genes (DEG), where 97 were up-regulated and 76 were down-regulated. Functional classification of gene ontology (GO) among the DEG was performed, and the most representative category between the upregulated genes is kinase activity. On the other hand, the most representative categories between the down-regulated genes are oxidoreductase activity, metal ion binding, and transmembrane transport. At least ten genes encoding transcription factors are differentially expressed. In silico analysis shows that at least 2,178 genes in T. rubrum have the hacA binding consensus region in their promoters, representing almost 25% of the T. rubrum genome (Bitencourt et al., 2020). However, few genes among DEGs have this consensus region, suggesting an indirect regulation performed by other transcription factors that HacA putatively regulates. Therefore, these data together indicate that HacA in T. rubrum is involved in many relevant biological processes and suggest its potential to be a target in fungal therapy.

Acknowledgments: Our work is supported by FAPESP (proc. No. 2019/22596-9), CNPq, CAPES, and FAEPA

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POSTER PRESENTATION

STRESS TOLERANCE OF CONIDIA OF METARHIZIUM ROBERTSII PRODUCED ON ZINC SULFATE SUPPLEMENTED MEDIUM

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Fungi for agricultural use are subject to a variety of physical and chemical insults, collectively referred to as stresses. The fungal response to stress conditions may be due to growth and metabolism impairments. It is important to understand the physiology of stress responses to alleviate the detrimental influences when applying the fungus in the field for insect control. Metal ions such as zinc play a role in the regulation of yeast metabolism, in which these cells need these metals to maintain the structural integrity of the cell and organelles. They also act in cell-cell interactions, the flocculation phenomenon, gene expression, cell division and growth, the mechanisms of nutrient uptake, the enzymatic action of metabolism, osmo-regulation, and maintenance of energy and cell survival. In addition, yeast cells need zinc as stress protectors in the face of environmental stressors. The present study aimed to evaluate the role of zinc sulfate to improve the entomopathogenic fungus Metarhizium robertsii against the harmful effects of the environment. Conidia were produced on potato dextrose agar (PDA) medium or on PDA medium supplemented with zinc sulfate at 0.5, 1.0, 1.5, and 2.0 g/l. The stress tolerances were evaluated of conidia produced in media supplemented or not with zinc sulfate to UV radiation, osmotic stress (potassium chloride), and heat. Zinc sulfate did not influence the radial growth of the mycelium or the conidial tolerances to heat and osmotic stress, which were similar to the controls. However, conidia produced on 1.5 and 2.0 g/l zinc sulfate were more heat tolerant than conidia produced on sole PDA medium. In conclusion, although it is well known that zinc sulfate improves stress tolerances of Saccharomyces cerevisiae during fermentation, zinc sulfate did not improve conidial tolerances to all stress conditions in M. robertsii.

POSTER PRESENTATION

STUA-DEPENDENT TRANSCRIPTION REGULATION OF ESSENTIAL GENES FOR GLYOXYLATE CYCLE IN TRICHOPHYTON RUBRUM

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The metabolic flexibility of pathogens is an important aspect of virulence during a host-pathogen interaction and is essential to overcoming different host environments, especially when glucose is scarce (Chew & Lung Than, 2021). For successful colonization and invasion of keratinized tissues, dermatophytes, such as T. rubrum, need to rapidly adapt an alternative carbon metabolism mechanism for efficient nutrient uptake (Peres et al., 2016). In this sense, the glyoxylate cycle, an alternative pathway of the tricarboxylic cycle, allows the utilization of two carbons compounds in the absence of glucose, exerting a crucial role in fungal metabolic flexibility. Despite this cycle being absent in mammals and could be an attractive target for antifungal drugs (Chew & Lung Than, 2021), little is known about the molecular mechanisms triggering transcriptional regulation that governs the glyoxylate cycle in T. rubrum. A ?stuA RNA-seq data of T. rubrum revealed genes coding for the glyoxylate cycle that are downregulated during the growth in different carbon sources. Since StuA plays an important role in adaptation to environmental cues and virulence in fungi (Bitencourt et al., 2021), we evaluated by RT-qPCR the transcriptional response of genes encoding key enzymes of glyoxylate cycle: isocitrate lyase (TERG_01271) and malate synthase (TERG_01281) of the ?stuA and wild type T. rubrum strains during growth on keratin and co-culture with human keratinocytes, mimicking an infection-like scenario, for 24 and 48 h. Our results showed that both TERG_01271 and TERG_01281 were downregulated in ?stuA T. rubrum strain compared to wild type during growth in keratin. Additionally, the transcripts levels of both genes were downregulated when ?stuA T. rubrum was co-cultured with HaCat keratinocytes. The results suggest that a StuA-dependent transcription mechanism governs the transcriptional regulation of key enzymes of the glyoxylate cycle.

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POSTER PRESENTATION

ABSTRACTS

STRESS MECHANISMS AND RESPONSES IN FUNGI

ISFUS

SURVIVAL OF TRICHOLOMA VACCINUM IN ECTOMYCORRHIZOSPHERIC HABITAT: FACING THE LIVING AND THE NON-LIVING

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Ectomycorrhiza-forming fungi like Tricholoma vaccinum have been shown to be very important in the balance of the forest ecosystem. Their roles in supporting plants through nutrient allocation and/or through wading off disease-causing pathogens and their role in making better soils has been well documented. These fungi have shown their ability to survive challenging environment such as sites contaminated with xenobiotics with evidence of a robust evolutionarily distinct relationship with many terrestrial plants. To ascertain stress in plant host – mycorrhiza fungi interaction, simple co-culture experiments were carried out to measure biomass change as a function of fitness in the interacting partners. In these studies, we suggest the roles of specific genes predicted to code for effector proteins, that are important in the interaction of the fungi (T. vaccinum) with its plant host (Picea abies). We also show the expression of different GST genes in T. vaccinum while interacting with biotic and abiotic stressors. RNASeq experiments were then carried out to analyse transcriptomic changes in T. vaccinum while interacting with abiotic stressors like metal-containing seepage water and while interacting with biotic partners like the plant host.

Attention is being driven towards the use of ectomycorrhiza fungi in the treatment of contaminated waste lands, as it helps in the remediation of sites while supporting the growth of host plants on the site, allowing for complete recovery of soil health. Therefore our continued contribution to the understanding of how these super resources functions will make them a great tool for reforestation.

POSTER PRESENTATION

THE TRANSCRIPTION FACTOR STUA REGULATES THE LEVELS OF THE MAPK HOG1 RNA TRANSCRIPTS IN THE DERMATOPHYTE TRICHOPHYTON RUBRUM

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Trichophyton rubrum is the leading causative agent of dermatophytosis worldwide and is responsible for most persistent skin infections caused by dermatophytes. The pathogenesis of dermatophytosis relies on multiple and integrated inputs coordinated by fungi aiming to establish its development onto the host's skin surface (1). Fungi adapt to environmental conditions and interchange signaling pathways, such as the Mitogen-activated protein kinase (MAPK) pathway, to promote the activation of specific signals to attend to physiological requirements. MAPK pathway is a ubiquitous phosphorylationbased signaling cascade related to multiple physiological functions, such as pheromone signaling, starvation responses, cell wall integrity maintenance, and osmotic stress tolerance ability (2). Comprised in the MAPK pathway, the HighGlycerol Osmolarity (HOG) cascade is one of the most studied signaling processes regarding kinase activation. It relies on the activation of sequential kinases towards triggering a specific transcriptional program to keep physiological conditions, mainly in response to osmotic stress (3). In T. rubrum, we know little about how the MAPK pathway can influence such responses and regulate processes capable of driving virulence and pathogenesis. At last, the regulation exerted by transcription factors over the MAPK pathway seems to be a suitable way of unraveling the roles of these kinase pathway components. This study aimed to investigate the role of the transcription factor StuA on the transcriptional regulation of Hog1 MAPK in T. rubrum regarding metabolic reprogramming in glucose and keratin. By revisiting RNA sequencing data published by our research group, we observed that hog1 was differentially expressed after stuA genetic inactivation (4). We validated this through RTqPCR and confirmed that hog1 transcription is coordinated by the protein StuA. Interestingly, in the presence of glucose, there seemed to occur a rewiring of carbon flux to attend to metabolic conditions, whereas, in keratin, a nitrogen-rich source, the transcript levels of hog1 in the ?stuA strain appeared to be downregulated. Aiming to elucidate the role of StuA in MAPK recruitment

regarding host interaction, we mimic a skin infection by co-culturing T. rubrum with HaCaT keratinocytes cells. We observed that the absence of StuA resulted in a differential modulation of the hog1 transcripts, which suggests that such an effect could be directly related to the metabolic reprogramming of carbon flux towards attending to cell requirements in virtue of the host contact. As a followup study, our findings corroborate the role of StuA on the pathogenesis of T. rubrum, which could bring relevant insights into the pathogenesis of this species and towards the development of new antifungal targets for dermatophytosis worldwide.

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POSTER PRESENTATION

THE TRANSLATION ELONGATION FACTOR HAS A PIVOTAL ROLE IN THE FUNGAL STRESS RESPONSE TO WOOD-DERIVED COMPOUNDS

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Ligninolytic fungi can be considered extremophiles because of the hostility of their natural habitat (Morel-Rouhier et al., 2021). To develop, they need to access nutritive substrates, water, oxygen and a matrix to spread. Due to the structural organization of the wood material and its chemical composition, all these parameters could be limiting factors for fungal development and thus use of wood as a substrate. Moreover, wood contains extractives with antifungal properties (Valette et al., 2017). To understand how fungi cope with coumarins, a comparative proteomic approach was carried out on four fungal strains with different physiological features. For two of them, the effect of esculin was highlighted on the protein translational process. In particular, the two subunits of the elongation factor complex eEF1B are induced in the presence of esculin. By characterizing these proteins at the biochemical level, we suggest that the elongation factor complex could be a modulator of protein translation under stress conditions.

POSTER PRESENTATION

TRANSCRIPTOME META-ANALYSIS UNVEILS FUNCTIONAL ASSOCIATION BETWEEN ALTERNATIVE SPLICING AND FUNGAL STRESS RESPONSE TO MUTATIONS, DRUG EXPOSURE, AND NUTRITION SOURCES

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The regulation of gene expression in eukaryotes is knowingly responsive to varied stimuli through a network of competing and interwoven cell signaling pathways. This constitutes a central system associated with transistor functions in fungi, tuning physiological responses to environmental conditions. In this study, we have come up with a new perspective on the problem involving the gaps between structural genomes versus transcription rates versus proteomic diversity. Using a meta-transcriptomic approach, we compared RNA-Seq data with different viewpoints of the biology of the dermatophyte model species Trichophyton rubrum. Our results support the occurrence of Alternative Splicing (AS) of pre-mRNA transcripts in a large subset of genes for all conditions investigated: (1) growth in glucose- or keratin-supplemented medium (1,340 genes), (2) exposure to the antifungal agent Undecanoic Acid (UDA) (654 genes), and knock-out mutations in the genes coding for the transcription factors (TF) (3) ap1 (1,432 genes) and (4) stuA (1,940 genes). In the set of 17 libraries analyzed, 5,449 single AS events by Intron Retention (IR) were detected in 2,763 genes. Notably, this number represents 31.71% of the 8,713 genes found in T. rubrum. Therefore, IR might represent a causative source of diversity for the 10,418 proteins described in this species. Most importantly, we highlight AS as a stress-responsive system. Our results revealed that 2,539 genes were differentially expressed (DE) in (1), 501 of which were also splicingregulated (SR); in (2) 688 genes were DE, with 41 SR; 1,433 DE, with 259 SR in (3); and 3,590 DE, with 852 SR in (4). We then conclude that compensatory mechanisms regulating gene expression may also be post-transcriptionally modulated by splicing factors in fungi, not simply representing a control by TF at the DNA level. In fact, the number of SR was equivalent to DE genes in two of the four experiments: TF ap1 mutation and UDA exposure. We also ran an analysis to search for AS specific to TF. Interestingly, libraries from all four experiments were enriched with IR events in TF. A total of 123 IR events

were detected in 67 TF genes. The results revealed a clear overlap between splicing and TF responses in the regulation of gene expression. Gene Ontology classification of AS events showed that libraries were also considerably enriched with genes representative of molecular functions such as: kinase activity, oxidoreductase activity, proteolysis, transmembrane transport, and even translation, regulation of transcription, and mRNA processing functions. Altogether, these results broaden our current knowledge of the multiple-level control of fungal physiology during the stress response. We have shown that compensatory mechanisms involving the transcription and splicing machinery must act in an adaptive and concerted manner. Overlaps between these systems provide fine-tuning of gene expression in response to multiple stimuli. This mechanism is presumably responsible for shaping a large amount of the diversity observed in the proteome of fungal cells.

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To be judged for the Journal of Fungi Award.

POSTER PRESENTATION

ABSTRACTS

FUNGAL PHOTOBIOLOGY, CLOCK REGULATION, AND STRESS

ISFUS

EFFECT OF OZONE ON CONIDIAL SURVIVAL OF THE FUNGI TRICHOPYTON RUBRUM AND MICROSPORUM GYPSEUM

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Onychomycosis is a contagious infection of the nail bed and is recognized as the cause of up to 50% of all nail diseases. This sparked our interest in researching a new alternative treatment, which is a less invasive and more resolute method to treat the disease, employing cost-effective and easy-toapply techniques. The technique of ozone therapy was used in this experiment. Ozone (O3) is a highly unstable gas, produces a characteristic odor, and rapidly dissociates into oxygen when exposed to room temperature. The objective of this work was to evaluate the effectiveness of O3 in a controlled experimental laboratory study (in vitro) of the fungi Trichopyton rubrum (ATCC 28188) and Microsporum gypseum (ATCC 24102). The organisms were cultured on Sabouraud dextrose agar (SDA). In the study, Petri dishes were divided into two experimental groups: the untreated control group (CG) and the ozone group (O3), which was treated with ozone therapy. The same O3 dosimetry (4 mg/ml) was employed through direct application of gas to the petri plate using a standardized plastic bag. A variety of times were tested from 2 to 30 min with an interval of 2 min. The ozone group treated with O3 did not present germinal metabolic activity after a period of 24 h. Nevertheless, after 48 and 72 h, germinal growth was visible in both species studied, with greater resistance in the species M. gypseum. Ozone achieved a positive result in this study; however, studies need to continue and intensify to elucidate the effectiveness of the technique in onychomycosis and establish a standard dose and possible treatment protocol

EFFECT OF INHIBITORY PHENOLIC COMPOUNDS OVER THE CAROTENOIDS PRODUCTION BY THE OLEAGINOUS FUNGI MUCOR CIRCINELLOIDES

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Carotenoids are a group of pigments present in nature and can be found in bacteria, fungi, algae and plants. These biomolecules give yellow and red colors to vegetable and microbial biomass and are used commercially as food coloring and nutritional supplements. Recently, carotenoids antioxidant activity has been arousing interest of many industrial segments; however, its uses are still narrow due to high production costs. Therefore, the present study has the objective to evaluate the impact of phenol, a representative inhibitory compound for some microorganisms and present in many agroindustrial by-products and wastes – as vinasse – over the biochemical parameters during carotenoids production. Here, the influence of phenol (0-500 mg L-1) was evaluated in the biotechnological production and accumulation of total carotenoids by the fungi Mucor circinelloides URM 4182 using the culture medium and conditions reported by Carvalho et al. (2015). The results show a reduction in all the biochemical parameters analyzed (biomass, lipids and carotenoids) gradually according the increase in phenol concentrarion, achieving less than 70% in the parameter compared to the control for initial phenol concentrations of 500 mg L1 when compared to the control. Therefore, the results indicate that in industrial applications, where high quantities of carotenoids and lipids are necessary, should require growth medium, complex or synthetic, with lower concentration of these compounds.

POSTER PRESENTATION

ABSTRACTS

FUNGAL STRESS IN INDUSTRY

ISFUS

FROM AGRO-INDUSTRIAL BY-PRODUCTS TO BIOFUELS: USING RICE AND CASSAVA WASHING WATERS TO PRODUCE FUNCTIONAL LIPIDS AND BIODIESEL

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Currently many technologies are being developed with the objective to transform agro-industrial wastes and by-products into high added-value products, since they can be used as nutrient source for filamentous fungi growth and for obtaining lipid and lipase rich biomass, with many applications in the pharmaceutical, food, cosmetics and biofuel industries. Brazil has rice and cassava flour as important food source for the population, generating as main by-products the washing waters from both rice and cassava. It is a known fact that the fungal strain Mucor circinelloides URM 4182 is able to assimilate the soluble starch (Carvalho et al., 2018). This study had the objective to produce an oleaginous microbial biomass from rice and cassava washing water. The substrates were analyzed regarding total sugar content and ionic composition. In the analyzed samples were found sodium, potassium and calcium cations and chloride, phosphate, nitrate, nitrite and sulphate anions. The cultivation was performed with the inoculation of a suspension of 1x105 spores in 500 mL Erlenmeyer flasks with 100 mL of liquid waste (rice or cassava washing water) at 26 ºC, 250 rpm during 120 h. The starchy substrates were assimilated by the microorganism achieving average concentrations of 3.4 ± 0.3 g L-1 of biomass with 17.8 ± 1.2% of lipids for both mediums, due to its similar composition and sugar concentration (21.1-28.5 g L-1). The composition of the extracted lipid presented mostly the fatty acids palmitic and oleic (48.7 ± 2.1%), as well as polyunsaturated fatty acids like linoleic and gama-linoleic (24.6 ± 1.7%). The oil extracted was ethanolyzed applying 10% of H3PMo/Al2O3 as catalyst in a stainless-steel pressurized reactor at 200°C for 6 h. The analysis of nuclear magnetic resonance spectroscopy (RMN1H) indicates high levels of ethyl esters in both reactions (96.7% ± 0.4). The fungal biomasses were also subjected to the olive oil hydrolysis test at 37 °C for 10 min, reaching activity values of lipase of 119 ± 14 U g-1. The current results shows that the valorization of starchy byproducts by biotechnological routes using M. circinelloides is an attractive alternative for the production of renewable products of industrial interest, as lipids and lipases.

POSTER PRESENTATION

FUNGAL STRESS TOLERANCE - A STORY TOLD BY THE ENZYMES

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Filamentous fungi can be found in environments with different characteristics, especially under stressing conditions as high temperatures, reduced water availability, radiation, and hypoxia, oxidative and nutritional stress. The nutritional stress, considering the carbon and nitrogen sources, is one the most important to be considered. The fungal development depends on the carbon:nitrogen balance and the availability of these elements. This is a key point to be pondered during fungal cultivation aiming different purposes, as fungal biology investigation and biotechnology. Both submerged fermentation (SbmF) and solid-state fermentation (SSF) have been applied to investigate the production of biomolecules with several applications in different sectors. The former is the main fermentative process used, but it is necessary to consider that it is an artificial and stressing condition for the microorganism. As response, the fungus produces and secretes enzymes, especially hydrolases, with distinctive characteristics to overcome this situation. In the last years, we have worked with the production of fructofuranosidases, phytases and tannases by different species of filamentous fungi isolated from areas under stressing condition of temperature and nutrients as the sugar cane plantation site and Cerrado biome. These enzymes have presented interesting characteristics as thermal and pH stability, salt tolerance and high degree of glycosylation. For example, Rhizopus microsporus var. microposrus produced an alkali stable phytase acting at 65°C and pH 9.5, while the Aspergillus phoenicis ßfructofuranosidase was thermo-tolerant. The latter also produced a glucose and solvent-tolerant tannase. These facts signalize the importance of the tolerant hydrolases as an important adaptation for the fungal survival under stressing conditions.

ORAL PRESENTATION

GROWTH RESPONSE OF FUNGUS COLLETOTRICHUM GLOEOSPORIOIDES IN THE PRESENCE OF AZO-DYES APPLIED IN INDUSTRY

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The azo dyes are xenobiotics compounds applied in textile industry, being recalcitrant and highly toxic to animals and humans and which can be mineralized or discolored by the action of micro-organisms such as fungi.. The main of this work had as purpose to analyze the capacity of growth of fungus Colletotrichum gloeosporioides in solid and liquid medium, in the presence of the azo dyes Congo Red, Methyl Orange and Direct Black to further potential utilizations of this microorganism in the recalcitrant dyes degradation The fungus was grown in solid Vogel's minimal medium with addition of glucose 1% (m/V) and 2% agar (m/V) during 5 days at 28±2°C. Afterward, five pellets of this medium were transferred to the middle of Petri glasses with Vogel's minimal medium, glucose 1%, agar 2% and various aqueous dye solutions (0,001%. 0,01%, 0,1% e 1,0% (m/V)) additions and the best result was selected to the growth in liquid médium. C. gloeosporioides hyphaes were transferred, respectively, to erlenmeyers with 50 mL of Vogel minimal medium in two conditions, with and without glucose 1% addition, and the each aqueous dye solution of 0,01% (m/V) and incubated at 28±2°C during 15 days with constant agitation. Each condition, after this period of time, was interrupted, centrifuged and filtered, the biomass separeted of the supernatant and used for gravimetry and spectroscopy assessment. The results showed that azo dyes weren't toxic to the C. gloeosporioides, because it was capable of growing in the presence of all of them. Besides, it was observed that it has a better growth in the presence of 0,01% (m/V) aqueous dye solution and glucose 1% additions. The assessment in the absorption spectrum, it was possible to view that the fungus is also capable of modifying the bands of absorption of all dyes that have been tested in this study, indicating a possibility to degradation of this dyes. In conclusion, the microorganism studied has a potential to be an agent of azo dyes degradation.

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POSTER PRESENTATION

IDENTIFICATION OF TRANSCRIPTION FACTORS INVOLVED IN ASPERGILLUS NIDULANS ADAPTATION TO RECOMBINANT PROTEIN PRODUCTION

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Filamentous fungi, such as Aspergillus spp, can secrete significant amounts of proteins and are used as a platform for the production of industrially important enzymes, such as hydrolytic enzymes used in the biorefinery industry. However, in terms of recombinant protein production, particularly of non-fungal protein, there is still potential for improvement. In this regard, many strategies have been explored to increase the capacity of fungal cell factories production; however, as consumer demand grows and productivity remains low, identifying new genetic targets to promote increased enzyme secretion becomes increasingly critical. Thus, the rational use of transcription factors has emerged as an interesting and promising approach. Therefore, our aim was to evaluate the production/secretion of enzymes in strains of Aspergillus nidulans overexpressing a heterologous enzyme in which target transcription factors have been genetically manipulated. To identify these transcription factors, we used RNA-seq data from A. nidulans strains overexpressing three heterologous enzymes and we performed differential expression and GO enrichment analysis. Next, using CRISPR-Cas9, we knock-out these transcription factors in A. nidulans overexpressing an heterologous a-L-arabinofuranosidase and analyze their phenotype to determine if they have any effect on enzyme activity.

Based on RNA-seq data, we identified six transcription factors candidates – AN8772, AN9373, AN7913, AN3420, AN0094 and AN7592 – with predict function in golgi vesicle transport, protein refolding, cell redox homeostasis and proteolysis. We found that the mutants AN0094, AN8772, AN3420 and AN7592 did not showed differences in enzymatic activity (U/mg), while the strains ?AN7913 and ?AN9373 had a small reduction of activity. This result may indicate the influence of the transcription factors AN7913 and AN9373 in total protein secretion, and that perhaps an overexpression strategy of these transcription factors can be more successful. We concluded that transcriptomic data could be used to identify transcription factors with possible involvement in the protein secretion pathway of A. nidulans, and we intend to expand our research by conducting additional experiments to determine the role of these transcription factors in the secretion of biotechnologically relevant recombinant enzymes.

POSTER PRESENTATION

PHYSIOLOGICAL INVESTIGATION OF THE STRESS INDUCED BY CONTAMINATING BACTERIA IN THE CONTEXT OF YEAST INDUSTRIAL BIOTECHNOLOGY

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Industrial biofuel production is severely affected by lactic acid bacteria, especially from the genus Lactobacillus. These bacteria are in constant interaction with the fermenting yeast. As a result, fermentative efficiency is often compromised leading to a drop in industrial yield and productivity. Studies involving co-cultivation between S. cerevisiae and species of lactobacilli allowed the identification of mechanisms such as competition for nutrients present in the fermenting broth, and the production of organic acids by bacteria, which result in the decrease in the viability of the yeast and carbohydrate/ethanol conversion. Some studies have also suggested beneficial interactions among these two microorganisms. In order to study yeast and bacteria interactions during bioethanol fermentation, we have performed a series of co-cultivation experiments, mimicking the industrial process as far as possible, and we have collected data on the physiology of such co-cultures as well as on intra- and extracellular metabolites from both yeast and bacteria, including heterofermentative and homofermentative bacterial strains.

ORAL PRESENTATION

PROTEOMIC APPROACH ON OXIDATIVE STRESS IN FUSARIUM OXYSPORUM CULTURED WITH AMINO ACIDS

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Fusarium oxysporum is a worldwide distributed soilborne filamentous fungus and that affects crops economically important. The success of this phytopathogen depends on metabolic changes, which involve the secretion of enzymes and other proteins related to the degradation of plant tissue. On the other hand, this enzyme arsenal has been differentially explored due to great diversity of enzymes with biotechnological applications (Pessôa et al., 2017). Nevertheless, the production of various extracellular enzymes can be influenced by several factors, such as nitrogen sources. Moreover, the proteins that performed the acquisition and catabolism of these sources also are modulated (Miao et al, 2019). Aerobic organisms are continuously exposed to reactive oxygen species, generated by cellular metabolism, and produce different types of molecules with antioxidant properties. The ability to activate the stress response is necessary for fungi to survive environmental changes, such as growing conditions. Proteins are tools involved in the restoration of redox homeostasis, damage repair and cell survival; therefore are fundamental in the response to oxidative stress (Zadrag-Tecza et al, 2018). Thus, the aim of this work was evaluate how amino acids affect the intracellular protein profile and the secreted enzymes of Fusarium oxysporum URM 7401. Filamentous fungi was cultured on submerged bioprocess with 1% (w/v) of amino acids (glutamic acid, cysteine, histidine and isoleucine), as nitrogen sources. After 96 h of bioprocess, the crude extract was processed to enzyme activities assays (lipase, peptidase and xylanase) and the intracellular proteins from mycelium were extracted and analyzed using 2D electrophoresis. After gel analysis, spots were selected and submitted to mass spectrometry. The nitrogen sources changed the profile and quantities of the hydrolases. The highest productions of the peptidases were observed in histidine and isoleucine cultures; and cysteine and histidine showed the best xylanase production. The identified intracellular proteins presented sequences involved in resistance to stress processes. We also note that in all conditions some sequences were involved in metabolic adaptation as response to stimulus. Thus, elucidating the intracellular protein profile and the secreted enzymes of Fusarium oxysporum URM 7401 can help clarify its behavior under distinct 1% amino acids sources.

POSTER PRESENTATION

STRESSFUL AND STRESS-FREE APPROACHES FOR FUNGAL CONTROL

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Approaches for control of fungal pathogens and spoilage organisms traditionally rely on the use of chemical actives, e.g., antifungal drugs, fungicides, preservatives. However, fungal resistance is growing, while tightening legislation and consumer concern over chemicals use can further restrict the available arsenal. Although involving more than one chemical, combinations of agents that synergistically stress the fungi can reduce total chemicals usage. They may also help target different subpopulations in heterogeneous cell/spore populations, which may otherwise enable harmful fungi to persist after singleagent treatments. Passive, physical measures could offer an important, activesfree option for fungal control. Our recent work has characterised polymer materials that passively resist surface attachment by fungi, so blocking a key first step that precedes many of the problems that they cause. I will discuss selected approaches in the context of current challenges for fungal control.

Approaches for control of fungal pathogens and spoilage organisms traditionally rely on the use of chemical actives, e.g., antifungal drugs, fungicides, preservatives. However, fungal resistance is growing, while tightening legislation and consumer concern over chemicals use can further restrict the available arsenal. Although involving more than one chemical, combinations of agents that synergistically stress the fungi can reduce total chemicals usage. They may also help target different subpopulations in heterogeneous cell/spore populations, which may otherwise enable harmful fungi to persist after singleagent treatments. Passive, physical measures could offer an important, activesfree option for fungal control. Our recent work has characterised polymer materials that passively resist surface attachment by fungi, so blocking a key first step that precedes many of the problems that they cause. I will discuss selected approaches in the context of current challenges for fungal control.

ORAL PRESENTATION

ABSTRACTS

FUNGAL BIOLOGY IN EXTREME ENVIRONMENTS

ISFUS

ANTIMICROBIAL ACTIVITY AND POTENTIAL TOXIC EFFECTS ON FRESHWATER MICROALGAE EXPOSED TO TITANIUM DIOXIDE MYCONANOPARTICLES

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Titanium dioxide has UVA/UVB reflection and absorption capacity and adequate tolerance to human skin. Due to these characteristics, inorganic sunscreens have titanium dioxide nanoparticles (TiO2NP) in their complex composition. This metallic nanomaterial (MN) is produced on an industrial scale by thermal decomposition synthesis. However, the necessity to develop new approaches for the synthesis of this MN is imminent, since the methods used produce a large volume of toxic gases and requires a large amount of energy. Given this demand, green nanotechnology has among many objectives to reduce the presence of residues generated during the synthesis, implement sustainable processes and increase the biocompatibility of these compounds. Different organisms are proposed in this type of approach, however filamentous fungi are the most promising. In this context, the present work aims to study the antimicrobial activity of two myconanoparticles of TiO2 (bio-TiO2NP) biosynthesized by fungi Rhizopus arrhizus (IPT1011) and Aspergillus niger (IBCLP20), present in suspension and encapsulated in alginate, besides the synthetic TiO2 nanoparticle (P25) with 25 nm present in suspension, and also to evaluate potential toxic effects on the marine microalgae Chlorella minutissima and the freshwater microalgae Chlorella fusca. Bio-TiO2NP were characterized by UV-vis spectrophotometry, 'Dynamic Light Scattering' (DLS) and Polydispersity Index (PDI). Antimicrobial activity was evaluated by minimum inhibitory concentration (MIC) using the pathogenic bacteria Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The microalgae Chlorella minutissima and Chlorella fusca were used for cell density studies. The bio-TiO2NPs were synthesized with two different diameters 69.8 (IPT1011) and 90.1 nm (IBCLP20) and PDI <0.3. The bacteria were exposed to a concentration range from 1 to 500 µg/mL of bio-TiO2NPs, precursor metal (TiO2) or the synthetic nanoparticle (P25), but after 48 h of the exposure of the compounds in suspension, at no concentration was observed inhibition of the growth of pathogenic bacteria. Whereas bio-TiO2NP (IPT1011) immobilized in alginate at concentrations of 1, 5 and 10 g had antibacterial action by inhibiting

the growth of ±80 % of E.coli. In relation to phytotoxicity assays in microalgae, both species were exposed to bio-TiO2NP (IPT1011) and synthetic nanoparticle P25 at concentrations of 50 to 500 µg/mL and both species had a toxic response, but between bio-TiO2NP and synthetic nanoparticle P25, both in C. minutissima and in C. fusca, bio-TiO2NP showed less toxicity than the nanoparticle synthetic P25. In view of the possibility of replacing synthetic TiO2NP with biological ones, of fungal origin, represents a promising alternative that meets the 2030 Agenda for Sustainable Development in the Goals (12), ensure sustainable production and consumption patterns and (13) take urgent measures to combat climate change and its impacts.

POSTER PRESENTATION

DIVERSITY OF FILAMENTOUS FUNGI ISOLATED FROM CANANÉIA MANGROVE SEDIMENTS

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Mangrove is a transient coastal ecosystem situated between terrestrial and marine environments. To preserve, restore and better understand these ecosystems, researchers have been studying their microbiology, but research focusing on fungal communities is still limited. As is known, fungi play an important role in the decomposition of organic matter in this ecosystem and produce important bioactive natural products. The fungal diversity in this ecosystem deserves attention, especially those present in the sedimentary column, as they are poorly studied. In our study, we investigated the fungal community in four sedimentary mangrove sites in Cananéia, São Paulo, Brazil. Surface samples from sediments (~2 cm of superficial sediment) were collected in the summer of 2022. The samples were stored at -20 °C and processed for isolation and cultivation of the fungus after transport to the laboratory. Briefly, fungi were isolated from 10 g of each sample suspended in 45 mL of saline solution with Tween 80 and then serially diluted. Samples were cultured in Petri plates containing potato dextrose agar medium. Plates were incubated at 30 °C for 14 days. After the incubation period, fungi were identified according to their macro and micro morphological characteristics with the assistance of identification keys. Fifty-six strains were morphologically analyzed, they showed compatible with the description for genus, Aspergillus, Penicillium and Trichoderma. Based on percentage of occurrence, the most common genus was Aspergillus (38.46 %), Penicillium (30.76 %), Trichoderma (7.69 %) and 23.06 % were not identified. Taking this into consideration, the identification of these fungi is essential, as future studies may highlight the application of new biomolecules with biotechnological applications as emerging contaminants.

POSTER PRESENTATION

EFFECTS OF GREEN NANOSTRUCTURED CORROSION INHIBITORS ON MARINE FILAMENTOUS FUNGI

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Corrosion is an aggressive phenomenon that causes the deterioration of metallic structures. Corrosion inhibitors (CIs), such as 2-mercaptobenzothiazole (MBT) and sodium gluconate (SG), are widely used in protective anti-corrosion coatings which are released in the early life stages of such coatings. To overcome this problem, CIs have been immobilized in stimuli-responsive engineered nanomaterials (ENMs), such as layered double hydroxides (Mg-Al LDH or Zn-Al LDH). The mycotoxicity of such innovative solutions are still unknown. Therefore, the toxicity of such novel compounds and its precursors was assessed upon three filamentous fungi (Ffs), isolated from the marine environment, namely the Aspergillus niger (IB-CLP20), Penicillium polonicum (IB-CLP22) and MP1B (identification in progress). Ffs were exposed to 7 compounds, namely the innovative ENMs loaded with CIs (Zn-Al LDH-SG; ZnAl LDH-MBT; Mg-Al LDH-MBT), the CIs (MBT; SG) and the raw ENMs (Zn-Al LDH; Mg-Al LDH), using the concentrations 1.23, 3.7, 11.1, 33.3 and 100 mg·L?¹, for a period of 72 hours. The minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) were then assessed for each species and chemical. Tested compounds did not caused fungi growth inhibition, apart from 2-mercaptobenzothiazole (MBT). The concentration of 100 mg MBT·L?¹ presented a fungistatic effect on IB-CLP20, and a fungicide effect on IB-CLP22 and MP1B. These findings reveal that nanotechnological-based approaches can reduce the negative environmental impacts of conventional CIs on marine fungi. However, further studies on other microorganisms are encouraged.

POSTER PRESENTATION

FRUITING BODY SPECIFIC SC4 HYDROPHOBIN GENE PLAYS A ROLE IN SCHIZOPHYLLUM COMMUNE HYPHAL ATTACHMENT TO STRUCTURED GLASS SURFACES

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Genes encoding hydrophobins play distinct roles at different stages of the life cycle of fungi, and they foster hyphal attachment to surfaces. The hydrophobin Sc4 is known to provide a hydrophobic membrane lining of the gas channels within Schizophyllum commune fruiting bodies. Here, we cultivated non-fruiting, monokaryotic S. commune 12-43 on glass surfaces that could be verified by micrography. Differential gene expression profiling of ten hydrophobin genes by quantitative PCR showed significant up-regulation of sc4 when S. commune was attaching to glass surfaces, confirmed also with RNA-Seq data analysis. Another silicate, namely quartz sand, was investigated, and induction of sc4 was seen as well. The up-regulation of the hydrophobin gene sc4 may indicate involvement in S. commune hyphal attachment to glass as well as quartz surfaces. We propose that the covering of hyphae by Sc4 allows for direct interaction with the hydrophobic surfaces, and that differential functions of specific hydrophobin genes depending on the surface interface is involved. This study could help with the clarification of the biological functions of this gene. Therefore, the analysis serves as a basis for understanding S. commune interaction with glass surfaces.

POSTER PRESENTATION

FUNGAL VOLATILES HAVE PHYSIOLOGICAL CONSEQUENCES

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Volatile organic compounds (VOCs) emitted by fungi cause familiar musky odors and have been theorized to cause or contribute to the symptoms associated with a controversial medical diagnosis called “sick building syndrome.” This presentation will review some of the aftermath of flooding associated with hurricanes and present relevant findings on molds, mold toxins, VOCs, and sick building syndrome. Our laboratory has pioneered the use of genetic model systems to study the physiological effects of VOCs and has developed a Drosophila eclosion assay to investigate the toxigenic activity of fungal volatiles. Low concentrations of “mushroom alcohol” (1-octen-3-ol) cause movement deficits and loss of dopaminergic neurons in Drosophila. On the other hand, some fungal volatiles have shown unexpected beneficial effects on plant growth and as safe fumigation agents whereby volatiles emitted by certain Trichoderma species inhibit growth of the oomycete potato blight pathogen. The physiological consequences of fungal intraspecific and interspecific signaling in the gas phase merits greater attention from mycologists and other scientists.

ORAL PRESENTATION

FUNGI IN METAL-RICH ENVIRONMENTS: SURVIVAL AND SUBSTRATE TRANSFORMATIONS

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Geomycology can be defined as the impact of fungi on geological processes, including bioweathering of rocks and minerals, metal and metalloid transformations, and element and nutrient cycling. Many geomycological processes, all dependent on hyphal growth form and chemoorganotrophy, are of relevance to metal pollutant fate in the environment altering metal mobility through, e.g. mineral dissolution, metal accumulation and biomineralization. Wherever fungi are found, transformations of metals and minerals are a key component of their activity, with biomineralization occurring in natural and human-influenced habitats. As with biominerals formed in other biological systems, the most common fungal biominerals are oxides, carbonates, phosphates, sulfates and sulfides, with a particular association of oxalates with fungi. Many fungal biomineralization processes can result in the production of nanoscale mineral and metallic products. Nanoscale elemental particles of gold, silver, palladium, selenium and tellurium can be produced by a wide range of fungal species. Nanominerals include oxides, carbonates, phosphates, selenides, tellurides and sulfides that can incorporate a range of metals including Cu, Cd, Zn, Mn, Ni, Fe, Pb, and Sr. More recently, it is realised that fungal nanoparticles may have benefits to the organism because of their catalytic properties. Nanozymes are inorganic nanoparticles that exhibit enzyme-like properties in redox reactions, and include metals and metal oxides, most of their catalytic reactions mimicing oxidase, peroxidase, catalase, and superoxide dismutase. Because fungi can mediate the formation of reactive nanoparticles, including metal oxides, these biomaterials are receiving deserved attention because of their hitherto unappreciated role in biogeochemical cycles for rare earth elements, metals and metalloids, and the significance of such biogenic nanomaterials in the evolution of the lithosphere and the biosphere. This contribution will highlight the concept of fungal biomineralization, the occurrence and significance of important fungal biominerals in natural, extreme and synthetic environments, its role in certain stress responses, and the applied potential of fungal biomineralization in nanobiotechnology.

ORAL PRESENTATION

MANGROVE FUNGI MAY BE A CANDIDATE FOR MICROPLASTICS BIODEGRADATION?

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The constant microplastics accumulation mainly in the marine environment offers potential damage to the ecosystems and to the public health. Besides that, with the need to investigate alternatives and explore methods to reduce their pollution, microorganisms have been shown to be able to biodegrade microplastics, neutralizing the compounds and incorporating them into biogeochemical cycles. For this reason, filamentous fungi provide greater practical and promising applicability to bioremediate microplastics from polluted environments. However, few studies evaluate adequate strategies to eliminate or mitigate their effects on the environment. Likewise, there are few reports of better biodegradation conditions pointing to the difficulty of obtaining qualified strains for this activity. Accordingly, this study evaluated the ability of four filamentous fungi from marine environments to biodegrade polyethylene (PE) microplastics. For this, each fungal isolate was cultured in 250 mL Erlenmeyer flasks containing 50 ml of minimal growth medium infused with 5 mycelium discs and 0.05 g of PE (250 µm). Incubation was carried out for a period of 28 days at 30°C under agitation at 150 rpm. Replicas were kept and sampled after 7, 14, 21 and 28 days. At the end of each period, the assays were evaluated for cell growth and residual microplastic. The tests revealed that, under the conditions and filamentous fungi tested, Aspergillus sp. obtained the best result with 48.1% PE mass reduction. The other fungi, Rhizopus sp. and Penicillium sp., showed a loss of 38%, 37.3% and 21.6% for unidentified lineage, respectively. In all samples there was a loss of fungal biomass, however, compared to controls, the fungi in treatment accumulated a slightly higher biomass. Penicillium sp. Showed the lowest biomass loss with 2%, followed by Aspergillus sp. and Rhizopus sp. with 10% and 32.6%, respectively, while the maximum weight loss was 80.5% for unidentified strain. These results indicate that fungal isolates can actively contribute to the biodegradation of microplastics under oligotrophic conditions. However, other techniques and analytical parameters are needed to prove effective biodegradation, so these strains can be used in future studies.

POSTER PRESENTATION

MARINE MYCOSILVER NANOPARTICLES SYNTHESIS: SCREENING, OPTIMIZATION AND ANTIMICROBIAL ACTIVITIES

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Twelve different strains of marine fungi were evaluated according to their capability to produce silver nanoparticles (AgNP). Among 12 selected strains, 10 were able to biosynthesize AgNP. All AgNP obtained were preliminary characterized by dynamic light scattering (DLS), zeta potential (Pz) and polydispersity index (PDI). Four of them, produced by Penicillium citrinum IBCLP11, Penicillium sclerotigenum IB-CLP17, Aspergillus niger IB-CLP20 and Penicillium polonicum IB-CLP22 showed best results parameters selected. These four strains were studied for optimization AgNP synthesis evaluating the influence of different physico-chemical parameters, such as, variations concentrations in AgNO3, biomass, agitation, temperature and pH. Antimicrobial capacity was also evaluated. The AgNP produced by the Penicillium citrinum IB-CLP11, Penicillium sclerotigenum IB-CLP17, Aspergillus niger IB-CLP20 and Penicillium polonicum IB-CLP22 strains have surface plasmonic resonance band (SPR) in the wavelength range of 410-450 nm, size between 1-100 nm, load with module value up to the limit of 30 mV, and polydispersity index (PDI) less than 0,3. In the antibacterial action studies, all 4 AgNP were able to inhibit the growth of Pseudomonas aeruginosa IPT322, Staphylococcus aureus IPT246 and Klebsiella pneumoniae IPT412 in concentrations equal to or greater than 50 µg mL-1. In relation to the antifungal action, Aspergillus niger IPT295 and Penicillium funiculosum IPT423 were the most sensitive, presenting minimum inhibitory concentration (MICs) from 20 to 40 µg mL-1. Regarding the

influence of different parameters on AgNP biosynthesis, AgNO3 concentrations higher than 1,0 mM triggered the increase of nanomaterials size and the AgNP IB-CLP22 presented instability once to infer to 1 month of storage. All strains presented biosynthesis capabilities when the biomass value was between 75.0-150.0 g L-1. In the evaluation of the agitation influence in the process of AgNP formation, in both static and agitated mode the four lines produced AgNP, however, the AgNP biosynthesized in static mode presented larger size when compared to processes carried out with agitation of up to 150 rpm. A higher aggregation state of AgNP produced at 200 rpm was also detected. In addition, AgNP IB-CLP11, IB-CLP17 and IB-CLP22 produced at 200 rpm precipitated after 3 months of storage. All AgNP formation occurred at temperatures between 25-35° C. However, an instability of AgNP IB-CLP11 and IB-CLP22 nanomaterials was observed at temperatures above 30 ° C. At pH above 5.5, no AgNP formation occurred for any of the selected strains. AgNPs produced at pH 4.5 showed better stability. In this context, the research allowed new insights about the best condition to produce AgNPs in an approach to an eco-friendly, low-cost, and bio-based solution. Thus, this study addresses two Sustainable Development proposed by the United Nations promoting access to industrial innovation (Goals 9) and encouraging the use of oceans, seas and marine resources for sustainable development (Goal 14).

POSTER PRESENTATION

SCREENING OF MANGROVE FUNGI STRAINS FOR POLYSTYRENE MICROPLASTIC BIODEGRADATION

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Over the last decade microplastics have been considered emerging contaminants, since they are widely distributed and show adverse effects on multiple ecosystems. Some microorganisms can eliminate or mitigate the effect of these materials on the environment, among them, filamentous fungi are one of the most capable of degrading microplastics, since they are described as an organism present on the mineralization of various pollutants. However, only a few filamentous fungi have been isolated and identified. Also, the microplastic interactions with fungi have not been fully elucidated, especially those of marine origin. Given this, the possibility of using marine organisms represents a promising alternative that could meet the 2030 Agenda for Sustainable Development. Thus, the present research aimed to prospect filamentous fungi from Mangrove and Apicum with the ability to biodegrade polystyrene microplastics. For this purpose, we used polystyrene with a size of 250 µm, sterilized with 70% alcohol and irradiated under UV, as a polymeric material to observe the fungal biodegradation capacity. Three fungi belonging to the Mycological Collection of the Laboratory of Mycology and Applications of Biotechnology and Nanotechnology (MicoBioNanoTec) of the Biosciences Institute - UNESP were selected for evaluation of the biodegradation potential. Therefore, the prospection of fungi consisted of inserting 5 discs of fungal mycelium into 250 mL Erlenmeyer flasks containing 50 mL of minimal growth medium and adding 0,05 g of polystyrene microplastics. Accordingly, the incubation period corresponded to 28 days at a temperature of 30 °C and agitation at 150 rpm. The samples were kept for 7, 14, 21 and 28 days to evaluate cell growth and determination of residual microplastic. Among the fungi used, Penicillium sp., Rhizopus sp. and an unidentified lineage, the first one showed greater biomass growth in the treatment samples compared to the control without microplastic, additionally showed a trivial reduction of residual microplastic, signaling a possible beginning of the biodegradation action of the plastic polymer. On the unidentified lineage, an abrupt decline in biomass was observed after the 14th day, suggesting an inability to use polyethylene as an energy source and the use of minimal growth medium in the first weeks of the test. These results demonstrate preliminary data on the biodegradation of polystyrene microplastic by filamentous fungi of marine origin. In this way, further research is needed to prove a new and effective way of mitigating these materials, consisting of the use of different fungal strains and the optimization of the investigated methods.

POSTER PRESENTATION

STABLE HYBRIDS IN THE POPULATIONS OF BLACK, EXTREMELY HALOTOLERANT YEAST HORTAEA WERNECKII – A NEW FUNGAL ADAPTIVE STRATEGY TO EXTREME ENVIRONMENTS?

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In the era of anthropocene, particularly in the last 20 years, the Earth is experiencing many changes, from global warming to increased drought and salinisation, as some of more visible and agronomically important consequences. Among the best adapted microorganisms to these particular changes are halophilic and halotolerant black yeasts, inhabiting hypersaline waters of solar salterns worldwide. In this talk I am going to focus on the dominat, extremely halotolerant black yeast Hortaea werneckii, that became a model organism for the sudy of specific adaptive strategies and molecular responses to high salinity in eukaryotes. Over the yeasr we identified as the main adaptations responses on the level of cell wall remodelling and pigmentation, related signalling, in particular high osmolarity glycerol (HOG) pathway, membrane transporters and fatty acid composition of membranes related to membrane fluidity, energy-related mechanism, in particular pentose pathway, synthesis of compatible solutes, from glycerol to mycosporines, and production of protective layers of extracellular polysaccharides. To gain additional insight into cellular adaptations to salt stress, we exposed a progenitor strain of H. werneckii for over seven years (800 generations) to hypersaline conditions. This experimental evolution was followed by phenotypic characterization and whole-genome sequencing that revealed accompanying aneuploydic genotypic changes. Genome sequencing of the reference strain revealed a recent whole genome duplication. Additional sequencing of more than 60 strains, that originated from different geographic areas and ecological niches, followed by population genomic analysis, unraveled the presence of both haploid and diploid strains and a new type of reproduction provisionally named »stable parasexuality«, that enables formation of stable hybrids in this asexual and clonally reproducing black yeast.

ORAL PRESENTATION

ABSTRACTS

IONIZING RADIATION, ULTRAVIOLET RADIATION, HEAT, AND OTHER STRESSES IN FUNGAL BIOLOGY

ISFUS

ANTIFUNGAL EFFECT OF OZONE THERAPY IN ENDODONTIC TREATMENT

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Microbial resistance and the persistence of contamination in root canals, especially from fungi, lead to failure in endodontic treatment. In this context, the evaluation of non-drug antimicrobial therapies, such as ozonation therapy, may be a good alternative, since it is a topical, non-invasive, low-cost treatment with unlikely microbial resistance. The aim of this study was to evaluate, in vitro, the antifungal effect of ozonation therapy in endodontic treatment. Thirty-six bovine roots were instrumented, in a mechanized way, contaminated with Candida albicans (ATCC 10231) and divided into 4 groups: Control Group (GC; n=9): irrigation treatment with saline solution; Chlorhexidine Group (GCHX; n=9): irrigation with 0.2% chlorhexidine, for 5 min; Ozonized Oil Group (GOO; n=9): irrigation with ozonized oil (2400 ppm), for 5 min; and Water Ozonized Group (GWO; n=9): irrigation with ozonized water at a concentration of 75 µg/mL, for 5 min. Microbiological samples were obtained before (T1) and immediately after (T2) the interventions. Microbial reduction values were analyzed by Kruskal Wallis Statistical tests and Dunn's test, as post hoc ($p<0.05$). The results showed total eradication of microorganisms in the group treated with chlorhexidine ($p<0.05$) and ozonized water ($p<0.05$) and fungal reduction of approximately 1.5 log ($p=0.0044$), between T1 and T2, in the group treated with ozonated oil. We can conclude that the use of ozonized oil in endodontic treatment promoted effective microbial reduction in root canals contaminated with C. albicans, while the use of chlorhexidine or ozonized water promoted fungal eradication.

POSTER PRESENTATION

MYCELIAL GROWTH ON CONGO RED SUPPLEMENTED MEDIUM INDUCES PRIMING AGAINST UV-B RADIATION, BUT NOT TO OSMOTIC, OXIDATIVE, AND THERMAL STRESSES

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The use of entomopathogenic fungi to reduce pest population density and consequent crop damage plays a key role in sustainable pest management programs. Metarhizium spp offer an environmentally friendly alternative; however, their use has been limited by their low tolerance to abiotic stresses, such as solar ultraviolet radiation. Metarhizium robertsii has been employed as a model to study fungal pathogenesis in insects, and its tolerance to different stressors has been extensively studied. Priming is the time-limited pre-exposure of an organism to stress that leads to an increased adaptive response to subsequent exposures. Congo red is a water-soluble diazo dye used to induce cell wall integrity stress in fungi. It induces morphological changes and weakens the cell wall at sublethal concentration; therefore, it can be used as a stressor to induce priming against other stress condition. Here Metarhizium robertsii was grown on 1) potato dextrose agar medium (PDA = control), on 2) PDA medium supplemented with (200 µg/mL) Congo red (CR), and 3) on minimal medium (MM), which is a condition that always causes priming to several stresses. Conidia produced in these three conditions were evaluated against osmotic, oxidative, heat, and UV-B radiation stress. Conidia produced on CR produced conidia more tolerant only to UV-B radiation but not to osmotic, oxidative, and heat stress. Therefore, we demonstrate that priming in M. robertsii confers tolerance to UV-B radiation but not for heat, oxidative, or osmotic stress.

POSTER PRESENTATION

THE EFFECTS OF ANTIFUNGALS ON THE PROTEIN PROFILE OF FUSARIUM OXYSPORUM URM 7401

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Fusarium oxysporum is a trans-kingdom pathogen that infects humans, animals, and plants (Sáenz et al., 2020). This fungus is notable for infecting a wide variety of plant species and causing significant economic losses (Sharma et al., 2016). The main concern with this genus is its resistance to multiple classes of antifungals, particularly azoles (Al-Hatmi et al., 2016, p.). The purpose of this study was to examine changes in the protein profile of Fusarium oxysporum URM 7401 grown in antifungal-supplemented medium. F. oxysporum URM 7401 was grown in media containing 2 µg/mL of amphotericin B, 64 µg/mL of fluconazole, or no antifungals (control). Six times were used for incubation: 3, 6, 9, 12, 24, and 48 hours. Intracellular proteins were extracted and then quantified using the Bio-Rad® RC DC Protein Assay kit. A total of 1.16 µg of protein from each sample was applied to 12% acrylamide gels for SDSPAGE and the bands were analyzed using Gel DocTM EZ. The gels revealed differences in band molecular weights between the conditions tested. In general, most bands were shared across the three gels, totaling 66 common bands. Fluconazole exposure resulted in more bands in common with the control condition (22) than with amphotericin B exposure (11). Amphotericin B exposure, on the other hand, promoted more bands in common with fluconazole exposure (11) than with the control condition (7). For fungal exposure to amphotericin B, more unique bands (4) were obtained in the times of 6, 24 and 48 hours of cultivation. Similarly, more unique bands were obtained for F. oxysporum URM 7401 exposed to fluconazole in 6 hours of incubation. Moreover, differences in intensity were observed for bands appearing in more than one time of incubation. Our findings indicates that the presence of antifungals and time of exposure modulates the protein profile of F. oxysporum URM 7401. Further studies are necessary to determine which proteins are being differentially regulated.

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POSTER PRESENTATION

TOLERANCE OF METARHIZIUM SPECIES TO MENADIONE-INDUCED OXIDATIVE STRESS

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Oxidative stress is caused by reactive oxygen species (ROS), including superoxide ion, hydrogen peroxide, and hydroxyl radicals. Entomopathogenic fungi are predisposed to ROS induced by heat and UV-A radiation when outside the insect host. When inside the host, they are subject to phagocytic cells that generate ROS to eliminate invading pathogens. The oxidative stress tolerance of several Metarhizium species was studied based on conidial germination on a medium supplemented with a strong superoxide-generating agent – menadione sodium bisulphate. The conidial germination was evaluated 24 h after inoculation on potato dextrose agar (PDA) (control) or PDA supplemented with menadione with seven different concentrations i.e., 0.05, 0.07, 0.09, 011, 0.13, 0.15, and 0.17 mM. The plates were maintained at 26 ?C in the dark. Conidia of M. acridum (3341), M. album (2082), and M. anisopliae (7847) isolates were the least tolerant to oxidative stress. Conidia from the three M. robertsii (23, 2560, and 2575), two M. acridum (324 and 3609), one M. brunneum (1187), and one M. anisopliae (4343) isolates had moderated tolerances. Conidia from two M. robertsii (2547 and 2134), two M. brunneum (5626 and 7711), and two M. anisopliae (4570 and 5749) were the most tolerant to oxidative stress caused by menadione. In conclusion, the phylogenetic relatedness of the species did not influence tolerance to oxidative stress, for example, isolates of M. anisopliae were placed in the three groups with low, moderate, and high tolerances to oxidative stress. M. brunneum isolates were placed in two groups with moderate and high tolerances. M. acridum isolates were placed in two groups with low and moderated tolerances.

POSTER PRESENTATION

TOLERANCE TO UV-B RADIATION OF THE ENTOMOPATHOGENIC FUNGUS METARHIZIUM RILEYI FOR DEVELOPMENT AS A MICROBIAL AGENT FOR MANAGEMENT OF MAIN LEPIDOPTERAN SPECIES IN SOYBEAN AND COTTON CROPS

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Soybean, corn and cotton crops are afflicted by several noctuid pests, therefore, the development of bioinsecticides can provide the control of these pests with the sustainability of the system. The fungus Metarhizium rileyi has the greatest potential, since its epizootics decimate caterpillar populations in the absence of fungicide applications. The low survival of insect-pathogenic fungi when used for insect control in agriculture, however, is mainly due to the deleterious effects of ultraviolet radiation and heat from solar irradiation. In this study, fourteen isolates of M. rileyi were studied and compared with isolates ARSEF 324 and ARSEF 2575 of Metarhizium acridum and Metarhizium robertsii respectively, which sensitivity to UV-B radiation had been previously studied. Conidial suspensions were exposed at room temperature (ca. 26 °C) to 847.90 mWm2 of Quaite-weighted UV-B using two fluorescent lamps TL 20W12 RS (Philips, Eindhoven, Holland). The plates containing the conidial suspensions were irradiated for 1, 2, and 3 h, providing doses of 3.05, 6.10, 9.16, and 12.21 kJ m2, respectively. Remarkable variability in conidial UV-B tolerance was found among 14 isolates of M. rileyi. Isolate CNPSo-Mr 150 was the most tolerant isolate (germination above 80% after 2 h exposure), which was comparable to ARSEF 324 (germination above 90% after 2 h exposure), the most tolerant Metarhizium isolate. The least tolerant isolate was CNPSo-Mr 597 (germination below 5% after 2 h exposure). Nine isolates were similar with ARSEF 2575 (germination above 50% after 2 h exposure). Concluding, the majority of M. rileyi isolates can endure 1 or 2 h of UV-B radiation exposure. However, after 3 h exposure caused great reduction of germination below 40% for all isolates, except for CNPSo-Mr 150 and ARSEF 324.

To be judged for the Journal of Fungi Award

POSTER PRESENTATION

ABSTRACTS

FUNGAL STRESS IN AGRICULTURE

ISFUS

ASPHYXIATION OF METARHIZIUM ROBERTSII DURING MYCELIAL GROWTH PRODUCES CONIDIA WITH INCREASED STRESS TOLERANCE VIA INCREASED EXPRESSION OF STRESS-RELATED GENES

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Little is known about the impact of hypoxia and anoxia during mycelial growth on tolerance to different stress conditions of developing fungal conidia. Conidia of the insect-pathogenic fungus Metarhizium robertsii were produced on potato dextrose agar (PDA) medium under normoxia (control = normal oxygen concentrations), continuous hypoxia, and transient anoxia, as well as minimal medium under normoxia. The tolerance of the conidia produced under these different conditions was evaluated in relation to wet-heat (heat stress), menadione (oxidative stress), potassium chloride (osmotic stress), UV radiation, and 4-nitroquinoline-1-oxide (=4-NQO genotoxic stress). Growth under hypoxic condition induced higher conidial tolerance of M. robertsii to menadione, KCl, and UV radiation. Transient anoxic condition induced higher conidial tolerance to KCl and UV radiation. Nutritional stress (i.e., minimal medium) induced higher conidial tolerance to heat, menadione, KCl, and UV radiation. The gene hsp30 and hsp101 encoding a heat shock protein was over expressed under anoxic condition. In conclusion, growth under hypoxia and anoxia produced conidia with higher stress tolerance than conidia produced in normoxic condition. The nutritive stress generated by minimal medium, however, induced a much higher stress tolerance. This condition also caused the highest level of gene expression in the hsp30 and hsp101 genes. Allowing to conclude that there has been a greater adaptation to stress of the conidia produced under nutritive stress, hypoxia and anoxia.

POSTER PRESENTATION

MICROBIAL SYNERGISTIC ANALYZES OF SELECTED STRAINS FOR THE BIODEGRADATION OF THE HERBICIDE AMICARBAZONE

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Agriculture has currently gained an even greater prominence in the economy of our country, and as a consequence there is a greater use of pesticides, which accumulate in the soil generating great environmental impacts (LOPES and ALBUQUERQUE, 2018). Several methodologies have been developed and used in order to mitigate the environmental impacts caused by xenobiotics. Among the most diverse technologies, we can highlight those that use microorganisms in a process known as bioremediation, which involves the use of organisms capable of biodegrading polluting compounds (ALENCAR, 2020). Unfortunately, when it comes to the incessant search for increased production, the impacts have not been studied enough, thus leading to an increased damage mainly to the soil microbiota, which is responsible for soil stability with cultivation, and, when degraded, can lead to low productivity (SILVA et al., 2019). Thus, the objective of the present work was to evaluate the use of microbial strains in the bioremediation of an agricultural soil artificially contaminated with the herbicide amicarbazone, used in the cultivation of sugarcane (Saccharum officinarum). The present work was based on the hypothesis that the interactions within the soil microbiota is capable of degrading the herbicide, therefore promoting the reduction of environmental impacts. In our assays, the respirometry methodology of Bartha and Pramer (1965) was used to quantify the herbicide biodegradation from microbial respiration, using a field concentration of 200 mg/L. From this, three microbial strains were selected and tests were carried out with them that evaluated, through synergistic consortia, the definitive capacity to biodegrade the studied herbicide through colorimetric tests. It was possible to verify first, from the CO2 curves emitted, during the biodegradation analysis process, that the soil microbiota degraded the herbicide under controlled field conditions. It was also possible to verify that, through the synergistic tests, the selected strains were definitely efficient in the degradation of amicarbazone from the analysis of discoloration of DCPIP (2,6-dichlorophenolindophenol).

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POSTER PRESENTATION

PRIMING RESPONSIVENESS OF METARHIZIUM ROBERTSII TO MAGNETIC AND ELECTRIC FIELD

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Little is known about the phenotypic effects caused by magnetic and electric fields on fungal mycelial growth and on conidial tolerance to different stress conditions. In this study, conidia of the insect-pathogenic fungus Metarhizium robertsii were produced on 1) potato dextrose agar medium (PDA = control), 2) under nutritional stress on minimal medium (MM), and on PDA medium under 3) magnetic field (MF) and 4) electric field (EF). The tolerances of conidia produced on these conditions were evaluated in relation to oxidative and osmotic stress, heat, and UV-B radiation. Apparently, the cultures of the fungus grown on the PDA medium under magnetic and electric fields were similar to the fungus grown on the control PDA medium; however, the three treatments produced more conidia than the fungus produced on the minimal medium. M. robertsii conidia produced under magnetic and electric fields were more tolerant to oxidative and osmotic stress, heat, and UV-B radiation. Both treatments, MF and EF, produced conidia with similar tolerances to all stress conditions, except for osmotic stress where conidia from MF were more tolerant than conidia from EF. On the other hand, conidia produced under magnetic and electric fields were less tolerant than conidia produced on minimal medium which causes nutritional stress.

ORAL PRESENTATION

THE CONIDIAL CONSCIOUSNESS TO REIKI: PRIMING RESPONSIVENESS OF METARHIZIUM ROBERTSII CONIDIA PRODUCED AFTER REIKI

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Reiki was developed in Japan by Dr. Mikao Usui in the 1920s and is a form of energy healing, a type of alternative medicine. Reiki practitioners use a technique called palm healing or hands-on healing through which a “universal energy” is said to be transferred through the palms of the practitioner to the patient to encourage emotional or physical healing. Reiki means “universal life energy” and is considered a subtle electromagnetic biofield therapy that can influence and promote changes in the recipient organisms. A Reiki treatment consists of a sequence of hand positions placed either on the patient or within a few centimeters from the patient. The Reiki practitioner is considered a passive conduit for the universal life energy and unable to transmit directly or otherwise manipulate the energy flow. We believe that the Reiki interaction in fungi may present surprising results, because according to Nicholas Money, fungi have conscious cells capable of learning, recognizing, and modifying their responses according to environmental stimuli. This research was premised on the fact that Metarhizium robertsii senses heat, light radiation, magnetic radiation, and electric field radiation, and that when we grow the fungus under these conditions, the fungus produces progeny (conidia) more tolerant to different types of stress. Studies show that fungi and other living organisms have the ability to store “priming” experiences as a memory that would allow them to prepare their responses to conditions of future stress, in the case of intergenerational priming, promoting stress memory for the first generation of offspring, consequently our hypothesis is that Reiki during fungal growth will

produce a progeny more tolerant to several types of stresses. Nothing is known about the phenotypic effects caused by Reiki on fungal mycelial growth and on conidial tolerance to stress conditions. In this study, conidia of the insectpathogenic fungus Metarhizium robertsii were produced on 1) potato dextrose agar medium (PDA = control); 2) under nutritional stress on minimal medium (MM), a condition that always produces conidia of M. robertsii more tolerant to many types of stresses; and 3) on PDA medium that received Reiki from the second to the fourth day of growth. The tolerances of conidia produced in these conditions were evaluated in relation to oxidative and osmotic stress, heat, and UV-B radiation. M. robertsii conidia produced after Reiki were significantly more heat tolerant, and somewhat more tolerant to osmotic stress and UV-B radiation than the control on PDA that did not receive Reiki.

POSTER PRESENTATION

UV SENSITIVITY OF BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE ISOLATES UNDER INVESTIGATION AS POTENTIAL BIOLOGICAL CONTROL AGENTS IN SOUTH AFRICAN CITRUS ORCHARDS

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Seven indigenous entomopathogenic fungal isolates were identified as promising biocontrol agents of key citrus pests including false codling moth, Thaumatotibia leucotreta Meyrick (Lepidoptera: Tortricidae), citrus thrips, Scirtothrips aurantii Faure (Thysanoptera: Thripidae) and citrus mealybug, Planococcus citri (Risso) (Hemiptera: Pseudococcidae) under laboratory conditions. Even though field trials using the two most virulent isolates (Beauveria bassiana G Ar 17 B3 and Metarhizium anisopliae FCM Ar 23 B3) against soil-dwelling life stages of T. leucotreta were positive, foliar application against citrus mealybugs and thrips, has been disappointing. Thus, the UV sensitivity of the seven initial promising isolates (four B. bassiana and three M. anisopliae) in comparison with two commercial isolates (M. anisopliae ICIPE 69 and B. bassiana PPRI 5339) and their formulated products were investigated in this study. All isolates investigated were highly sensitive to UV radiation, and a 2 h exposure to simulated full-spectrum solar radiation at 0.3 W/m2 killed conidia of all tested isolates. Nonetheless, variability in susceptibility was found amongst isolates after exposure for 1 h. The most virulent M. anisopliae isolate, FCM Ar 23 B3, was the most susceptible to UV radiation with < 3% relative germination, 48–51 h post-exposure. Whilst isolates of the two mycoinsecticides showed similar susceptibility to UV radiation, their formulated products (vegetable oil and emulsifiable concentrate) were tolerant, when tested for 1 h. These findings indicate that a suitable UV protectant formulation of these fungi or a different application strategy will be required for success against P. citri and S. aurantii.

ABSTRACTS

STRESS IN FUNGAL PATHOGENESIS

ISFUS

FAPESP CAPES

A MYCOLOGICAL HISTORY OF SUSTAINABLE DEVELOPMENT: BIOLOGICAL CONTROL: EARLY BLIGHT OF APPLES THE GONATOBOTRYS/ALTERNARIA MODEL.

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Aeromycological and epidemiological research for a better phytosanitary approach to Rosacea orchards in the region of Oulmes (Middle Atlas) at an altitude of 800 m has enabled us to list using standard trapping methods or the use of more sophisticated ones, such as the Burckard sensor, the a entire aerial mycological flora as well as other microorganisms that have been listed in the area. In our samples from the apple orchards, an unexpected fungus, Alternaria alternata (Keissler), in the area was regularly harvested in abundance. This mushroom was accompanied by a whitish/orange mycelium on the surface. An identification of this whitish/orange strain made it possible to identify the genus Gonatobotrys sp. A species of Gonatobotrys simplex has been identified. Later, after many careful and biometric observations, a second endemic species was identified, Gonatobotrys africana (Najim et al; 1984) (CBS 465 84).

Their particularities are to develop on Alternaria alternata as mycoparasites. The couple Gonatobotrys / Alternaria gives us a model of choice to research mycoparasitism on the one hand and all the physiopathological, biochemical, and genetic mechanisms on the other hand, the purified substance they called

"Mycotrophein" from the fungus Arthrobotrys musiformis. This substance would probably be a growth factor necessary for contact biotrophic fungi such as Calcarisporium parasiticum (Hwang et al. 1985).

Our survey shows that "mycotrophein" contains some amino acids, including the proline and phenylalanine, these products added in the culture medium provides development of the mycoparasite; however, its development is lightly modified.

This indispensable substance for growth of the Gonatobotrys sp. is therefore a small peptide or a combination of some amino acids.

The Gonatobotrys / Alternaria mycoparasitism brought to light following this study has opened up several research topics on the systematics of these fungi, including biology, physiology, biochemistry, a whole host of new and interesting aspects, socio-economically and particularly sustainable development. An opening on the path of biological control using a simple model or Gonatobotrys, which is mycoparasite, by developing on Alternaria slows down its progress; however, a refinement of the system and a better biochemical knowledge can help to better target phytosanitary treatments in time and space.

Multidisciplinary work on this mushroom "couple" would provide more fundamental and applied scientific information on the mechanisms of growth and development on both partners. At the practical level, applications could be exploited in the field of biological control of fungal infections, both for plants and for human.

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POSTER PRESENTATION

ADJUVANT N-ACETYLCYSTEINE AS CANDIDATES FOR THE TREATMENT OF CRYPTOCOCCUS NEOFORMANS INFECTION

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Cryptococcosis is an opportunistic systemic mycosis caused by Cryptococcus spp. and mainly affects immunosuppressed patients, such as those living with acquired immunodeficiency syndrome (AIDS), organ transplant recipients, and individuals under treatment with immunosuppressants and patients with hematologic diseases (Schmiedel and Zimmerli, 2016) . The treatment of this disease is by the combination of three antifungals: amphotericin B, 5-flucytosine and fluconazole. However, these drugs have many disadvantages such as high nephrotoxicity, a marketing ban in some countries and fungal resistance (Yoon et al., 2019; Chang et al., 2017; Morschhäuser, 2016). Since the launch of new classes of antifungals on the market is precarious and the research and development of a new drug are expensive and take decades, one of the solutions to find possible new drugs to combat fungal infections is drug repositioning which consists of using a drug already approved by regulatory bodies for another therapeutic purpose (Emmert-Streib et al., 2013; Langedijk et al., 2015). The objective of this work was to evaluate N-acetylcysteine as an antifungal candidate to be used in the fight against infection caused by C. neoformans. The results of this work demonstrated that N-acetylcysteine can interfere in the formation of the polysaccharide capsule, reduce the size of those already formed and hinder the entire architecture of these structures. The results indicate that N-acetylcysteine is a strong adjuvant for the treatment of cryptococcosis, since, in addition to the desired antifungal effect, it can also modulate the main virulence factor - the polysaccharide capsule.

POSTER PRESENTATION

ANTIMICROBIAL PHOTODYNAMIC THERAPY OF FUNGI THAT CAUSE ONYCHOMYCOSIS: AN IN VITRO STUDY

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Onychomycosis is a fungal infection of the nail plate. It is one of the most common diseases in the field of dermatology and has a high incidence in the general population. The available treatments for onychomycosis have limited use due to side effects, drug interactions, and contraindications, requiring an alternative treatment. In recent years, antimicrobial Photodynamic Therapy (aPDT) has been recognized as an alternative treatment option for several infectious diseases, including dermatological infections. However, the ideal parameters have not been fully elucidated for a safe and effective therapy to treat onychomycosis. Thus, this study aimed to evaluate the effectiveness of aPDT, in vitro, on the fungi Trichophyton rubrum and Microsporum gypseum, which cause onychomycosis. In the in vitro tests, the fungi T. rubrum (ATCC 28188) and M. gypseum (ATCC 24102) were divided into 13 experimental groups: Control group (C group ¬– no treatment); Methylene Blue group (MB group– treated with methylene blue); group R100 (treated only with LED at a dose of 100 J/cm2) and groups T10, T20, T30, T40, T50, T60, T70, T80, T90, and T100 (treated with LED at doses of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 J/cm2. For the treatment, Methylene Blue was used at a concentration of 100 µM, using 30” of pre-irradiation and a LED device with a wavelength of 630 nm (100 mW; 2.93 cm2) as a light source. T. rubrum germination was reduced by aPDT beginning at an energy density of 10 J/cm2 and reaching its maximum inhibitory effect at 60 J/cm2. For M. gypseum, the inhibition rate was proportional to the energy density, with higher doses more effective, achieving the best results at 100 J/cm². These results suggest that aPDT could be an alternative treatment to combat onychomycosis, although further investigation is needed to determine whether such efficacy could be achieved in vivo.

POSTER PRESENTATION

ASPERGILLUS FUMIGATUS CELL WALL: PAST AND FUTURE

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The cell wall of Aspergillus fumigatus remains a mysterious organelle. In spite of many progresses with biochemical and genetic approaches, knowledge of the fungal cell wall biosynthesis remains poor. One of the difficulties is that we know now that the cell wall is not an inert exoskeleton. Changes in the environment and stress induce continuous modifications of the cell wall with some molecules which favor the pathogenesis of the fungus whereas others induce an efficient response of the host. We can expect to see a revival of cell wall research in the near future with the surge of more and more precise biophysical methods like cryo-EM or ssNMR to better perceive the structure of the cell wall polysaccharides and of their biosynthetic enzymes in situ in cellulo. In addition, parthenogenetic uninucleate conidia showed different cell wall structures and this heterogeneity in the spore population is needed for survival as seen by the tolerance of some individuals of the conidial population to the drugs. The heterogeneity between all individuals of a colony makes global analysis of mycelia in toto not always relevant. Solutions are however lacking to date since we are far away from a single cell approach in “cellwallomics” like with other omics.

ORAL PRESENTATION

COMPLEX MODIFICATIONS OF EXTRACELLULAR VESICLES IN RESPONSE TO CELLULAR STRESS

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Extracellular vesicles from microbes have diverse roles in biology. Fungal extracellular vesicles were first characterized by Marcio Rodrigues and colleagues in 2007 and studies have since linked extracellular vesicles to the pathogenesis of diverse human mycoses. We have demonstrated, using multiOMICS approaches, that the packaging of the rich array of molecules within fungal extracellular vesicles varies upon cell stress. The human fungal pathogen Histoplasmosis capsulatum has been the focus of our work with fungal stress on extracellular vesicle biology. Stressors ranging from nutrient availability to antibody, especially Histoplasma-specific monoclonal antibodies, binding cell surfaces significantly modify the payloads of the extracellular vesicles in both a qualitative and quantitative manner. For example, the loading and release of extracellular vesicles from Histoplasma markedly varies between yeast cells cultivated in poor (RPMI) or rich (BHI or Ham's F12) mediums. Additionally, Histoplasma yeast cell uptake by macrophages pre-treated with extracellular vesicles derived from cells treated with or without monoclonal antibody is regulated in an antibody concentration-dependent manner. Further, mAb induced global changes in the composition of H. capsulatum membranes. The findings on fungal stress and loading of extracellular vesicles are linked to pathogenesis. This work will describe our findings on the effects of stressors on extracellular vesicles loading, and demonstrate the biological consequences of this remarkable process.

ORAL PRESENTATION

CYCLOSPORINE EFFECTS ON CRYPTOCOCCUS NEOFORMANS

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Increase in the number of fungal infections is closely associated with a significant increase in immunocompromised patients mainly due to immunotherapy, transplants, oncological and hematological diseases, surgical procedures, acquired immunodeficiency syndrome (AIDS), and immunosuppressive therapy (Araujo et al. 2021). In the group of immunosuppressants, cyclosporine is a drug widely used as a potent immunosuppressive agent, especially in transplant patients (Christopher et al. 1989). This group of patients is more likely to develop opportunistic fungal infections. Among them, there are species of the genus Cryptococcus spp., capable of causing mycoses in humans, known as cryptococcosis, a disease with 223,100 clinical cases per year and approximately 181,000 deaths. The high rates of incidence and mortality caused by cryptococcosis, associated with the absence of an effective therapy, have led to the search for new diagnostic and therapeutic alternatives to control these diseases (Araujo et al. 2021). Therefore, our group evaluated the action of cyclosporine against Cryptococcus neoformans. Our recent studies showed that cyclosporine can inhibit the growth of C. neoformans. In addition, the yeast loses its regular spherical morphology and presents altered morphology, with buds that do not separate from the mother cell (Almeida-Paes et al. 2021). Based on the experiments performed, our results suggest that the drug may interfere with the final process of cell division. Our findings reveal that this drug is also capable of altering the capsular diameter and increasing the amount of chitin in the budding region. More studies are being carried out to understand the mechanism by which cyclosporine alters the cell biology of C. neoformans.

POSTER PRESENTATION

DETECTION OF THE ANTIFUNGAL POTENTIAL OF A COMPOUND PRODUCED BY PSEUDOMONAS AERUGINOSA AGAINST CANDIDA SPECIES

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Fungal infections of immune-suppressed patients have become more frequent. Among these infections, Candidiasis is one of the most common life-threatening systemic fungal infections. These infections are challenging to treat because of the inconvenience of effective antifungal drugs. Therefore, the insufficiency of and developing resistance to these drugs may be a potential problem for future antifungal treatment. This frequent increase in drug-resistant fungi has directed attention towards alternative therapies from natural sources. In the present study, an antifungal compound was detected and produced by Pseudomonas aeruginosa HS 28 (isolated from a clinical sample), which was identified conventionally and by 16S rDNA analysis. This antifungal compound exhibited good inhibitory activity against Candida species, such as C. albicans, C. glabrata, C. tropicalis, C. krusei. This compound retained its stability at a high range of temperatures and varying pH. Moreover, its bioactivity was retained when treated with organic solvents, chloroform vapors, metal salts, and different surfactants/detergents. Growth kinetics analysis illustrated that the maximum production of antifungal compounds occurred in the log phase of growth and extended well into the late log phase. Furthermore, the highest saturation of this protein was achieved at the concentration of 60% ammonium sulphate. The overall results indicated the promising antifungal potential of the compound produced by Pseudomonas aeruginosa HS 28.

ORAL PRESENTATION

EFFECT OF ACID PH ON CELL MODULATION AND PATHOGENICITY OF THE FUNGUS PARACOCCIDIOIDES BRASILIENSIS

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Paracoccidioidomycosis (PCM) is the most prevalent systemic mycosis in Latin America and is caused by fungi from the Paracoccidioides genus The interaction between Paracoccidioides spp. and its extracellular environment is of great importance for the successful establishment of the fungus in the host. Although pathogenic fungi respond to different stresses, such as high temperatures, ROS/RNS, hypoxia, and the limitation of macro and micronutrients, little is known about how fungi human pathogens respond to low pH levels. Mainly when lower pH conditions are encountered in host tissue, inside phagocytic cells, granuloma formation, and nutrient deprivation. In this sense, the objective of this work is to evaluate the adaptation of Paracoccidioides brasiliensis in response to environments with low pH. For this, P. brasiliensis was cultivated at pH 4 or pH 6,5 for 7 days and we evaluated cell viability, gene expression, modulation of cell wall components, laccase activity, susceptibility to stressors, and proteolytic activity of secreted proteases. Yeast grown at low pH has a decrease in its proliferative capacity, but it manages to change the pH of the medium to make the environment favorable for its growth. Low pH regulates the expression of genes involved with melanin synthesis pathways and stimulates melanization of P. brasiliensis cells. Melanization can be seen visually in the fungus. Furthermore, the activity of Laccase, the enzyme involved in melanization, increased when the fungus was cultivated under acidic pH conditions. Pigmented yeasts had an increase in chitin labeling and a decrease in mannan labeling, thus increasing the expression of essential cell wall components and decreasing the exposure of PAMPs. In addition, fungi cultivated for 24 and 48 hours at low pH showed susceptibility to Congo Red (3 µg/mL) and Calcofluor White (1.5 µg/mL) wall stressors, however, they did not show susceptibility to osmotic and oxidative. Finally, it was evaluated whether

yeasts increase their proteolytic capacity when grown at low pH. We observed that P. brasiliensis, grown at acidic pH, showed higher activity of secreted aspartic proteases compared to the control. These proteases were able to cleave BSA (Albumin), collagen, and hemoglobin, and their activity was inhibited in the presence of pepstatin A. These data demonstrate that aspartyl proteases are modulated by low pH conditions. Together, these findings contribute to the understanding of how P. brasiliensis can survive acid pH stress by affecting melanin expression and regulating the expression and activity of aspartic proteases.

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POSTER PRESENTATION

FUNGAL UNISEXUAL AND PSEUDOSEXUAL REPRODUCTION

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Our studies have revealed novel modes of sexual reproduction involving either one or two parents, resulting in production of clonal progeny and illustrating the capacity for sexual reproduction to generate diversity de novo. Our findings reveal parallels in modes of selfing sexual reproduction shared with plants and animals. Our studies also provide insights into the evolutionary trajectory of sexual reproduction in eukaryotes and may provide insights into how sex first evolved. Our findings suggest that there may have been an evolutionary epoche in which there was sexual reproduction before there were mating types or sexes.

Our work is supported by NIH R01 grants AI39115-24 and AI50113-17 and by the CIFAR program Fungal Kingdom: Threats & Opportunities.

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ORAL PRESENTATION

HISTOPLASMA RESPONSE TO HOST-IMPOSED COPPER POISONING

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Histoplasmosis (HPM) is an endemic and neglected fungal disease with high incidence and mortality in Latin America including Brazil. HPM is caused by the dimorphic fungus Histoplasma capsulatum. The nutrient copper (Cu) is essential for the survival of organisms, but in high amounts, it can be toxic. The host uses Cu-limitation and/or Cu-poisoning mechanisms as microbicidal strategies. As pathogens, this fungus must present molecular mechanisms for adaptation and survival under these conditions found in the infection sites. Both low-availability Cu uptake machinery and toxicity-fighting machinery have been described as relevant aspects in the pathogenesis of fungi and bacteria. In the case of H. capsulatum, studies have shown that the Cu uptake machinery is important for survival at late infection, after the peak of host adaptive immunity. However, our data demonstrate that, at early infection, Cu detoxification machinery is activated and plays a role in fungal virulence. Transcript measurements revealed that the copper efflux pump (CRP) is induced during macrophage (MO) infection independently on IFN treatment. Also, MO pretreatment with copper decrease fungal burden. Corroborating, CRP silencing decreases Histoplasma survival in high copper as well as virulence in macrophage and mouse model. The high copper regulator ACE1 is upregulated in rich copper environment as well as during first 24 h of macrophage infection regardless IFN activation. Assays with GFP reporter strains showed that, in macrophages, ACE1 is induced in the first 24 h and decreases at 48h. On the other hand, the low copper regulator MAC1 is repressed at early infection and induced at later times. Additionally, ACE1 silenced fungal cells presents increased susceptibility to copper and decreased virulence in macrophages. ACE1 knockdown resulted in the non-induction of CRP1 and in the dereppression of the copper uptake transporter CTR3. Our data suggests that H. capsulatum undergoes a transient high copper condition at early MO infection and that fungal adaptation is dependent on the action of ACE1, which activates copper detoxifying machinery and inactivates copper uptake mechanisms.

ORAL PRESENTATION

INTERACTIONS OF THE EMERGING FUNGUS CANDIDA AURIS WITH ACANTHAMOEBA CASTELLANII REVEAL PHENOTYPIC CHANGES WITH DIRECT IMPLICATIONS ON THE RESPONSE TO STRESS AND VIRULENCE

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Acanthamoeba castellanii (Ac) is a free-living amoeba known as a ubiquitous environmental host for viruses, bacteria and fungi, as well as an opportunistic important human pathogen. Studies show that predation by these amoeba can select traits that favor the virulence of their endosymbionts. As an emerging pathogenic fungus and a major current public health problem, due to its resistance and persistence in the environment, our group aims to understand the mechanisms of interaction of Candida auris (Ca) with Ac. Our results have revealed that recognized Ca-resistant and Ca-sensitive phenotypes (MMC1 and MMC2 strains, respectively) interact distinctly with Ac, with different association rates, depending on factors such as time and multiplicity of infection (MOI). Both phenotypes were able to reproduce within Ac, and maintaining their viability even under predation, would provide a multifactorial response to stress, with possible changes in the metabolism and in the expression of virulence attributes. Initially, the Ca recovered after interactions with Ac or its secreted fractions had its response to different stress conditions (oxidative, osmotic, high temperature and pH extremes) compared to the control fungus. We mainly observed that the Ca MMC1 strain showed greater tolerance to oxidative and osmotic stress upon interaction with Ac; however, it is noteworthy that the MMC2 strain showed greater tolerance both to oxidative and osmotic stress, as well as to higher temperatures, such as 37 and 42oC. Regarding drug susceptibility, analyzes with the MMC1 strain of Ca confirmed its extreme

resistence, with an inhibitory concentration (MIC) of Amphotericin B > 32 ??g/mL g/mL, while after incubation with Ac, there was a reduction to 4??g/mL g/mL As for the MMC2 strain, incubation with Ac was able to double the MIC (from 2 ??g/mL /mL to 4??g/mL g/mL). Finally, to characterize possible changes in virulence, we evaluated the effects of the amoeba-Ca interaction in the lepidopteran model of Galleria mellonella. For the MMC1 strain, which demonstrated higher resistance to Ac pressure, larvae infected with Ca recovered upon incubation with Ac had a lower mortality rate when compared to the control group, indicating a possible attenuation in virulence, which will be further investigated. In the most sensitive strain (MMC2), there was a reversal of the phenotype, with higher mortality for the larvae infected with Ca-recovered upon the interaction with Ac. Thus, we observed that the MMC2 strain, being more sensitive and therefore more susceptible to predation, would be more prone to selective pressure, with consequent adaptation to stress and modification of its susceptibility to antifungal agents and virulence. Therefore, we conclude that these possible phenotypic changes, caused by the contact with Ac and adaptation to stress, indicate the urgent need for strategies for the microbiological control of emerging pathogens and their environmental hosts, as a possible pillar in the concept of One Health.

POSTER PRESENTATION

LIPID METABOLISM IN CALCINEURIN-INDUCED STRESS RESPONSE IN PARACOCCIDIOIDES SP

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The Paracoccidioides braziliensis is a thermodimorphic fungus that causes paracoccidioidomycosis, a human systemic mycosis endemic in Latin America. The dimorphic transition from mycelium to yeast, is triggered by temperature rise from 25oC to 37oC and both virulence and pathogenicity factors of yeast cells are expressed during this phase. Despite its importance, little is known about the intracellular mechanisms or the metabolic reprogramming behind dimorphism of P. braziliensis, that is, the biochemical stress responses that are required for virulence set up of yeast cells. Using fluorescence microscopy, we present the intracellular localization of calcineurin, a Ca2+-calmodulindependent phosphatase that regulates dimorphism and proliferation of P. braziliensis, and its relationship with lipid bodies in yeast cells. We provide evidence that calcineurin might be involved in the regulation of the production/consumption of lipid bodies of P. braziliensis under stress conditions and hence, in the adaptation/virulence of this fungus to a challenging environment.

Our work is supported by Fapesp, CAPES, CNPq.

ORAL PRESENTATION

OXIDATIVE STRESS OF ENVIRONMENTAL AND ANIMAL STRAINS OF NANNIZZIA GYPSEA

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Nannizzia gypsea is the commonest geophylic agent of animal dermatophytosis. This study compares the oxidative stress of N. gypsea strains isolated from moss, sand, and a dog. Catalases have an antioxidant function, being able to convert hydrogen peroxide, which is lethal to the fungus, into water and oxygen, thus allowing the fungus to escape the host's immune response (Nakamura et al., 2012). The sensitivities of the isolates to hydrogen peroxide were measured by agar plate diffusion assay, in which fungal cells were mixed with a warm SDA medium to a final concentration of 1 × 106 macroconidia/ml of culture medium. The experiment was done in triplicate. After agar solidification in Petri dishes (90 mm diameter), four holes of approximately 5 mm diameter were punched in each plate, and 65 µl of hydrogen peroxide 30% was added. Plates were incubated at 25°C and inhibition zones of fungal growth were measured after 7 days of incubation (Cruz et al., 2020). All isolates in this study were susceptible to 5% H2O2 and only one from sand showed a significant difference when compared to the dog. N. gypsea was inhibited in the presence of hydrogen peroxide. Since this fungus is a extracellular pathogen its survival does not strongly depend on resistance to phagocytosis. Although dermatophytes are related to more superficial lesions, the production of catalase must be confirmed. A recent study showed that M. canis had low production of this enzyme (Ramos et al., 2020). N. gypsea plasticity may be crucial for the development of therapeutic strategies and control of dermatophytosis.

POSTER PRESENTATION

REPURPOUSING OF DRUGS: NICLOSAMIDE SHOW INHIBITORY ACTIVITY AGAINST SPOROTHRIX BRASILIENSIS

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Sporotrichosis, one of the main subcutaneous mycoses in Latin America, is a mycosis caused by fungi of the genus Sporothrix (Macedo-sales et al. 2020). Difficulties are observed regarding the treatment of sporotrichosis due to the reduced number of antifungal agents, in addition to the refractoriness of some cases and the existence of potential resistant strains. The drug repositioning has been shown to be a possible tool for the discovery of new treatments with already known drugs (Liu et al. 2016). During the COVID-19 pandemic, the Medicines for Malaria Venture (Geneva, Switzerland) made available a collection of drugs for drug repositioning called COVID Box,

containing 160 different substances. Our group evaluated the antifungal potential of these compounds against Sporothrix brasiliensis and as a result observed that the drug Niclosamide was able to inhibit fungal growth, demonstrating fungicidal activity. The Niclosamide shows promise for new studies on S. brasiliensis (Almeida-Paes et al. 2021) .

POSTER PRESENTATION

RESPONSES OF PATHOGENIC FUNGI TO STRESSES ENCOUNTERED IN THE HOST AND POLYMICROBIAL ENVIRONMENTS.

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The focus of our research is on stress-sensing and signalling mechanisms in human pathogenic fungi and their importance in commensalism and virulence. This research focus is split between two themes ; the mechanisms employed by pathogenic fungi, in particular Candida spp, to survive host-imposed stresses during infection, and the anti-fungal mechanisms employed by bacterial competitors within polymicrobial environments.

Regarding host-imposed stresses, following uptake by phagocytes, C. albicans is exposed to a toxic cocktail of reactive oxygen and nitrogen species, cations, heavy metals, antimicrobial peptides and digestive enzymes all within a nutrient poor environment. Recently, we have found that acquisition of the macronutrient phosphate, is essential for C. albicans to survive the phagosomal environment. In the first half of this talk I shall give an overview of the mechanisms governing phosphate acquisition in C. albicans, and how we can exploit this knowledge to screen for new antifungal compounds.

Regarding our work on stresses encountered in polymicrobial environments, we are specifically deciphering the mode of action of anti-fungal effectors elicited by the Type VI secretion system - which is widely used by bacteria to fire diverse effector proteins into neighbouring target cells. In the second part of the talk, I shall present our work on deciphering the mode of action of two antifungal effectors, Tfe1 and Tfe2, which are elicited by the Serratia marcescens Type VI secretion system to compete against competitor fungal cells.

ORAL PRESENTATION

THE EFFECT OF ACQUIRED ANTIFUNGAL DRUG RESISTANCE ON STRESS TOLERANCE AND VIRULENCE OF CANDIDA PARAPSILOSIS AND CANDIDA AURIS

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Candida species are a major cause of life-threatening bloodstream infections worldwide. Although Candida albicans is responsible for the vast majority of infections, the clinical relevance of other Candida species, like C. parapsilosis and C. auris has also emerged over the last twenty years. Among these species, fluconazole resistance is commonly encountered and cross-resistance to azoles and other types of antifungals can also develop during therapy. In our previous work, we studied the effect of antifungal drug resistance development (fluconazole, posaconazole and voriconazole) on viability, stress response, and virulence of both C. auris and C. parapsilosis. Antifungal resistant strains were generated from susceptible clinical isolates of C. auris (0381, 0387) and C. parapsilosis (CLIB214) and compared under various conditions. Antifungal cross resistance between fluconazole and voriconazole was a general occurrence in both species, whereas all C. auris 0387 originated triazole evolved strains also acquired resistance to posaconazole. From C. auris 0381 originated strains, POSevo showed severe growth defect in the presence of cell wall and membrane perturbing agents, while both the FLUevo and VORevo strains displayed increased CDR1 expression, which was associated with TAC1b mutations. All 0387 derived strain were less susceptible to cell wall perturbing agents and osmotic stressors. Furthermore, a loss of function mutation in the ortholog of BCY1 was identified in all three 0387-derived strains suggesting the potential role of the cAMP/PKA pathway in the antifungal resistance development of C. auris. The POSevo strain further harbored an ERG3 point mutation. Likewise point mutation in ERG3 was also identified in C. parapsilosis POSevo strain and similarly to C. auris, increased efflux pump activity was also identified in C. parapsilosis FLUevo and VORevo strains due to amino acid substitutions in Mrr1p. C. auris 0381 azole-evolved strains showed a slight increase in virulence, while the virulence of 0387-derived strains significantly decreased in a mice model of systemic candidiasis. Contrary virulence of C. parapsilosis was only slightly affected by fluconazole and voriconazole adaptation, while it significantly decreased after posaconazole adaptation suggesting antifungal drug dependance. In the case of C. parapsilosis the development of echinocandin resistance was also correlated with attenuated virulence in vivo likely due to significant alterations in the exposure of inner cell wall components. Our data suggests that contrary to C. parapsilosis, acquired antifungal resistance not necessary leads to fitness loss in C. auris as in vivo results indicates that triazole treatment might even increase the pathogenic potential of this species.

ORAL PRESENTATION

UNDERSTANDING THE ROLES OF SIRTUINS IN ASPERGILLUS FUMIGATUS

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Sirtuins are NAD+-dependent lysine deacetylases that participate in the regulation of acetylation status of many proteins [Narita, T., et al. 2019]. Protein acetylation dynamics has been correlated to pathogenesis in some fungi [Li, Y., et al. 2019], moreover lysine deacetylase inhibitors have been proposed as potential antifungal agents [Bauer, I and Graessle, S., 2021]. Here, we describe the phenotypic profile of A. fumigatus sirtuin knockout strains (?AfSirA, ?AfSirB, ?AfSirC, ?AfSirD, ?AfSirE and ?AfHstA) and demonstrate their potential protein targets and effects in gene expression by acetylome and transcriptome analysis of an A. fumigatus strain with the six predicted sirtuins deleted (SIRTko) and ?AfSirE strain, reported as hypovirulents in Galleria mellonella model. Our results indicate that sirtuins are not essential for cell viability, but they are involved in cell wall biosynthesis, growth, protease secretion, and secondary metabolites biosynthesis. Acetylome analysis revealed that sirtuins might regulate the acetylation of at least 126 proteins, including histones and seven genetic determinants of virulence, such as miosyn; G-protein complex beta subunit; a putative UDP-galactopyranose mutase and septin. Transcriptome analysis indicates that sirtuins also directly or indirectly regulate genes involved in secondary metabolic processes as corroborated by metabolome profiles of mutant strains. In addition, we have seen that aminoacid and lipid metabolism pathways are enriched in the set of different gene expression. Here we show that sirtuins are involved in various biological processes by maintaining protein acetylation balance. In that way, understanding the biology of these enzymes and their regulation in A. fumigatus may open perspectives for the development of new targets to use as antifungal drugs.

POSTER PRESENTATION

ABSTRACTS

STRESS IN POPULATIONS, FUNGAL COMMUNITIES, AND SYMBIOTIC INTERACTIONS

ISFUS

BIOLOGICAL CONTROL OF PHYTOPATHOGENIC FUNGI THROUGH PAIRED CULTIVATION WITH PHYLLOSTICTA CITRICARPA

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The adversities generated by the use of pesticides on environmental quality and human health have been more emphasized (ARAÚJO and ORLANDA, 2014), affect production stability and their continuous and unbridled use can lead to the acquisition of resistance by phytopathogens. Therefore, an alternative is biological control through microorganisms. Fungi are important candidates for controlling phytopathogens because they colonize a similar ecological niche, competing for nutrients, producing antagonistic substances and inducing resistance (ELIAS, 2015). Endophytes are known to inhibit producing metabolites and compete for space. The non-occurrence of studies on phytopathogens with control potential motivates the development of new research. In this study, isolates of fungal diseases relevant to agriculture were selected in order to perform the paired plate culture test. They are: Sclerotinia sclerotiorum (White mold), Rhizoctonia solani (Root rot) and Fusarium verticillioides (Root rot).

The microorganisms were grown on plates containing PDA culture medium and 5 mm mycelium discs on the opposite side of Phyllosticta citricarpa. Plates with Phyllosticta citricarpa were inoculated 7 days before the phytopathogen, simultaneously and plates where the phytopathogens were inoculated 7 days before and in the control individually. The percentage of growth inhibition was performed in order to estimate the difference between the average growth of the phytopathogen and the average growth of Phyllosticta citricarpa (EDGINTON et al., 1971; JUNG, 2012). Area values were calculated by AutoCAD software and submitted to statistical data analysis. It was possible to determine that the production of metabolites is a factor that inhibits growth or structures of reproductive resistance, as in the case of Sclerotinia sclreotiorum. When the phytopathogens are inoculated before Phyllosticta citricarpa there is an advantage of mycelial growth and mutually, however when inoculated simultaneously Phyllosticta citricarpa inhibits the growth of Sclerotinia sclerotiorum and Fusarium verticillioides. Therefore, Phyllosticta citricarpa presents control potential and opens new gaps for the exploration of its fungicidal potential.

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POSTER PRESENTATION

SECRET LIFE INSIDE PLANTS: THE ROLE OF FUNGAL ENDOSYMBIONTS

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Traditionally, associations between plants and microorganisms have been mainly studied from the angle of crop diseases, and therefore, not much is known about the molecular mechanisms underlying such relationships. To fill this knowledge gap, we have characterized a wide range of endosymbiotic bacteria and fungi living inside plants. We were mainly interested in microbes that protect their host against pathogens (biocontrol) and/or stimulate plant growth (biofertilization). This study examined the poorly investigated hostendosymbiont relationships in the ericacean plant Vaccinium macrocarpon (cranberry). Among the isolated fungal strains with biocontrol and biofertilization abilities is a so far unrecognized species that we call provisionally 'Endophytic Champignon 4' (EC4), which forms a phylogenetical sister group of the ascomycete genus Codinaeella. We sequenced the nuclear genome and transcriptome of EC4, which revealed genes with the potential to suppress a notorious plant pathogen, promote plant growth, and mediate communication with its host via proteins referred to as 'effectors' and known from other systems. In summary, we have identified and characterized at a molecular level the first Vaccinium endophyte. The knowledge gained on molecular communication involved in biocontrol and plant-growth promotion of fungal endosymbiosis has the potential to guide us towards long-term sustainable agriculture.

To be judged for the Journal of Fungi Award.

POSTER AND ORAL PRESENTATION