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Cover Art: "Stressed Aspergillus"

By: Drauzio Eduardo Naretto Rangel

About the Symposium

Understanding how fungi cope with stress has important implications and consequences for fundamental as well as applied sciences. Hence, a diverse group of experienced and innovative researchers presented at the Second International Symposium on Fungal Stress.

General Information

The world is at the cross roads in relation to many issues: human health, climate change, food production and spoilage, etc. Fungal biology, and fungal stress in particular, can help to inform and/or solve many of these issues. For instance, fungi can be used for bioremediation; to replace synthetic pesticides by using them for biological control of insect pests; fungi can be used to produce biofuels, novel antibiotics, enzymes, and useful chemicals; soil-dwelling fungi may enable production of crops in arid land; and foods can be manipulated to prevent proliferation of fungal xerophiles; fungi grow on many substrates and can help to recycle organic waste. In addition, fungal pathogens represent a serious threat for crops and animals, including humans. Thus, understanding how fungi deal with stress during growth or while infecting a host will help to optimize the use of fungi in biotechnological applications and to fight fungal diseases.

This conference was held in Brazil because this nation is at the cross roads of work on fungal stress. There is a long tradition of studies on metabolism of trehalose, a unique and universal fungal stress metabolite; biofuel production (including pioneering work on ethanol stress); entomopathogenic fungi to control insect pests (especially in relation to manipulation of stress metabolism); fungi in food production and food spoilage.

This symposium was designed to facilitate interactions between world leaders from Brazil and other countries, and between established researchers and young students/ scientists including undergraduates, graduates, and postdocs from Brazil and elsewhere. This will catalyze future interactions between Brazilian groups and those from other countries. The first ISFUS (ISFUS 2014), held in São José dos Campos, SP, Brazil has thus far resulted in the publication of more than 30 collaborative articles, including papers in leading journals such as ISME Journal and Environmental Microbiology, as well as a special issue of Current Genetics that was devoted to ISFUS 2014, which published 18 papers.

For the International Speakers, it is a unique opportunity to see first-hand the great scientific level in Brazil and to discuss their work with other colleagues with similar

interests. At the same time, the distinguished group of researchers and the synthesis of science present should lead to cross-cutting discussions and stimulate new lines of research in relation to current world issues; both pure and applied. Small but highly focused meetings can provide the best quality of interaction between speakers, students, and other scientists, and the size of the meeting (no more than 200 attendants) is designed to maintain a degree of intimacy and thereby optimize interactions between all delegates (speakers, those presenting posters, and others, international and national; young and established scientists). The long lunch break and social activities provide many opportunities for all the delegates to interact, share ideas, and make lasting professional contacts. Hence, the model of the meeting should produce a high number of collaborations afterwards.

The most striking difference in this symposium is that it will generate at least 35 original or review articles in a special issue in the journal Fungal Biology written by the speakers and the students that attend the meeting.

ISFUS 2017 offers a unique education opportunity and interdisciplinary insights for undergraduate and graduate students as well as to postdocs, mid-career researchers, and university professors in the context of fungal stress. This symposium is also registered at Universidade Federal de Goias (ppgbrph and ppgmtsp) as graduate course work where the masters and PhD students can take this course work as graduate credits. There are nine primary undercurrents within the symposium, though these are not mutually exclusive:

1. **Fungal biology in extreme environments;**
2. **Stress mechanisms and responses in fungi: molecular biology, biochemistry, biophysics, cellular biology;**
3. **Fungal photobiology in the context of stress;**
4. **Role of stress in fungal pathogenesis (in plant- and animal systems);**
5. **Fungal stress in agriculture: including biological control of insect pests;**
6. **Fungal stress in the industry: including biofuel and food production.**
7. **Ionizing radiation and fungal stress**
8. **Astrobiology.**
9. **Fungal stress and its implications on Bioremediation.**

These topics were selected in the context of the research-related and applied issues of the day (see above).

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Light in the fungal world: a signal from the environment and a stress for the cell

Luis M Corrochano * [1]

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Light is the ultimate source of energy for life but, in addition, is both a signal from the environment and a damaging agent for all organisms. An excessive exposure to light results in different types of stresses that the cell should be able to cope in order to survive. Light can be used as a signal from the environment to increase reproductive success, but it can be harmful due to damage to DNA by UV radiation. Most fungi use light as a signal to regulate development, modulate growth, and promote the synthesis of protective pigments, like carotenoids. Fungal photoreceptors sense blue and red light and, after light reception, activate the transcription of genes that lead to the accumulation of the proteins needed for the cellular responses to light. The damage to DNA caused by UV radiation is corrected by blue-light sensing photolyases. Most fungi use proteins similar to WC-1 and WC-2 from *Neurospora crassa* for sensing blue light. In *N. crassa* and other fungi these two proteins form a photoreceptor and transcription factor complex (WCC) that binds to the promoters of light-regulated genes to activate transcription. The activation by light of genes for enzymes that participate in pigment biosynthesis leads to the activation of metabolic pathways that should help to protect the cell from excessive light. A comparison of the set of photoreceptor genes in the genomes of selected fungi gives clues on the origin of fungal vision and its evolution across the fungal kingdom. In *N. crassa* other photoreceptors play a secondary role in coordination to the main photoreceptor, the WCC. In Mucoromycotina fungi multiple *wc* genes originated after a whole-genome duplication and have specialized. For example, in *Phycomyces blakesleeanus* one of the *wc* genes encodes a photoreceptor, MadA, that is required for all responses to light. In *Mucor circinelloides*, on the contrary, each Wc-1 protein plays a specific role in photoreception. Protection from UV radiation is provided by photolyases, but none is encoded in the *P. blakesleeanus* genome despite an efficient photoreactivation activity. Cryptochromes evolved from photolyases, and the DNA repair activity of the *P. blakesleeanus* cryptochrome suggests that it represents and early stage in the evolution of photolyases from DNA repair enzymes to photoreceptors.

Keywords: Photobiology, UV radiation, Phycomyces blakesleeanus, Neurospora crassa, WC complex, photolyase, cryptochrome

*Speaker

Lagers yeasts and the delicate balance between hybrid genomes and brewery's stress

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Lager yeasts (Saccharomyces pastorianus) have been employed in brewery industry for the fermentation of Lager beers, a mass product consumed worldwide. Despite its industrial importance, poorly is known about how S. pastorianus deals with brewery stress (worts with high carbohydrate concentration, low pH, high hydrostatic pressure and dissolved CO2). Additionally, the stress effects induced by brewery's propagation systems, which employ worts with high nutrient and oxygen concentrations for cell biomass production is also little understood. In this sense, we evaluated the major stress-associated biological mechanisms that are modulated by brewery's fermentation and propagation conditions by using a transcriptomic and systems biology analyses. Our data indicated that, during propagation, genes associated with DNA repair and meiosis mechanisms are overexpressed, especially those linked to homologous recombination (HR) pathway. The biological implications of meiosis and HR activation in S. pastorianus is discussed considering the hybrid genome of this species.

Keywords: Lager Yeasts, Hybrid Genomes, Systems Biology, Brewery, Yeast Propagation, Oxidative Stress, Fungi

*Speaker

Functional caracterization of transcription factors/proteins regulating stress response in Neurospora crassa

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The fungus *Neurospora crassa* has been widely used as a model organism for the understanding of fundamental aspects of eukaryotic biology. The knowledge of its genome sequence has allowed the identification of the proteins required for gene regulation, such as the transcriptional regulatory proteins. The availability of a set of deletion strains, each carrying a deletion in a specific ORF encoding a transcription factor, allows the screening for genes linked to a particular phenotype. We have been using this mutant strains set to identify transcription factors/proteins that either directly or indirectly regulate reserve carbohydrate metabolism in *N. crassa*. Functional studies of some transcription factors have allowed us to characterize signaling pathways not yet characterized in *N. crassa* and have revealed divergent roles when compared to others model organisms. In addition, some transcription factors are involved in different cellular processes, including stress response. In this talk, I will be presenting the results related to the characterization of some transcription factors/proteins that we have been studying in the fungus *N. crassa*.

Keywords: Neurospora crassa, gene expression, CHIP, RT, qPCR

*Speaker

Interventions of glycerol expand the water-activity limit for life: ecology and biophysics of extreme fungal xerophiles

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Glycerol is a chemically simple and yet biologically complex substance which is produced by many microbes (especially yeasts and fungi) as a compatible solute. It can reduce intracellular water activity and thereby regulates cell turgor, and can also protect macromolecular systems against various types of stress. However, at high concentrations glycerol can itself become a stressor. A series of studies were carried out, using fungal xerophiles as model systems, to (i) unravel the various stress mechanisms by which glycerol can inhibit fungi, and determine whether glycerol can also (ii) enhance biotic activity of fungi and (iii) enable fungal germination and hyphal growth beyond the established 0.605 water-activity limit for life. At high concentrations, glycerol reduces water activity to beyond the known limit for xerophile growth and metabolism and, at molar concentrations, also acts as a chaotropic stressor; this polyol does, however, enhance the rate of fungal germination – and reduce the water activity minimum for germination and growth – of extreme fungal xerophiles to $<$ 0.590 water activity. The findings were considered in context of key questions relating to terrestrial ecosystems, biotechnology, and the astrobiology field.

Keywords: Fungal xerophiles, Aspergillus penicillioides, Xeromyces bisporus, glycerol, water activity, chaotropicity, limit for life, habitability

*Speaker

Metabolic changes in Paracoccidioides spp. during host infection.

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Introduction: Invasive fungal diseases pose a serious and growing health problem and are a major cause of death worldwide. The genus *Paracoccidioides* comprises human thermal dimorphic fungi, which causes paracoccidioidomycosis (PCM) an important systemic mycosis in Latin America. Pathogens encode a wide spectrum of multifunctional proteins interacting to and modifying proteins and other molecules in host cells. Thus, the identification of genes and proteins involved in host-pathogen interactions are important for the elucidation of virulence factors, mechanisms of infection among other aspects. In this sense, we have been employing *in vitro*, *ex vivo* and *in vivo* large-scale expression integrated approaches to study the *Paracoccidioides* response to host conditions. **Methods:** The *in vitro* models comprehend fungal deprivation of carbon sources and hypoxia. In the context of microbial pathogenesis, it is accepted that hypoxia and carbon deprivation occur at sites of infection. Additional *ex vivo* (macrophages and neutrophils) models were employed, as well as *in vivo* infection of mice and recovering of alveolar lavage fluid. **Results/Discussion:** Acquiring of alternative metabolic pathways, expressing virulence and defense molecules, are the fungus response to these hostile environments. We have also assessed the actual contribution of gene products for the survival of *Paracoccidioides*, by using RNA antisense. The work has been providing a big arsenal of molecules that could be a good target for blocking *Paracoccidioides* proliferation inside phagocytes and tissues.
Supported by: CNPq, FAPEG.

Keywords: Paracoccidioides spp., infection, proteomics, RNAseq, models of infection

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Light dependent regulation of cellulase gene expression and secondary metabolism in Trichoderma reesei

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Trichoderma reesei is one of the most prolific producers of plant cell wall degrading enzymes. We showed that carbon metabolism including cellulase gene regulation are targets of the light signaling pathway. Additionally, secondary metabolism is altered depending on light in *T. reesei*.
Analysis of the photoreceptor ENV1 revealed an evolutionary conserved mechanism to integrate stress responses with light response in Hypocreales. This finding highlights the importance of stress response in diverse interconnected regulatory processes in *T. reesei* like light dependent regulation, enzyme expression, metabolite production and chemical communication in nature.

Keywords: Trichoderma reesei, cellulase, light response, enzyme production, gene regulation, secondary metabolism

*Speaker

Illuminated fungi during mycelial growth produce conidia with increased stress tolerance

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Visible light exposure during growth influences primary and secondary metabolism, sporulation, sexual and asexual development, and pigment production in many fungal species. However, little is known about the phenotypic effects of light during mycelial growth on the tolerance of the developing fungal conidia to different stress conditions. Conidia of the entomopathogenic fungi *Aschersonia aleyrodis*, *Beauveria bassiana*, *Isaria fumosorosea*, *Lecanicillium aphanocladii*, *Metarhizium anisopliae*, *M. brunneum*, *M. robertsii*, *Simplicillium lanosoniveum*, *Tolypocladium cylindrosporum*, and *T. inflatum*, and were produced on potato dextrose agar (PDA) medium under continuous visible light, on PDA medium in the dark, and on under nutritional stress (= Czapek medium without sucrose - MM) in the dark. The conidial tolerance of these species produced under these different conditions were evaluated in relation to: A) wet-heat 38 or 45 ∘C depending on the species tolerances, B) to menadione, a potent inducer of reactive oxygen species, C) to osmotic stress caused by potassium chloride, D) to UV radiation, and E) to genotoxic stress caused by 4-nitroquinoline 1-oxide (4NQO). Several fungal species were more stress tolerant when conidia were produced under visible light as compared with conidia produced in the dark; for instance light induced higher tolerance of *A. aleyrodis* to KCl and 4NQO; of B. bassiana to KCl and 4NQO; of *I. fumosorosea* only to UV radiation; of *M. anisopliae* to heat and menadione; of *M. brunneum* to menadione, KCl, UV radiation, and 4NQO; of *M. robertsii* to heat, menadione, KCl, and UV radiation; and of *T. cylindrosporum* to menadiona and KCl. Nevertheless, conidia of *L. aphanocladium*, *S. lanosoniveum*, and *T. inflatum* produced under visible light never responded with increased tolerance to any stress conditions. When conidia were produced under nutritional stress in the dark a much higher tolerance to the majority of stress conditions were found particularly for *Beauveria* and *Metarhizium* species. For example: nutritional stress induced higher tolerance of *B. bassiana* to menadione, KCl, UV radiation, and 4NQO; of *I. fumosorosea* to KCl and 4NQO; of all *Metarhizium* species to heat, menadione, KCl, and UV radiation; of *T. cylindrosporum* to menadiona and UV radiation; of *T. inflatum* to heat and UV radiation. Again, conidia of *L. aphanocladium*, and *S. lanosoniveum* produced now under nutritional stress never responded with increased tolerance to any stress conditions. *Aschersonia* did not produce conidia on MM. Visible light is, therefore, an important factor that induces higher stress tolerance in some insect-pathogenic fungi, but nutritional stress always surplus the conidia with a more intense stress tolerance than conidia produced under visible light.

*Speaker

Keywords: entomopathogenic fungi, cross resistance, photobiology, nutritional stress, UV, B radiation, heat stress, oxidative stress, osmotic stress, genotoxic stress

The cause-effect relationships between the oxidative stress and fungal culture degeneration

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Filamentous fungi including mushrooms frequently and spontaneously degenerate during subsequent culture maintenance on artificial media, which shows the loss or reduction abilities of asexual sporulation, sexuality, fruiting and production of secondary metabolites, thus leading to economic losses during mass production. To better understand the underlying mechanisms of fungal degeneration, different fungal species were employed for comprehensive analyses. We found the linkage of oxidative stress to culture degeneration, mitochondiral DNA from the sectors undergoes non-enzymatic glycation, and treating healthy mycelia with H2O2 significantly increased the frequency of colony sectorization and reproduced the glycation pattern shown in sectors. Taken together with the verifications of cell biology and biochemical data, a comparative mitochondrial proteome analysis revealed that, unlike the healthy wild type, a spontaneous fluffy sector culture of *Aspergillus nidulans* demonstrated the characteristics of mitochondrial dysfunctions. Relative to the wild type, the features of cytochrome *c* release, calcium overload and up-regulation of apoptosis inducing factors evident in sector mitochondria suggested a linkage of fungal degeneration to cell apoptosis. However, the sector culture could still be maintained for generations without the signs of growth arrest. Up-regulation of the heat shock protein chaperones, anti-apoptotic factors and DNA repair proteins in the sector could account for the compromise in cell death. Our studiesy not only shed new lights on the mechanisms of spontaneous degeneration of fungal cultures but will also provide alternative biomarkers to monitor fungal culture degeneration.

Keywords: Fungal culture degeneration, oxidative stress, mitochondria, apoptosis

*Speaker

Antimicrobial Photodynamic Inactivation and Photodynamic Therapy for fungal infection

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The fungi are saprophytes widely distributed in nature and potentially pathogenic to plants and animals. With the advent of immunosuppressive therapy and increased life expectancy, the incidence of fungal infections has increased as well as. Additionally, new fungal species causing infections are appearing and the tolerance of fungi to antifungal drugs is more present. Therefore, the necessities to the accurate fungal species identification and the development of new strategies to control these pathogens are needed to improve the current therapeutic armamentarium. This study was conducted with *Neoscytalidium* spp. and *Fusarium* spp. isolates. Both are filamentous fungi able to cause superficial mycoses, onychomycosis and opportunistic deeper mycoses. A collection of clinical isolates morphologically identified as *N. dimidiatum* or *N. dimidiatum* var. *hyalinum* were genotyped and their susceptibility to commercial antifungal drugs was tested. The two fungal varieties (dematiaceous and hyaline) were separated by sequence types and high concentrations of commercial antifungal drugs were required to inhibit the fungal growth. So, there is an urgent need in developing new antifungal therapies against these pathogens. Antimicrobial Photodynamic Inactivation (API) appears as a promising alternative to antifungal treatment. API involves the use of a photosensitizer (PS) that preferentially accumulates in the target cells and is activated by exposure to light of suitable wavelength. Activation of the PS in the presence of molecular oxygen induces the generation of reactive oxygen species (ROS), mainly singlet oxygen, that are capable of damaging the fungal biomolecules, killing the microbial cell. In this study, the effects of API with the phenothiazine PS methylene blue (MB), toluidine blue (TBO), new methylene blue (NMBN) and the pentacyclic phenothiazinium S137 were evaluated on both, arthroconidia and microconidia of *Neoscytalidium* spp. and *Fusarium* spp., respectively. The physicochemical properties of PS were observed, as well the API effects on the fungal biomolecules (lipids, proteins and DNA) of microconidia and arthroconidia. Finally, the virulence of *Fusarium* spp. was evaluated in the invertebrate model *Galleria mellonella* as well the *in vivo* antimicrobial photodynamic therapy with MB, TBO, NMBN and S137. Thus, API with the phenotiazinium PS MB, TBO, NMBN and S137 is a promising therapy against fungal infections.

Keywords: Neoscytalidium spp., Fusarium spp., Multilocus sequence typing, antimicrobial photodynamic inactivation, photodynamic therapy, Galleria mellonella, virulence.

*Speaker

Mediation of stress responses by root-associated microorganisms – examples from agriculture and forestry.

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Plant roots are exposed to highly diverse soil microbial communities containing both beneficial symbionts, as well as potential pathogens. Different plant species select non-random communities of fungi and bacteria from the surrounding soil that have effects on their health and growth, and biotic and abiotic stress plays a central role in these interactions. In my lecture, I will discuss the ways in which different types of mycorrhizal fungal symbionts reduce the nutritional and non-nutritional stress on their plant hosts, but also the ways in which the fungi themselves respond to different sorts of abiotic stress. Different types of biotic stress also play important roles in interactions between fungal pathogens causing plant disease and bacterial antagonists with potential as biological control agents. Genomic and transcriptomic approaches involving high throughput sequencing of DNA and RNA from fungi and bacteria colonizing roots are starting to yield interesting information about these interactions and examples involving the fungal plant pathogen *Rhizoctonia solani* and bacterial antagonists belonging to the genus *Serratia* will be discussed. The different mycorrhizal fungi colonizing tree roots appear to exert a strong influence on the associated bacterial microbiome and single root microbiome studies and 13C-RNA based stable isotope probing provide convenient ways to examine responses to different environmental perturbations. Better understanding of these stress-related responses will enable us to manage root and rhizosphere microbial communities to achieve sustainable improvements in plant health and yield.

Keywords: biocontrol, rhizosphere, mycorrhiza, Rhizoctonia, Serratia

*Speaker

Regulation of Aspergillus nidulans CreA-mediated catabolite repression by Fbx23 and Fbx47, F-box subunits of the SCF ubiquitin ligase complex

Gustavo Goldman * [1]

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Carbon catabolite repression (CCR) is a process that selects the energetically most favorable carbon source in an environment. CCR represses the use of less favorable carbon sources when a better source is available. Glucose is the preferential carbon source for most microorganisms because it is rapidly metabolized, generating quick energy for growth. In the filamentous fungus *Aspergillus nidulans*, CCR is mediated by the transcription factor CreA, a C2H2 finger domain DNA-binding protein. The aim of this work was to investigate the regulation of CreA. CreA depends in part on *de novo* protein synthesis and is regulated in part by ubiquitination. The attachment of one or more ubiquitin molecules by SCF (Fbox) complexes to protein substrates targets them for subsequent degradation by the 26S proteasome, allowing the control of numerous cellular processes. This work shows that the Fbox proteins Fbx23 and Fbx47 proteins are involved in CCR under de-repressing (xylan) or repressing-conditions (glucose). Furthermore, this study also described the interaction partners for Fbx23 and CreA. In the presence of xylan, the SCFbx23 complex is connected to CreA through a weak interaction mediated by GskA. Fbx23 interacts with casein kinase, which in turn phosphorylates CreA at serine S262, causing CreA to remain in the nucleus. Accordingly, deletion of *fbx23* induces repression, followed by a reduced protein secretion and xylanase activity. This was in contrast to Fbx47 and casein kinase B, where the absence of the respective genes resulted in a CCR de-repressed phenotype. Taken together, these results indicate previously unidentified functions of this important transcription factor. These novel functions serve as a basis for additional research in fungal carbon metabolism with the potential aim to improve fungal industrial applications. Financial support: FAPESP and CNPq, Brazil

Keywords: Aspergillus nidulans, catabolite repression, CreA, Fbox

*Speaker

Oxidative Stress and Aging: Learning from Yeast Lessons

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Although aging is likely to be a multifactorial process, several evidences show that oxidative stress is connected to life span. Many questions remain unanswered: oxidative stress does indeed contribute to ageing; are reactive oxygen species (ROS) only destructive agents or regulators of stress response and ageing; is it the absolute level of oxidative stress or the response to oxidative stress, or a combination of both, that determines life span? Interest in the factors that determine longevity has increased since the life expectancy has increased and the world leading causes of death are age-related diseases, such as cancer and neurodegenerative diseases. The use of the yeast *Saccharomyces cerevisiae* as an experimental model in biochemical studies has enabled the understanding of basic cellular and molecular processes. Even taken into consideration the vast differences in complexity between yeast and humans, the study of oxidative stress response in yeast has provided key insights into the pathways that modulate human longevity. The entire genome sequence of yeast has been elucidated and it is amenable to genetic modifications, which facilitates the identification of drug targeting genes or stress response pathways. The reduced genetic redundancy favors the visualization of the effect of the deleted or mutated gene. *S. cerevisiae* has similar antioxidant responses to mammals and over 25% of human-degenerative disease related genes have close homologues in yeast. By manipulating growth conditions, yeast cells can survive only fermenting (low ROS levels) or respiring (increased ROS levels), which facilitates the elucidation of the mechanisms involved with acquisition of tolerance to oxidative stress. Furthermore, the yeast databases are the most complete of all the eukaryotic models. In this work we highlight the value of *S. cerevisiae* as a model to investigate the oxidative stress response and its potential impact on aging and age-related diseases.

Keywords: Saccharomyces cerevisiae, oxidative stress, aging, age, related diseases

*Speaker

New functions for an old protein: examining the role of FREQUENCY in clock regulation, nutritional sensing and stress responses in the phytopathogen Botrytis cinerea

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Circadian clocks are molecular devices that allow organisms to anticipate daily cyclic challenges, temporally modulating different processes. Thus, plant defense mechanisms against pathogens have been reported to vary daily in *Arabidopsis thaliana*. Although the plant–pathogen interaction is a two-sided story, nothing was known regarding circadian regulation of pathogenic traits. Thus, we characterized a functional circadian clock in the phytopathogenic fungus *Botrytis cinerea*. By using different plant and Botrytis clock-null mutants, we demonstrate that the interaction between this pathogen and its host varies with the time of day, being the *B. cinerea* circadian clock key in regulating this outcome. In Neurospora, the FREQUENCY (FRQ) protein is the main component of the circadian oscillator, a role that is also conserved for the Botrytis ortholog BcFRQ1. Surprisingly, in this fungus, this protein appears to play extra-circadian roles, as it plays a critical function in asexual/sexual decisions. Nevertheless, developmental phenotypes triggered by the absence of BcFRQ1 can be reversed by nutritional cues, placing this protein at the crossroad between circadian and metabolic regulation. In addition, we have observed how light/dark regulation impact the ability of this fungus to deal with cell-wall stressors, by a mechanism that is still under study. FONDECYT 1171151

Keywords: circadian, clock, FRQ, phytopathogen

*Speaker

Biorremediation by fungi in a stressfull environment: application of white rot fungi in the degradation of recalcitrant organic pollulants

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Throughout the past century industrial and farming activities have released many organic pollutants, such as polycyclic aromatic hydrocarbons, synthetic dyes and pesticides into the environment. The accumulation of these persistent chemicals in soil and water is harmful to both the environment and human health. Removing these pollutants from the environment in an ecologically responsible, safe, and cost-effective way is a top concern for the land management agencies. Bioremediation using various microbial organisms is one way of doing this. In the last years the capability of white rot fungi (WRF) to biodegrade several recalcitrant pollutants has generated a considerable research interest in this area of industrial/environmental microbiology. The model species among the WRF for many bioremediation studies has been *Phanerochaete chrysosporium*, but other species have been studied for the same purpose. The ability of WRF to degrade pollutants appears to be related with the capability to producing non-specific lignin degrading enzymes, especially peroxidases and laccases, as well as to the intracellular cytochrome P450 system. WRF have demonstrated capability to transform several recalcitrant organic pollulants and can be an alternative to reduce the ecological problems caused by the accumulation of these products in nature.

Keywords: bioremediation, cytochrome P450, white rot fungi

*Speaker

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Antimicrobial photodynamic treatment as an alternative to control fungal pathogens

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The control of pathogenic fungi, both in the clinical and agricultural areas, has been facing some serious problems, such as the selection of fungi resistant to conventional antifungal agents and the few classes of currently available and effective antimicrobials, which have stimulated the development of new strategies to control pathogenic fungi, including the light-based approach antimicrobial photodynamic treatment (APDT). The selective accumulation of a photosensitizer (PS) in the target microbial cell followed by the activation of antimicrobial activity upon exposure to light at an appropriate wavelength provides the mechanistic basis of APDT. Exposure to light triggers a photochemical process that can produce several reactive oxygen species (ROS), such singlet oxygen (1O2), hydroxyl radical and/or peroxides, leading to non-specific oxidative damage and death of the pathogen cell without significant harm to the host. In comparison with currently used antifungals which have a single mode of action, the multiple targets of reactive oxygen species reduce the chance of selecting tolerant microorganisms. This talk will focus on physiological and molecular effects of *in vitro* and *in vivo* APDT with different PS such as phenothiaziniums, coumarins and furocoumarins both on human and plant-pathogenic fungi. Efforts that have been made to use the APDT in agriculture will also be discussed and a special focus will be given on the photodynamic inactivation of conidia of the plant-pathogenic fungi *Colletotrichum abscissum* (former *Colletotrichum acutatum sensu* lato) both on leaves and petals of the plant host *Citrus sinensis*. The effects of the APDT on the plant host will be presented as well.

Keywords: Antimicrobial photodynamic treatment, photoantimicrobials, oxidative stress

*Speaker
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Resistance of an Antarctic cryptoendolithic fungus to radiation

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The cryptoendolithic black fungus *Cryomyces antarcticus* spreads in the ice-free area of the McMurdo Dry Valleys, Antarctica, which is the best Terrestrial analogue for Mars. Conditions on rock surface are often incompatible with life, and it lives within porous rocks as its last chance for survival. The almost complete isolation over a timescale of evolutionary significance led to the evolution of a unique, extremely adapted and resistant, genotype. Stress tolerance goes well beyond the conditions, already limited by its extreme natural environment. These include extremes of temperature, both low and high, desiccation, vacuum, salinity, UV radiation and even space conditions. To define how the fungus responds to UV and ionizing radiation of astrobiological relevance, *C. antarcticus* was irradiated with Hg low pressure lamp UVC (254 nm), Polychromatic UV lamp (200-400nm), gamma rays (60Co), Heavy ions (He), Deuteron and X-rays densely-ionizing deuterons (2H) and sparsely-ionizing X-rays, in the frame of different experiments (LIFE, BIOMEX, STARLIFE). After exposition survival was revealed by both cultural and molecular tests; DNA damage by PCR techniques, metabolic activity by MTT and XTT approaches and ultrastructural damage by Electron Transmission Microscopy. The fungus was able to survive even high doses with minimal or no DNA alteration; this bewildering stress tolerance is mainly due to melanin that also confer the astonishing capacity to convert ionizing radiation to metabolic energy. Radio resistance is, therefore, not an exclusive prerogative of prokaryotes such as *Deinococcus radiodurans*.

Keywords: Antarctica, DNA damage, ionizing radiation, survival

*Speaker

Oxidant states improve production and quality of conidia in entomopathogenic fungi

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Mycoinsecticides formulated with conidia from entomopathogenic fungi (**EF**) control insect plagues in crop fields, either as alternatives or as enhancers of chemical insecticides. The main species of **EF** belong to the genera *Metarhizium, Beauveria* and *Isaria*. Conidia are mostly produced by solid cultures (**SC**), in which culture conditions determines higher levels or better productivities. Conidia from agar cultures (inoculants) or as final products from **SC**, face different type of stresses both in culture media or in open fields after application as biologic agents. **EF** subjected to a moderate and controlled stress can acquire higher tolerance to the same and even other types of stress (cross protection), although there is only a narrow time in the culture where the mycelia are competent to acquire the maximal trait of cross protection. Application of oxidant pulses by atmospheric modification (from 16% to 40% oxygen concentration) modulates the improvement of either conidial yields or stress tolerance by **EF** (osmotic, UV radiation, temperature). Some strains improve infectivity parameters against insects (larvae or adults). After determining the best culture condition, it is possible to achieve a simultaneous enhancement of the majority of these quality characters for every strain. Modification of atmosphere during cultures affects the production of reactive oxygen species (ROS), which are cellular signals triggering conidiation, although beyond a critical threshold they produced oxidative stress and molecular damage during fungal growth. There are also specific responses mediated by overexpression of genes coding antioxidant enzymes of those involved in the biosynthesis of compatible solutes. These metabolic adjustments to cope with oxidant states explain some part of the cross protection mechanisms, in addition to the infectivity improvements for some strains. These are some recent strategies showing how culture conditions modify production and quality of conidia by **EF**, which are currently used as alternatives in biological control.

Keywords: Entomopathogenic fungi, conidiation, stress tolerance, antioxidant response, solid cultures

*Speaker

Molecular interactions between entomopathogenic fungi and their insect host: insights into both cuticle and hemolymph battlefield

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Entomopathogenic fungi invade their insect hosts by penetrating through the cuticle, and then colonize and proliferate throughout the host by replicating as hyphal bodies. To help breaching the insect cuticle, fungi produce a variety of degrading enzymes; and during the invasive process many strains secrete toxic compounds (mainly secondary metabolites) that facilitate fungal invasion. After microbial invasion, insects trigger two types of innate immune reactions: the cellular and the humoral responses. The latter includes the induction of several antimicrobial peptides (AMPs), lectins, and the prophenoloxidase cascade. Transcription of AMPs is regulated mainly by the Toll signal transduction pathway; the resulting peptides are then secreted into the hemolymph to prevent microbial proliferation. It is well known that an increased sensitivity to oxidative stress both on the conidial germination level and during host invasion is triggered in fungal cells to cope this situation. In this presentation, several components involved in an arms race between insects and fungal pathogens will be described, and some molecular mechanisms involved in such interaction will be discussed.

Keywords: Beauveria bassiana, Metarhizium anisopliae, invertebrate pathology, gene expression, oxidative stress

*Speaker

New Generation Aminoglycoside Fungicides

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Certain natural product aminoglycosides produced by Actinomycetes are among the oldest and most successful anti-infective drugs. Mainly antibacterial, the majority of fungi are not affected by these aminoglycosides. The long-term and excessive use of traditional aminoglycosides in medicine and agriculture has bred resistance – rendering widely used ones ineffective as medically useful antibiotics. The attachment of alkyl and other hydrophobic groups to traditional antibacterial kanamycins, neomycins, and tobramycin creates amphiphilic aminoglycosides with altered antimicrobial properties. Recently discovered amphiphilic kanamycins are antifungal, but not antibacterial, and they inhibit fungal growth by perturbing plasma membrane functions. With less potential for promoting bacterial resistance, low toxicities against plants and mammals and production by scalable and green methods, amphiphilic aminoglycosides are promising next generation crop fungicides. They are also examples of reviving obsolete drugs into useful therapeutic and crop protective agents.

Keywords: fungicide, amphiphiic aminoglycosides, crop protection, plant disease

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Characterization of black fungi iron homeostasis

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The polymorphic fungi *Fonsecaea pedrosoi* and *Cladophialophora carrionii* are the main etiological agents of Chromoblastomycosis (CBM), a chronic cutaneous mycotic infection, which occurs mainly in humid tropical regions. Those fungi grow as mycelium in environment and in infected tissues, but may differentiate into sclerotic cells only in host tissues. Fungal pathogens must be able to acquire nutrients during infection. Iron is an essential micronutrient, although its excess is toxic. Also, host immune response decreases the metal availability in order to starve invaders. On this way, fungal virulence is dependent on the ability to acquire iron during infectious process. Some virulence factors of *F. pedrosoi* and *C. carrionii* have been described. However, the data available related to iron uptake and homeostasis mechanisms are still scarce. The two Chromoblastomycosis agents have orthologous genes relate to iron reductive and non-reductive pathways. The genome data mining found genes encoding to two iron permeases/oxidase systems, as well as, siderophore biosynthesis and uptake. O-CAS assays revealed that both fungi secret ferricrocin. Mass spectrometry analysis confirmed that and, also, pointed that intracellularly *F. pedrosoi* and *C. carrioni* produces ferricrocin. Additionally, iron scarcity induces the transcript level of iron uptake genes while iron availability decreases it. Of special note, both iron permeases were upregulated upon iron starvation. The expression data of iron homeostasis regulators HapX and SreA suggested they are in a negative feedback loop. Growth assays indicated both black fungi are able to uptake iron from potentially host sources, such as hemoglobin, ferritin and transferrin. In conclusion, these fungi present iron uptake mechanisms that may play important roles in host conditions.

Keywords: Pathogenesis, iron uptake, host iron sources

*Speaker

Thermotolerant and Ethanol-Resistant Saccharomyces cerevisiae Strains: Isolation, Molecular Characterization and Evaluation of gene Expression of Stress related Genes

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The use of *Saccharomyces cerevisiae* strains able to growth and ferment at both higher temperatures and ethanol concentration than the strains currently used is an excellent alternative to improve the efficiency of industrial ethanol production. For instance, the fermentation at high temperatures could eliminate the competition with invading microorganisms, such as wild yeasts and bacteria. For the ethanol resistant strains, an increase in sugar concentration at the beginning of fermentation could contribute for elevating ethanol concentration at the end of the process. The use of these strains could significantly optimize the process resulting in positive perspectives for the Brazilian ethanol production. Therefore, the aim of this study is the isolation and molecular characterization of thermophilic and/or ethanol-resistant yeast strains during the ethanol production process through fermentation analysis, genotyping and the study of genes related to resistance under stress conditions. We selected four ethanol-resistant strains and five thermotolerant wild yeasts during the ethanol producing process. Fermentative capacity, growth rates and ethanol production of these strains were evaluated, showing high performance in these conditions when compared to industrial yeast Pedra-2. The expression of stress genes *OLE1, HSP26* and *YHR087W*, were also evaluated showing a strong correlation with the acquisition of ethanol and temperature tolerance. Global gene expression will be evaluated in these strains and could contribute for the identification of genes conferring these characteristics. Furthermore, our study can contribute for the identification of new strains that could be immediately used in the ethanol plants.

Keywords: Saccharomyces cerevisiae, ethanol production, thermotolerant yeast

*Speaker

Tolerance of conidia and blastospores of Metarhizium spp. and Beauveria bassiana to heat and UV-B radiation

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The current study investigated the tolerance to heat and UV-B radiation of conidia and blastospores of *Metarhizium anisopliae* s.l. (IP 363), *Metarhizium robertsii* (IP 146), *M. acridum* (ARSEF 324) and *Beauveria bassiana* s.l. (IP 361 and GC 307). Suspensions of conidia and blastospores (103 propagules mL-1) were exposed to 45 ∘C; conidia were exposed for 0 (control), 60, 120, 240 or 360 min, while blastospores, presumably more sensitive to heat, were exposed for 0 (control), 15, 30, 45, 60, 90, 120 or 150 min. After exposure, an aliquot of each suspension was inoculated and spread on PDAY medium with chloramphenicol (0.055% w/v) in Petri dishes, and incubated for 7 days at 27 ∘C and RH $\geq$ 80%. Colonies were then counted for calculation of the relative percent of Colony Forming Units (CFU). Conidia of *M. anisopliae* IP 363 were more tolerant to heat than blastospores at 60 or 120 min exposure, whereas blastospores of *B. bassiana* CG 307 were more tolerant than their conidia. Additionally, propagules of ARSEF 324 were exposed to heat for 0 (control), 1, 2, 4, 6, 8, 16, 24, 32, 40, 48, 56 or 64 h. From 1 to 4 h exposure, conidia and blastospores of ARSEF 324 did not differ in tolerance to heat; however, from 6 to 48 h exposure conidia were more tolerant than blastospores, and at 56 and 64 h exposure conidia and blastospores were equally susceptible to heat. ARSEF 324 conidia were more tolerant to heat (79.1%) than conidia of IP 363 (55.5%), IP 146 (1.5%), GC 307 (0%), and IP 361 (0%) at 2 h exposure, as well as blastospores after 60 min exposure, with mean percent CFU of 100%, 12.3%, 30.7%, 55% and 0%, respectively. Fungal suspensions were also inoculated and spread on PDAY in Petri dishes, and exposed to UV-B radiation for 0 (control), 1.33, 2.67, 4.01, 5.35, 6.69 or 8.03 kJm-2, at 743.75 mW m-2 of Quaite weighted irradiance. After irradiation, the dishes were incubated for 7 days at 27 ∘C and RH $\geq$ 80%. No difference in mean relative percent CFU between conidia and blastospores of a same isolate was observed, demonstrating that both propagules were equally susceptible to UV-B. Tolerance to UV-B of blastospores of IP 146, IP 363, IP 361, CG 307 and ARSEF 324 did not differ among them. In conclusion, the tolerance of blastospores in contrast to conidia may vary among fungal isolates, and blastospores with marked natural tolerance to heat and UV-B radiation may be promising for biological control of arthropods.

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Keywords: entomopathogenic fungi, thermotolerance, ultraviolet radiation, fungal propagules.

Light, fungal development and coordinated secondary metabolism

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Differentiation and secondary metabolism are correlated processes in fungi that respond to various external triggers including light. *A. nidulans* development includes light promoted asexual conidia formation or the formation of sexual fruiting bodies in the soil as resting structures. Such overwintering structures are associated with a specific bioactive secondary metabolism presumably for defense against other organisms of the habitat. The control network for the coordination of differentiation and secondary metabolism includes several layers of regulation. Proteins for posttranslational and transcriptional control can physically interact to each other. Several methyltransferases, which affect through chromatin modification secondary metabolism and differentiation, interact to the velvet family of transcriptional regulatory proteins, which are similar to mammalian NF-B. The interplay of this complex fungal network of different posttranslational and transcriptional control mechanisms will be discussed.

Keywords: Fungal Development, Secondary Metabolism, Posttranslational Modification

*Speaker

Photoregulation of metabolism and antifungal sensitivity in the mold pathogens Aspergillus and Fusarium.

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We have previously shown that light induces asexual development and stress resistance in the mold pathogen *Aspergillus fumigatus*. Transcriptomic analysis has revealed less obvious features of the *A. fumigatus* photoresponse, particularly those processes that are suppressed by light. This includes several genes involved in cellular respiration, the light repression of which could be tied to changes in reducing power of the fungus *in vitro*. Ergosterol biosynthetic genes were similarly down regulated in light, and this likely accounts for an observed increase in sensitivity of *A. fumigatus* to sterol-targeting antifungals in illuminated culture. Interestingly, there is a good correspondence between *light-repressed* and *hypoxia-induced* genes in *A. fumigatus*, suggesting that light could serve as a proxy signal for the oxygen concentrations in the environment (e.g. dark signifies hypoxia). Consistent with this idea, light was found to inhibit growth under hypoxic conditions. We reasoned that the influence of light on drug sensitivity or metabolism may be relevant in the context of ocular infections, in which the organism is readily exposed to light. Accordingly, we sought to determine whether the photoregulation of such pathways was conserved in species commonly associated with fungal keratitis, namely *Fusarium oxysporum* and *F. solani*. For both organisms, the influence of light on respiratory function and ergosterol biosynthesis was conserved, suggesting that light may be a salient factor in the treatment outcome of such infections.

Keywords: Aspergillus fumigatus, photobiology, metabolism

*Speaker

Melanized fungi are resistant to both sparsely and densely ionizing radiation

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Melanin is a ubiquitous pigment with unique physicochemical properties. The resistance of melanized fungi to cosmic and terrestrial ionizing radiation suggests that melanin also plays a pivotal role in radioprotection. In the initial study, we compared the effects of acute (delivered at a high dose rate) densely-ionizing deuterons and sparsely-ionizing X-rays on two microscopic fungi capable of melanogenesis. We utilized the fast-growing pathogenic basiodiomycete forming an induced DOPA-melanin, *Cryptococcus neoformans* (CN); and the slow-growing environmental rock-inhabiting ascomycete synthesizing a constitutive DHN-melanin, *Cryomyces antarcticus* (CA); melanized and non-melanized counterparts were compared. CA was more resistant to deuterons than CN, and while CN was more resistant to X-rays. The irradiated cells were subjected to a panel of metabolic assays – XTT, MTT and ATP. Deuterons increased XTT activity in melanized strains of both species, while the activity in non-melanized cells remained stable or decreased. For ATP levels the reverse occurred: it decreased in melanized strains, but not in non-melanized ones, after deuteron exposure. Our data show, for the first time, that melanin protected both fast-growing and slow-growing fungi from high doses of deuterons under physiological conditions. The follow-up study utilized the transmission electron microscopy (TEM) to assess the morphological changes in the irradiated CN cells. TEM demonstrated the removal of polysaccharide capsule by radiation in both melanized and non-melanized cells and considerable damage to the cell wall and organelles in the non-melanized cells only. These observations will help in creating melanin-based radioprotectors for space travel and radiation therapy of cancer.

Keywords: C.neoformans, C.antarcticus, melanin, gamma radiation, deuterons, alpha particles, metabolic assays, TEM

*Speaker

The contribution of the Cell Wall Integrity Pathway to virulence and to fumiquinazoline production in Aspergillus fumigatus

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Aspergillus fumigatus is an allergen of mammals and an important opportunistic pathogen that causes invasive pulmonary aspergillosis in immunosuppressed individuals. The ability to handle different stress conditions is essential to the survival and virulence of the pathogen inside the host. Therefore, environmental changes are sensed by invading microorganisms and transduced through signaling transduction pathways that lead to cell adaptation strategies. The Cell Wall Integrity (CWI) pathway is a signaling cascade primarily activated in fungal cells under conditions of synthesis and/or remodeling of the cell wall. In *S. cerevisiae*, CWIP is launched by the activation of the Protein Kinase C (*PKC1*), which interplays with a MAP kinase cascade (MAPK) leading to the phosphorylation of the associated *RLM1* transcription factor. We investigated the function of different genes of the *A. fumigatus* CWI pathway. Delta *rlmA* and *pkcA(*G579R) mutant strains exhibits an altered cell wall organization in addition to defects related to vegetative growth and tolerance to cell wall-perturbing agents. A genetic analysis indicated that *rlmA* is positioned downstream of the *pkcA* and *mpkA* genes in the CWI pathway. As a consequence, *rlmA* loss-of-function leads to the altered expression of genes encoding cell wall-related proteins. RlmA positively regulates the phosphorylation of MpkA. The delta *rlmA* strain had attenuated virulence in a neutropenic murine model of invasive pulmonary aspergillosis. Our results suggest that RlmA functions as a transcription factor in the *A. fumigatus* CWI pathway acting downstream of PkcA-MpkA signaling and contributing to the virulence of this fungus. In addition, the CWI is an important hub for fungal secondary metabolites production. Recently, we observed that CWI components impacts on the production of fumiquinazolines (Fq). FqC is the major Fq produced by *A. fumigatus* which accumulation was associated with conidia formation. Here we show that *pkcA*(G579R) and delta *rlmA* mutant strains produce lower FqC (24.7% and 27.9%, respectively) and that FqC concentrations were 10.5- fold lower in the delta *mpkA* strain. This decrease is accompanied by global down-regulation in mRNA expression of the Fq cluster genes during the asexual development. We propose that the PkcA-MpkA-RlmA circuit directly participates in the regulation of FqC accumulation in *A. fumigatus*.

Keywords: Aspergillus fumigatus, cell wall integrity, fumiquinazolines

*Speaker

Mutual tolerances of fungi and bacteria within stressed microbial communities

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Two of the important groups of micro-organisms in soil are the procaryotic bacteria and mycelial molds. In the biorefining experiments with the UMC (Undefined Mixed Cultures), we have studied the interactions of the microbial groups. The molds can modify the hostile environments from a distance by spreading on the surfaces and interfaces. By modifying the niches from outside, they also neutralize, balance and hydrolyze their future surroundings. Hyphal structures or mold mycelia facilitate fast transport of nutrients to the active sites.
Many microbiological environments are often seen as battlegrounds of various strains for attaining dominance. However, in the variable conditions, it is the versatility which makes any microbial community more resourceful. Moreover, the repulsive actions could be limited in scale and with respect to dimensions in order to maintain the overall balance. The microbial growth is often restricted by salt, sugar, pH conditions, preservatives or other characteristics of the niches. The long reach of the molds by the mycelia facilitates the degradation and further metabolism of the substrate material.
One mold strain was isolated from the bottom of Lake N´asij´arvi, in Tampere, Finland. One *Penicilllium* sp. strain was a laboratory contaminant. The challenged bacterial strains were *Echerichia coli* 99 and *Staphylococcus aureus* 178 from HAMBI Microbiological Culture Collection, University of Helsinki, Finland, and dairy or environmental isolates of *Bacillus* sp. One *Bacillus* strain originated from the Lake N´asij´arvi sediment, and two others from the Negev desert soil (Israel). The results indicated limited growth inhibition of the molds by some of Gram-positive strains. This partial inhibition or attenuation could reflect the mutual benefits attained *in vivo* and it has many potential applications.

Keywords: Micobial community, fungal interactions with bacteria, metabolic versatility, soil microbiome, food preservation, niches

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CO2 sensing in Aspergillus fumigatus

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The concentration of carbon dioxide (CO2) is 0.033% in the environment and can reach up to 5% in the host (about 150-fold higher). Thus, during the infection process, *Aspergillus fumigatus* must adapt to different CO2 levels. Carbonic anhydrases (CAs) are ubiquitous enzymes, found in all organisms, that catalyse the reversible hydration of CO2 to bicarbonate (HCO3-), maintaining efficiently CO2/HCO3- homeostasis. These enzymes were already related to CO2 sensing in other fungi. *A. fumigatus* has four CA-encoding genes, named *cafA-D*. *cafA* and *cafB* are constitutive and strongly expressed, whereas *cafC* and *cafD* are weakly expressed, but CO2-inducible genes. Only the double mutant *cafAcafB* is unable to grow at 0.033% CO2 and this growth defect can be restored by high CO2 concentrations (5%). *A. fumigatus cafA*, *cafB*, *cafC*, *cafD* and *cafAcafB* mutant strains are fully virulent in a low-dose murine infection, suggesting that the CAs are not required for development and virulence of the *A. fumigatus* in the mammalian host. On the other hand, this fungus modifies the expression of some genes when is transferred from an atmosphere of 0.033% CO2 to one of 5% CO2 (data not published), suggesting the importance of these genes to the *A. fumigatus* virulence. The *cipC* gene (Afu5g09330) is involved in this adaptation process and is important for *A. fumigatus* virulence, making it a target for study of new therapies for treatment of invasive aspergillosis. Other genes such as those encoding tyrosinase (Afu3g01070), HMG-CoA synthase (Afu8g07210), oxidoreductase (Afu2g00750), α 1,3-glucanase (Afu1g03352), among others, also had their expression altered when *A. fumigatus* was transferred to 5% CO2, however, the importance of these genes to the virulence was not established yet.

Keywords: Aspegillus fumigatus, virulence, carbonic anhydrase, cipC

*Speaker

Studies on the Biological Characteristics of Beauveria bassiana Bb10 from Biocontrol Agents and 4 Beauveria bassiana isolates of Silkworms origin

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In order to explore the relationship between the strain of *Beauveria* as biocontrol agents in forest and the isolates from silkworms, and also to supply the basic information of assessing the safety to silkworm production after using the biocontrol agents of *Beauveria* in the Sericulture region. One biocontrol strain of *Beauveria* and four isolates of silkworms origin from Xiangzhou,Yizhou, Huanjiang and the other from Xiangzhou, Guangxi Zhuang Autonomous Region, South of China were respectively collected. The culture medium of PDA was used to isolate, identify and compare with the biological characteristics of the above isolates. The results were showed that the isolate of Bb9 from Sicun town of Xiangzhou and the biocontrol agent of Bb10 are both of *B. tenella*. Bb11 isolate from Yizhou belonged to small *B. bassiana*, and the silkworm *Beauveria* Bb3 from Zhongping town of Xiangzhou and the Bb12 isolate from Huanjiang are both belonged to *B. bassiana*. These five isolates had different biological characteristics in colony morphology, vegetative growth, sporulation and virulence to silkworm. The isolates Bb9 and Bb10 grew the fastest.Their colony were of flocculence look. But the conidiospores of Bb10 are bigger than that of Bb9. The isolates of Bb10 and Bb11 had the highest spore quantity. The isolate Bb3 had the highest virulence to silkworms, while both Bb9 and Bb10 had weaker virulence to silkworms. The above results implied that the origin of white muscardine pathogen of in Guangxi rearing areas is very complicated. There are certain similarity between the strain of *Beauveria* from the partial silkworm area and the biocontrol agents. It will be the basis for discriminating pathogens and control of silkworm white muscardine. (*This research was supported by the earmarked fund for Modern Agro-industry Technology Research System CARS-22-ZJ0205)

*Speaker

Keywords: Beauveria bassiana, Biological Characteristics, Biocontrol agent, isolate of Silkworm, Virulence

Regulation of antimicrobial peptide genes via insulin-like signaling pathway in the silkworm, Bombyx mori

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Antimicrobial peptides (AMPs) are important effector molecules of insect humoral immunity, and expression of AMPs is mainly regulated by the Toll and immune deficiency (IMD) pathways. FoxO, a key downstream regulator of the insulin-like signaling (ILS) pathway, has been recently reported to be involved in the regulation of AMPs in *Drosophila* melanogaster. In the present study, we investigated AMP expression and the regulation pathway controlled by starvation in the silkworm *Bombyx mori*. We discovered that antibacterial activity in the hemolymph of *B. mori* larvae was induced by starvation, and AMP genes (*BmCecB6*, *BmAtta1*, *BmLeb3* and *BmDefB*) as well as the ILS target genes (FoxO, *InR* and *Brummer*) were strongly activated in the fat body by starvation. Moreover, phosphorylation of Akt kinase was reduced in the Bm-12 cells after starvation, suggesting that the ILS pathway was inhibited. We then showed that more FoxO protein was present in the cytoplasm than in the nucleus of Bm-12 cells under normal conditions, but more FoxO was detected in the nucleus after cells were starved for 8 hours, indicating that FoxO was activated by starvation. In summary, our results indicated that starvation can induce AMP gene expression in *B. mori* via the ILS/FoxO signaling pathway.

Keywords: FoxO transcription factor, antimicrobial peptide, ILS, starvation, Bombyx mori

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Evaluation of the fungistatic and fungicidal effects of essential oils on microorganisms causing dermatomycosis

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There is great search for new treatment alternatives for dermatomycoses, mainly natural products. Onychomycosis is the most prevalent and treatment is prolonged, costly and causes adverse reactions. The aim of this work was to evaluate the antifungal activity of the essential oils of *Rosmarinus officinalis* (rosemary), *Lavandula angustifolia* (lavender), *Cinnamomum cassia* (cinnamon) and *Melaleuca alternifolia* (melaleuca) against the main fungi that cause dermatomycosis. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (CFM) were determined against fungal strains of the species *Trichophyton rubrum*, *T. mentagrophytes var. Interdigitale*, *Candida albicans*, *Candida parapsilosis*, *Fusarium spp* and *Scytalidium spp.* Fluconazole and terbinafine were used as reference medicines. The results obtained were cinnamon with MIC and CFM from 62.5 to 125 μg / mL against *T. mentagrophytes*, from 15.62 and 31.25 μg / mL for *T. rubrum*, from 31.25 and 62.5 μg / mL for *C. albicans* and *C. parapsilosis* and from 62.5 to 125 μg / mL for *Fusarium spp* showed resistance to antifungal agents tested. Lavender and melaleuca had a fungicidal effect only against yeasts and *T. rubrum* with MIC and CFM ranging from 2000 to 4000 μg / mL and in contrast to the other dermatophytes showed only fungistatic effect. Rosemary did not present antifungal activity. It was concluded that cinnamon showed a fungicidal effect against all evaluated microorganisms.

Keywords: Antifungals. Essencial oils. Dermatophytes. Yeasts. Onychomycosis.

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Study of the interaction between methylene blue and toluidine blue on the photoinitiation of Sacharomyces cerevisae

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Yeast Saccharomyces cerevisiae, in addition to its commercial purposes for the production of food products such as wine, bread and beer, is the eukaryotic model organism and widely used in scientific research. Due to its safe handling, easy isolation of mutants, short life cycle allow rapid cell growth, and in-depth knowledge about its genome structure and development along with appropriate molecular tools make S. cerevisiae an excellent organism model. This work evaluated a photographic inactivation of Saccharomyces cerevisiae induced by methylene blue and toluidine blue. Both methylene blue and toluidine blue inhibit most yeast growth after photodynamic treatment. However, cell death does not exceed 80% in both cases when treated at concentrations below 100uM. When incubated in the dark only toluidine blue showed a cytotoxic effect on the yeasts. The staining of the yeasts with the two photosensitizers, revealed by microscopic analysis that methylene blue blends the cell membrane very well, whereas toluidine blue color intracellular structures. It was possible to observe that when the combination of the two dyes occurs in the photoinitiation, the photodynamic activity of the methylene blue dye is improved. We observed that the two dyes completely inhibited yeast growth after photodynamic treatment. The planarity of methylene blue as well as the ability of toluidine blue to make hydrogen bonds may be responsible for the results obtained in this work.

Keywords: photoinactivation, methylene blue, LED

*Speaker

Utilization of heme by Paracoccidioides lutzii: analysis of the cell wall proteome after exposure to hemoglobin and heterologous expression of Pga7, a probable hemoglobin receptor

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In humans, most iron (Fe), an indispensable metal for most biological systems, is complexed to the cofactor heme, present in hemoglobin, a molecule that is exploited by pathogens as an iron source during infectious processes. *Paracoccidioides* spp., a complex of thermodymorphic pathogenic fungi, are the etiological agents of paracoccidioidomycosis (PCM), and are able to use hemoglobin in a receptor(*Pb*Rbt5)-mediated process. However, experimental evidence points to the existence of a complex system with the presence of other proteins. In the present work, we demonstrated the similarity between PAAG_02225 (*Pb*Pga7), from *Paracoccidioides lutzii*, and the sequence of the heme/hemoglobin receptor Pga7 from *Candida albicans,* and expressed *Pb*Pga7 in *Escherichia coli*. In addition, nanoUPLC-MSE was employed to analyze *P. lutzii* cell wall proteome. The treatment of *P. lutzii* with hemoglobin promotes induction of potential adhesins and defense-related enzymes against reactive oxygen species, which indicates that these proteins may be important for the pathogen to access hemoglobin by adhering and lysing erythrocytes, besides counteracting the toxicity generated by heme/hemoglobin released from erythrocytes, allowing the uptake and use of these molecules. The results obtained in the present work reinforce the complexity of the interaction event between pathogen and host and, in addition, contribute to broaden the understanding of the biology of *Paracoccidioides* spp.

Keywords: Paracoccidioidomycosis, Iron, nanoUPLC MSE, Adhesins, Host pathogen interactions

*Speaker

FUSARIC ACID PRODUCED BY NITROGEN STRESS FUNGAL

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The determination of the biochemical parameters that interfere in the production of a secondary metabolite is fundamental for the knowledge of the ideal conditions for this biosynthesis. Several studies have been carried out on the influence of different carbon and nitrogen sources on the production of fusaric acid in fungus cultures.
The fusaric acid acts in the metabolism of *Zea mays,* isolated mitochondrias, inhibiting the electrons flow between the succinate-desidrogenase and the coenzyme Q, the activity of ATPase/ATP-sintase and probably also inhibiting, the alpha-cetoglutarato desidrogenase. Many cultures such as corn, rice, wheat, sorghum, banana, barley and passion fruit are affected by *Fusarium* spp.
In this work, we sought not only to determine the level of fusaric acid produced at the end of fungus growth, but also to monitor the production of this mycotoxin throughout the whole culture of the microorganism.

The objective of this work was to evaluate the relation between carbon and nitrogen ideal for producing the micotoxin Fusaric acid by *F. oxysporum* f.sp. cubense.

Extraction of Fusaric acid: The *F. oxysporum* f. sp. cubense, was grown on oat liquid medium and homogenized with 100 mL of a methanol solution: K2HPO4 1%, pH 3.0, and centrifuged (10000 x g) for 20 minutes. The resulting supernatant was adjusted to pH 3.0 with HCl 2N. It was proceeded to the extraction by three times with 80 mL of methyl cloride. The resulting extract had its volume reduced to 40 mL in rota-vapour. Following , 25 mL of an aqueous solution of NaHCO3 (5%) was added and a new extraction was performed twice. The fraction that contained NaHCO3 was adjusted to pH 3.0. The extraction with methyl chloride was proceeded two more times, when finally, the methyl chloride was removed under vacuum at 40 C in rotatory evaporator. HPLC determination: The obtained residue was resuspended in the mobile system and used in HPLC. The eluent system possessed the following composition: toluene, ethyl acetate, formic acid (50:40:20 vol, vol, vol). A Licrosorb-18 (ODS) column of 10 cm, with flow of 1.25 ml/min,c at temperature of 25 C was used. The UV detector at 210 nm, was used in the liquid chromatograph model CG-420. The standard fusaric acid was obtained from Sigma Co.

The *Fusarium oxysporum* f.sp. cubense, demonstred to be a regular producer of fusaric acid. The results showed that the fusaric acid had its beginning of synthesis around 72 hours of fungus cultivation, and the beginning of the synthesis corresponded to the availability in the culture system of 1.6 g% of total carbohydrates and 0.3 g % protein. Therefore, the C:N ratio was of the order of 5:1. The exponential phase of production occurred between 72 and 120 hours of the

*Speaker

growth process.

The low availability of nitrogen in the culture system was a decisive factor for this mycotoxin synthesis. The change in coloration of the culture medium, which changes from light beige to red-wine color over time, is indicative of the production of secondary metabolites. The decrease in the nitrogen level, which signals the onset of fusaric acid production, represents a deviation in the metabolism of the microorganism, and therefore the secondary metabolism.

Keywords: Fusaric acid, nitrogen stress, secondary metabolism.

Heterologous Expression of Hyperthermophilic Enzymes in Aspergillus nidulans

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Thermostable enzymes are effective catalysts for industrial applications. However, the main challenge is the enzymes production high yield and low costs. The aim of this study was to perform a comparative analysis of different hyperthermophilic enzymes in *Aspergillus nidulans*. The target genes were xylanase GH10 (*them1*) arabinanase GH43 (*them4*), arabinofuranosidase GH51 (*them5*), endoglucanase GH12 (*tcel1*), endoglucanase GH12 (*tcel6*) and processive endoglucanase GH5 (*tcel4*), cloned under control of a glucoamylase promoter. The *tcel1*, *tcel4* and *tcel6* genes were cloned from *Pyrococcus furiosus*, *Pyrococcus abyssi* and *Caldivirga maquilingensis*, and *them1*, *them4* and *them5* were cloned from *Thermotoga petrophila*. The hyperthermophilic enzymes recombinant production was analyzed by SDS-PAGE indicating low secretion levels. In order to analyze the expression of the target genes, the mRNA was measured by qPCR and showed that most the thermophilic genes were well expressed in *A. nidulans*. We selected the Them1 recombinant strains for further studies based on its higher activity. First, the *them1* gene expression was analyzed under different maltose concentrations (0.01, 0.1, 1.0 2.0 and 5.0%). The results showed lower transcript levels at lower maltose concentrations (0.01 and 0.1%), however, higher (_˜50%) enzymatic activity. The results indicated that controlling the expression of the target gene by varying the concentration of the promoter inducer is an effective strategy to improve the secretion of hyperthermophilic enzymes, and suggests an improvement of secretion of target enzymes when there is no overload of proteins in the ER. We are currently analyzing some unfolded protein response (UPR) genes in the recombinant strains.

Keywords: Aspergillus nidulans, hyperthermophilic enzymes, glycoside hydrolases, heterologous expression, enzymes secretion, UPR

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Understanding the production of recombinant proteins in Aspergillus nidulans by global proteome profiling

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This work investigated extra and intracellular proteomes from *Aspergillus nidulans* recombinant strains producing the arabinofuranosidase and cellobiohydrolase from *Aspergillus fumigatus*, in order to understand aspects and bottlenecks of heterologous proteins production. After mass spectrometry analysis, 250, 441 and 424 intracellular proteins were identified in the strains Anid_pEXPYR (control strain), Anid_AbfA and Anid_Cbhl (both recombinant strains), respectively. More specifically, the profiles of the recombinant strains Anid_AbfA and Anid_Cbhl were similar, although the latter strain secreted more recombinant enzyme than the former. In this context, the main upregulated processes in the recombinant strains were energy pathway, amino acid metabolism, ribosome biogenesis, reticulum and oxidative stress. In addition, a faster maltose uptake was observed in the control strain when compared to the recombinant strains, what may reflect the slower growth of the latter group. The secretome analysis of both heterologous strains performed at 72 h of growth showed the downregulation of biomass-degrading enzymes, suggesting the presence of the mechanism Repression under Secretion Stress (RESS) that is associated with the high load of unfolded and misfolded proteins in the endoplasmic reticulum. Finally, odd number of cysteines and the number of N-glycosylation sites in the primary sequence of heterologous proteins might directly impact the difference in their secretion levels. These findings provide insights of highlighted mechanisms involved in the high secretion of recombinant proteins in *A. nidulans*, as well as in the rational manipulation of target genes for fungal strains engineering.

Keywords: Aspergillus nidulans, cellobiohydrolase, arabinofuranosidase, heterologous expression, protein secretion

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Identification of hydroxamate siderophores involved in iron acquisition in the human pathogenic fungus Cladophialophora carrionii

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Polymorphic fungus *Cladophialophora carrionii* is the second most common etiological agent of Chromoblastomycosis (CBM), a chronic cutaneous disease, which occurs mainly in humid tropical and subtropical areas. CBM is very difficult to treat and prone to recurrence because the scarcity of effective antifungal agents. Moreover, the chronic lesions may be predisposed to develop into non-invasive squamous cell carcinomas. Some virulence factors of *C. carrionii* have been described, however, the data available regarding the molecular biology of these fungi are still scarce in the literature. The ability of pathogenic microorganisms to assimilate host micronutrients, which include iron, is a key aspect of infection. As an essential nutrient, iron is a vital constituent of all organisms, required for various metabolic processes, including electron transport and redox reactions, except for some bacteria. In the context of infection, the host decreases the metal availability to the pathogen, hampering the lesion advance; while the pathogen activates mechanisms for obtain the metal to survive in infected tissues. Therefore, the fungi developed homeostatic mechanisms to acquire adequate quantities of iron, avoiding the toxicity associated with free iron, and regulating its use. *In sílico* analyses were performed, revealing the presence of orthologous genes involved in siderophore-assisted iron mobilization in *C. carrionii*, such as biosynthesis and transport siderophores. Conserved domains were found in the predicted protein sequences according to the suggested function. Transcription of the two siderophore biosynthesis related genes (*sidA* and *sidI*) were differentially expressed in iron-deficiency response. The overlaid-chrome azurol S (O-CAS) assay revealed that *C. carrionii* produces siderophores of hydroxamate type under iron-deficient conditions. Siderophores of hydroxamate type also were identified in supernatants of *C. carrionii* under iron-deficient

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conditions through ferric iron perchlorate method. Reversed-phase HPLC and mass spectrometry analysis identified ferricrocin siderophores intra- and extracellularly in *C. carrionii*. Lastly, siderophores produced by both pathogens were able to restore the growth of the *A. nidulans sidA* mutant lineage. Altogether, the results demonstrated *C. carrionii* is a hydroxamate producer and that this system is used during iron-deprivation. **Financial support:** CNPq, CAPES, FAPEG.

Keywords: chromoblastomycosis, gene expression, ferricrocin, mass spectometry, siderophore

Analysis of molecules involved in nitrogen metabolism of the human pathogenic fungus Paracoccidioides brasiliensis

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The *Paracoccidioides* genus is composed of thermodimorphic fungus that causes paracoccidioidomycosis (PCM), an endemic human systemic mycosis in Latin America. These organisms grow as mycelium in temperatures below 28 ∘C and as yeast form in temperatures above 37 ∘C. Nitrogen is an important element in this microorganism's nutrition that participates in the synthesis of proteins, nucleic acids and others biomolecules. In this regard, nitrogen uptake and metabolism are essential to growth and fungal establishment. When nitrogen levels and sources such as glutamine and ammonia concentration are limited, pathogenic fungus use a regulation system called Nitrogen Catabolite Repression that induces the expression of genes encoding permeases and enzymes required for the catabolism of secondary nitrogen sources, such as formamidase, gamma-glutamiltranspeptidase and urease. Gamma-glutamiltranspeptidase (Ggt) is an enzyme that catalyzes the first reaction of glutationa degradation and it has been the target of several studies about nitrogen starvation in various fungi. It has been observed that the expression of the gene encoding this enzyme was induced in limiting conditions of nitrogen and was repressed when the availability of nitrogen was high. Urease (Ure) is an enzyme that catalyzes the degradation of urea in ammonia and carbonic acid. This enzyme is already known as a virulence factor in some fungi, such as *Cryptococcus neoformans*, and also has been the target of studies about nitrogen starvation. This study aims to express the proteins gamma-GT and urease of *Paracoccidioides brasiliensis*, isolate *Pb*18, in *Escherichia coli* bacterial heterologous system and functionally characterize the recombinant proteins regarding its function in nitrogen starvation. The gene coding for Ggt and Ure were cloned in pET32a expression vector, and the respective clones were used in *E. coli* pLysS cells transformation. The recombinant proteins produced were shown to be catalytically active. The characterization of the recombinant proteins will elucidate its behavior during nitrogen starvation, add knowledge about the enzymes gamma-GT and urease of *Paracoccidioides brasiliensis*, isolate *Pb*18, and contribute to the understanding of host-pathogen relationship, biology and virulence of this important pathogenic fungus. Financial Support: CNPq, CAPES, FAPEG

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Keywords: Nitrogen Catabolite Repression, cloning, enzymatic activity, gamma glutamiltranspeptidase, urease, Paracoccidioides

Fungicidal Syringomycin E as an Organic Seed Protectant Against Pythium ultimum

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Syringomycin E (SRE) is a natural fungicide produced by certain strains of *Pseudomonas syringae* . In organic farming, disease control is difficult because synthetic chemical fungicides are not allowed. The limited availability of disease-free organic-compatible seeds is a particularly serious problem. The goal of this research is to develop SRE as a seed treatment for organic farming. Bioreactor production of SRE by a new SRE-overproducing *P. syringae* strain G10 yielded 50 mg L-1 of SRE in 40 h. SRE was purified by chromatography using an ʹAKTA avant 150 system and organic compatible solvents. The minimum concentrations of organic compatible SRE needed to inhibit the soil-borne (damping-off) pathogen *Pythium ultimum* 50% and 90% was 31.3 and 250 μg mL-1 SRE, respectively. SRE-coated cucumber seeds germinated at the rate of 72% in soil naturally infested with *P. ultimum* while non-coated seeds had a germination rate of 0%. In summary, organic SRE was not phytotoxic for organic cucumber seeds and provided protection from infection by germinating *P. ultimum*. This study reveals that organic compatible SRE has potential as a seed treatment fungicide for organic farming.

Keywords: fungicide, syringomycin, seed treatment, organic agriculture, Pythium ultimum

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Expression analysis of genes encoding metacaspases in the aquatic fungus Blastocladiella emersonii

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Metacaspases are caspases related proteases that exists in protozoa, fungi and plants where regulates cell death and are involved in stress response. Metacaspase genes can be found in different quantities according to the organism, indicating function specialization. In the aquatic fungus *Blastocladiella emersonii* it was observed that genes encoding metacaspases were induced during sporulation when exposed to cadmium, a toxic metal. In this study, we analyzed the expression of ten metacaspase genes of *B. emersonii* during sporulation in physiological conditions and when exposed to cadmium. We performed real time RT-PCR assays using cDNA samples from *B. emersonii* sporulating cells at 30, 60, 90 and 120 min after deprivation of nutrients and its released zoospores in physiological conditions. We also used cDNAs obtained from RNAs extracted from *B. emersonii* cells exposed to 50 and 100 μM CdCl2 at sporulation stage. Relative gene expression was evaluated using 2-$\Delta\Delta$CT method. We observed that some genes encoding metacaspases are upregulated during later sporulation and a few are in zoospores. Additionally, all of them were induced when cells were exposed to cadmium. These data suggest that some *B. emersonii* metacaspases are involved in sporulation process. Additionally, most of them seem to possess important functions on the mechanism of cadmium stress response in this fungus.

Keywords: primitive fungi, cadmium, metacaspase, sporulation

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Heterologous expression of proteins related to copper metabolism in Paracoccidioides spp.

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Introduction: Paracoccidioidomycosis (PCM) is an important mycosis of Latin America, caused by thermodymorphic fungi of the genus *Paracoccidioides*. Homeostasis of metals such as copper, zinc and iron is important for the survival of the fungus in the host environment. In this context, copper is an important cofactor for several enzymes, such as superoxide dismutases and cytochrome c oxidase, and it also plays a structural role in several proteins. The excess free copper in the cell promotes the accumulation of reactive oxygen species, causing damage to nucleic acids, lipids and proteins. Thus, organisms must maintain cytoplasmic concentrations of these micronutrients at non-toxic levels, just sufficient for cell growth and vital metabolic processes. The metabolism of this metal is strictly controlled by high and low affinity systems. ATX1, CTR2 and CTR3 are genes responsive to copper metabolism in *Paracoccidioides* spp. **Methods:** Oligonucleotides were designed for the respective genes of interest, amplification of cDNA was performed by PCR, and the product was purified and cloned in pET32a using In-Fusion HD Cloning (Clontech). **Results:** After heterologous expression, the recombinant proteins were confirmed by LC-MS / MS. The mice will be immunized to obtain polyclonal antibodies to the proteins cited above. Subsequently assays for the protein localization in the fungus cell will be performed. **Conclusions:** The characterization of ATX1, CTR2 and CTR3 can provide subsidies regarding the relevance of the copper metabolism to *Paracoccidioides* spp.

Keywords: Paracoccidioides spp, paracoccidioidomycosis, copper metabolism

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Involvement of CAT and GST enzymes in multiple stress tolerance of an evolutionary engineered S. cerevisiae strain

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An evolutionary engineered ethanologenic industrial strain of *S. cerevisiae* was selected by its high acetic acid tolerance on adaptation to increasing concentrations up to 12g/L acetic in rich medium. The selected strain exhibited higher tolerance (i.e. up to 2-fold higher viability values depending on the stressor) to multiple stresses: saline, freezing-thawing, osmotic, formic acid, phenolics, oxidative and ethanol. Assuming that resistance to oxidative stress could be an indicative of broad stress tolerance, intracellular ROS concentrations were measured in both strains, and ROS basal levels were significantly between parental and adapted strains. A programmed cell death mechanism, triggered by acetic acid and mediated by ROS, would explain the different stress tolerances observed between strains. To search for a biochemical basis for the different ROS levels in the isogenic strains, the main antioxidant enzymes defenses involved in oxidative stress (i.e., catalase, CAT; superoxide dismutase, SOD; glutathione transferaste, GST) were measured in cell-free extracts of both strain cells after being submitted to different oxidative conditions. While the different CAT and GST specific activities found could explain the differences in ROS levels in the strains, no significant differences were observed for SOD. When aerobic growth was measured during 4 days in the presence of up to 100mM acetic acid, the adapted strain showed a shorter lag phase and higher biomass productivity than the parental one. Moreover, fermentative capacity measured as bioethanol production of the adapted clone was similar in the presence of acetic acid 80mM and in its absence in the fermentation medium.

Keywords: multiple stress tolerance, evolutionary engineering, ROS

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Bioethanol production from agroindustrial byproducts by two yeast species adapted by evolutionary engineering to tolerate bioprocess stresses

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Scheffersomyces stipitis and *Saccharomyces cerevisiae* yeast strains were adapted by evolutionary engineering to increasing concentrations in the fermentation medium of a non-detoxified hydrolyzate of lignocellulosic residues of jojoba (*Simmondsia chinensis*). Cell viability and ethanol production were measured in order to evaluate the adaptation of the strains to the presence of the stressors product of the dilute acid treatment of the lignocellulosic substrate commonly used in the industry. The stress tolerance increase (determined as viability of the adapted clone vs. the parental strain) was 79% in the case of *S. cerevisiae* and 98% for *S. stipitis*. As for ethanol production, in fermentation experiments containing 90% hydrolysate supplemented with salts, vitamins and amino acids, the engineered strains produced 1.7 and 3 times more ethanol than the parental strains, for *S. cerevisiae* and *S. stipitis*, respectively. These findings show that the use of strains produced by evolutionary engineering allows the design of an ethanologenic process that does not require the toxic elimination steps prior to the beginning of the fermentation, which results in a simplification of the process and, consequently, lower production costs.

Keywords: biofuel, bioethanol, stress tolerance, evolutionary engineering

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Virulence, stress tolerance and conidial production of Isaria javanica isolates

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Abiotic factors, such as UV radiation, heat, osmotic and oxidative stress are major constraints for the successful exploitation of entomopathogenic fungi as biocontrol agents, as they may hinder conidial production, viability, virulence and, consequently, effectiveness. The selection of resilient strains represents an important step in developing robust mycopesticides. The virulence toward *Bemisia tabaci* nymphs, tolerance to abiotic stresses, and conidial production on rice grains were assessed for 11 *Isaria javanica* strains from different geographic regions, which were deposited to the Fungal Culture Collection at 'Embrapa Arroz e Feijão' (CNPAF). Significant differences among isolates were found for virulence, as the lethal median concentration (LC50) varied from 1.10 (CNPAF 22) to 3.03 (CNPAF 23) x 106 conidia/mL. For osmotic stress, the median effective doses (ED50) to reduce the relative conidial viability remained 6.52 (CNPAF 21) – 8.87 (CG 1228) M of NaCl, while for oxidative stress the ED50 ranged from 3.09 (CNPAF 14) to 4.72 (CNPAF 23) mM of H2O2. The effective time to kill 50% conidia (ET50) due to heat at 45 °C spanned from 0.48 (CNPAF 18) to 1.25 (CG 1228 and CNPAF 22) hours. Regarding UV-B tolerance, ED50 fell within the range of 3.86 (CNPAF 22) – 7.86 (CNPAF 21) kJ/m2. The isolates CG 1228 and CNPAF 23 attained the highest conidial production. Our results revealed that i) high conidial yield and oxidative tolerance is correlated with low UV-B resilience, ii) high LC50 and heat tolerance is correlated with low osmotic tolerance. We envision that genetic recombination through protoplast fusion using the best strains may provide an improved hybrid with multi-stress tolerance and high spore production and virulence.

Keywords: Entomopathogenic fungi, Hypocreales, Whitefly, Abiotic stress, Phenotyping.

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The heat shock transcription factor HsfA is required for the thermal adaptation in Aspergillus fumigatus and plays a role in response to cell wall stress

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Aspergillus fumigatus is a filamentous saprophyte and a human opportunistic fungal pathogen responsible for several human respiratory diseases including the invasive pulmonary aspergillosis. Thermotolerance is one of the key virulence determinants of this fungus, being a prerequisite for the establishment of infection and maintenance of the pathogen in the host sites of infection. In *Saccharomyces cerevisiae*, the transcription factor Hsf1 is responsible for the transcription of several heat shock proteins, such as Hsp90, Hsp70, Hsp60 and Hsp40 chaperones, which are part of the cellular program for heat adaptation. In addition, it has been observed a coordinated function for this transcription factor in the maintenance of cell wall integrity in yeast and in the pathogen *Candida albicans*. Here we investigated the functions of the Hsf1 homolog in *A. fumigatus, hsfA*, both in the heat shock adaptation and cell wall stress. As observed in other fungal organisms, *hsfA* is an essential gene in *A. fumigatus*. *hsfA* expression is induced by temperature stress, mainly at 48 °C, and also in cell wall stress induced by congo red and caspofungin. The mRNA abundance of *hsfA* is also significantly increased in response to cell wall stress induced by caspofungin in the *pkcA*G579R, delta *mpkA* and delta *rlmA* mutant strains which encode components the *A. fumigatus* Cell Wall Integrity (CWI) pathway. Western blot experiments have shown that HsfA protein abundance is also up-regulated under the same stress conditions. Interestingly, this increase in HsfA expression is accompanied by an increased in the Hsp90 molecular chaperone expression. These results show the importance of HsfA transcription factor in *A. fumigatus* and point out to the existence of a concise relationship between thermotolerance and CWI, possibly through the Hsp90 chaperone interaction with the CWI pathway components.

Keywords: Aspergillus fumigatus, heat shock, heat shock transcription factor, Hsf1.

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The CWIP of Aspergillus fumigatus is related to the production of Fumiquinazoline C, a secondary metabolite potentially cytotoxic to macrophages and soil amoeba Dictyostelium discoideum

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Fungi remarkably produce a variety of secondary metabolites as a consequence of different environmental stimuli. These compounds can ultimately provide fitness attributes to the producing organism. Recently, we characterized two components of the *A. fumigatus* cell wall integrity pathway (CWI), *pkcA* and *rlmA* and observed that in addition to the cell wall related-phenotypes, the perturbation of the signaling circuit coordinated by the PkcA-MpkA-RlmA module impacts on the production of fumiquinazolines (Fq). FqC is the major Fq produced by *A. fumigatus* which accumulation was associated with conidia formation. Here we show that *pkcA*G579R and delta *rlmA* mutant strains produce lower FqC (24.7% and 27.9%, respectively) and that FqC concentrations were 10.5- fold lower in the delta *mpkA* strain. This decrease is accompanied by global down-regulation in mRNA expression of the Fq cluster genes during the asexual development. Aiming to understand if other cell stresses could influence the production of FqC, we performed a screening using different mutants and found that the deletion of the transcription factor SebA, (primarily involved in heat shock and oxidative stress) overproduced FqC (about 4.5-fold increase) indicating that *sebA* is a negative regulator of FqC production. *A. fumigatus* is sensitive to FqC and this tolerance is decreased in the CWI pathway mutants and increased in the delta *sebA* strain. In addition, FqC can induce pore formation on the membrane of macrophages and highly stimulates the secretion the pro-inflammatory cytokine TNF-a by this cell type. We also used the soil amoeba Dictyostelium *discoideum* to study the phagocytic interaction of this organism with conidia from the delta *sebA* strain. Interestingly, conidia of the delta s*ebA* were significantly less phagocytized by *D. discoideum* and the opposite occurred when conidia from the CWI pathway mutants were tested. Our results suggest that Fq production is regulated at different levels in *A. fumigatus* and that FqC can serve as a defense

*Speaker

compound against other microorganisms or soil predators. Although we could not detect FqC in the lung of infected mice, this molecule is potentially toxic to fungi and mammalian cells.

Keywords: Fumiquinazoline C, Aspergillus fumigatus

Bioaccumulation of lithium in Pleurotus ostreatus mycelia

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Basidiomycetes have the capacity of bioaccumulate metals from environment for reducing stress caused by toxic elements. High concentration of lithium is toxic and could be bioaccumulated by mycelia as a strategy to reduce this ion nearby. Lithium has been used to treat bipolar and Alzheimer's disorders and mycelial bioaccumulation could improve drug bioavailability. The objective of this study was to determine lithium concentration - from LiCO source - that affects mycelial growth and bioaccumulation in *Pleurotus ostreatus*. LiCO was added to the liquid culture medium until obtaining final concentration of zero, 5, 10, 15, 20, 25, 30, 40, 50, 100 or 200 mg L of lithium. Mycelia were inoculated in liquid culture medium and kept static at 28 ∘C for 15 days, in the dark. Mycelia were separated by centrifugation, washed and dried at 60 ∘C until constant mass. Lithium was extracted using hydrolysis with HNO and HO and determined on a flame atomic absorption spectrophotometer. Lithium addition of 40 mg L in culture medium reduced 87% of mycelial production in relation to control although it provided a higher bioaccumulation of lithium (1575.29 μg g) in mycelia. LiCO caused fungal stresses activating the metal bioaccumulation process which in turn reduce the salt concentration in the environment. This study opens a new perspective for development of high value-added biotechnological products and alternative forms of delivering lithium or other pharmacological salts.

Keywords: Basidiomycete, Mycelial bioaccumulation, Lithium carbonate.

*Speaker

Effect of co-culturing of basidiomycetes on mycelial biomass production, laccase activity and RBBR decolorization

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The co-cultivation of fungi promotes complex interactions that can cause physiological and biochemical changes, favoring the synergistic and more efficient action of extracellular enzymes. Thus, co-cultivation can be a strategy for the degradation of dyes. In this work the co-cultivation of the basidiomycetes *Lentinus crinitus*, *Pycnoporus sanguineus*, *Trametes* sp. and *Pleurotus ostreatus*, and the production of laccase, mycelial biomass and decolorization of remazol brilliant blue R (RBBR) were observed. The fungi were grown alone and in co-cultivation (in pairs) in malt extract without and with RBBR (0.01%) supplementation, kept at 28 ∘C, in the dark for 15 days. The decolorization was monitored every 24 hours (592 nm) and the laccase determined on the 15th day. Co-cultivation favored the production of laccase in both RBBR-free and dye-supplemented environments, but the presence of RBBR reduced fungal growth. The *Trametes-Pleurotus* co-culture increased laccase activity in RBBR-free medium and was 14% higher than *Trametes* and 84% higher than *Pleurotus* in mono-culture. The production of laccase in the RBBR medium with *Pycnoporus-Pleurotus* combination was 12% higher but the growth decreased 59% in relation to the RBBR-free medium. This indicates increased growth-independent laccase production suggesting that RBBR acted as an inducer of laccase. In all cultures there was a reduction of the RBBR color, however, it was higher in the co-cultures. *Lentinus* in mono-culture caused the greatest decolorization (66%), followed by *Trametes-Pleurotus* (60%) and *Lentinus-Pleurotus* (55%), all after 15 days. Each combination and species can provide different interactions and responses of laccase production, biomass and dye decolorization.

Keywords: Basidiomycetes, Anthraquinone, Ligninases

*Speaker

Standardization and optimization of high throughput activity of the key enzymes of the glyoxylate cycle malate synthase and isocitrate lyase.

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The great majority of microorganisms are able to grow on non-fermentable carbon sources such as acetate, ethanol or fatty acids, because they have the glyoxylate cycle, an anaplerotic pathway that allows the biogenesis of carbohydrates using two carbon compounds. The two key-enzymes for this pathway are isocitrate lyase and malate synthase. Both are very important for establishing infection of microorganism, including *Paracoccidioides* spp.. Here were described micro-assays based methods to evaluate *Paracoccidioides* isocitrate lyase and malate synthase activities as an alternative to onerous macro-assays methods. The search for optimizations to micro-assays was performed using the Response Surface Quadratic Model. The reactions volumes for micro-assays were reduced to 100 μL from 1 mL used in the macro-assays. Specific activities for both enzymes were increased in the micro-assays, which were 2 fold more sensitive than macro-assay. The micro-assays were reproducible, being faster and cheaper than macro-assays. Those assays could be easily amenable to use of recombinant enzymes from any fungi and many types of microorganisms. The standardization of micro-assays enables to perform types of investigations that require higher throughput, for instance, drug screening and/or characterization, and/or studies in which only a small amount of biological material is available. It is expected that those methods will stimulate others to use these assays to screen compounds to the discovery new generation of safe and effective therapeutics for the treatment of human diseases.

Keywords: Paracoccidioides spp., glyoxylate cycle, isocitrate lyase, malate synthase, Standardization, high throughput activity.

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Characterization of gene expression profile of Hsp70 family of Trichoderma asperellum during mycoparasitism and thermal stress

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Trichoderma is a genus that involves species commonly used to act in biocontrol of phytopathogens, it acts on phytopathogenic fungi through mycoparasitism. The variation of soil temperature and the mycoparasitism are stress conditions routinely imposed to *Trichoderma* and consequently there is a response to this generated stress. This stress response occurs through changes in cellular metabolism, activating its defense mechanisms that include the performance of heat shock proteins (HSPs). The objective of this work was to characterize the Hsp70 family of *Trichoderma asperellum* and evaluate the importance of this protein family during mycoparasitism and in situation of thermal stress. Firstly, we performed comparative amino acid sequence analysis to identify all proteins of Hsp70 family in *T. asperellum* using proteome data available at JGI site. We observed 12 different proteins corresponding to Hsp70 family in this fungus. From these, we selected initially three Hsp70 proteins to evaluate the corresponding gene expression. To evaluate gene expression during mycoparasitism we performed paired culture between *T. asperellum* and *S. sclerotiorum* in MYG medium at 28 ∘C in a 12-hour photoperiod, during the three phases of mycoparasitism: pre-contact, contact and post-contact. For evaluation of HSP70 expression in thermal stress, the vials containing mycelium in TLE medium were conditioned for 48 hours at 28 ∘C and then conditioned at 38 ∘C for 0.5, 1, 2, 4 hours. Total RNA was isolated from mycelial cells, quantified and integrity checked using agarose-formaldehyde electrophoresis gel. We observed that *hsp70* genes analyzed showed different patterns of expression during mycoparasitism, indicating that these genes are important during the biocontrol performed by *T. asperellum*. We also verified that these hsp70 genes are responsive to heat stress.

Keywords: HSP70, thermal stress, mycoparisitism

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Analysis of the role of heat shock proteins of the HSP70 family of Trichoderma harzianum during mycoparasitism

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The filamentous fungus *Trichoderma harzianum* is part of a genus of fungi that acts as agents of biological control, and that do not represent danger to humans. It is widely used against some phytopathogens, such as *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, and is considered an excellent strategy for pest control in plantations. When this fungus is in contact with another one during mycoparasitism, this relationship causes a stress, which induces a cellular response through the production of several proteins. This work aims to analyze the participation of heat shock proteins of the HSP70 family of *Trichoderma harzianum* in the process of mycoparasitism. For this study, *Trichoderma harzianum* ALL42 isolate was used in direct confrontation assays against *S. sclerotiorum*. Three biological replicates were performed and *Trichoderma* mycelia were removed in three conditions: pre-contact, contact and post-contact with the phytopathogen. RNA extraction, cDNA synthesis and real-time RT-PCR were then performed to evaluate the level of expression of three hsp70 genes during mycoparasitism. We observed an increase in the levels of HSP70 expression during mycoparasitism indicating that these proteins are important for this process and during the interaction of *T. harzianum* with phytopathogens.

Keywords: Trichoderma harzianum, S. sclerotiorum, biological control, mycoparasitism.

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Analysis of expression of HSP90 and HSP104 genes during elevated temperatures and mycoparasitism in Trichoderma spp.

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Trichoderma spp. are potent biocontrol agents because of its mycoparasitism ability. Due to its extensive use, Trichoderma spp. are exposed to different environments and phytopathogens, which can provoke different stress responses. Heat shock proteins (HSPs) work as chaperones assisting folding of newly synthesized proteins and refolding of degraded proteins being constitutively expressed or also induced by stress conditions. In this study, we evaluated the relative expression of genes encoding HSP90 and HSP104 in Trichoderma harzianum and Trichoderma asperellum during heat shock stress and mycoparasitism against Sclerotinia sclerotiorum to better understand HSPs function and importance in these conditions. We performed real time RT-PCR assays using samples from Trichoderma spp. cultures that were grown at 28 °C and then exposed at 38 °C for 1h, 2h and 4h or grown in dual cultures against S. sclerotiorum during three interaction moments: pre-contact, contact and pos-contact. We observed that when Trichoderma spp. were exposed to elevated temperature, HSP90 and HSP104 expression were highly induced at the first hour, then there was a decreasing in this induction at 2h and 4h. This is also reported in other organisms and suggests that the induction is temporary. During mycoparasitism, Trichoderma spp. showed a distinct relative expression profile for HSP90 and HSP104 genes, suggesting that these HSPs are important for Trichoderma spp. in the process of phytopathogens recognition. Financial Support: CNPq

Keywords: heat shock proteins, Trichoderma harzianum, Trichoderma asperellum

*Speaker

The Aspergillus fumigatus smiA gene is required for vegetative growth sporulation and cell wall maintenance

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Aspergillus fumigatus is a human opportunistic pathogenic fungus responsible for the incidence of the vast the majority of invasive pulmonary aspergillosis in immunosuppressed patients. The *A. fumigatus* infection is multifactorial and the mechanisms underlying this process are not completely understood. Inside the host, fungi species respond to the environmental stimuli through signal transduction cascades, translating the external signals to proper responses. Among the several strategies involved in the regulatory system that controls their cellular responses is the Cell Wall Integrity (CWI) pathway, which is mainly responsible for the *de novo* synthesis and/or remodeling of fungal cell wall. It is known that *Sacharomycces cerevisae* gene *SMI1* (*KNR4*) acts modulating the activity of CWI pathway, regulating cell wall synthesis, in addition to be essential to the cells to cope with many sorts of cell wall stress. It also interacts with several genes of the yeast CWI pathway. To evaluate the function of *smiA* gene in cell wall integrity and remodeling in *A. fumigatus,* a delta *smiA* mutant strain was isolated. The deletion strain presented severe growth defects and very decreased conidiation on minimal and complete medium. The colonies of this mutant are white indicating complete absence of conidia pigmentation. Delta *smiA* shows defects in asexual structures such as abnormal conidiophore. To verify whether *smiA* is involved in cell wall maintenance we tested the wild type, mutant and complemented strains in the presence of cell wall stress conditions. The mutant strain was more sensitive to all compounds tested such as congo red, calcofluor white, caspofungin, caffeine and SDS, suggesting a role for *smiA* in cell wall integrity. Interestingly, these delta *smiA* defects were not recovered by the addition of sorbitol as osmotic stabilizer. The caspofungin paradoxical effect was also absent in the mutant strain. In addition, delta smiA strain is completely avirulent in the *Galleria mellonella* model when compared with wild and complemented strains. Taken together our data suggest that *smiA* plays an important role in cell wall maintenance, being closely involved in *A. fumigatus* sporulation, proliferation and growth, in addition to be determinant for the CWI pathway proper operation. Financial support: FAPESP, CAPES

Keywords: A. fumigatus, smiA null mutant, cell wall integrity, defective growth

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Chalcone derivatives as potential antifungal agent against Sclerotinia sclerotiorum

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Sclerotinia sclerotiorum (Lib.) de Bary (phylum Ascomycota) is a ubiquitous necrotrophic fungus that is a common pathogen in a wide range of crop plants worldwide. In this study a series of 21 chalcones with various substituents were synthesized and examined in vitro against *S. sclerotiorum*. Sensitivity of each isolate to chalcones was assessed using a mycelial growth assay at concentrations of 6.12, 12.5, 25, 50, 100 and 200 μg/mL in potato dextrose agar (PDA). Most of the synthesized compounds showed potent inhibitory activity with minimum inhibitory concentrations (MICs) ranging from 125 to 7.5 μg/mL. The chalcones treatment led to pronounced alterations in mycelial morphology, cellular ultrastructure, membrane permeability under transmission electron microscope, and also reduced the ergosterol content of fungi. In addition, quantitative RT-PCR was used to measure transcription levels of the *ERG6* gene involved in ergosterol biosynthesis. The *ERG6* gene was repressed in the presence of MICs of chalcone, indicating that interference with ergosterol synthesis caused cell membrane disruption. Hence, chalcones and their derivatives may be the potential candidate to investigate as a safe antifungal agent against *S. sclerotiorum*.

Keywords: Chalcone, Sclerotinia sclerotiorum, antifugal

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NITROGEN CATABOLITE REPRESSION IN PARACOCCIDIOIDES spp: AN IN SILICO APPROACH

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Paracoccidioides spp are the etiologic agents of paracoccidioidomycosis (PCM). These fungus are thermo-dimorphic and have the ability to transit between mycelia and yeast forms at temperatures around 37◦ C. The nitrogen metabolism is essential for the growth and establishment of the fungus in host tissues, where this nutrient is scarce. Also, the nitrogen-dependent pathways is related to fungus survival and pathogenicity. After internalization by host, microorganisms are subjected to an environment with extreme nutrient-limited conditions. Nitrogen Catabolic Repression (NCR) are regulatory mechanisms of nitrogen uptake when the preferred nitrogen sources as ammonia or glutamine are scarce in the environment. Thereby, when non-preferences sources are the only nutritional sources for fungal pathogens, the cell will trigger through the NCR mechanism the regulation and expression of enzymes and permeases that will act in the catabolism of secondary nitrogen sources. This study aims to characterize NCR *in silico* in *Paracoccidioides spp.* Analysis *in silico* of NCR in *Paracoccidioides spp* have been elucidated in this model, where the GATA family transcriptional factor areA*Pb* were identified as a possible activator of NCR, which shares homology with areA of *Aspergillus nidulans* and Nit-2 of *Neurospora crassa*, that are described in the literature acting in the regulation of NCR. These GATA-proteins have Zinc finger domain with key function in NCR induction. Among the identified proteins regulated by NCR is the general amino acid permease (GAP), which is considered the NCR marker and its regulation is dependent of GATA transcriptional factor. This protein was identified by *in silico* analysis in *Paracoccidioides* spp. This NCR mechanism is important for the growth, behavior and dissemination inside host. Altogether, these results can improve the knowledge regarding *Paracoccidioides* biology and nitrogen metabolism, that can be related to *Paracoccidioides*-host interaction.

Keywords: Nitrogen starvation, Paracoccidioides spp, nitrogen uptake

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INFLUENCE OF SALINITY ON THE DEVELOPMENT OF ISOLATED FUNGI FROM THE ARAÇA BAY SEDIMENT, SAO SEBASTIAO, SP.

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This work aimed to determine the influence of salinity on the growth of the isolated fungi from the Araça Bay sediment, Sao Sebastiao, SP. Salinity is an environmental parameter that is directly related to the distribution of the species in the mangrove ecosystem, being a stress factor for an allochthonous species. The macro and micromorphological growth and size of 4 genera of fungi isolated from the Araçá Bay sediment, *Aspergillus* sp., *Penicillium* sp., *Curvularia* sp. and *Aureobasidium* sp. Due to the variation of the salinity measured *in situ* in the collected samples, the fungal gowth was tested, in duplicate, for salinity values of 30, 15 and 0 using the PDA culture media (Potato Dextrose Agar), inoculated for 7 days at 25∘C. Regarding the size of the colonies, it was found that in salinity 30 had the growth of *Penicillium* sp. was approximately 75% lower than the standard. The size of the colonies of *Curvularia* sp. and the *Aspergillus* sp. and *Aureobasidium* sp. were 50 and 20% smaller than the standard, respectively. Only *Aureobasidium* sp. showed higher growth in salinity 15, while for other genera smaller sizes were observed in almost 50%. Micromorphologically all genera presented mature and sporulated structures at the lowest salinity. The fungi tested in this study showed a decreased growth in high salinity, but this factor didn't inhibit its development. It is possible to find these microorganisms in a lower density in saline environments, but it's more favorable for the growth and development of fungi in brackish environments of the mangroves.

Keywords: Mangrove, salinity, morphology.

*Speaker

Characterization and gene expression analysis of sHspsof Trichoderma harzianumduringmycoparasitism.

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Characterization and gene expression analysis of sHspsof *Trichoderma harzianum*duringmycoparasitism.

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The genus *Trichoderma* has a significant importance in agriculture, sincethese fungi acts as antagonists against several phytopathogenic fungi, such as *Fusarium, Rhizoctonia, Phytium* and *Sclerotinia*. Knowing their performance as mycoparasite, species of the genus Trichoderma, are used to act in the biocontrol of phytopathogens. This relationship imposes a stress on this microorganism during mycoparasitism, and of course, a response to the stress generated. This response occurs through changes in cell metabolism, which include the performance of heat shock proteins (HSPs), which is a primary protection response.Heat shock proteins, as a class, are among the most expressed cellular proteins in all species. In this work, we evaluated the relative expression of genes encoding two sHSPs (HSP23 and HSP42) in Trichoderma harzianum and Trichoderma asperellum during mycoparasitism against Sclerotinia sclerotiorum to better understand HSPs function and importance in these conditions. Real time RT-PCR experiments were realized using samples from Trichoderma spp. cultures grown in direct confront assays against S. sclerotiorum during three interaction moments: pre-contact, contact and pos-contact. We observed that hsp23 and hsp42 genes showed different patterns of expression during mycoparasitism, indicating that these genes are important during the biocontrol performed both by T. harzianum and T. asperellum species.

Keywords: T. harzianum, Mycoparasitism

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Metarhizium acridum colonies developing from conidia produced under light are more tolerant to UV-B radiation than those arising from conidia produced in the dark

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Light is an important stimulus regulating many biological processes in fungi. In the entomopathogen *Metarhizium* spp., it is known that light induces the production of conidia with increased tolerance to UV-B (280-315 nm) radiation. Also, we have previously shown that exposing young colonies to light will rapidly increase their tolerance to UV-B in *Metarhizium acridum*. However, there is no information in the literature regarding any differences existing between colonies arising from conidia produced under light and those arising from conidia produced in the dark. We plate-inoculated both light- and dark-produced conidia and incubated them in the dark for 12, 15, 18, 21 and 24 hours before exposing the plates to UV-B radiation. Relative survival was calculated by counting colony forming units on the UV-B-exposed plates and dividing them by the number of colonies counted on non-exposed control plates. We also photographed the plates in order to evaluate any difference in growth speed or pattern. Overall, we observed that conidia produced under light will develop into colonies that are more tolerant to UV-B radiation when compared to colonies arising from conidia produced in the dark. The discrepancies in UV-B tolerance are better observed for the 12- and 15-h time points, when the differences in relative survival are statistically significant ($P < 0.05$). For the 18- and 21-h time points, the difference in tolerance diminishes and is no longer statistically significant, although we could still see a trend towards higher tolerance for the colonies arising from light-produced conidia. Finally, 24-h-old colonies showed no difference in UV-B relative survival or growth delay. We propose a model in which light induces the expression of genes responsible for UV tolerance and the resulting proteins accumulate in conidia, remaining functional through germination and colony development. If we assume that accumulation occurs at the protein level, it is possible that answering this problem will require the use of proteome-based experiments and we hope our results will stimulate the use of proteomics to further elucidate light responses in fungi. Acknowledgements: We sincerely thank the State of São Paulo Research Foundation (FAPESP) for a Ph.D. scholarship to Guilherme Brancini (2015/24305-0)

Keywords: Metarhizium, uv radiation, light, tolerance

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Analysis of Trichoderma harzianum response to aluminium stress

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The genus Trichoderma corresponds to filamentous fungi, anamorphous, found in several environments. They have a high level of tolerance to the adverse conditions found in the environment, which favors their performance in the biocontrol of mycoparasites. The cerrado soils present a high aluminum content, with a high acidity, pH ranging from 4 to 5. To analyze the mechanisms of adaptation and tolerance of Trichoderma harzianum (isolated ALL42) to aluminum, we performed fungus growth in different media using crescent concentrations of aluminium chloride, ranging from 0.05 to 2.0 mg/mL. Subsequently, the dry weight of each sample was measured in order to determine the fungal mass obtained with the addition of the metals. The results showed that T. harzianum presents a significant tolerance to aluminum up to 1.25 mg/mL BDA medium, while in the MYG and MEX medium, the tolerance decreases from 0.2 mg/mL. We also analyzed the effect of aluminum under the secretion of proteins in the medium and we observed that the protein secretion decreases according to the increase of the concentration of the metal. This study demonstrates that T. harzianum presents greater tolerance to aluminum when grown in BDA medium. These data suggest that the constitution of the culture medium is influencing how Trichoderma tolerates the metal. Understanding the ability of Trichoderma to adapt and tolerate aluminium will allow us to use this fungus more efficiently as a biocontrol agent in the most diverse types of environments.

Keywords: aluminium, stress, Trichoderma

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Production of mycelial pellets by alkane-grown Beauveria bassiana display oxidative stress and cell surface alterations

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B. bassiana is able to grow on insect-like hydrocarbons improving its virulence. In this study we described the physiological and molecular processes involved in fungal growth, nutritional stress response and cell surface alterations found in alkane-grown conidia. Fungi were observed by optical microscopy, transmission electron microscopy (TEM) and atomic force microscopy (AFM), and their hydrophobicity was measured on the cell surface. Additionally, the expression pattern of several genes associated with oxidative stress, hydrophobicity and peroxisomal biogenesis was analyzed by qPCR.
We found a novel type of growth in alkane-cultured *B. bassiana*, similar to mycelial pellets described in other alkane-free fungi, which were able to germinate in media without a carbon source. Optical microscopy and TEM showed that pellets were formed by hyphae cumulates with an apparent surface thickening. Cell surface appeared to be more hydrophobic and exhibited different surface topographies as was observed by AFM. We also found a significant induction in several genes encoding for catalases, superoxide dismutases, hydrophobins and peroxins.
Additional studies are being conducted to better understand the relationship between alkane growth adaptation and fungal cell changes, in order to improve the efficacy of fungal penetration through the cuticle and thus enhance virulence against insect pests.

Keywords: Mycelial pellets, Beauveria bassiana, oxidative stress, hydrophobic cell surface, alkanes

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Analysis of the viability of fungal isolates from Cerrado soil after storage in sterile water

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Cerrado biome is known for its great biodiversity that also includes microorganisms. The preservation of a fungal strain or an entire collection, aiming preservation and studying, is an important aspect of mycology. Stored strains should remain viable for long periods and do not accumulate mutations that cause morphological or physiological changes. Castellani's method, (storage in sterile water) shows good results for fungi preservation in alternative to techniques like cryopreservation or lyophilization methods and also have low cost. In this work we evaluated the viability of preservation in sterilized water of filamentous fungi isolated from Cerrado soil. Slices of 0.5 cm diameter from culture media containing fungi were placed in sterile tubes filled with 2 mL of sterile distilled water. After storage, the growth diameter of each fungi were measured on plates containing culture medium. From the 39 fungal isolates that were analyzed, 29 resisted at least 6 months of storage, 16 resisted for at least one year and 5 were able to grow 2 years after storage. Diameter of growth varied depending on the culture media used and also viability were different among the strains isolated. Besides, an *Aspergillus sp.* strain showed little variation in growth along 2 years of storage. Fungi from native Cerrado soil were more resistant to storage than from pasture or crop soil. Results showed The results show that most fungal isolates from Cerrado can be preserved up to 6 months using Castellani's method and it is to reduce costs caused by continuous subculture method using during the study of the microbiota from this important biome.

Keywords: mycology, Cerrado, storage, Castellani's method, biodiversity

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Increased tolerance to UV-B radiation of entomopathogenic fungi by addition of riboflavin to the culture medium

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Techniques of biological control have become important objects of study once they are based in biodegradable elements. Meanwhile, in the open field, where abiotic stresses cannot be controlled, the survivor of biocontrol agents may be negatively affected. The UV-B radiation can damage the genetic content of organisms and lead it to death. Riboflavin, a B complex vitamin, has key-roles in DNA repairing. This study evaluated the UV-B tolerance of conidia of *Metarhizium robertsii* ARSEF 2575, *Beauveria bassiana* ARSEF 9588, and *Isaria javanica* CNPAF 22 produced on potato dextrose agar medium (PDA) supplemented with riboflavin. The isolates were cultivated on PDA or PDA plus 0.01% riboflavin (PDA+Rb). Conidia were suspended in 0.01% Tween 80 solution and inoculated on PDA supplemented with yeast extract plus chloramphenicol and benomyl, and exposed to UV-B radiation: 3.9, 5.9 or 7.8 kJ m-2 at 866.7 mW m-2 of Quaite weighted irradiance. The plates were then incubated for 48h at 27 ± 1 ºC, and the relative germination (%RG) of conidia was assessed. *M. robertsii* conidia produced on PDA+Rb were significantly more tolerant than conidia produced on PDA at all three UV-B doses tested: %RG was 14%, 32% and 11.5% higher, respectively. *B. bassiana* showed similar results; the %RG of conidia produced on PDA+Rb was 16.5%, 34.8% and 14.1% higher at the same three doses. Finally, *I. javanica* conidia produced on PDA+Rb had %RG 9.2%, 27.8%, and 14.7% higher than their conidia produced on PDA, respectively at 3.9, 5.9 or 7.8 kJ m-2. Riboflavin had a photoprotective effect on conidia of entomopathogenic fungi, observed for the first time in *B. bassiana* and *I. javanica* strains. This study suggests that the use of riboflavin to produce conidia may raise their efficiency as biocontrol agents.

Keywords: Abiotic stress factors, Hypocreales, Mycopesticides, B2 vitamin, Photoprotectants.

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Impact of short-term temperature challenges on the larvicidal activities of the entomopathogenic watermold Leptolegnia chapmanii against Aedes aegypti, and development on infected dead larvae

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The oomycete *Leptolegnia chapmanii* is a promising pathogen for biological control of *Aedes aegypti*. The larval stage of this mosquito can survive in small water sites that are exposed to temperature variations during the day. One of the major challenges for application of *L. chapmanii* to control mosquitoes is the susceptibility of its propagules to natural abiotic stresses. The present study evaluated the effects of short-term heat or cold stresses on the virulence against *A. aegypti*, production of encysted zoospores (cysts) and oogonia of *L. chapmanii*. Cysts of ARSEF 5499 were exposed to temperature regimes between -12 ∘C and 40 ∘C for 4, 6 or 8 h, and their infectivity was tested against third instar larvae (L3) at 25 ∘C. In addition, the production of cysts and oogonia was monitored on L3 killed by infection and exposed to the same temperature regimes; their larvicidal activity was also examined. Cumulative mortality of larvae dropped significantly in proportion to the increased time exposure of cysts to -12, 0, 35 or 40 ∘C. The virulence of cysts was less negatively affected by fast thawing than by slow thawing from the frozen state. The zoosporogenesis was highest between 5 ∘C and 30 ∘C in compared to freezing or high temperatures (-12, 35 or 40 ∘C), but was significantly lower at 25 ∘C (positive control; 1.5 × 104 cysts/larva). The production of oogonia on dead larvae was stimulated by exposure to freezing and low temperatures; no oogonia was observed when infected larvae were exposed to higher temperatures (≥ 25 ∘C). This study described the susceptibility of *L. chapmanii* cysts to short-term exposure at differents temperatures, and indicated which ones improved the effectiveness of *L. chapmanii*. The application of this patogen for *A. aegypti* control may be challenging in natural enviroments with extreme temperature variation.

Keywords: Temperature stress, mosquito, Saprolegniales

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Gene expression analysis during microsclerotial development of the entomopathogenic fungus Metarhizium robertsii ARSEF 2575

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The main goal of this study was to understand the microsclerotia (MS) metamorphosis in *M. robertsii* ARSEF 2575, based on the expression pattern of genes markers of oxidative stress (superoxide dismutases, *sod*, and catalases, *cat*), peroxisome biogenesis (peroxins, *pex*) and superficial hydrophobicity (hydrophobin, *ssga*). ARSEF 2575 was cultured in an agitated (250 rpm) liquid medium with carbon:nitrogen ratio 30:1 for production of MS. At 1, 6, 12, 18, 24, 48, 72 or 96 h, aliquots were collected and examined for observation of MS formation process using optical microscopy. Additional aliquots were stained with 3,3-diaminobenzidine (DAB) for evaluation of peroxidase activity. Samples cultured for 24, 48, 72 or 96 h were macerated with liquid nitrogen to obtain total RNA using the Trizol method. The cDNA was then synthesized. The expression of nine genes was studied by qPCR: *Mrsod1*, *Mrsod*2, *MrcatA*, *MrcatB*, *Mrpex5*, *Mrpex7*, *Mrpex14/17*, *Mrpex19*, *MrssgA* and *Mrgpd* (housekeeping gene). The results revealed that aggregation of hyphae was not seen before 6 h incubation, and from 6 to 18 h incubation aggregates were compacted and melanized. DAB staining revealed peroxidase activity. *Mrsod*1 ($F2{,}6 = 5.117$, $P = 0.050$); *Mrsod*2 ($F2{,}6 = 5.203$, $P = 0.048$); *Mrcat*A ($F2{,}6 = 6.900$, $P = 0.028$), and *Mrpex*5 ($F2{,}6 = 4.597$, $P = 0.061$) were upregulated at 96 h compared to 24 h incubation, with expression levels up to 3 fold induction. At 48 or 72 h, no upregulation was detected for any of the genes studied. We concluded that an oxidative stress scenario with an increment of peroxidase activity and peroxisomal proliferation is induced during metamorphosis of ARSEF 2575 in the fourth day of microsclerotial development.

Keywords: Microsclerotia, Oxidative stress, Peroxisomes

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Beauveria bassiana produces microsclerotia-like propagules with active peroxisome biogenesis

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Entomopathogenic fungi are able to produce several types of propagules such as aerial conidia in solid media, or blastospores, submerged conidia and microsclerotia in liquid cultures. Microsclerotia are usually melanized compact hyphal aggregates that are tolerant to desiccation. As far as we known, there are no reports about the production of melanized microsclerotia by *Beauveria bassiana* isolates. The aim of this study was to describe microsclerotial growth in *B. bassiana* and to start studies about the molecular and physiological mechanisms implicated in the production of these propagules. *B. bassiana* strain GHA was cultured in complete liquid medium for 4 days at 26°C with vigorous agitation (250 rpm). Microsclerotia-like structures were separated by centrifugation and processed for microscopic observation and real time qPCR analysis. Under this culture condition, *B. bassiana* was able to produce compact brownish hyphae aggregates that were able to germinate and produce viable conidia after desiccation. Optical microscopy images showed similarity in form, structure and size with microsclerotia reported elsewhere from other entomopathogens, and staining with 3,3-diaminobenzidine (DAB) revealed high peroxidase activity. Genes encoding for peroxisome biogenesis factors, named peroxins, showed high expression levels (up to 17-fold induction), i.e., *Bbpex5*, *Bbpex7*, *Bbpex14/17* and *Bbpex19* genes in 4-days cultures compared with conidia used as starting inocula. Additional studies are being carried out to elucidate the relationship between microsclerotia formation and peroxisomal biogenesis, as same as stress tolerance, cell surface alterations and virulence against insect hosts.

Keywords: Entomopathogenic fungi, microsclerotia, gene expression, peroxins

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Ultrastructural analysis of heat-stressed Metarhizium anisopliae s.s conidia

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High temperatures delay the germination of *Metarhizium* spp. conidia or may make them unviable, depending on the isolate or lineage and the heat-exposure time. The current study evaluated the effect of heat on the ultrastructure of *Metarhizium anisopliae* s.s. IP 119 conidia. Conidial suspensions of *M. anisopliae* in Tween 80 0.01% solution were adjusted to 2 × 108 conidia ml-1 and exposed for 0 h (control), 6 or 8 h to 45 ± 0.5 ∘C in a water bath. After exposures, samples were fixed overnight in 2.5% Glutaraldehyde, 0.2% picric acid in 0.1M cacodylate buffer pH 7.2, and then, post-fixed in osmium tetroxide, and washed with distilled water and dehydrated in a gradual series of acetone solutions. After dehydration, the samples were pre-infiltrated and infiltrated with EPON resin (Electron Microscopy Sciences). Ultrathin sections of 70 nm of the samples were taken in an ultramicrotome and placed on 300 msh copper screens, and then, contrasted with uranyl acetate and lead citrate. After drying, the sections were analyzed and photographed under a Transmission Electron Microscope (TEM) (Jeol JEM 2100) at the Laboratory of High Resolution Microscopy (LabMic) from Universidade Federal de Goiás (UFG). The results showed a marked disorganization in the cytoplasm of the conidial cell when exposed to heat for 6 and 8 h in comparison to the control cells which were suspended in unheated aqueous solution. Both heated and unheated conidia presented cell membrane apparently undamaged. In conclusion, heat exposure may promote disorganization within the conidial cell, and it can be inferred that these changes, along with other factors, may lead conidia to delayed germination or unviability.

Keywords: High temperature, ultrastructure, entomopathogenic fungi.

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Studies on tick eggs protein profile after females' fungal exposure

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The use of entomopathogenic fungi to control ticks is an interesting approach since these fungi have good dispersal ability, penetrate through host cuticle and also show specificity for different arthropods, contributing to the preservation of non-target organisms. Unveiling ticks' immune response after entomopathogenic fungal challenging is remarkably important to obtain significant improvements in the use of fungi against this pest. The present study aimed to analyze changes in the protein profile of eggs from *Rhipicephalus microplus* tick females exposed to *Metarhizium* through the identification of peptides/proteins associated to arthropods' immune response. Tick females were exposed to the fungus *Metarhizium anisopliae* senso lato ARSEF 782 on the first day after detachment of bovine host. Unexposed females formed control group. Eggs from day 5 of oviposition were analyzed on polyacrylamide 2D gels and spots were identified by mass spectrometry (MALDI-TOF-TOF). 52 proteins were identified in *R. microplus* eggs; four serine inhibitors proteases are possibly related to an adaptive response, since they were only present in the gel of eggs from females previously exposed to the fungus. Detect differences in the protein profile of eggs from tick females previously exposed to *M. anisopliae*, focusing on proteins that may be related to the tick immune response, enhances the understanding of tick capacity to act against fungal infection. Accordingly, it is suggested that tick immune response to fungi may exceed female's organs, changing the protein profile of eggs and possibly of larvae.

Keywords: Metarhizium anisopliae, Rhipicephalus microplus, mass spectrometry

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Tolerance of conidia and blastospores of Metarhizium acridum to UV-B radiation

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Metarhizium acridum is a fungus which infects and kills orthopterans, and it has been used for biological control of this pest. *Metarhizium* species are susceptible to abiotic stresses, but *M. acridum* is known as distinctly tolerant to ultraviolet (UV) radiation. UV-B radiation may affect the viability of entomopathogenic fungi in open fields and reduce the efficacy of bioproducts. Fungal conidia are infective forms naturally found in the environment, whereas blastospores are found in infected host haemocoel. The present study compared the tolerance of conidia and blastospores of *M. acridum* ARSEF 324 to UV-B radiation. Aliquots of 50 μL of conidia or blastospores suspension (103 propagules mL-1) were inoculated and spread on PDAY medium in Petri dishes (90 × 10mm). The inoculated dishes were covered with a cellulose diacetate film (0.13 mm) to block UV radiation below 290 nm (UV-C and short wavelength UV-B), or covered with aluminum foil to block all radiation (control). Propagules were exposed to 1.11, 2.22, 3.33, 4.44, 5.56, 6.67, 7.78 or 8.85 kJ m-2 at 743.45 mW m-2 of UV-B Quaite-weighted irradiance, and the dishes were then incubated for seven days at 27 ± 1º C with RH ≥ 80%. At day 7, the colonies (colony-forming units, CFU) of each dish were quantified, and the percentage of CFU of treated propagules, in relation to the control group, was calculated. The relative percentage of CFU of blastospores or conidia decreased from 100%, at the lowest dose, to virtually 0%, at the highest dose. No significant difference of tolerance was detected between conidia and blastospores in each UV-B dose tested. Blastospores were as much tolerant to UV-B as conidia, suggesting that this propagule may be a promising candidate for use in biological control programs.

Keywords: Entomopathogenic fungi, propagules, ultraviolet radiation

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Heat tolerance and conidial production of multiparticulate formulation of microsclerotia of Metarhizium anisopliae s.s.

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Granular formulation of entomopathogenic fungi is a promising alternative to conidial suspensions in biological control programs of arthropod pests. Pellets are granules with small spherical units, with medium size, formed by agglomeration of fine powders. Pellet formulations protect the propagules from abiotic stresses, facilitate their application and storage, and allow the development of conidia that infect target hosts. In the current study, pellets containing microsclerotia (MS) of *Metarhizium anisopliae* s.s. IP 119 were produced, and the production and viability of conidia at 27 °C or 32 °C evaluated. IP 119 was cultured for production of MS in an agitated liquid medium, at 250 rpm, for 4 days at 27 ± 1 °C, with a carbon:nitrogen ratio 30:1. The biomass was processed, and the pellets formulated with microcrystalline cellulose or vermiculite. Pellets were sprinkled on the surface of 2% water-agar medium and incubated in the dark at 27 ∘C (optimum) or 32 ∘C (stress) for 10 or 15 days; the conidia produced were quantified, and their viability evaluated. The results showed that the pellets with MS of IP 119 produced conidia at both temperature conditions; however, the production of conidia was reduced at 32 °C in comparison to the production at 27 ∘C ($F = 33.7$; $P = 2.67$ e-05), for pellets with cellulose or vermiculite ($F = 5$; $P = 0.04$). The mean viability of conidia produced from cellulose or vermiculite formulations was 100%, regardless the temperature and incubation period investigated ($P = 0.7$). In conclusion, the pelletsof IP 119 with inert microcrystalline cellulose or vermiculite produced conidia effectively at optimum temperature or at heat stress condition.

Keywords: Entomopathogenic fungi, temperature, microcrystalline celulose, vermiculita.

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Molecular targets of argentilactone in Paracoccidioides spp. identified by chemoproteomic

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Paracoccidioidomycosis (PCM) is the cause of several deaths from systemic mycoses. The etiological agents of PCM, belong to the genus Paracoccidioides spp., restricted to regions of Latin America. The infection is acquired through inhalation of conidia that primarily lodge in the lungs and may disseminate to other organs. Treatment of PCM is commonly performed with the administration of antifungal of the azole class, sulfonamides and amphotericin B. The toxicity and side effects of the antifungal, added to the long treatment time has driven research for new bioactive compounds. Argentilactone, compound isolated from a Brazilian Cerrado plant, *Hyptis ovaliofolia*, has been suggested as potent antifungal, inhibiting dimorphism of *P. brasiliensis* and the enzymatic activity of isocitrate lyase, key enzyme of the glyoxylate cycle. Furthermore, it has no cytotoxicity and genotoxicity in fibroblast cells at concentrations that inhibit the fungus. This work was developed due to the importance of elucidating the mode of action of argentilactone. The chemoproteomics approach, by affinity chromatography, was the methodology used to explore the interactions between proteins of *P. brasiliensis* and argentilactone. A total of 333 proteins was identified and classified functionally, being the most representative, related to amino acid metabolism, energy and detoxification. Argentilactone inhibited the enzymatic activity of malate dehydrogenase, citrate synthase and pyruvate dehydrogenase. Furthermore, induced the production of reactive oxygen species and arrested the cell cycle in G0/G1 phase. Therefore, argentilactone has shown to be a promising antifungal.

Keywords: Paracoccidioides, antifungal, targets, argentilactone, chemoproteomic

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Light during mycelial growth produce conidia of Metarhizium robertsii with increased germination speed and virulence

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Light conditions during fungal growth are known to cause several physiological adaptations in conidia. In this study, conidia of the entomopathogenic fungi *Metarhizium robertsii* were produced on: 1) potato dextrose agar (PDA) medium in the dark; 2) PDA medium under white light (4.98 W m–2); 3) PDA medium under blue light (4.8 W m-2); 4) PDA medium under red light (2.8 W m-2); and 5) minimum medium (Czapek medium without sucrose) supplemented with 3% lactose (MML) in the dark. The conidial production for each treatment as well as the speed of conidial germination and virulence to the insect *Tenebrio mollitor* (Colleoptera: Tenebrionidae) were evaluated. The fungus grown under blue light produced more conidia than the fungus grown in the dark. The conidial production of the fungus grown under white and red light was similar. The MML afforded the least conidial production. Conidia produced on MML or on PDA medium under white or blue light germinated faster than conidia produced on PDA medium in the dark or under red light. Conidia produced on MML and PDA medium under white light were also more virulent than conidia produced on any other treatment. In conclusion, the white light treatment during mycelial growth produced conidia with faster germination speed and were more virulent to the insect, with similar result of conidia produced on MML.

Keywords: entomopathogenic fungi, photobiology, nutritional stress, virulence, germination speed, conidial production

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