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# Oral Presentations

## IMPACT OF NUCLEOTIDE SUGAR TRANSPORTERS ON THE STRESS RESPONSES OF THE PLANT PATHOGENIC FUNGUS *LEPTOSPHAERIA MACULANS*

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Nucleotide sugars are the building blocks for the polysaccharides that make up fungal cell walls as well as for glycan modifications on other molecules. Glycosylation requires the import of nucleotide sugars into either the golgi apparatus or endoplasmic reticulum via specific transporters, prior to their use as substrates for glycosyltransferases. Apart from in the model yeast *Saccharomyces cerevisiae*, no comprehensive analysis has been conducted on the transporters of nucleotide sugars within fungi. *Leptosphaeria maculans* is the major pathogen of *Brassica napus* (canola), causing blackleg disease that develops as lesions of leaves and later cankers at the base of the plant. Little is known about the molecular basis of how this fungus causes disease; however, it is highly likely that the interaction between the cell wall or other glycosylated molecules of the fungus with the plant contributes to disease outcomes. To test this, all eight nucleotide sugar transporters (NSTs) were mutated in *L. maculans* using the recently developed CRISPR-Cas9 system, and phenotypes tested in the mutant strains. The mutants exhibit a range of phenotypes related to stress response and plant pathogenesis. This analysis provides new insights into the roles of NSTs in fungal biology.

Key Words: plant pathogen; nucleotide sugar transport; *Brassica napus*; cell wall

# CALCIUM SIGNALLING DYNAMICS DURING DIVERSE STRESS RESPONSES IN *CANDIDA ALBICANS*

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*Candida albicans* lives as a commensal yeast in humans but, in certain patient groups, causes chronic mucosal infections or life-threatening systemic disease. The formation of penetrative hyphae is a hallmark of *C. albicans* tissue disruption and invasion. We have shown that an essential charactertistic of invasive hyphae is their ability to steer as they grow and mutants that cannot regulate their direction of growth fail to colonise and damage host tissue. In our investigation of the mechanisms of directional regulation, we have identified parallels with other elongating cell-types, such as neurons, including small GTPase activity, a Paxillin-like system and the involvement of calcium signalling. We have developed the first real-time calcium reporter and analysis software in *C. albicans* and are using it to investigate not only the role of calcium in directional responses, but more broadly to understand which cell stress responses elicit a calcium signal, which calcium transporters are involved in stress responses and which signaling pathways facilitate the responses we see. Our early results show that some stresses, such as a sudden increase in pH, elicit dramatic increases in cytplasmic calcium spiking, but others inhibit calcium spiking for many minutes, suggesting that calcium signalling plays a minimal role in the immediate recovery phase after specific types of shock. Our work is revealing novel insights into how *C. albicans* regulates calcium homeostasis and how cytoplasmic calcium changes in response to different external stimuli.

Key Words: *Candida albicans*; calcium signaling, stress responses, homeostasis, directional growth.

# STRESS ADAPTATION OF A FUNGAL PATHOGEN TO COMPLEX NICHES IN ITS HUMAN HOST

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*Candida albicans* is a major fungal pathogen of humans. It inhabits the gastrointestinal and urogenital tracts of healthy individuals, but when when host defences become compromised it causes oral and vulvovaginal infections, and it can disseminate to cause life-threatening infections of the bloodstream and internal organs in severely immunocompromised patients. The ability of *C. albicans* to colonise these divergent host niches is dependent upon robust stress responses, metabolic adaptation and immune evasion. The molecular mechanisms by which *C. albicans* responds to specific stresses and nutrients *in vitro* is reasonably well understood. However, host niches are complex (in that fungal cells are subjected simultaneously to combinations of host signals) and they are dynamic (in that these signals change over time). We find that this complexity and dynamism can yield non-additive and unexpected fungal behaviours, at least when when compared to the responses to individual stresses and nutrients *in vitro*. Furthermore, *C. albicans* appears to have developed anticipatory behaviours in which it to modulate the exposure of major epitopes at its cell surface in response to specific host inputs, thereby allowing the fungus to evade subsequent detection by innate immune cells. These anticipatory behaviours, which promote stress resistance as well as immune evasion, influence fungus-host interactions and *C. albicans* pathogenicity.

Key Words: *Candida albicans*; combinatorial stresses responses; nutrient adaptation; immune evasion; emergent behaviours; anticipatory behaviours; fungal pathogenicity

## DYNAMIC TRANSCRIPTIONAL RESPONSES OF FUNGAL PATHOGENS TO MACROPHAGE PHAGOCYTOSIS

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Pathogenic fungi have evolved diverse mechanisms to survive host immune surveillance and attack. Studies aimed at dissecting the interactions between fungal pathogens and host phagocytes have successfully identified several fungal responses important for virulence. However, most previous studies are limited in temporal resolution and focused mainly at later time points of infection, and consequently, the immediate and temporal responses of fungal pathogens upon phagocytosis by immune cells are still not well defined. In this work, we set out to map genome-wide active transcription changes of a number of human fungal pathogens in a high-resolution time course experiment right after macrophage phagocytosis. Through a Comparative Functional Genomics approach, we have identified common and unique physiological pathways employed by different pathogens for surviving macrophage attacks. We are in the process of identifying and characterizing transcription regulators responsible for controlling the responses and will confirm their roles in virulence.

Key Words: transcription response; stress response; fungal pathogens; macrophage; infection.

## THE BROAD SPECTRUM METABOLIC REPROGRAMMING INDUCED BY CYCLOSPORINE A IN *PARACOCCIDIOIDES BRASILIENSIS* MAY REVEAL NOVEL DRUG TARGETS

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The cyclic peptide Cyclosporine A (CsA) produced by the secondary metabolism of the fungus *Tolypocladium inflatum* is famous for its immunosuppressive properties and was a breakthrough in organ transplant surgery. In Nature, CsA is thought to be a part of a molecular defense strategy of this soil organism against competitors or predators in its ecological niche. Not surprisingly, CsA severely affects the cellular and molecular behavior of an enormous amount of fungal species. Among them is the South American human pathogen *Paracoccidioides sp*. This organism is a thermodimorfic fungi also found in the soil. It has been predicted to infect up to 5% of Brazil's population, or about 10 million people. CsA acts through its association with specific proteins called cyclophilins and inhibits the signaling protein calcineurin by inducing the formation of a stable ternary cyclophilin-CsA-calcineurin complex. Inhibition of both enzymes is fundamental for the molecular mechanism underlying CsA action in all eukaryotes studied so far. Calcineurin is a calcium/calmodulin dependent serine/threonine phosphatase. Its inhibition is probable responsible for most of CsA's immunosuppressive and anti-fungal effects. On the other hand, cyclophilins belong to a family of peptidyl-prolyl-cis-trans-isomerases that have a plethora of functions in cell physiology and are less well studied in fungi.

We therefore used CsA as a tool to identify biochemical and/or metabolic processes that, once disturbed, inhibit fungal growth. We were able to show that CsA blocks dimorphism and proliferation of *Paracoccidioides brasiliensis* yeast and mycelia and that these effects are mediated by calcineurin. Mass spectrometry experiments were carried out to define the protein profile of *P. brasiliensis* yeasts grown for 24 hours with CsA, revealing 86 proteins with changed protein levels. We are currently investigating which cellular processes are critical for cell homeostasis and thus profiling promising drug targets. The broad metabolism reprogramming of *Paracoccidioides brasiliensis* by CsA apparently produces a state of starvation in yeast cells at the same time it prevents triacylglycerol catabolism.

Key Words: *Paracoccidioides brasiliensis*, metabolism, calcineurin, cyclophilin, proteome

# STRESSING OUT OR STRESSING IN: INTRACELLULAR PATHWAYS FOR SAPK ACTIVATION

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The yeast stress-activated MAP kinase (SAPK) Hog1 functions within the well-characterized High Osmolarity Glycerol (HOG) pathway, which responds to hyper-osmotic stress. The SAPK Mpk1 functions within the similarly well-characterized Cell Wall Integrity (CWI) pathway, which responds to cell wall stress. However, it is now clear that both Hog1 and Mpk1 respond to a wide variety of additional stressors, but the pathways through which specific stress signals are transmitted to the SAPKs are not known. Our recent findings have begun to address two important and related questions. First, do various stressors activate a SAPK through common pathways initiated at the cell surface, or through alternative, intracellular inputs? Second, how does an activated SAPK mount a specific response appropriate to the particular stress experienced? Our work has uncovered the mechanisms by which two stressors, the toxic metalloid arsenite and genotoxic stress, stimulate Hog1 and Mpk1, respectively. We found that these stresses activate the SAPKs through intracellular inputs that modulate their basal phosphorylation, rather than by activation of the protein kinase cascades known to stimulate them. Both stresses act through targeting, in different ways, the tyrosine-specific or dual-specificity protein phosphatases that normally maintain the SAPKs in a low activity state. This work reveals that basal activity of SAPKs is important to allow their activation by intracellular inputs that modulate that activity. Additional studies with the arsenite metabolite methylarsenite (MAs), which reacts with cysteine thiols in proteins, suggest that this stressor modifies the output of activated Hog1 in multiple ways. First, MAs blocks glycerol biosynthesis, which is normally stimulated in response hyper-osmotic stress, by reaction with the glycerol-

3-phosphate dehydrogenase. Second, MAs blocks the stable association of Hog1 with its tyrosine phosphatases, potentially allowing for other Hog1 interactions. Third, MAs reacts with Hog1 itself in a manner that may alter its target specificity. Thus, specific stressors can activate SAPKs through non-canonical intracellular pathways that may influence molecular interactions and signaling output of the SAPK. Moreover, some stressors also modify SAPK targets or even the SAPK itself in ways that influence signal output.

Key Words: *Saccharomyces cerevisiae*, stress, SAPK, arsenite, genotoxic damage, protein phosphatases

## CIRCADIAN CLOCK REGULATION OF MRNA TRANSLATION IN *NEUROSPORA*

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Our long-term goal is to understand the fundamental mechanisms by which the circadian clock, important in human health and drug metabolism, regulates rhythmic gene expression and thus cell function and metabolism. Most of the focus on understanding clock control of gene expression has been at the level of transcription. However, in many systems, there are examples of specific proteins that show a circadian rhythm in levels, while levels of the associated mRNA are relatively constant throughout the day. This suggests that the clock may regulate mRNA translation, but which proteins cycle in abundance in cells over the day, and the mechanisms of clock control of translation, are not known. Using *Neurospora crassa* as a model organism, we discovered that the circadian clock, through regulation of specific kinases and phosphatases, regulates the phosphorylation state and activity of eukaryotic elongation factor 2 (eEF2) and cap-dependent eukaryotic initiation factor 2α subunit (eIF2α). Both eEF2 and eIF2α peak in activity during the night, coincident with reduced stress and high energy levels. Circadian ribosome profiling, coupled with transcriptome analyses from WT and eEF2 and

eIF2α kinase mutants revealed that clock control of translation elongation and cap-dependent initiation leads to rhythmic translation of specific mRNAs, rather than acting globally. Work is currently in progress to identify and validate elements that confer specificity. In addition, recent preliminary data from mass spectrometry suggested that the circadian clock controls the amount of ribosomal proteins associated with functional ribosomes (ribosome heterogeneity). Experiments are in progress to confirm these data, and to determine the functional consequences of rhythmic ribosome heterogeneity on mRNA translation.

Key Words: Circadian clock, mRNA translation, translation initiation, translation elongation, ribosome, *Neurospora crassa*.

## 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* 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 in 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. On the other hand, 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 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.

Key Words: entomopathogenic fungi; cross resistance; photobiology; nutritional stress; UV-B radiation; heat stress, oxidative stress, osmotic stress, genotoxic stress

# MELANIZED FUNGI AS RADIOPROTECTORS AND DETECTORS OF IONIZING RADIATION

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There is a growing need for novel and effective prophylactic treatments and radioprotective materials to protect soldiers and enable them to sustain military operations in radiation-contaminated environments. Melanins are pigments that are ubiquitous in nature. These pigments confer a survival advantage to melanized fungi in extreme environments. The resistance of melanized fungi to cosmic radiation in Mir Spacecraft and the presence of numerous melanized fungal species in the damaged nuclear reactor at Chernobyl suggest that melanins also play a pivotal role in protection from ionizing radiation. We used clonogenic survival assays, transmission electron microscopy, and a variety of metabolic assays to demonstrate that melanin prototected such diverse fungi as *Cryptococcus neoformans, Cryomyces antarcticus and Friedmanniomyces endolithicus* against both sparsely and densely ionizing radiation.

Living organisms, especially plants and microorganisms, possess remarkable regulatory flexibilities that allow them to thrive under different external conditions and to survive harsh environments. Programing of genome structure and gene expression landscape plays a central role in cellular adaption to long-term extracellular stimuli. It has been reported that melanized fungi have the ability of growing into and decomposing “hot particles” that are contaminated with various long-lived radionuclides emitting beta or gamma radiation at the damaged Chernobyl Nuclear Plant site. Furthermore, only fungal species isolated from sites with elevated radiation showed the distinct stimulatory growth effects in response to different radionuclides. Those observations strongly suggest that chronic exposure to radiation selects those fungi to cope with and adapt to the low dose radiation (LDR) environment by developing new physiological and genetic features, such as melanin production, enhanced growth and conserved genome structure. We designed and built up the radiation apparatus that was used to study radioadaptation, radiostimulation and to measure radiotropic growth of fungal cells. The melanized and non-melanized *C. neoformans* and *Wangiella dermatitidis* cells

were constantly exposed to 1000 µSv gamma field at a 60 µSv/hr dose rate. We observed the radiation stimulation in *C. neoformans* yeast cells and both radiation stimulation and radiotropism in *W. dermatitis* conidia growth. We conclude that the unique ways melanized fungi interact with ionizing radiation could be potentially utilized in multiplicity of applications such as radioprotection and radiation detection.

Key Words: melanin, ionizing radiation, *Cryptococcus neoformans, Cryomyces antarcticus, Friedmanniomyces endolithicus,* radiostimulation, radiotropism, radioprotection

## WHY USE *SACCHAROMYCES CEREVISIAE* TO INVESTIGATE THE MOLECULAR MECHANISMS OF HUMAN DISEASES?

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The yeast *Saccharomyces cerevisiae* is among the best-studied experimental organisms. Although apparently yeast and humans have little in common, yeast cells share many basic biological properties with human cells. Furthermore, a considerable number of yeast and human genes perform the same roles in both organisms, meaning that the expression of a human gene is able to replace for that of the yeast. One of those conserved genes is *SOD1*, which codes for Cu, Zn superoxide dismutase. Around 20% of familial Amyotrophic Lateral Sclerosis (fALS) cases are attributed to heterozygotic mutations in the *SOD1* gene. Although much has been known about fALS-Sod1 homodimers, the investigation of Sod1 heterodimerization remains poorly unexplored in the literature. In this study, an imaging approach named Bimolecular Fluorescence Complementation (BiFC) was used to analyze the heterodimeric combination of WT and the mutant Sod1 proteins (A4V, L38V, G93A and G93C) as well as to investigate the effects of Sod1 heterodimers on aggregation, antioxidant activity and dynamics properties. The BiFC allows us to image and quantify the inclusions formed by WT/mutant heterodimers by fluorescence microscopy in human neuroglioma cells (H4) and in yeast, containing either one copy of WT hSod1-VN and one copy of mutant hSod1-VC. In this study, *sod1Δ* yeast cells were specifically used to better evaluate the

effect of the human Sod1 expression, without the endogenous Sod1 influence. Moreover, yeast cells have long served as an advantageous model to study oxidative stress response. Exponential-phase glucose-grow yeast cells only ferment and, consequently, show low levels of reactive oxygen species (ROS), which increase in chronological aged cells. In both experimental models, we confirmed the intracellular WT/mutant heterodimer formation by observing the Venus fluorescence intensity. In mutant Sod1 heterodimers, a larger number of inclusions per cell were observed in comparison to WT and mutant homodimers. This effect was particularly strong for the A4V mutant, which significantly increases in the percentage of cells with inclusions. Moreover, by using Fluorescence Recovery After Photobleaching (FRAP), the inclusions formed by the Sod1 WT/A4V showed a reduced mobility and the most stable aggregate in comparison to the other Sod1 mutants analyzed. The presence of BiFC-tagged human Sod1 inclusions were also observed and quantified in *sod1*Δ cells submitted to chronological aging. In addition, our results in yeast cells showed the WT/mutants hSod1 activity significantly decreased after aging compared to the cells containing the WT homodimer. Altogether, our study sheds light into the effects of fALS Sod1 mutations on inclusion formation, dynamics and antioxidant response, opening novel avenues for investigating the role of fALS Sod1 mutations in pathogenesis.
Key-words: Sod1, fALS, *Saccharomyces cerevisiae*

## BIOTIC STRESS IN YEAST: RESPONSES TO THE PRESENCE OF COMPETING SPECIES

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Stress responses in microorganisms have primarily been investigated with regards to physical or chemical factors that impact the ability of microbial species to grow and/or to reproduce. In yeast, and primarily the yeast *Saccharomyces cerevisiae*, this has led to the accumulation of an impressively large set of data describing the physiological and molecular response of populations and individual cells to stresses such as changes in osmotic pressure, temperature, salt concentration,

and nutrient availability. The data sets include large scale, high-throughput screenings, the systematic physiological characterisation of individual strains, and a full complement of system-wide omic data, from genomics to transcriptomics, proteomics and metabolomics. Together, these data probably provide the most systematic and widest evaluation of stress responses in any biological system. Yet, it can be argued that these physical and chemical stresses are less significant in terms of evolutionary relevance than stresses that are due to the presence of competing or interacting microorganisms. However, only very limited data regarding the nature and impact of, and the molecular responses to, such biotic stresses has been generated. This is likely due to several factors, including the complexity of controlling and evaluating multispecies cultures, an under-appreciation of the relevance and importance of microbial interactions in the natural life-cycle of all microbial species, the difficulty of producing reproducible data in highly dynamic and sometimes unpredictable multispecies systems, the absence of tools to evaluate such systems in high-throughput modes, and the general focus on single species systems in most laboratory and biotechnological applications. Here we present an integrated approach combining laboratory-based evolution using biotic selection pressure and synthetic ecology approaches in combination with genome sequencing and transcriptome analysis of multispecies culture to better understand such biotic stress responses in multispecies cultures. The data highlight a number of mechanisms by which yeast respond to stresses and challenges in multispecies systems, which include the adaptation of metabolism to better use nutrient resources, changes in cell wall composition and cell wall properties, and the importance of direct physical ontact between cells in regulating the response to the presence of other species.

Key Words: Yeast; biotic stress; multispecies cultures; *Saccharomyces cerevisiae*; *Lachancea thermotolerans*; metabolic regulation; nutrient utilization; physical contact; co-flocculation.

# GEOMYCOLOGY: METALS , MINERALS AND FUNGI

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"Geomycology" is an important part of "geomicrobiology" and can be defined as the impact of fungi on geological processes, including bioweathering of rocks and minerals, metal and metalloid transformations, and element and nutrient cycling. Fungi are important geoactive agents in soil, rock and mineral surface layers, whether free-living or in symbioses with phototrophs, and also significant biodeteriogens in the built environment. As such, many are resistant to stressful environmental conditions and exhibit a variety of morphological and physiological responses to, e.g. toxic metals, pollutants, extremes of temperature and pH, UV irradiation and desiccation. Many geomycological processes, all dependent on hyphal growth form and chemoorganotrophy, are of relevance to metal pollutant fate in the environment altering metal mobility through such processes as mineral dissolution, metal accumulation and biomineralization. Fungi (including lichens) are ubiquitous members of rock-inhabiting communities where they cause a range of effects ranging from discolouration, staining and biofouling to structural and chemical alteration of the substrate. Biodeteriorative processes involve a combination of physical and biochemical mechanisms, such as tunnelling or penetration, expansion or contraction of biomass, and the excretion of metal-complexing or mineral solubilizing metabolites. Conversely, growth of microbial biofilms and production of various minerals through biomineralization phenomena, can lead to the formation of stable crusts, patinas and varnishes that can stabilize surfaces and protect from further abiotic weathering. Fungi, including lichens, have been suggested to be the most significant organisms in nature that can biodeteriorate rocks and minerals. This presentation will emphasize some important activities of fungal systems in the transformation of metal(loid)s such as Se, Te, Pb, U, Mn, Ca and Co where the formation of insoluble phosphate, oxide, carbonate or oxalate minerals can contribute to physical disruption, staining and discolouration, but also rock coating development and bioprotection. These processes can also provide a means of metal immobilization for bioremediation or element biorecovery. Some biometal(loid)s/biominerals are formed at the micro-

and nanoscale providing further interest for the development of useful products, including novel electrochemical biomaterials. Finally, the biodeteriorative properties of fungi regarding the destruction of mineral-based building materials, including concrete, which may have consequences for nuclear decommissioning and radionuclide containment, will be outlined.

## LIGHT STRESS AND THE COORDINATED DEVELOPMENT AND SECONDARY METABOLISM IN *ASPERGILLUS*

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Fungal growth and differentiation and the concomitant secondary metabolism occur in response to internal and external signals that are sensed through receptors and transported by highly controlled signal transduction pathways. The resulting genetic transcriptional control includes interaction of different genetic networks, which are organized chronologically and hierarchically and contain several feedback functions. Transcription is coupled to posttranslational histone modifications as epigenetic control affected by additional signal transduction pathways. Fungal differentiation linked to specific secondary metabolites requires additional posttranslational control mechanisms including attachment and removal of ubiquitin family modifiers, which alter protein function or cellular localization, and initiate degradation through ubiquitin 26S proteasome and autophagy pathways. This leads to a choreography of changes in transcription, translation, posttranslational histone modifications and protein stability, followed by proteomic changes, which we study in *Aspergillus nidulans*.

Key Words: *Aspergillus nidulans*; development, secondary metabolism, protein homeostasis

# ANTIMICROBIAL PHOTODYNAMIC TREATMENT: WHERE WE ARE AND WHERE WE WILL GO?

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Classical approaches for controlling plant-pathogenic fungi can be impaired by the development of pathogen resistance, and limited availability of new and effective antifungal agents. Recent increases in consumer awareness of and/or legislation regarding environmental and human health, and the urgent need to improve food security, are driving increased demand for safer antimicrobials. There is, therefore, a need for a step change in the approaches for controlling pre- and post-harvest diseases and food-borne human pathogens. The use of light-activated antimicrobial substances for the so-called photodynamic treatment of diseases is known to be effective in a clinical context, and maybe equally effective for use in agriculture, to control plant-pathogenic fungi and bacteria, and to eliminate food-borne human pathogens from seeds, sprouted seeds, fruits, and vegetables. Here, we take a holistic approach to review recent findings on: (i) the ecology of naturally-occurring, (ii) photodynamic processes including the light-activated antimicrobial activities of some plant metabolites, and (iii) fungus-induced photosensitization of plants, against the backdrop of existing knowledge. The inhibitory mechanisms of both natural and synthetic light-activated substances, known as photosensitizers, are discussed in the contexts of microbial stress biology and agricultural biotechnology. Special attention is given to the use of photoantimicrobials for the control of plant-pathogenic fungi.

Key words: Antimicrobial strategies, Global food security, Photoantimicrobials, Photodynamic inactivation of microbes, Plant-pathogenic fungi.

# MITIGATING STRESS IN INDUSTRIAL YEASTS

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The yeast, *Saccharomyces cerevisiae*, is the premier fungal cell factory exploited in industrial biotechnology. In particular, ethanol production by yeast fermentation represents the world's foremost biotechnological process, with beverage and fuel ethanol contributing significantly to many countries economic and energy sustainability. During industrial fermentation processes, yeast cells are subjected to several physical, chemical and biological stress factors that can detrimentally affect ethanol yields and overall efficiency of production. These stresses include ethanol toxicity, osmostress, pH and temperature shock, as well as biotic stress due to contaminating microorganisms. Several approaches to mitigate yeast stress during industrial fermentations can be undertaken and these will be discussed in this presentation. For example, certain physiological cell engineering approaches involve careful control over mineral nutrient bioavailability either during fermentations or by preconditioning seed cultures prior to fermentor inoculation. Results will be discussed showing how the levels of key minerals such as magnesium and zinc can play important roles in alleviating stress on *S. cerevisiae* cells caused by ethanol and heat shock. This presentation will highlight the importance of furthering our understanding of key aspects of yeast stress physiology and the beneficial impact this can have more generally on enhancing industrial fungal bioprocesses.

Key Words: yeast, fermentation, ethanol production, stress factors

# THE ROLES OF LIGHT IN POST-TRANSCRIPTIONAL GENE EXPRESSION CONTROL AND TOLERANCE TO UV RADIATION IN *METARHIZIUM ACRIDUM*

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*Metarhizium acridum* is an important entomopathogenic fungus currently being used for biological control of insect pests. Environmental stressors such as ultraviolet radiation and heat require the fungus to be as stress-tolerant as possible. It was previously observed that visible light increases *M. acridum* tolerance to ultraviolet radiation and to heat. Here we employ a combination of transcriptomics (mRNA-Seq) and high-throughput proteomics to understand how light regulates gene expression and protein accumulation. Twenty-four-hour-old cultures grown in the dark were briefly exposed to visible light for 5 min and returned to dark conditions for different periods according to the technique employed: 0, 10, 25, 55, and 115 min for mRNA-Seq; and 10, 25, 55, 115, and 235 min for proteomics. Cultures kept in the dark (no light exposure) were used as controls (DD). Fold change was calculated relative to DD for each time point. Transcripts and proteins were considered regulated if their abundance changed at least two-fold relative to DD. Light exposure resulted in 1128 genes (11.3% of the genome) being regulated at the transcript level. The number of proteins changing in abundance was only 57. Combining the two datasets, only 34 proteins were regulated both at the transcript and the protein levels. Because only 34 transcripts/proteins were commonly regulated in both datasets, we were left with 23 proteins that changed in abundance in the absence of mRNA regulation and also 1094 regulated transcripts for which there was no protein change. Among down-regulated proteins, we observed subunits of eIF3, deoxyhyousine hydroxylase, and ribosomal proteins. This indicates that light reduces translational activity, which is one potential explanation for the reduced number of regulated proteins. We also observed that a photolyase was up-regulated both at the transcript and the protein level. We characterized the role of this enzyme and

observed it is essential to *M. acridum* survival under UV-B radiation. Taken together, our results show that while light regulates gene expression to a large extent, it also reduces translational activity, thus making many changes at the mRNA level null at the protein level.

Key Words: *Metarhizium*; light; proteomics, transcriptomics; translation

The present work was supported by a State of São Paulo Research Foundation (FAPESP) Ph.D. scholarship to GTPB (2015/24305-0) and a research grant to GULB (2016/11386-5).

## THE *ASPERGILLUS FUMIGATUS CRZA* AND *ZIPD* TRANSCRIPTION FACTORS ARE INVOLVED IN CALCIUM METABOLISM AND THE CASPOFUNGIN PARADOXICAL EFFECT

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*Aspergillus fumigatus* is an opportunistic fungal pathogen that causes invasive aspergillosis (IA), a life-threatening disease in immunocompromised humans. The echinocandin caspofungin, adopted as a second-line therapy in combating IA, is a β-1,3-glucan synthase inhibitor, which, when used in high concentrations, reverts the anticipated *A. fumigatus* growth inhibition, a phenomenon called the "caspofungin paradoxical effect" (CPE). The CPE has been widely associated with increased chitin content in the cell wall due to a compensatory upregulation of chitin synthase-encoding genes. Here, we demonstrate that the CPE is dependent on the cell wall integrity (CWI) mitogen-activated protein kinase MpkA$^{MPK1}$ and its associated transcription factor (TF) RlmA$^{RLM1}$, which regulate chitin synthase gene expression in response to different concentrations of caspofungin. Furthermore, the calcium- and calcineurin-dependent TF CrzA binds to and regulates the expression of specific chitin synthase genes during the CPE. These results suggest that the regulation of cell wall biosynthetic genes occurs by several cellular signaling pathways. In addition, CrzA is also involved in cell wall organization in the absence of caspofungin. Differences in the CPE were also observed between two *A. fumigatus* clinical isolates, which led to the identification of a novel basic leucine zipper TF, termed ZipD. This TF functions in the calcium-

calcineurin pathway and is involved in the regulation of cell wall biosynthesis genes. This study therefore unraveled additional mechanisms and novel factors governing the CPE response, which ultimately could aid in developing more effective antifungal therapies

Key words: caspofungin, *Aspergillus fumigatus*, calcium metabolism

Financial support: FAPESP and CNPq, Brazil.

## FROM "HUNTING BAGS" WITH ROS TO ANTIOXIDANT SURFACE-ACTIVE PROTEINS: THE STRESS PALETTE OF *TRICHODERMA* WARS WITH OTHER FUNGI

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The war is the most common mode of interactions in fungal communities. Inevitably any combat is a stress. Saprotrophic fungi and many biotrophs (deriving nutrients from living organisms in symbiosis) are capable of killing their partners or neighbors continuing to feed on their remains and then using their foraging grounds. Consequently, carnivory and herbivory fungi have evolved a multitude of defense mechanisms that allow them to protect their habitat from aggressive invaders. Above this, the obligate (mycoparasitism) and facultative fungivory appear to be essentially more widespread than it was previously considered. The increasing numbers of the genome-wide studies offer an indirect proof of the long evolutionary history of interfungal relations: many reports detect the relatively high frequency of lateral gene transfers (LGT) between fungi including genetic exchanges even between closely related taxa. Undoubtedly, this behavior suggests common intimate contacts between the cells of the LGT donors and recipients that may be achieved through endoparasitism of one fungus on another. As fungi have primitive bodies with limited attributes, their battles, defenses and other interactions strongly depend on chemical warfare. In this study, we have

investigated the combative interaction between the two environmentally opportunistic biotrophic hypocrealean fungi. Contrary to numerous cases of a 'deadlock' reaction when the growth of contacted fungi remains arrested, *Trichoderma guizhouense* can overgrow *Fusarium oxysporum*, cause sporadic cell death and inhibit its growth. We will present the details of the transcriptomic analysis of this interaction and show that *T. guizhouense* undergoes a succession of metabolic stresses while *F. oxysporum* responded relatively neutrally but used the constitutive expression of several toxin-encoding genes as a protective strategy. Because of these toxins, *T. guizhouense* cannot approach this combatant on the substrate surface and attacks *F. oxysporum* from above. The success of *T. guizhouense* is secured by the excessive production of hydrogen peroxide ($H_2O_2$), which is stored in microscopic bag-like guttation droplets hanging on the contacting hyphae. The deletion of NADPH oxidase *nox1* and its regulator, *nor1* in *T. guizhouense* led to a substantial decrease in $H_2O_2$ formation with concomitant loss of antagonistic activity.

Interestingly, in our settings, the surface of guttation drops had a visible film that probably provided the reservoir for the solvent necessary for the biochemical reactions involving hydrolytic enzymes and $H_2O_2$ on the aerial hyphae. *F. oxysporum* hyphae are hydrophilic, and the formation of guttation droplets by *T. guizhouense* on them would, therefore, require a surface-active protein. In support of this mechanism, we found the upregulation of one hydrophobin-encoding gene, and its expression was NOX1-dependent. In the Tgui$_{nox1OE}$ strain, one additional hydrophobin gene was upregulated. To verify this hypothesis we have labeled most upregulated hydrophobins of *T. guizhouense* with red and yellow fluorescent proteins. These secreted proteins self-assemble in the interface and thus may be involved in the formation of the film on the surface of the guttation capsule. Our data indicate the array of stress-triggered defensive mechanisms that require hydrophobins as crucial acting molecules.

Last not least, we envision the role of NOX proteins in the antagonism of *T. guizhouense* as an example of metabolic exaptation evolved in this fungus because the primary function of these ancient proteins was probably not linked to combative interfungal relationships. In support of this, *F. oxysporum* showed

almost no transcriptional response to *T. guizhouense* Δ*nox1* strain indicating the role of NOX/H2O2 in signaling and communication.

Key Words: Cryo-SEM, Exudate, *Fusarium oxysporum*, guttation droplets, interfungal combative interactions, hydrophobins, hydrogen peroxide, *nox* genes, transcriptional response, *Trichoderma guizhouense*, superoxide

## FUNGAL STRESS DATABASES - CONSTRUCTIONS AND APPLICATIONS

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In the last 10 years, we together with my colleagues and collaborating partners constructed some fungal stress databases to help the work of mycologists performing a wide range of research projects in the field of fungal stress biology. In general, these databases incorporated either fungal stress response protein orthologs found in various species or fungal stress physiology data. Considering the currently available stress databases, Fungal Stress Response Database (FSRD) version 2 (FSRDv_2; http://internal.med.unideb.hu/fsrd2/?p=consortium) is based on 1985 fungal stress response proteins, which were characterized in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, *Cryptococcus neoformans*, *Ustilago maydis* as well as in some *Aspergillus*, *Fusarium* and *Candida* species. FSRDv_2 includes altogether 43725 stress protein orthologs identified in 41 fully sequenced genomes of 39 fungal species (with 3 genomes for *Aspergillus niger*). Fungal Stress Database (FSD; http://www.fung-stress.org/) was constructed to store fungal stress physiology data recorded in stress agar plate experiments. FSD currently includes 1412 photos taken on agar plate colonies of 17 *Aspergillus* species, grown under various (oxidative, high-osmolarity, heavy metal and cell wall integrity) stress conditions. These fungal stress databases have facilitated comparative genomics and comparative fungal stress biology research projects especially in the Aspergilli, and the set of stress proteins with verified functions in FSRDv_2 has

also been used in homology search and annotation work carried out in newly sequenced fungal genomes including several *Aspergillus* and *Penicillium* spp. as well as the near-obligate nematode endoparasitic fungus *Drechmeria coniospora*. In addition, mapping stress protein expansions and reductions has become an important part of some more recent fungal life-style and evolutionary biology studies. It is noteworthy that bioinformatics-based data extracted from fungal stress databases have initiated a number of „wet lab" projects, which have helped us to gain a better understanding of the remarkable cadmium tolerance and osmophility observable in some *Aspergillus* spp. Nevertheless, future maintenance or even expansion of fungal stress databases clearly needs the joint support of the community of fungal stress biology experts. Starting discussions within the community, *e.g.* on the standardization of the collection and archiving of fungal stress physiological data, also seems to be recommendable.

Key words: fungi, stress biology, stress response, databases, FSRD, FSD, homology search, Aspergilli

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## HETEROGENEITY OF STRESS-RESISTANCE OF FUNGAL CONIDIA

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The function of spores is to disperse fungi to new areas and to get them through

difficult periods. This also makes them important vehicles for food contamination. This contribution aims to explore the connection between prevention of fungal food spoilage to spore biology and modeling studies. It introduces the fungal spore as a vehicle of survival and distribution and discusses its variety and stress resistance. Spores (conidia) are not static particles; they show maturation and change in time. How long spores survive in the food production environment is not known, but the presence of water is important. At the limits of growth, spore germination, which is also highly responsive on environmental cues, becomes unpredictable and shelf life becomes variable. To prevent spoilage of processed foods and drinks, industry often challenges their products based on the worst-case spoilage scenario.
For this, knowledge of the range of stress resistance of a population of spores is very important. The variation between fungal strains, thus intraspecific variation, with respect to stress resistance of spores is another scarcely studied area. Here, data are provided about the intraspecific variation of conidial stress resistance of numerous strains of three important food-related fungi; *Aspergillus niger*, *Penicillium roqueforti* and *Paecilomyces variotii.*

Key Words: fungal spores; conidia; heterogeneity; germination; stress resistance; compatible solutes

## ROS DETOXIFICATION AND SENSING DURING FUNGAL GROWTH AND DIFFERENTIATION

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Our group has contributed to establish the role of reactive oxygen species (ROS) as ubiquitous signals that regulate cell physiology and development. Using the fungi *Aspergillus nidulans* and *Neurospora crassa*, we have studied the regulated production of ROS by the NADPH oxidases, the perception of ROS by the SakA and MpkC MAP kinase pathways and the redox sensing transcription factor NapA, and the detoxification of ROS by catalases and peroxiredoxins. I will discuss the

roles of SakA, MpkC, NapA and peroxiredoxins in ROS sensing not only in response to oxidative stress but also during carbon utilization and normal development, focusing on the role that these proteins have in the production of asexual spores and their viability and fitness.

Key Words: Stress MAPKs, SakA, MpkC, Yap1, peroxiredoxins, conidiation, sexual development, spore viability, spore fitness.

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## HOW TO MEASURE CELLULAR STRESS, AND WHAT IS STRESS ANYWAY?

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Cellular stress occurs when physical forces act on the microbial system in such a way that impairs its ability to function. This said, there is no consensus on exactly how we define the concept of stress, or even how we measure it (Hallsworth, 2018). Measures of growth parameters such a biomass and germination are commonly made at a single time-point and then used as indicators of stress. However, such values are in reality compound representations of parameters such as inoculum size, proportion of viable cells, length of lag, and subsequent growth rate. We hypothesized that length of the lag phase, during which cells can adapt to new conditions, is a reliable measure of cellular stress (Hamill et al., in preparation). We carried out a study of fungal xerophiles (*Aspergillus penicillioides* JH06GBM, JH06THJ, JH06THH; *Eurotium amstelodami* FRR 2792; *Eurotium echinulatum* FRR 5040; *Eurotium halophilicum* FRR 2471; *Eurotium repens* JH06JPD; *Xerochrysium xerophilum* FRR 0530; *Xeromyces bisporus* FRR 0025, FRR 1522, FRR 2347, FRR 3443), *Saccharomyces cerevisiae* 14 and 77, and several bacteria. In some cases, lag phase was not proportional to germination- or exponential growth rates. Conversely, there were strong correlations for some cultures; and in other cases, growth rates varied greatly with stressor concentration even if lag phase remained unchanged (Hamill et al., in preparation). Collectively, these

findings indicate that neither lag phase nor single-point determinations of growth parameters can be relied on as measures of cellular stress.

We went on to study NaCl-saturated brines such as saltern crystalliser ponds, inland salt lakes, deep-sea brines and liquids-of-deliquescence on halite that are commonly regarded as a paradigm for the limit of life on Earth (Lee et al., 2018). Typically, NaCl-saturated environments contain all domains of life and perform complete biogeochemical cycling. Despite their reduced water activity, ~0.755 at 5 M NaCl, some halophiles exhibit optimum growth/metabolism in these brines. Furthermore, the recognised water-activity limit for microbial function, ~0.585 for some strains of fungi (Stevenson et al., 2017; Hallsworth, 2019), lies far below 0.755. NaCl-saturated environments contain biomass-dense, metabolically diverse, highly active and complex microbial ecosystems; and this underscores their moderate character. NaCl-saturated brines are biologically permissive, fertile habitats that are thermodynamically mid-range rather than extreme. Indeed, were NaCl sufficiently soluble, some halophiles might grow at concentrations of up to 8 M (Lee et al., 2018).

Stress is an inextricable aspect of life, so it is no surprise that with respect to microbial cells we do not have a concept that describes being non-stressed. Ironically, a cell that exhibits optimal growth also has reduced energy generation, is less resilient to change, and can have poor competitive ability. Furthermore, rapid growth is associated with a high level of oxidative damage and compromised vitality of the system (Hallsworth, 2018). Stresses induced by temperature, pH, water activity, chaotropicity, reactive oxygen species, dehydration-rehydration cycles, ionizing radiation, and changes in turgor or other mechanical forces are well-known. There is also a paucity of information on why the cellular system ultimately fails under extremes of stress, and it is even debatable whether any microbe can ever be completely stress-free. However, cells that exhibit optimal rates of biotic activity are likely to exhibit low ecological fitness compared with those that are moderately stressed; in other words, stress can enhance microbial vitality, vigour and resilience (Hallsworth, 2018). Whereas terms such as ‘rapid-growth stress’, ‘nutrient stress’ and ‘biotic stress’ span a range of logical categories, their modes-of-action do usually involve a biophysical component. ‘Stress’ is sometimes applied mistakenly to describe the effects of toxic substances that have target site-specific modes-of-action (e.g. antibiotics) rather than and do not inhibit the cell via

any type of stress-mediated mechanism. Stress can impact all levels of biology (from biomacromolecules to ecosystems), is a potent driver for evolutionary processes and - it could be argued - is an inherent property of life itself.

Keywords: astrobiology; cellular stress mechanisms and responses; chaotropicity; Earth's biosphere; halophile ecology; hurdle technology; microbial lag phase; limits for life; vigour and vitality; water activity.

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## WHEN IN ROME, DO AS THE ROMANS DO: BLACK FUNGI THAT SHARE NICHES WITH PHOTOTROPHS HAVE MULTIPLE PHOTORECEPTORS

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Sunlight, the major source of energy, drives life but in excess it provokes UV-induced DNA damage, accumulation of reactive oxygen species, desiccation, osmotic stresses and so on. Biological strategies to survive excess light include protection, avoidance and active utilisation. Phototrophic organisms including cyanobacteria, green algae and plants use all three strategies. Fungi occupying

phototrophic niches may profit from the surplus photosynthetic products which also obliges them to cope with the same light-induced stresses. One way of handling these stresses would be to use similar signalling pathways to sense the presence and to interact with their phototrophic partners. Obviously, the levels of adaptation and responses to light will depend on the environment as well as fungal genotype and phylogenetic position. Black fungi – a polyphyletic group – accumulate the dark pigment DHN melanin in their cell walls and often occupy light-flooded habitats from phyllosphere to rock surfaces. Here we compare two sequenced melanised fungi of different lifestyles in their response to light.

The Leotiomycete *Botrytis cinerea* is an aggressive pathogen that primarily infects the above-ground parts of plants. It possesses large numbers of photoreceptors that respond to a broad spectrum of light. As a consequence, light controls morphogenesis in which it induces conidiation for disease spreading and represses sclerotial development for survival and/or sexual recombination. Cellular components involved in photo-perception and regulation of morphogenesis, stress responses and virulence have been identified and appear to regulate propagation, survival and infection. These include phytochromes, a group of photoreceptors which are particularly enriched in the Leotiomycetes and that mediate coordinated responses to light and elevated temperatures. Assuming that photo-regulation may be equally important for fungi that live in mutualistic relationships with phototrophs either by forming composite organisms or biofilms, we investigate the role of light in the rock-inhabiting Eurotiomycete *Knufia petricola*. Like other black yeasts, *K. petricola* grows slowly, does not form specialised reproductive structures and constitutively produces DHN melanin as well as carotenoids. Combining *K. petricola* with the cyanobacterium *Nostoc punctiforme*, we developed a model system for studying biofilm formation and bio-weathering. A genetic toolbox to manipulate this model system is being developed. *K. petricola* strain A95 possesses ten putative photoreceptors, more than found in filamentous Eurotiomycetes suggesting that light plays an important role for abiotic and biotic interactions in extremo-tolerant and symbiosis-capable fungi.

Keywords: *Botrytis*; carotenoids; *Knufia*; melanin; *Nostoc*

# PECULIAR GENOMIC TRAITS IN THE STRESS-ADAPTED CRYPTOENDOLITHIC ENDEMIC ANTARCTIC FUNGUS *FRIEDMANNIOMYCES ENDOLITHICUS*

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The highly melanized fungus *Friedmanniomyces endolithicus* is endemic to the Antarctic, occurring exclusively associated with endolithic microbial communities in the ice-free areas of the Victoria Land, including the McMurdo Dry Valleys, accounted as the coldest and most hyper-arid desert on Earth and the Martian analogue on Earth. The natural niche where the fungus thrives is characterized by high UV irradiation, low temperatures, and oligotrophy; only few other microorganisms are able to settle in these conditions. Black meristematic fungi are recurrent colonizers of these communities and, among them, *F. endolithicus* is undoubtedly the most widespread and frequently isolated, indicating a high degree of adaptation to the prohibitive environmental conditions of this area. Recently, its responses to sub-optimal temperature were investigated and concern a downregulation of proteins expression without a consequent heat-shock protein production, maybe as adaptation to its permanently cold habitat. Yet, the nature of its extremotolerant specialization has never been investigated in detail. There are some evidences that stress adapted organisms exhibit a whole genome duplication as for *Deinococcus radiodurans*; the same was observed for fungi, as *Hortaea werneckii*. A duplicate gene burden makes them more resistant to the injuries of different stress. In this study *F. endolithicus'* genome was sequenced by using the Illumina Miseq; genome assembly and annotation were performed with Funannotate utilizing Augustus, GeneMark.hmm-ES and EVM. Gene functions were also assigned by matches to the Pfam, MEROPS and CAZy. Further, the assembly was compared to other genome sequences available of *F. simplex, Acidomyces richmondensis, Hortaea thailandica* and *H. werneckii* as representative of fungi occurring in extreme environments, to highlight the genomic traits of the hyper-adapted fungus *F. endolithicus*.

The genomes here compared differ for major aspects, including the size and number of predicted genes, which were much higher in *F. endolithicus* and *H. werneckii*. In all cases the genomes and predicted proteomes contained more than 90% of identifiable fungal Universal Single-Copy Ortholog genes and in *F. endolithicus* more than 60% were duplicated. Our initial investigation have identified similiarity in genome assembly between *F. endolithicus* and the duplicated *H. werneckii* and results may be interpreted as relatively hybridization or whole genome duplication in the endolithic fungus triggered by the exposure to Antarctic prohibitive conditions.

Key Words: Antarctic; cold stress; genome size; nutritional stress; osmotic stress; UV-B radiation; whole-genome duplication

## REGULATION OF CONIDIATION AND CAROTENOID BIOSYNTHESIS BY THE VELVET COMPLEX IN *NEUROSPORA CRASSA*

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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. 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.

The *velvet* regulators are members of a family of proteins with a conserved domain that help to coordinate growth, differentiation and secondary metabolism in fungi. In *Neurospora* crassa the *ve-1* mutant has defects in aerial hyphal growth, conidiation and reduced carotenoid accumulation. We have detected the presence of VE-1, VE-2 and the methyltransferase LAE-1 in vegetative mycelia where they form a protein complex, and during conidiation. In addition, we noted that VE-1 was absent in aerial hyphae grown in the dark despite the presence of *ve-1* mRNA. The absence of VE-1 in aerial hyphae in the dark is due to protein degradation through the proteasome with a key role for the adaptor protein FWD-1. We propose that the light-dependent regulation of VE-1 stability modifies the components of the *velvet* complex resulting in changes in the transcriptome during conidiation.

Key Words: Neurospora crassa, blue light, velvet domain, protein stability, proteasome

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## FUNGAL OPTOGENETICS: OBTAINING AN ACCURATE PICTURE OF LIGHT-SENSING AND GENERATING TOOLS TO REPROGRAM CELLULAR FUNCTION

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The filamentous fungus *Neurospora crassa* perceives and responds to blue light through a transcriptional heterodimer named White Collar Complex (WCC), which contains a LOV (Light Oxygen Voltage) domain capable of detecting blue wavelengths, which promotes a conformational change that leads to dimerization,

resulting in strong transcriptional activation, in a light-intensity dependent manner. We have also adopted optogenetic approaches to further delve into Neurospora's light-responses. In doing so, we were able to genetically program 2D-images in this organism. Thus, we can project a photograph on top of a Neurospora carrying a luciferase reporter under the control of a light responsive promoter and obtain back a bioluminescent pattern mimicking the original image: a live canvas in which images are genetically processed and reconstituted with real-time dynamics. This technology provides a great way to assess transcriptional dynamics, and also explore the properties of genetic circuits, circadian systems, and transcriptional memory.

In addition, through the development of Neurospora-based optogenetic switches we have successfully implemented a blue-light responding transcriptional system in *Saccharomyces cerevisiae*. Therefore, in yeast, now we can efficiently induce gene expression over 1000-fold and control biotechnological relevant phenotypes such as flocculation by switching on/off the lights. Thus, we can reprogram cellular traits, utilizing light as an orthogonal signal.

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## TELOMERE LENGTH MAINTENANCE IN YEAST: NATURE VS NURTURE

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Telomeres are nucleoprotein structures located at the ends of chromosomes and are essential for chromosome function and stability. Telomere length regulation is central to telomere function. Our lab has identified ~500 telomere length maintenance (TLM) genes that, when mutated, lead to either telomere lengthening or shortening. In humans, telomeres shorten as a function of age. Short telomeres are known determinants of cell senescence and longevity, and shorter telomeres are seen in older people. Moreover, psychological stress has been also associated with shorter telomeres. Thus, it appears that telomere length may be regulated both by genetic, as well as environmental factors.

We have examined whether different environmental signals promote changes in telomere length. We have shown that exposure to ethanol induces telomere elongation, while exposure to caffeine or high temperature shorten telomeres in Saccharomyces cerevisiae. The availability of a complete list of genes affecting a single phenotype allows us to explore the interphase between nature (genes) and nurture (environment).

## HETEROGENEITY AND STRESS RESPONSE

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Invasive fungal infections kill more than one million people each year. Many of these infections start with the inhalation of fungal spores from the environment. The environmental conditions during sporulation can vary widely, imposing many different kinds of stress. Germination often occurs in conditions that are very different from those of sporulation, especially in the case of pathogenic fungi whose environmentally produced spores break dormancy and germinate within the lungs of mammalian hosts. We are using the filamentous fungal pathogen of humans *Aspergillus fumigatus* to investigate the influence of sporulation conditions on germination potential. Our results show that fungal conidia are not homogeneous and that the level of heterogeneity is influenced by stress during sporulation.

## A NOVEL LIGHT-SENSING PATHWAY INVOLVING HYDROGEN PEROXIDE AND A PEROXIREDOXIN CONNECTS ILLUMINATION TO OXIDATIVE STRESS IN BAKER'S YEAST

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Unlike other fungi, baker's yeast *Saccharomyces cerevisiae* lacks dedicated photoreceptors. However, blue light still causes pronounced oscillations of the general stress-associated Zn-finger transcription factor Msn2 into and out of the nucleus. We have shown that this poorly understood phenomenon is initiated by a peroxisomal oxidase that converts light into a hydrogen peroxide ($H_2O_2$) signal that is sensed by the peroxiredoxin Tsa1 and its dedicated reductase thioredoxin, which in turn counteract Msn2 phosphorylation. Interestingly, a homologous peroxisomal oxidase was previously implicated in blue light toxicity in mammalian cells suggesting that the pathway may be conserved. The exact mechanism by which Tsa1 controls downstream signaling events is, however, still unclear but our data point to that the activity of both peroxiredoxin catalytic cysteine residues inhibit the activity of the conserved nutrient signaling kinase protein kinase A (PKA) upon $H_2O_2$ to allow Msn2 nuclear concentration. Conversely, peroxiredoxin hyperoxidation interrupts the $H_2O_2$ signal and drives Msn2 oscillations by superimposing on PKA feedback regulation. All in all, our data identify a mechanism by which light could be sensed in cells lacking dedicated photoreceptors. In particular, the use of $H_2O_2$ as a second messenger in signaling is common to Msn2 oscillations and to light-induced entrainment of circadian rhythms and suggests conserved roles for peroxiredoxins in endogenous rhythms.

**Key Words:** light sensing, *Saccharomyces cerevisiae*, baker's yeast, $H_2O_2$, oxidative stress, peroxiredoxin, kinase signaling, nutrient signaling, protein kinase A

# STRIVING FOR A LIFE IN HARMONY IN THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

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Microorganisms are permanently challenged with hazardous environmental conditions that restrict their potential for survival and reproduction. To overcome this threatening many of them evolutionarily opted for a life in symbiosis. Fungi from the Glomeromycota phylum engaged in a life in mutualistic symbiosis with plant roots more than 450 million years ago. Since then, plants provide fungi with carbohydrates and in turn become an improved inorganic fertilization. It is, however, only recently recognized that AM fungi are not just mere colonizers of the root and that symbiosis implies to overcome the default surveillance system of the plant for the adaptation to a life inside the plant.

With our discovery of the first AM fungal effector protein SP7 (Kloppholz et al., 2011) the paradigm that AM fungi are naïve colonizers of plants and that the symbiosis is exclusively controlled by the plant was challenged. In general AM fungi could use at least three type of effectors to interact with the plant: i) effectors to avoid being recognized; ii) effectors for suppressing defense responses elicited after recognition iii) effectors that that allow manipulation of the plant cell metabolism. We have now studied in detail the molecular mechanisms by which SP7 and the members of the SP7-like family entirely rewire the plant cell program. We hypothesized that the conserved domain structure of this group of proteins despite their sequence divergence would point to a conserved biochemical function on different specific plant targets. Results from microarray analyses, localization and interaction studies have converged to show that this might be indeed the case and that fungal accommodation involves a plant rewiring exerted on conserved nodes required to cope with abiotic stresses.

Kloppholz, S., Kuhn, H., and Requena, N. (2011): A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. Curr. Biol. 21: 1204-1209

# THE *NEUROSPORA CRASSA* COT1 KINASE – A REGULATOR OF POLAR GROWTH: INTERACTIONS WITH MOB2A, TYPE 2A PHOSPHATASE AND THE STRESS RESPONSE-RELATED PROTEIN GUL1

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COT1 is the founding member of the highly conserved nuclear Dbf2-related (NDR) Ser/Thr kinase family and plays a role in the regulation of polar growth and development in *Neurospora crassa* and other fungi. Osmotic, oxidative as well as other stress conditions result in the partial suppression of the defects observed when COT1 function is impaired, demonstrating a functional link between COT1 and stress response. Changes in COT1 phosphorylation state have been shown to affect hyphal elongation, branching, and conidiation. As part of its functional nature, COT1 interacts additional proteins. These include co-activators, phosphatases and downstream effectors. MPS1 binding (MOB) proteins act as co-activating subunits which are required for NDR kinase function. MOB2A and MOB2B physically and genetically interact with COT1 and phosphorylation has also been suggested to play a role in the regulation of these co-activators. By analyzing MOB2A phosphomimetic mutants we were able to demonstrate the potential significance of MOB2A phosphorylation on the physical interaction with COT1. Furthermore, in spite of some overlapping functions, we determined that MOB2B cannot compensate for MOB2A's role in conidiation and germination. One of the regulators of COT1 is type 2A protein phosphatase (PP2A). PP2As are heterotrimers comprised of a catalytic and scaffolding protein along with an interchangeable regulatory subunit involved in determining substrate specificity. Inactivation of the *N. crassa* PP2A regulatory subunits *rgb-1* and *b56* conferred severe hyphal growth defects. Partial suppression of defects observed in the *rgb-1*$^{RIP}$strain (but not in the Δ*b56* mutant) was observed in *cot-1* phosphomimetic mutants, demonstrating that altering COT1 phosphorylation state can bypass, at least in part, the requirement of a functional RGB1 subunit. Reciprocal co-immunoprecipitation analyses, using tagged COT1, PPH1 (the catalytic subunit of

PP2A), RGB1, and B56 subunits established that these proteins physically interact. Another component of the COT1 complex that also undergoes phosphorylation is GUL1 (the homologue of yeast Ssd1p). GUL1 is predicted to be an mRNA-binding protein involved in translational regulation of cell wall remodeling proteins and in stress response. Deletion of *gul-1* results in partial phenotypic suppression of the *cot-1* (ts) mutant and affects transcript abundance of multiple genes in the COT1 pathway, including those involved in processes such as cell wall remodeling, nitrogen and amino acid metabolism, as well as almost 300 genes of yet unknown function. Using a GUL1::GFP chimera we found that under standard growth conditions GUL1 is mostly dispersed within the cytoplasm yet also produces aggregates that exhibit high mobility within the cell, which is dependent on a functional cytoskeleton. Some of these aggregates are associated with nuclei and in the proximity of the cell wall. Under stress conditions, a significant increase in GUL1 aggregate association with nuclei was observed. Using RNA Antisense Purification (RAP) and RNA Immunoprecipitation (RIP) experiments we have determined that GUL1 is a bona fide RNA-binding protein and can physically associate with several RNA species.

Key Words: Hyphal cell polarity; NDR kinase; PP2A; RNA-binding protein; cell wall remodeling; nutritional stress; cell wall biogenesis stress

## EXPLORING THE EFFECT OF MELANIZATION IN FUNGAL THERMAL PROPERTIES

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Melanin is known to protect fungi against temperature stress and absorb solar radiation energy with transduction into heat. Despite these known roles in heat capture and thermoregulation the effect of melanization on how a fungal mass interacts with thermal energy has never been evaluated. In this study, we perform

thermal analysis on melanized and non-melanized versions of *Cryptococcus neoformans* and *Wangiella dermatitidis*. Differential scanning calorimetry revealed that melanization was associated with an increase in yeast's specific heat capacity which means that more thermal energy is required to increase the temperature of the fungal mass. This increase in heat capacity appears to be related to the stability of cell-associated water by melanin. Moreover, melanized yeast samples exhibited higher heating and cooling rates than non-melanized cells suggesting differences in thermal conductivity. In conclusion, our data show that melanization can alter yeast's thermal properties. Such effects could translate into alterations in energy performance and/or in responses to heat fluctuations by providing a window of opportunity for cellular adaptations to thermal stress.

Key Words: heat capacity; thermal conductivity; blackbody radiation; heat capture; Cryptococcus neoformans; L-DOPA, melanin

## LIGHT AND TEMPERATURE REGULATION IN ASPERGILLUS NIDULANS AND ALTERNARIA ALTERNATA

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Bacteria often use two component systems (TCS) as phosphorylation relays to transmit environmental signals from the cell surface to the inner cell. In comparison, microbial eukaryotes use MAP kinase phosphorylation cascades, although TCS are commonly found in the fungal kingdom. Interestingly, in the case of stress-sensing, fungi use a composite signaling cascade comprised of a TCS plus a downstream MAP kinase cascade to trigger gene expression. Besides osmolarity or oxidative stress, fungi sense many other environmental factors, one of which is light. Light controls morphogenetic pathways but also the production of secondary metabolites such as penicillin[1,2]. In the case of light sensing, a signaling cascade appears to be unnecessary, because light does not stop at the cell surface. However, here we found that phytochrome-dependent light signaling in *Aspergillus nidulans* uses the stress-sensing signaling cascade to transmit the

signal from the cytoplasm into nuclei[3]. In a screening for *blind* mutants, the MAP kinase HogA/SakA was identified by whole-genome sequencing. The phytochrome FphA physically interacted with the histidine-containing phosphotransfer protein YpdA and caused light-dependent phosphorylation of the MAP kinase HogA/SakA and its shuttling into nuclei. In the absence of FphA, HogA/SakA still responded to osmotic stress but not to light. The HogA pathway thus integrates several stress factors and can be considered as a hub for environmental signals. Our work shows a link between light sensing and stress sensing in general. A similar link has been discovered for blue-light sensing in *Trichoderma atroviride*[4]. We found that phytochrome plays a dual role in *A. nidulans* and is also involved in temperature sensing. Temperature increases cause structural changes in the protein, which probably mimmick the changes upon illumination. *Alternaria alternata* contains also blue- and red-light photosensory systems and in addition functional opsin as green-light sensor. The interplay between the different light-sensing systems has been studied.


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## THE ROLE OF *CRYPTOCOCCUS NEOFORMANS CYS3* TRANSCRIPTION FACTOR IN SULFUR UPTAKE


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Cryptococcosis is a major fungal diseases caused by *C. neoformans*. In order to adapt and survive in diverse ecological niches, such as the animal host and environment, this opportunistic pathogen relies on its ability to uptake nutritional elements, such as carbon, nitrogen, iron, phosphate, sulfur and amino acids.

Genetic circuits play a role in response to environmental changes, modulating gene expression, which will adapt the microbial metabolism to the nutrients available, favoring the best energy usage and survival. Our group has been studying the sulfur assimilation and its implications on *C. neoformans* biology and virulence. CNAG_04798 was annotated as *CYS*3 in the genome of *C. neoformans*; it encodes a putative Bzip transcriptional factor, which has been considered an essential gene. However, we showed that *CYS*3 is not essential, in fact, its knockout led to sulfur amino acids auxotroph, which can be satisfied by supplementation with methionine and cysteine (Met/Cys). Our data indicates that Cys3 transcription factor is required during sulfur limiting condition. Deletion mutant (*cys*3Δ::*Neo*$^{R}$) has a poor growth rate in YEPD, even supplemented Met/Cys; however, growth is completely restored in SD + Met/Cys. Fluorescent microscopy in the wild type strain showed that GFP:Cys3 co-localizes with DAPI nuclear dye during growth in rich medium (41%) and co-localization drops down in SD + Met/Cys (17%). Also, western blot analysis showed that GFP:Cys3 is abundant in YEPD, but the supplementation with Met/Cys3 leads to protein degradation. GFP:Cys3 was immunoprecipitated and protein complexes were analyzed by MALDI-TOF and LC/MS. Three interacting phosphatases (CNAG_01744 GPP2, CNAG_04796 calcineurin-CNA1 and CNAG_03370 CNB2) were identified in a nutrient deprived condition. Yeast two-hybrid assays showed that Cys3 physically interacts with Cnb1 and Gpp2 and Cna1ΔC interacts with Cnb1 and Gpp2, confirming the mass spectrometry data. Deletion of the calcineurin complex and Gpp2 in a GFP:Cys3 background demonstrated that Cna1 and Cnb1 are required to maintain Cys3 high levels in limiting sulfur condition (YEPD) and that deletion of Gpp2 causes GFP:Cys3 fusion protein to persist in SD + Met/Cys3, a condition that Cys3 is degraded in the wild type. Also, in our work we showed that Cys3 is required for virulence in *Galleria mellonella* animal model.

Key Words: sulfur uptake, sulfur amino acid metabolism, *C. neoformans*, nutritional signaling

# COPING WITH TWO STRESSORS CONCOMITANTLY

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In “classic” stress biological experiments, the behavior of fungal cultures exposed to one type of environmental stress is compared to that of untreated cultures. Although this approach is useful to understand how fungi can adapt to a certain type of stress and how they can regulate this process, it does not reflect properly their behavior shown in their natural habitats. Under natural conditions, fungi commonly have to cope simultaneously with more than one type of stress. In the human body pathogenic fungi suffer from limitations in carbon source, iron, zinc or oxygen and they are frequently sense oxidative and nitrosative stress. In addition, they also have to tolerate the body temperature of the host organism, the pH of the infected tissues or cell organelles as well as the applied antifungal agents. The success of their pathogenesis can highly depend on how they tolerate the combinations of these stresses and not on how efficiently they can protect themselves against separate stresses. Therefore, studying how one stressor can modulate the stress response developed in the presence of another stressor can be very important. These studies can also help us to understand the background of the observed synergistic or antagonistic effects between two antifungal agents. The presentation - besides some other examples – focuses on how *Aspergillus fumigatus* responded to oxidative stress under iron starvation. The major conclusion includes: 1) Combination of withholding iron from pathogens and attacking them with reactive oxygen species is a highly efficient strategy to prevent infections. 2) Responses to single stress conditions do not reflect the behavior of fungi in a complex habitat. 3) The unique success of *A. fumigatus* as one of the most important opportunistic human pathogens among the Aspergilli may be explained with its successful adaptation to stress combinations, which occur typically within the human body.

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Key Words: *Aspergillus fumigatus*; transcriptomics, oxidative stress, iron starvation stress, combined stress

# Poster Presentations

## 1. STRESS MECHANISMS AND RESPONSES IN FUNGI: MOLECULAR BIOLOGY, BIOCHEMISTRY, BIOPHYSICS, AND CELLULAR BIOLOGY

### A NITRATE REDUCTASE INVOLVED IN NITRIC OXIDE PRODUCTION AND SPORULATION OF MUCOR CIRCINELLOIDES

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Nitric oxide is a cellular signaling molecule involved in the regulation of diverse biological processes in many organisms. In mammalian, plants and bacteria, nitric oxide is produced from L-arginine and molecular oxygen by nitric oxide synthases, which are structurally different. Some of these proteins share the dependence of nucleotide coenzymes such as NADPH, FAD, FMN, and tetrahydrobiopterin, a cofactor, that are essentials for enzimatic activity in certains isoform of these proteins. In these organisms, nitric oxide is involved in process such as neurotransmision, biotic and abiotic stress, and oxidative stress, respectively. In fungi, the nitric oxide has been detected in Aspergillus nidulans, Magnaporte oryzae and Neurospora crassa and its role is related with development and conidiation. In Phycomyces blakesleeanus, production of nitric oxide and tetrahydrobiopterine was corroborated and the rol of this molecule in the formation of macro sporangiophores was indirectly demonstrated. However, a gene responsible for nitric oxide synthase remains to be identified and some studies

suggest that this molecule is produced by non-enzymatic pathways. Mucor circinelloides is widely used for study many proccesses such as carotene synthesis and biodiesel production. However, nitric oxide synthesis has not been investigated in this Mucoral. Our aim was identify genes involved in nitrogen assimilation and nitric oxide production in the genome of M. circinelloides for acquire knowledge about the rol of this molecule in development and cell differentiation of this organism. We identified sequences, whose predicted proteins are related to nitrate reductases or nitric oxide synthases, and some of them were interrupted by homologous recombination in M. circinelloides. Moreover, we analized the nitric oxide production in this organism. Progress in the phenotypic and molecular characterization of the mutants will be presented. Our results demonstrate that 51822-sequence, which must be called niaD gene in the M. circinelloides genome, is coding for a nitrate reductase involved in nitrate assimilation and the sporulation. In addition, the intracellular production of nitric oxide was lower in mutant strains than wild type strain.

## A NOVEL FUNCTIONAL ROLE FOR TWO ATP-DEPENDENT DNA HELICASES IN NEUROSPORA CRASSA STRESS RESPONSE

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Previous work by our group identified by RNAseq the Neurospora crassa NCU03482 trancript in heat stress response. This ORF codifies a homologous protein to the human RuvBL1 protein, which together with its RuvBL2 paralog, has been identified as essential proteins components of several macromolecular complexes. They are reported to be involved in chromatin remodeling, telomerase assembly, gene regulation, mitotic spindle assembly, and in cancer in mammalian cells. The RuvBL2 ortholog in N. crassa is the product of the ORF NCU06854, and both proteins are annotated as RuvB-like helicases 1 and 2, belonging to the AAA+ ATPases family. In N. crassa, we named these proteins as RVB-1 and RVB-2. In

this work, we describe a novel functional role for these proteins in N. crassa stress response. In silico analysis of the rvb-1/2 5'-UTR regions identified three putative STRE motif (5'-CCCCT3'), which is present in the promoter region of stress responsive genes. Our results showed increased expression by western blot of both RVB-1/2-V5 proteins when mycelia were subjected to heat stress (45°C), pH stress [7.8 (alkaline) and 4.2 (acid)], and osmotic stress (NaCl), suggesting a novel functional role in stress response, not yet described for these proteins. Analysis of cellular location by fluorescent microscopy showed that the RVB-1/2-GFP fusion proteins are predominantly nuclear, and heat stress significantly increase their levels in the nuclei. We also characterized these proteins in a biochemical and biophysical manners. The proteins interact each other when co-expressed in E. coli, and the purified recombinant complex (RVB-1/2) exhibits in vitro ATPase activity. Analytical size-exclusion chromatography demonstrated that the RVB-1/2 complex displays different oligomeric states in the presence of nucleotides; in the presence of ATP, the complex predominates as hexameric form, but on the other hand, with ADP a dodecameric structure is predominant. To verify if the complex binds DNA, we performed EMSA, and our results showed a strong interaction with DNA, suggesting that these proteins may be involved in chromatin remodeling likely regulating gene expression under different environmental conditions in Neurospora crassa.

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## A POSSIBLE INVOLVEMENT OF P-BODIES AND STRESS-GRANULES IN CYCLOSPORIN-INDUCED GROWTH ARREST IN PARACOCCIDIOIDES BRASILIENSIS

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Paracoccidioides brasiliensis is a dimorphic fungus and the etiological agent of a deep mycosis, the paracoccidoidomicosis, a neglected tropical disease that afflicts endemic areas of Central and South America. Similar to other pathogenic fungi, the calcium/calmodulin dependent phosphatase calcineurin is responsible for the

regulation of several molecular mechanisms by which the fungi adapt to stress, and by doing so, calcineurin also controls virulence. Understanding the mechanisms by which calcineurin acts in P. brasiliensis could reveal some processes that make the fungus succeed inside the host and, hence, point to a potential pharmacological target to treat fungal diseases. Kozubowski et al. (2011) have shown that in Cryptococcus neoformans, during thermal stress, the subcellular localization of calcineurin is clustered with processing bodies and stress granules. One of the known function of these granules of ribonucleoproteins is to regulate protein expression by mRNA degradation and miRNA-mediated translational repression. In this work, using bioinformatics tools (multiple global and local alignments based of greedy algorithms together with Hidden Markov Models), we investigated the proteins that putatively compose P-bodies and stress granules that were found in differential levels in the proteome of P. brasiliensis yeasts grown for 24 h with cyclosporin, an inhibitor of calcineurin. The search for similarities using the databases RefSeq, UniprotKB/Swiss-Prot, UniprotKB/TrEMBL, KEGG and Pfam, revealed six proteins that are potentially associated with P-bodies: C0RX76 (uncharacterized), C1GEW2 (uncharacterized), A0A1D2JHN4 (ATPdependent RNA helicase DBP2), CIG3D9 (uncharacterized), CIGXZ4 (PremRNA processing factor 39) and C0SDT5 (NOP58), which could also be associated with ribosomal biogenesis. All these proteins are derived from single copy genes. The proteins: C0RX76, has 56.76% and 55.85% identity to Human and Zebrafish Cleavage and polyadenylation specificity factor subunit 5 respectively; CIGEW2 has 77.21% identity with U3 small nucleolar RNAassociated protein 10 from Aspergillus fischerianus and 62.47% identity to human RNA helicase p68; C1G3D9 has 30.91% identity to human PCBP3, 42.62% identity to Heterogenous nuclear rnp K-like protein 2 S. cerevisiae; CIGXZ4 retains 35.65%, 27.93% and 91.49% identity, against Homo sapiens, S. cerevisiae and Emmonsia crescens pre-mRNA processing factors 39 respectively and C0SDT5, 51.95% and 62.53% identical to the human and S. cerevisiae NOP58, which was the only protein extinguished by cyclosporin treatment. These preliminary results suggest that ciclosporin, likely via calcineurin, regulates protein expression of yeasts via ribosomal biogenesis and P-bodies assembly and/or composition. This analysis will ground future experiments to determine the intracellular localization of calcineurin in yeasts of P. brasiliensis

treated with Cycloheximide or after a heat shock, both conditions known to change the numbers of P-bodies and stress granules. The results could help to reveal the processes controlled by calcineurin in the attempt to propose a promising new target for the control of pathogenic fungi.

## ANTIMICROBIAL EFFECTS OF PHOTODYNAMIC TREATMENT WITH PHENOTHIAZINIUM PHOTOSENSITIZERS ON FUNGAL BIOMOLECULES.

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The photodynamic therapy has been proposed to inactivate microorganisms by using non-toxic dyes (photosensitizers) activated by the absorption of visible light to form reactive oxygen species, especially singlet oxygen, that can oxidize biomolecules and kill the cells. The antimicrobial photodynamic therapy (APDT) is coming as an efficient alternative to the treatment of microbial infections and to the biological control of plant diseases. Both diseases are a problem which is presently aggravated by the increasingly widespread diffusion of antibioticresistant microbial strains. APDT does not lead to the selection of mutant resistant strains, a clear benefit compared to antibiotic treatment. Fusarium oxysporum is a filamentous fungus widely distributed and pathogen of plants, animals, and humans. Crop diseases generate great economic losses in the production of fruit, vegetables, cereals, and cellulose; and the control of the progression of fusariosis in humans by single-agent antifungal therapy is problematic, leading to a high mortality rate, especially with immunocompromised patients. In the present study, we evaluated the effect of APDT with phenothiazinium photosensitizer methylene blue (MB), toluidine blue O (TBO), new methylene blue N (NMBN), and a novel pentacyclic phenothiazinium S137 on the biomolecules of Fusarium oxysporum microconidia. The effect of singlet oxygen produced during the photochemical process of APDT was evaluated by adding the ROS scavengers mannitol (hydroxyl radical), sodium azide (singlet oxygen) and glutathione (free radical

scavenger). Both glutathione and sodium azide reduced the damaging effect of APDT to the microconidia, but mannitol did not. Thus, singlet oxygen is the main ROS causing the oxidative stress in the microconidia. The microconidia membrane permeability was evaluated by propidium iodide (PI), once damage in cell membrane increases PI uptake. APDT increased microconidia membrane permeability. The oxidative stress on microconidia lipids and proteins were evaluated after APDT by the formation of malondialdehyde (lipid peroxidation major product) and protein carbonylation (carbonyl groups formation), respectively. Increases in lipid peroxidation at the microconidia were observed only after APDT with NMBN and S137. We observed an increase in protein carbonylation on microconidia after sublethal APDT. The oxidative damage on DNA was measured by voltammetric determination of 8-oxoguanine at screen-printed graphite electrodes in which, the high presence of 8oxoguanine detected in our experiment indicated that APDT caused damage on microconidia DNA. Thus, our study expands the understanding of photodynamic inactivation effects in the biomolecules of filamentous fungi and opens the interesting perspective of using APDT to control F. oxysporum.

## ASPERGILLUS FUMIGATUS TRANSCRIPTION FACTOR ZIPD PARTICIPATES IN CALCIUM-CALCINEURIN SIGNALING, REGULATING CELL WALL ORGANIZATION, VIRULENCE AND ANTIFUNGAL RESISTANCE

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Aspergillus fumigatus is a filamentous fungus that can cause disease in humans. Depending on a patient's immunological status, A. fumigatus can cause a distinct set of clinical disorders that extend from severe allergies to lethal disseminated infections. Invasive aspergillosis is the most common lifethreatening fungal diseases in immuno-compromised humans and mortality rates can reach 90%. The treatment of disseminated infections with antifungal drugs, including echinocandin cell wall biosynthesis inhibitors, is increasingly challenging due to the rise of drug-resistant pathogens. Sensing and withstanding the host environment is essential for A. fumigatus virulence. Calcium is an essential secondary messenger and modulates the conformation of calcium-binding proteins, such as calmodulin, which activates calmodulindependent enzymes including the calcineurin phosphatase. In response to stimuli that increase cytosolic calcium, calcineurin regulates the nuclear localization and activity of the CrzA transcription factor. The calcium responsive calcineurin-CrzA pathway influences cell morphology, cell wall composition, virulence, and echinocandin resistance. Recently, we have identified a novel basic leucine zipper ZipD transcription factor that played a role in the calciumcalcineurin pathway and was involved in the caspofungin paradoxical effect. Comparative transcriptomics revealed ZipD regulated the expression of genes involved in calcium metabolism, as well as the cell wall integrity and osmotic stress pathways. ZipD and CrzA modulated shared and unique gene networks, suggesting they participate in converged, and distinct, stress response mechanisms. CrzA and ZipD additively promoted calcium stress tolerance. However, ZipD also regulated cell wall organization and osmotic stress tolerance, contributing to evading immune activation, while promoting virulence and echinocandin resistance. This study emphasizes the complexity of the calcium/calcineurin network, revealing additional layers of regulation, which control the fungal response to multiple stresses requiring the cell wall remodeling. These functions in turn contribute to virulence and antifungal resistance. This reinforces the idea that components of the fungal cell wall and their regulatory mechanisms are potential drug targets for the development of novel combinational therapies for aspergillosis.

# AUTOPHAGY AND LIPID DROPLETS DURING NITROGEN AND AMINO ACIDS STARVATION IN PARACOCCIDIODES BRASILIENSIS

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The human pathogenic fungus Paracoccidioides brasiliensis is one of the etiological agents of paracoccidioidomycosis, an endemic deep mycosis prevalent in Latin American, mainly found in Brazil. This fungus transits from the mycelial form to the yeast form depending on temperature where it is found, that is, from environmental temperature of 25ºC to 37ºC as the host's lungs. Autophagy is reported as one of the cellular mechanisms involved in the differentiation and adaptation of eukaryotic cells in response of different stresses conditions, such as nutrient limitation, heat, and oxidative stress. Over the last years, autophagy has been correlated with various types of nutrient starvation. In P. brasiliensis, we have recently shown that autophagy is activated early in both during dimorphism of P. brasiliensis and in the adaptation of yeasts growing under glucose starvation. In the present work, yeasts of P. brasiliensis isolate 18 (Pb18), were submitted to nitrogen and / or amino acids starvation for 24, 48 and 72 hours, at 36ºC, to verify both lipid metabolism and activation of autophagy. Pb18 yeasts grown for 48 hours both in starvation of nitrogen and amino acids (-NA) or in starvation of amino acids (-A) showed reduced viability when plated in rich solid medium (YPD). On the other hand, the viability of Pb18 yeasts grown on nitrogen starvation (-N) was similar to that obtained for cells grown in the presence of this nutrient (control medium, C). Lipid metabolism and autophagy in Pb18 yeasts were analyzed, respectively, by staining of lipid droplets with oil red O and autophagic vacuoles by monodansylcadaverine (MDC). Pb18 yeasts grown in amino acids starvation showed, from 24 hours on, an increase both in the amount of lipid droplets and in the labeling of the autophagic vacuoles, suggesting that in P. brasiliensis there may be a correlation between these cellular processes.

# BIOSYNTHESIS OF ANTIOXIDANT AZAPHILONES IN TRICHODERMA

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Azaphilones, a large class of fungal secondary metabolites, mainly pigments, characterized by a pyrone-quinone structure, have antimicrobial, antiviral, antioxidant and anti-inflammatory activities. Many of their properties are explored by pharmaceutical and cosmetical industries, and medicine. In this study, we present a functional, genetic and biochemical characterization of a group of antioxidant azaphilones produced by mycotrophic Trichoderma guizhouense (Hypocreales, Ascomycota) during antagonistic interactions with Fusarium oxysporum f. sp. cubense 4 (Foc4) (Hypocreales, Ascomycota) and abiotic oxidative stress.

Generally, Foc4 is highly resistant against mycoparasitic attacks of the majority of Trichoderma spp. However, one species T. guizhouense (Harzianum Clade), can antagonize it by producing an excessive amount of reactive oxygen species (ROS), mainly hydrogen peroxide (H2O2) in addition to the array of secreted proteolytic and chitinolytic enzymes. The transcriptomic analysis of these interactions shows that T. guizhouense undergoes a succession of metabolic stresses. The success of T. guizhouense is secured by the excessive production of hydrogen peroxide (H2O2), which is stored in microscopic baglike guttation droplets hanging on the contacting hyphae. The up-regulated transcipts also pointed to the specific activity of the PKS cluster (OPB37942OPB37951). More interestingly, the expression of this cluster was triggered by the exposure to elevated concentrations of H2O2 and amphotericin. The deletion of the pks gene (OPB37945) and overexpression of the respective transcription factors (OPB37944 and OPB37950) from the same SM cluster demonstrated that indeed these genes were responsible for the production of the dark yellowish pigmentation noticed during the interaction between T. guizhouense and Foc4, but also other fungi. The purified compounds revealed the pyrone-quinone structure and antioxidant activity and were attributed to azaphilones. In this presentation,

we will demonstrate the putative biosynthetic pathway of the group of novel antioxidative secondary metabolites of filamentous Ascomycota and show that the production of azaphilones is most likely evolutionary conserved in these organisms.

## CELL WALL INTEGRITY PATHWAY CONTROLS THE ASEXUAL DEVELOPMENT OF ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is an opportunistic human pathogen that causes systemic infections, including invasive pulmonary aspergillosis in immunocompromised individuals. Survival of this fungus is highly dependent on the cell wall organization and function of its structural components. Cell wall integrity pathway (CWIP) is the primary signaling cascade that controls de novo synthesis of the cell wall in fungi. Asexual development in Aspergillus sp. includes the conidiation process which is linked to the emergence of unique morphotypes such as the conidiophores. How the cell wall is remodeled or what are the accompanying changes in the cell wall composition throughout asexual development is currently unknown. Since the abundant conidiation is a virulence determinant in A. fumigatus, here we investigate the function of the CWIP components PkcA, MpkA and RlmA in the asexual development of A. fumigatus. The CWIP mutants, pkcAG579R, ?mpkA, and ?rlmA, displayed reduced asexual sporulation and pkcA and rlmA transcripts are induced during synchronized asexual development in the wild-type strain. In addition, it was found increased phosphorylation of MAP-kinase MpkA in this same condition in the wild-type strain, but these genes had their expression reduced in mutants of CWIP, as well as the reduction of MpkA phosphorylation in these mutants, which leads to the conclusion that CWIP is induced both at protein and transcriptional levels during the conidiation. Additionally, we show by ChIPqPCR

that the CWIP associated transcription factor RlmA directly regulates the expression of key transcription factors involved in conidiogenesis in Aspergillus sp., such as brlA, abaA and rasB, as well as early regulators of conidiation, including flbB and flbC. Finally, we show by ChIP-qPCR that the CWIP associated transcription factor RlmA directly regulates the expression of chitinases and glucanases involved in remodeling of the cell wall during asexual development. Our results suggest that the asexual development is linked to the main players of cell wall integrity highlighting the importance of cell wall biosynthesis and/or remodeling during this unique process of filamentous growth.

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## DECIPHERING THE NEUROSPORA CRASSA LIGNIN SECRETOME

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Cellulose is an important fraction of plant biomass, and its access is impaired by lignin. Current methods to remove lignin includes harmful chemical/physical pretreatments, and replacing these methods with enzymes cocktails provides an alternative for lignin modification, without environmental impacts. Filamentous fungi are excellent cell wall modifiers, and Neurospora crassa efficiently degrades plant biomass, despite it is not consider a good enzyme producer organism. An analysis of its genome revealed at least 20 ORFs for lignolytic enzyme, including laccases and peroxidases. The aim of this work is to decipher the N. crassa lignin secretome and identify proteins that can be further incorporated in enzymic cocktails with commercial purposes. For this, we first stablished a time-course growth profile (1 to 4 weeks) for N. crassa WT strain, in VM-liquid medium, supplemented with sugarcane bagasse or sawdust (pretreated or not) or increasing amounts of purified lignin as carbon sources. The respective secretomes were analyzed by SDS-PAGE, and samples showing protein with different MW were detected for all growth conditions, being more accentuated for

sugarcane bagasse. Moreover, N. crassa VM-lignin resulting mycelia, the radial growth and the apical extension of basal hyphae were strongly reduced than those observed for sugarcane and sawdust, suggesting that this recalcitrant agent may promotes nutritional and oxidative stress. The former, the lignin as solely carbon source decreased fungal growth but still allows mycelia to occur, showing that N. crassa can modify and use lignin as carbon source. The latter, lignin modification leads to the ROS hydrogen peroxide production, causing cell oxidative damages. To identify lignin-modifying proteins, the N. crassa lignin secretome was determined by mass spectrometry (LC-MS/MS). Among the 115 proteins detected, 25 ORFs showed signal peptides, indicating that proteins are target to the secretory pathway. We also identified 42 hypothetical ORFs, two hemicellulose degrading enzymes, a dimethylaniline monooxygenase, and an alpha-L-arabinofuranosidase. Considering that some of these proteins were previously reported to be related to Miscanthus hemicellulose degradation, one might hypothesized that to modify lignin, the fungi need to first sense the environmental hemicellulose. These are preliminary results and need to be more investigated, in order to elucidate the role of these proteins in cell wall degradation.
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## EFFECTS OF CADMIUM, COBALT AND MANGANESE ON SPLICING AND BIOSYNTHESIS OF THE SPLICEOSOME IN THE AQUATIC FUNGUS BLASTOCLADIELLA EMERSONII

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In eukaryotes, splicing is an essential process, catalyzed by the spliceosome complex, which occurs co-transcriptionally, involving removal of non-coding regions (introns) and joining of coding regions (exons) of pre-mRNAs. In the aquatic fungus Blastocladiella emersonii, some stressful conditions, as cadmium exposure, can cause inhibition of splicing. The mechanism underlying this effect remains unclear. In the present study, we used RT-qPCR technique to investigate the level of spliced and unspliced transcripts in B. emersonii genes that are known

to be highly expressed under cadmium stress and genes that are known to be highly expressed under normal conditions, in order to better understand if the inhibition occurs randomly and therefore is more frequent on highly expressed, abundant, genes. We also performed gene expression assays using different concentrations of cobalt and manganese to verify if the inhibition is triggered by other divalent metals that share similar properties with cadmium. To determine the effect of the metals on the biosynthesis of the spliceosome, we analyzed the expression of some of its components: the snRNAs U1, U2, U4, U5 and U6 under the same conditions. Our results showed that splicing was inhibited in both groups of genes and the metals cobalt and manganese only slightly affected the unspliced levels of transcripts compared to cadmium. The snRNAs U1, U5 and U6 were strongly up-regulated in response to the cadmium treatment, while U2 and U4 were down-regulated. Cobalt treatment up-regulated U1 and U5 snRNA levels and manganese treatment down-regulated U2 snRNA levels. It seems that the levels of snRNAs are probably raised as a compensatory way to overcome the negative effects of splicing inhibition and to enable its survival in the hostile environment. Together, these data suggest that although these metals share common characteristics, the splicing process is more strongly inhibited by cadmium, a non-essential heavy metal.

## EVALUATION OF THE POTENCIAL OF APPLICATION OF CYCLODEXTRIN COMPLEXED WITH ELLAGIC ACID FOR THE TREATMENT OF ORAL CANDIDOSIS

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The increase in the prevalence of fungal infections worldwide and the rise in the occurrence of antifungal resistance suggest that the discovery of antifungal molecules is needed. The aim of this study was to contribute to the prospection of therapeutic alternatives, evaluating cyclodextrin complexed with ellagic acid (EA) for the treatment of oral candidosis. In order to reach the general aim, the following

specific objectives were determined: to complex the ellagic acid in cyclodextrin at effective concentration against Candida albicans; to chemically characterize the encapsulate, to determine the mechanisms of action (activity on wall or membrane); to evaluate the ability of ellagic acid to reduce virulence factors, such as exoenzymes production and adherence to epithelial cells; to evaluate the cytotoxicity of cyclodextrin complexed with AE; to test the EA activity in Drosophila melanogaster model infected with C. albicans and its toxicity; and to evaluate the in vivo effectiveness for the treatment of oral candidosis in murine model. The obtained data was statistically analyzed (level of significance 5%). Cyclodextrin formed soluble inclusion complexes with EA. SEM and FTIR analyses confirmed the formation of inclusion complex. The percentage of elagic acid in complexed of EA was 15.25 ± 0.49%. The cyclodextrin complexed with EA showed the same antifungal activity of pure EA with MIC value of 25 µg/ml for C. albicans ATCC 18804 and 50 µg/ml for C. albicans SC 5314. Treatment with sub-MIC on C. albicans revealed increased MIC value in the presence of sorbitol for ATCC 18804 (50 µg/ml), suggesting activity on cell wall. Results suggest no effect on cell membrane. No effects of EA on epithelial cell adherence and production of extracellular enzymes were detected. Moderate cytotoxicity was observed for a concentration of 250 µg/ml. Protective effect of EA at 3.2, 6.4 e 32 µg/ml was observed in D. melanogaster model, resulting in survivals of 45, 33 and 34% in comparison to the 21% in the control group. At these concentrations (3.2, 6.4 e 32 µg/ml) ellagic acid was not toxic for D. melanogaster, neither at a higher concentration (200 µg/ml). In vivo tests in murine models indicated reduction in epithelial invasion after treatment complexed with EA for 24 and 48 hours when compared to control. In conclusion, complexed with EA showed inhibitory effect on C. albicans in vitro, with moderate toxicity. Protective effect against fungal infection was observed in D. melanogaster model. In murine model of oral candidosis, complexed with EA reduced fungal epithelial invasion.

## GETTING FAT, BUT GROWING SLOW: THE ROLE OF CELL CYCLE ARREST ON LIPID DROPLET SYNTHESIS

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Proliferating cells execute a mitotic-cell cycle program that coordinates metabolic processes, including lipid homeostasis, to ensure proper cell growth and division. Entrance into the cell cycle (G1/S) is only licensed if triacylglycerol hydrolysis is activated by the major regulator of the cell cycle, the cyclindependent kinase CDK1/Cdc28. When subjected to stress, several microorganisms such as green algae, and the yeast Yarrowia lipolytica and Saccharomyces cerevisiae halt growth concomitant with accumulation of triacylglycerol in lipid droplets (LDs). To test whether accumulation of triacylglycerol is due to diminished growth and cell cycle arrest we blocked the cell cycle at G1, S or G2/M phases, by known chemicals, and measured the LD content. Using BODIPY fluorescence staining, we observed that arrest at all cell cycle phases induce the LD content, being arrest by alpha-factor the more pronounced. Next we screened a library of mitotic cell cycle mutants to identify the genes involved on regulation of LD content during cell cycle progression. We found 26 hits with significantly higher LD content (>15%) than wild type cells. Most hits belong to G1/S and M phases. From these hits we investigated further whether the transcription factor Swi4p is involved in LD homeostasis. Swi4p together with Swi6p, form the SBF complex (Swi4/Swi6 Binding Factor) which in Saccharomyces cerevisiae orchestrates progression from G1 to S phase, time where lipolysis is activated. Their high lipid droplet content was further confirmed by thin layer chromatography. Both mutants displayed approximately 50% more triacylglycerol content, but the content of steryl esters was the same as WT. We show that swi4? mutant has a slow growing phenotype and is unable to accumulate more LD when treated with cell cycle inhibitors. As AMPK/Snf1p and TORC1 coordinate LD synthesis, we tested whether Swi4p regulation is dependent of the activity of those two kinases. Surprisingly, using the swi4? mutant background, both rapamycin treatment (TORC1 inhibitor) and snf1? deletion have additive effects on LD accumulation. These findings suggest that different types of stress result in cell cycle arrest and triacylglycerol accumulation, but each one through mechanisms that are specific to the stressor agent.

# GLOBAL GENE EXPRESSION OF TRICHODERMA HARZIANUM IN RESPONSE TO ALUMINUM

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Excessive amounts of the metal aluminum (Al) should be considered a concern to the agricultural industry in areas where the pH is below 5.0, as it can affect several cellular processes, impairing crops development. The region of Cerrado is the second largest biome in Brazil, after Amazon, covering about 21% of the country's territory. Cerrado soils are generally acid and poor in nutrients, requiring some treatments before usage for cultivation. Some microorganisms, as the fungus Trichoderma harzianum, are commonly applied in seeds to promote plant growth, since it may enhance nutrient uptake, control pathogens and induce stress resistance in plants. In this study, we investigated the effects of aluminum (in concentration similar those found in Brazilian Cerrado soil) in the global gene expression profile of T. harzianum, in order to better understand the applicability of the fungus in this region. Our results showed that of the 13,932 genes predicted by the reference genome, 6,364 were differentially expressed in the presence of the metal, being 3,239 up-regulated and 3,125 down-regulated. Gene Ontology categorization and enrichment indicated that the biological processes related to nucleobase-containing compound biosynthetic process, RNA biosynthetic process and glycosyl compound metabolic process were mainly up-regulated, and carbohydrate metabolic process, organonitrogen compound biosynthetic process, electron transport chain, cellular amide metabolic process and regulation of protein modification process were mainly down-regulated. Molecular functions as transcription regulator, drug binding, DNA binding, ligase and GTPase binding were upregulated while protein dimerization, hydrolase and electron transfer were down-regulated. The cellular components category COP9 signalosome was strongly up-regulated as the ribonucleoprotein complex and the respiratory chain complex were down-regulated. Together, these results suggest a great readjustment in the expression of the genes and possibly in the synthesis of

proteins of T. harzianum in the presence of aluminum in concentration similar to the existent in the Brazilian Cerrado soil. The data obtained in this study provides possible targets for biotechnological improvement of the fungus survival and application in this biome.

## GLYCEROL-3-PHOSPHATE DEHYDROGENASE GFDB IN THE OXIDATIVE STRESS DEFENSE OF ASPERGILLUS NIDULANS

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Similar to the baker's yeast Saccharomyces cerevisiae, the genome of the filamentous fungus model organism Aspergillus nidulans also accommodates two genes encoding two isoforms of glycerol-3-phosphate dehydrogenase, which are called GfdA and GfdB. The aim of this study was the phenotypic characterization of the ?gfdB gene deletion strain in A. nidulans. The functional characterization of the gfdA gene was published by Fillinger at al. (2001), and the ?gfdA strain showed a considerable, osmoremediable growth defect, which was indicative of likely disturbances in the biosynthesis of cell wall (Fillinger at l. 2001). The ?gfdB (AN6792) gene deletion mutant was constructed by the Double-Joint PCR method of Yu et al. (2001). The ?gfdB strain also showed somewhat reduced growth on minimal agar medium containing glucose as the sole carbon source [colony diameters: 5.1±0.2 cm (?gfdB) and 6.3±0.2 cm (control), P?0.001, n=3] at 37 °C (incubation time 5 d). Importantly, A. nidulans produced solely erythritol and mannitol sugar alcohols in submerged cultures (Witteveen et al. 1990) as indicated by NMR measurements. The alditols were identified by 1H and 13C NMR spectroscopy, in agreement with literature data. The NMR spectra were recorded at 298 K on Bruker Avance II and Avance NEO spectrometers operating at 500

and 700 MHz 1H frequency, respectively. Meanwhile erythritol production was not disturbed in the ?gfdB strain (273±12 µmol g DCM-1 vs. 251±91 µmol g DCM-1, recorded in the control strain) mannitol was significantly overproduced in the mutant strain even under unstressed culture conditions (632±50 µmol g DCM-1 vs. 429±115 µmol g DCM-1, n=3, P?0.05) as measured by HPLC using a hydrophilic interaction liquid chromatography (HILIC) method for separation. Unexpectedly, the ?gfdB gene deletion strain possessed higher sensitivity to oxidative stress inducing agents like diamide, tert-butyl hydroperoxide (tBOOH) as well as hydrogenperoxide when compared to the control strain. As oxidative stress response has been associated with secondary metabolite production, we also compared the sterigmatocystin productions of the strains tested, and significant increases in the mycotoxin production have been found in the mutant strain. Significant differences were also observed in the reactive species productions of the strains; it was higher in the gfdB strain in comparison to the control either in the presence or in the absence of 0.6 mM tBOOH. Some specific antioxidant enzyme activities, including glutathione peroxidase (GPx), glutathione reductase (GR), catalase and superoxide dismutase (SOD) were also measured in order to gain an overview on the oxidative stress defense systems of the mutant and control strains. The deletion of the gfdB gene increased the specific GR and SOD activities meanwhile the catalase and the GPx activities were lower than those found in the control strain. Moreover, the specific SOD, catalase and GPx activities decreased in the gfdB strain under tBOOH stress.

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## HISTOPLASMA CAPSULATUM IS POISONED WITH COPPER AT EARLY STAGES OF MACROPHAGE INFECTION

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As a pathogen the fungus Histoplasma capsulatum collect micronutrients from the host tissues to survive and grow during the infection. In contrast, to combat infections and limit the proliferation of the pathogens, hosts may either decrease or increase the exposure of the invading pathogen to nutritional resources. Copper (Cu) toxicity has been reported as a host defense mechanism. Immune system phagocytic cells, such as macrophages, accumulate Cu in the phagosome to poison invaders. On the other hand, fungal cells promote changes in the expression of genes encoding components of the copper withholding and detoxification machinery such as the CRP, a copper membrane transporter that pumps out the metal. In this study, expression of genes putatively related to copper homeostasis was investigated in yeast cells of H. capsulatum incubated in high copper and low copper conditions. Genes related to copper uptake such as CTR3, CTR1 and MAC1 were induced in low copper treatment. On the other hand, genes related to copper detoxification were more expressed in copper availability, among them a copper efflux pump CRP1. The expression profile of copper related genes was also accessed when fungal cells were infecting macrophages. After 24h of infection, fungal cells up-regulated genes of Cu detoxification and down-regulated genes of Cu uptake suggesting H. capsulatum is exposed to a high copper environment in phagosomes. The influence of copper availability in fungicidal activity of macrophages was evaluated. Macrophage precultivated with with 30uM CuSO4 presented a lower fungal burden when compared with untreated ones. Considering that ATP7A is a copper transporter that increases Cu concentration inside phagosomes, the effect of ATP7A silencing in macrophages was analyzed. ATP7A silencing not only increases in fungal burden in macrophages but also decreases CRP1 transcript levels in yeasts. Altogether, these data strongly suggest that H. capsulatum experience a Cu-poisoning milieu during infection and that ATP7A is responsible for metal accumulation in phagosomes. Additionally,

qRT-PCR data suggests that ATP7A-mediated copper increasing is dependent, at least in part, on IFN-? activation. CRP1 silenced fungus strains were generated. CRP1 silencing increases fungal sensitivity to Cu. Also, knockdown mutants presented a decreased virulence in a macrophage infection model. Altogether the data shows that macrophages utilize Cu toxicity as a microbicidal mechanism against H. capsutalum, which in turn counteract Cu-accumulation by increasing CRP1 expression to pumps the metal out the cell.

## IMPACT OF TWO-COMPONENT HISTIDINE KINASE DRK1 ON PARACOCCIDIOIDES BRASILIENSIS CELL WALL MORPHOGENESIS

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Dimorphic fungi of Paracoccidioides spp. genus, P. lutzii and P. brasiliensis are the causative agents of Paracoccidioidomycosis (PCM), a systemic mycosis endemic in Latin America with high prevalence in Brazil, concerning mainly rural workers. On the environment (25°C), this fungus presents as mycelium and when inhaled by the host (37°C), reverts to yeast form. This ability of switch mycelium to yeast is essential to disease establishment and propagation. It is known that different genes are expressed according to the fungus phase and, in the last years, genes related to mycelium-yeast transition (M-Y) were identified. In dimorphic fungi species, a dimorphism-regulating histidine kinase (DRK1) is mainly expressed in yeast phase. Recently, we characterized the expression of DRK1 gene in P. brasiliensis, the study showed its role in dimorphism over a histidine kinase inhibitor (iDRK). The assay demonstrated that the fungus remained as mycelium even when cultivated at 37°C. Histidine kinases represents great importance regulating virulence and cell survival, and the investigation of associated signaling pathways may aid the discovery of potential molecular targets resulting on the development of drugs with antifungal activity, once being

present in prokaryotes, plants, bacteria and fungi but absent in mammal cells. Previous studies with Sporothrix schenckii demonstrated that SsDRK1 is also required for cell wall composition and integrity. We performed a spot assay in which yeast cells were first incubated for 24 h with varying concentrations of iDRK and subsequently spotted on YPD agar. The results showed that iDRK alone is not responsible for diminishing cell viability. Next, we used an intermediate concentration of iDRK (25 µg/ml) and incubated P. brasiliensis yeast cells for 24h, followed by spotting on YPD agar containing varying cell wall disturbing agents, such as Congo Red, Calcofluour White and sodium chloride. Interestingly, we observed reduced cell viability on samples submitted to iDRK, suggesting that PbDRK1 is related with cell wall morphogenesis. In order to evaluate genes involved with cell wall morphogenesis we performed qPCR analysis and observed differences between iDRK treated samples and non-treated controls. Further information in necessary in order to understand the mechanisms involved in cell wall morphogenesis and PbDRK1 potential signaling pathway related to it. The characterization of DRK1 and its signaling pathway will help the understanding of mechanisms involved with dimorphism, virulence and possibly PCM development.

## IN VITRO ACTIVITY OF NANO SILVER-HYDROXIAPATITE AGAINST A CLINICAL ISOLATE OF CRYPTOCOCCUS NEOFORMANS

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Nanostructured materials have been developed to be applied in health areas as alternative treatments for control of infectious diseases, exhibiting high antimicrobial activity and biocompatibility. Therefore, these nanocompounds can be linked with hydroxyapatite, a biological material present in teeth, bones, increasing the biocompatibility and possible synergistic antimicrobial effect. Moreover, the association with silver (Ag) is an alternative once it present a huge interaction with fungal cell to growth inhibition: producing free radicals, cell wall damages, DNA and RNA interactions. Cryptococcus neoformans is encapsulated

yeast that can cause a wide range of opportunistic infection, superficial until deep, which are difficult to treat, due to its resistance to drugs commonly used in clinical practice. The aim of this work was to assess the antifungal activity of silver-loaded hydroxyapatite (Ag/HAP) nanocomposites (NCs) under different concentrations of Ag (4% and 8%), pure Ag and pure HAP synthesized by a one-pot microwave-assisted solvothermal method, against a clinical isolate of Cryptococcus neoformans. To determine the minimal inhibitory concentration (MIC) of the NCs, the susceptibility test was conducted by the microdilution method, based on document M27-A3 - Clinical and Laboratory Standards Institute, all in on triplicate. The isolate was tested against two Ag/HAP nanocomposites concentrations (4% and 8%), pure Ag and HAP, in comparation to antifungal activity. The highest antifungal activity was observed for the NC with 8% Ag, with MIC of 15.6 µg/mL followed by 4% Ag and pure Ag with the same MIC 125 µg/mL. In relation with HAP, it did not show any activity, already expected. So, the synergistic effect occurred, because, when Ag and HAP was associated, the antifungal potential increased. The action of Ag/HAP NCs against Cryptococcus neoformans appear as new therapeutic approach for fungal disease. This study contributes to the knowledge of interactions between nanocomposites and fungal cell, showing a potential target present in Cryptococcus neoformans.

## LEUCINE ZIPPER BASIC DOMAIN TRANSCRIPTION FACTORS (BZIP) ATF1, ARE DEPENDENT ON SAKA (HOG1), AND ARE INVOLVED IN THE ADAPTIVE RESPONSE TO OSMOTIC AND CELL WALL STRESS IN ASPERGILLUS FUMIGATUS.

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Invasive aspergillosis is predominantly caused by Aspergillus fumigatus, a saprophytic, soil-dwelling filamentous fungus that produces conidia as means of

dispersal that can be inhaled by humans. The ability to sense and adapt to the surroundings is essential for the survival of A. fumigatus within a mammalian host. A. fumigatus adaptations to stresses experienced within the human host are a prerequisite for the survival and virulence strategies of the pathogen. The central signal transduction pathway operating during hyperosmotic stress is the high osmolarity glycerol mitogen-activated protein kinase cascade. A. fumigatus MpkC and SakA, orthologues of the Saccharomyces cerevisiae Hog1p, constitute the primary regulator of the hyperosmotic stress response. We have identified and characterized null mutants for four A. fumigatus basic leucine zipper proteins transcription factors. The atfA and atfB have comparable expression levels with the wild-type in ?mpkC but are repressed in ?sakA and ?mpkC ?sakA post-osmotic stress. The atfC and atfD have reduced expression levels in all mutants post-osmotic stress. The atfA-D null mutants displayed several phenotypes related to osmotic, oxidative, and cell wall stresses. To evaluate the physical interaction of AtfA-D:GFP with SakA:3xHA of A. fumigatus, co-immunoprecipitation (Co-IP) assays were performed during induction of CR or sorbitol for 10 min. The results show that AtfA, AtfB, AtfC and AtfD interact directly with SakA, showing the importance of TFs during the transcription of genes that may respond to different stresses. In addition, the AtfA:GFP, was constitutively present in the nucleus, regardless of the treatments performed. However, the other AtfB-D:GFP proteins translocated to the nucleus after 10 or 30 minutes of treatment with different stressors. Double deletions were made, and we can observe that there is a possible genetic interactions among these null mutations. The ?atfA and ?atfB were shown to be avirulent and to have attenuated virulence, respectively, in both Galleria mellonella and a neutropenic murine model of invasive pulmonary aspergillosis.

## MATURITY FAVORS LONGEVITY AND DOWNREGULATION OF AGING GENES IN S.CEREVISIAE SUBMITTED TO HIGH HYDROSTATIC PRESSURE

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A strong relationship between the activation of regulatory pathways in response to stress, longevity, and aging has been pointed out in many studies. The causes of aging are multifactorial, affecting a series of cell mechanisms, and these regulatory pathways among eukaryotes are surprisingly well-conserved. The maintenance of an efficient stress response mechanism can be a potential strategy to extend longevity and to delay aging and the appearance of ageassociated diseases. Addressing longevity and aging in the yeast Saccharomyces cerevisiae is a well-accepted approach since the pathways involved in energy balance, damage accumulation, stress response, genomic integrity, apoptosis are preserved in this model organism. Thus, the purpose of this study was to evaluate the effects of the stress caused by high hydrostatic pressure (HHP) on mother and daughter cells of S. cerevisiae and the favoritism of maturity for longevity in the response to this specific stress. To achieve these goal, mature and young cells were separated through affinity chromatographic method. Mother cells or mature cells had their cell surface marked with biotin and streptavidin containing magnetics microbeads and, after incubation in rich medium in glucose, generated unlabeled daughter cells or young cells. Thus, the culture containing labelled mother cells and unlabeled daughter cells was added to a magnetic column where the labeled cells were retained and the unlabeled eluted into a test-tube. HHP of 50 and 100 MPa was applied and yeast survival, as well as gene expression related to stress response, longevity and aging were analyzed. As expected, S. cerevisiae mother cells presented a higher survival rate than the daughter cells. In contrast, genes that encode the antioxidant enzymes catalase, CTT1, and superoxide dismutase, SOD2, were upregulated in daughter cells and they were not significantly altered in mother cells. The TOR1 gene, a transcriptional regulatory gene, inducer aging and apoptosis, and RAS2, proliferative growth inducer and coding a G protein were upregulated in daughter cells and downregulated in mother cells. The accumulation of reactive oxygen species (ROS) were also measured, showing that mother cells had higher levels of ROS than the daughter cells. Therefore, mother cells presented a better survival to HHP stress than the daughter cells, but this was not related to the upregulation of the antioxidant system. On the other hand, there was an implication on the downregulation of apoptosis and acceleration of aging, showing that maturity plays an important role in the HHP stress response.

# METABOLISM OF ISOPRENES AND STEROL IN PARACOCCIDIOIDES BRASILIENSIS

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Paracoccidioides brasiliensis is a thermodymorphic pathogenic fungus and the etiological agent of the systemic mycosis paracoccidioidomycosis, endemic in South America. Calcineurin, a serine and threonine phosphatase activated by calcium and calmodulin, important in the process of morphogenesis and adaptation of fungi both under physiological conditions or in response to environmental stresses, controls proliferation, dimorphism, but non-survival in P. brasiliensis, as shown by our group. The total proteome of yeasts of P. brasiliensis cultured in the presence and absence of a calcineurin inhibitor, cyclosporin A (CsA), was previously produced by our group as an approach to reveal biochemical processes potentially regulated by this phosphatase involved in cell adaptation and stress control responses. Proteomic analysis revealed an apparent reprogramming in the lipid metabolism of yeasts maintained for 24h with 1 microg.ml-1 of CsA. In this work, we aimed to characterize the enzymes Isopentenyl-diphosphate delta-isomerase (IPP isomerase) and HMG-CoA synthase, which showed reduced levels in CsA-treated yeast cells, 2,15 and 6,71 fold, respectively. These proteins are from the mevalonate biosynthesis pathway, responsible for the synthesis of sterol isoprenoids, with the end product ergosterol in yeast, and non-sterolic isoprenoids such as dolichols, the ubiquinone side chain, farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP ). FPP and GGPP are involved in cell signaling and proliferation processes. FPP is also a precursor for molecules such as the dolichols involved in signal transmission from the neuronal membrane, and ubiquinone an electron carrier from the mitochondria. Ergosterol is involved in several biological functions, such as fluidity, regulation, growth, infection process and distribution of the membrane's integral proteins. By bioinformatic analysis, we show that IPP isomerase and HMG-Coa

synthase are conserved in approximately (70-96%) and (80-94%) fungi, (52-55%) e (3850%) in mammals (Human), respectively. The HMG-Coa synthase enzyme has the domain HMG_CoA_synt_N and HMG_CoA_synt_C and the enzyme IPP isomerase possesses the Nudix hydrolase domain which is characteristic of hydrolase activity. While in humans there are one mitochondrial and cytosolic isoforms of the HMG-CoA synthase, this enzyme is encoded by a single gene in P. brasiliensis, which shares 46% and 49.79% identity at the amino acid level, respectively, with human's versions, suggesting that the P. brasiliensis enzyme may have these two intracellular locations and may be bivalent in the mevalonate biosynthesis and ketone bodies pathway. The possibility of differential splicing-generating isoforms from this single gene isn't ruled out. Considering the importance of lipids in the metabolism of the fungus and in particular the intermediates of the mevalonate-farnesol-ergosterol pathway in maintaining fungal integrity, this work may reveal targets other than ergosterol for biochemical/pharmacological intervention to control populations of interested fungi. This work aim at evaluating the possible effects of the treatment of P. brasiliensis cells with statins (as inhibitors of the HMG-Coa reductase enzyme that prevents the synthesis of Mevalonic Acid, a substrate of IPP isomerase, which had levels reduced by CsA), and the impairment of the inhibition of isoprenes biosynthesis and sterol metabolism in the biology of P. brasiliensis under normal and stress conditions.

## MOLECULAR CHARACTERIZATION OF ASPERGILLUS NIDULANS CLINICAL ISOLATES

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In addition to Aspergillus fumigatus being the main etiological agent of Aspergillus-related infections, other Aspergillus spp., such as A. nidulans, have been found to

also have a high infection rate in a condition-dependent manner. Whereas A. fumigatus virulence attributes and their relation to host infection are relatively well studied, the virulence of A. nidulans clinical isolates (Cis) remain largely uncharacterised. The aim of this work was therefore to carry out a detailed phenotypical and physiological characterization of two A. nidulans clinical strains that were isolated from two different patients. The first patient suffered from breast carcinoma and pneumonia and the second patient had cystic fibrosis with subsequent lung transplantation. In a first instance, growth phenotypes of both Cis in the presence of different nutrients was carried out and compared to the reference strain FGSC A4. A reduction in growth (dry weight) was observed for both CIs in the presence of glucose whereas they grew better in the presence of either ethanol, casamino acids or different lipids. Subsequently, metabolomics was carried out of both CIs and FGSC A4 strain when grown in the presence of glucose, acetate, ethanol or mucin as single carbon sources. Principal Component Analysis and Hierarchical Clustering Analysis clearly showed that clinical isolates clustered apart from the reference strain and from each other in all tested carbon sources, indicating that they are metabolically different. The percentage of quantitatively significantly different metabolites common to both clinical isolates and different from the reference strain was 43%, 60 %, 50% and 31% in the presence of glucose, acetate, ethanol and mucin, respectively. These results suggest that in the presence of glucose and mucin, both clinical isolates are metabolically more similar to the reference strain than when grown in the presence of acetate and ethanol. Moreover, the genomes of the three strains were sequenced and compared. A total of 19,271 SNPs (single nucleotide polymorphisms) were identified in the CIs when compared to the FGSC A4 reference strain. These results indicate substantial genomic and metabolic differences between A. nidulans strains. Remodeling of the metabolism might have favored a more efficient use of alternative carbon sources such as ethanol, amino acids and lipids which could ultimately affect the ability of these strains to colonise mammalian hosts and cause disease.

## STUDY OF THE PATHWAYS RELATED TO THE BIOSYNTHESIS OF THE BIOACTIVE SECONDARY METABOLITES OF THE FUNGUS EXSEROHILUM

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Secondary metabolites produced by the fungus Exserohilum rostratum may present activity against microorganisms and previously data from our group showed the induction of cellular proliferation on mammalian cells, such as on human umbilical vein endothelial cells (HUVECs). Considering the perspective of biotechnological application of this fungal species by the synthesis of bioactive compounds previously identified and coded as F1 and F2, the objective of the present study was describe the pathways involved in the biosynthesis of these secondary metabolites. Mutants defective to the production of these compounds were generated by random mutagenesis mediated by transformation using the bacteria Agrobacterium tumefaciens. The bacterium carrying the insertion DNA was co-cultured with the fungus by inoculation of a suspension of spores and bacteria 1:1 (v/v) on a nylon membrane in solid inducer medium (IM) for 72 h, at 28°C in the dark. Thirty mutants strains were selected and grown in a potato dextrose agar (PDA) containing glufosinate-ammonium at 28°C. For the analysis of the secondary metabolites biosynthesis, all the mutants were cultivated in PD medium for 15 days at 28°C under shaking (150 rpm). The culture supernatants were submitted to a solid phase extraction (SPE) using C18 cartridges with methanol as organic solvent. After being dried in Speed-Vac system, the organic extracts were analyzed by high performance liquid chromatography (HPLC) using a C18 column and methanol as mobile phase for the detection of the compounds F1 or F2. Seven mutants did not produce the bioactive compounds and were selected for future analysis. The next steps will be performed with qPCR analysis in order to identify the mutant that have only one insertion fragment. The localization of the insertion studied by Tail-PCR, allowing the detection of the genes and the identification of the possible proteins associated to the biosynthesis of these bioactive secondary metabolites.

## THE TRANSCRIPTION FACTOR HSFA AND THE HSP90 CHAPERONE ARE IMPORTANT FOR THE THERMOTOLERANCE AND THE MAINTENANCE OF THE CELL WALL INTEGRITY OF ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is a major opportunistic pathogen of mammals and the main causative pathogen of invasive pulmonary aspergillosis (IPA) in immunocompromised individuals. Thermotolerance is one of the key virulence determinants of this fungus, being a prerequisite for the establishment of infection and persistence of the pathogen inside the host. In Saccharomyces cerevisiae, the Hsf1 transcription factor is responsible for regulating 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, in yeast and in the fungal pathogen Candida albicans, Hsf1 has been associated with the maintenance of the cell wall integrity (CWI). Here, we aimed to investigate the role played by HsfAHsf1 in both thermotolerance and in the cell wall integrity. We found that hsfA is an essential gene in A. fumigatus. So, we constructed a xylP::hsfA conditional mutant which was used for phenotypic characterization. hsfAHSF1 is fundamental for thermal adaptation, viability and important for cell wall stress and oxidative stress tolerance. A RNA-Seq analysis revealed that genes related to cell wall biosynthesis and maintenance are induced during heat shock and HsfA possibly acts on the regulation of the expression of such genes. We also observed that during temperature and cell wall stresses, hsfA genetically interacts with PkcA and MpkA (the apical protein kinase and the MAP kinase of the CWI pathway, respectively), and also with SakAHOG1, the MAP kinase of the High Osmolarity Glycerol (HOG) pathway. Furthermore, experiments using mutants of genes of the CWI pathway (pkcAG579R, ?rlmA and ?mpkA) containing the luciferase reporter gene under the control of the hsp90 promoter showed that hsp90 expression is highly induced both in thermal and cell wall stresses, and that this expression is increased in the loss of function of the CWI pathway genes. These results highlight the importance of HsfA and Hsp90 for the adaptation of the fungus to heat shock and cell wall stress, and point out to the existence of a concise relationship between thermotolerance and CWI and HOG pathways.

# VACUOLAR TRANSPORTERS AND VESICLE-MEDIATED TRANSPORTERS INVOLVED IN TRICHODERMA HARZIANUM RESPONSE TO METAL STRESS

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Trichoderma harzianum is a common saprophyte fungal used as biological control agent due its mycoparasitism ability besides being helpful in plant growth. T. harzianum lives in soil and plants roots and it is tolerant to different abiotic stresses, such as pH, salinity, heat, and the presence of metals. This study aimed to contribute to the understanding of metal tolerance mechanisms employed by this fungus under cadmium and aluminum stresses. Therefore, results from RNA sequencing of T. harzianum mycelium grown in potato medium [20% potato, 2% dextrose] supplemented with 1.0 and 2.0 mg/mL of CdCl2, and 1.5 and 3.0 mg/mL of AlCl3 were used to verify the main biological processes involved in the response to these metals by using Gene Ontology (GO) enrichment analysis. Being both the capture of metal ion in vacuoles and the exocytosis of it from the cell important metal tolerance mechanisms in fungi, we studied proteins involved in vacuolar transport and vesicle-mediated transport categories observed as up-regulated in RNAseq experiments. The sequence of each studied protein was obtained by searching their identification codes (IDs) on Joint Genome Institute Trichoderma harzianum TR274 v1.0 database and they were used to characterize the proteins families and important domains on InterPro v.73. All the analyzed proteins from vacuolar transport category - IDs: 784226, 785401, 833355, 835208, 844400 - were common to all stress conditions analyzed. According to our research, four of them belong to Snf7 family, which is part of the ESCRT-III complex and it is involved in the transport from the endosome to the vacuole or lysosome in cells by multivesicular body (MVB) pathway. Different from the others, the protein 784226 was predicted to belong to NEDD4/Bsd2 family, which in turn is the interaction between an endosomal membrane protein (NEDD4), that in humans it

is related to regulation of metals in neurons, and a molecule (Bsd2) responsible to target proteins into the MVB. On the other hand, the proteins from vesicle-mediated transport category in CdCl2 1.0 mg/mL - IDs 716618, 750357, 814699, 817543 and 84079 - were related to Trafficking Protein Particle Complex/Subunit 6B, Adaptor protein complex AP-3, Coatomer/beta subunit, AP complex/subunit beta and Vacuolar protein sorting-associated protein 53, respectively. In AlCl3 1,5 mg/mL, the families found in vesiclemediated transport category - IDs 726428, 729382, 816455, 844174 - were Sec1-like protein, Transport protein particle (TRAPP), Clathrin adaptor and 729382 showed a Sec39 domain. Finally, the families found in AlCl3 3.0 mg/mL vesicle-mediated transport category - IDs: 728364, 751408, 784386, 785956 and 826412 - were Exocyst complex component Exo70, Trafficking protein particle complex/subunit 2, Snf8/Vps36, Synaptobrevin and Sec1-like protein, respectively. These results show a different gene response to different metals and concentrations indicating that the mechanisms of metal transport through vesicles can be performed by different sets of proteins depending on the metal and its concentrations. On the other hand, vacuolar transport mechanism seems to be similar regardless of metal type or its concentration.

## ZINC HOMEOSTASIS IN THE HUMAN PATHOGEN FONSECAEA PEDROSOI

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Fonsecaea pedrosoi is a human pathogenic fungus belonging to the order Chaetothyriales, which harbours dematiaceous fungi, characterized by depositing a large amount of melanin in the cell wall. F. pedrosoi is the main etiologic agent

of Chromoblastomycosis (CBM), a mycosis of global distribution. The infection occurs by the inoculation of hyphae or conidia in a previously established cutaneous lesion, which allows dissemination through the subcutaneous tissue. CBM is a recalcitrant disease and difficult to treat.

Therefore, new treatment options are necessary to improve the quality of life of the patients. One of the promising fronts is the understanding of the process of obtaining metals essential for survival in hostile environments, such as those found during infection. Among the metals of major importance for maintenance of basic activities in an organism, zinc is the second in terms of association with enzymes and total cellular distribution. During the infectious process, the host manipulates the metal levels in order to contain the infection, a process called nutritional immunity. In this context, micro-organisms may be subject to metal deprivation or intoxication. Our initial studies performed in order to understand the mechanisms of zinc homeostasis in F. pedrosoi are presented here. F. pedrosoi zinc sensibility was accessed in vitro by growth in chemically defined minimal media containing a broad range of different zinc concentrations. F. pedrosoi was able to grow on concentrations up to 2.5 mM of zinc and also in zinc deprivation generated by the addition of DTPA and TPEN, an extracellular and an intra-/extracellular zinc chelator, respectively. Such versatility is probably due to the zinc homeostasis mechanisms conserved in pathogenic fungi. Namely, the CDF and ZIP family of proteins are capable of quickly move zinc out or inside the cell by deploying a variety of transporters found in the cytoplasmatic and organelles membranes. Previous in silico analysis performed by our group identified orthologs of those proteins in F. pedrosoi genome. Gene expression analysis demonstrated that the mRNA levels of the ZrfB ortholog, a high affinity zinc importer, is induced during zinc depravation. Biolistic transformation coupled with Double Joint PCR was also performed to obtain a knockout mutant strain for the ortholog of the main transcription factor known to regulate zinc homeostasis in pathogenic fungi: Zap1. Following selection in hygromicin containing media, colonies were tested for the presence of the selection marker as well as the absence of zafA. Two ?zafA clones were chosen and are currently under analysis. Meanwhile, an in silico folding test was performed and possible zinc binding motifs and protein conformation was obtained. F. pedrosoi seems to hold a ZafA structurally different from the orthologs already described in literature.

Interestingly, F. pedrosoi ZafA structure is shared among members of the Chaetothyriales order. This is the first report of molecular mechanisms related to metals homeostasis in causative agents of CBM. The results will provide information on fungal biology as well as the possible relationship between zinc and F. pedrosoi virulence.

# 2. FUNGAL PHOTOBIOLOGY, CLOCK REGULATION, AND STRESS

## CIRCADIAN REGULATION OF A MYCOPARASITIC INTERACTION BETWEEN BOTRYTIS CINEREA AND TRICHODERMA ATROVIRIDE

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Circadian rhythms coordinate organisms' activities with daily rhythms and, are controlled by a circadian clock: a molecular machinery that allows organisms to keep track of time and synchronize their biology with the daily environment. In the past years, evidence has risen to show that circadian clocks not only regulate organism's inner processes, but it also impacts the outcome of organismal interactions.

In this study, the circadian regulation of a mycoparasitic interactions between Trichoderma atroviride and Botrytis cinerea were studied. B. cinerea, a wellknown phytopathogen, possess a functional circadian clock that enhances its virulence during the night. On the other side, T.atroviride's putative circadian clock hasn't been studied yet, although it possesses clock homologous genes: blr1, blr2 and tafrq (homologs to Neurospora crassa's wc1, wc2 and frq).

First, we evaluated the role of T. atroviride's frequency gene by generating a knockout strain ?tafrq and a tafrq overexpressor strain, OE::tafrq. Both strains were grown in PDA medium and their conidia were collected at 3, 5 and 7 days of growth and quantified. Compared to wt strain, ?tafrq strain presents a delay in conidia production, which is accelerated in the OE::tafrq strain. Secondly, to assess the functionality of T. atroviride's circadian clock, we developed a luciferase reporter strains to follow tafrq & taFRQ oscillations under free running conditions. Reporter strains were grown on different culture media under a 12:12 LD cycle for 3 days, and its bioluminescence was recorder for 5 days using a CCD camera. A TaFRQLUC translational reporter showed daily oscillations of protein levels and the same was observed with a transcriptional reporter containing a frq

minimal promoter. Taken together, these results show a role of tafrq in T. atroviride conidiation and provides evidence of a functional circadian clock.

Regarding to the circadian regulation of the mycoparasitic interaction, wt and clock mutant strains of both fungi were confronted on antagonistic assays at 20°C under LL, DD and LD conditions. Confrontations were evaluated at 7 and 14 days, measuring the growth area of both fungi, the produced necrosis halos and the ability of T. atroviride to overgrow B. cinerea. All T. atroviride strains (wt, ?blr1 & ?tafrq) overgrew B. cinerea, with maximum overgrowth at DD conditions. ?blr1 possess the greatest mycoparasitic and ?tafrq the weakest, suggesting that light and BLR1 inhibits the mycoparasitic behavior of T. atroviride, while tafrq may have the opposite effect. On the other hand, B. cinerea strains (wt, ?bcwcl1, ?bcfrq1) displayed different resistance abilities. ?bcwcl1 is easily overgrown by T. atrovitide wt strain, while ?bcfrq1 in all conditions generates an intense necrotic area, suggesting that the absence of bcfrq1 make B. cinerea more susceptible.

In conclusion, T. atroviride possess a functional circadian clock and it's components like those of B. cinereas, influence the outcome of a mycoparasitic interaction between them.

## OUTCOME OF BLUE, GREEN, RED, AND WHITE LIGHT ON METARHIZIUM ROBERTSII DURING MYCELIAL GROWTH ON CONIDIAL STRESS TOLERANCE AND GENE EXPRESSION

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Fungi sense light and utilize it as a source of environmental information to prepare against many stressful conditions in nature. In this study, Metarhizium robertsii was grown on: 1) potato dextrose agar medium (PDA) in the dark (control); 2)

under nutritive stress in the dark; and 3) PDA under continuous (A) white light; (B) blue light lower irradiance = LI; (C) blue light higher irradiance = HI; (D) green light; and (E) red light. Conidia produced under these treatments were tested against osmotic stress and UV radiation. In addition, a suite of genes usually involved in different stress responses were selected to study their expression patterns. Conidia produced under nutritive stress in the dark were the most tolerant to both osmotic stress and UV radiation, and the majority of their stress- and virulence-related genes were up-regulated. For osmotic stress tolerance, conidia produced under white, blue LI, and blue HI lights were the second most tolerant, followed by conidia produced under green light. Conidia produced under red light were the least tolerant to osmotic stress and less tolerant than conidia produced on PDA medium in the dark. For UV tolerance, conidia produced under blue light LI were the second most tolerant to UV radiation, followed by the UV tolerances of conidia produced under white light. Conidia produced under blue HI, green, and red lights were the least UV tolerant and less tolerant than conidia produced in the dark. The superoxide dismutases (sod1 and sod2), photolyases (6-4phr and CPDphr), trehalosephosphate synthase (tps), and protease (pr1) genes were highly up-regulated under white light condition, suggesting a potential role of these proteins in stress protection as well as virulence after fungal exposure to visible spectrum components.

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# 3. FUNGAL STRESS IN INDUSTRY

## EFFECTS OF FUNGAL SILVER NANOPARTICLE EXPOSURE ON THE ROUTINE METABOLISM OF PALAEMON PANDALIFORMIS AND DANIO RERIO

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Effects of fungal silver nanoparticle exposure on the routine metabolism of the aquatic organisms Palaemon pandaliformis and Danio rerio Metal nanomaterials (NM) are currently widely used in several industrial segments. Silver nanoparticles (AgNP) are the most described due to their effective antimicrobial action. AgNP can be obtained by physical and chemical processes, but recently, the biological synthesis using mainly filamentous fungi (Ff) has been described as innovative, since its process is considered ecofriendly and the resulting nanoparticles have higher biocompatibility when compared to synthetic ones. In view of this new alternative to obtain AgNP, studies are necessary to evaluate its effective antimicrobial activity and potential toxicity, in order to avoid compromising public and environmental health. Exposition of aquatic invertebrate and vertebrate organisms to assess the toxicity and risks of nanomaterials support the development of environmental laws that can serve as tools to manage and preserve the trophic balance of the environment. In this context, invertebrate Palaemon pandaliformis (shrimp) and vertebrate Danio rerio (zebrafish), used as a general toxicity model, were selected for this study. For AgNP biosynthesis, the Penicillium citrinum IBCLP11 strain isolated from mangrove sediments of Baia do Araçá and belonging to the Culture Collection of the Institute of Biosciences of Campus do Litoral Paulista (UNESP) was used. The study of the toxic effect of biological AgNP IB-CLP11 occurred by exposing in vivo P. pandaliformis and D.

rerio. For each condition studied, 7 individuals were used. The organisms were exposed to AgNP and their precursor metal AgNO3 in a concentration range of 10-1000 µg·L-1. The organisms were kept for 24 hours in 2L fish tanks, with constant temperature of 24 ºC, pH 6.7 and photoperiod of 12h light and 12h dark. By the end of this period, tests were carried out for specific oxygen consumption (O2) and total ammonia excretion using the Winkler and Nessler method. The specific consumption of O2 in the individuals exposed to biological AgNP showed no significant difference when compared to the control group up to the concentration limit of 100 µg·L-1 for P. pandaliformis and 250 µg·L-1 for D. rerio. The individuals exposed to AgNO3 presented a great change in their metabolic rate, with the concentrations used being lower than AgNP concentrations. For both organisms, there was an increase in the ammonia excretion rate. Numerous studies have evaluated the effects of synthetic AgNPs exposure to aquatic organisms; however, the use of micogenic AgNPs is an economical and ecological alternative that can be used as an instrument for future government decision-making, regarding the reduced environmental impacts associated with these nanocomposites.

## EVOLUTION OF ALDO-KETO-REDUCTASES ENZYMES (AKRS) IN YEASTS AND THEIR IMPORTANCE IN FERMENTATION FOR THE PRODUCTION OF SECOND GENERATION ETHANOL

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Faced with the imminent depletion of fossil fuels, the main energy source used globally, the use of renewable energy sources gains space. In this scenario, biofuel coming from second generation (2G) ethanol appears as a promising alternative. But some bottlenecks in 2G ethanol production process still need to be addressed for it to reach its economic viability. The use of bagasse and

sugarcane straw in fermentation brings new sugars that are not naturally consumed by industrial yeasts, mainly xylose.

In this study, through comparative genomics, we search for genes to maximize the production of 2G ethanol. Among the enzymes that engage in the consumption of xylose in yeasts there is Xylose Reductase (XR) in which, through previous works from our group, positive selection marks have already been found. As this enzyme is of great importance in the metabolic pathway of xylose in the production of 2G ethanol, this project aims to better understand the Aldo-Keto Reductase (AKR) enzyme superfamily, of which XR is a part. As the superfamily in question is present in several biological groups with different functions, its functional characterization in yeasts is sought through an evolutionary study, highlighting genomic differences between xylose fermenting and non-fermenting yeast, and thus better comprehending its role in the fermentation process of xylose.

Using comparative genomics associated with bioinformatics, from all available genomes of the Saccharomycotina subphylum (180 yeasts), the genes of the enzymes belonging to the AKR superfamily were identified through the HMM profile of AKR. Sequences obtained by using the HMM profile were found to be clustered in 9 gene families defined through OrthoFinder analysis. For phylogenetic reconstruction of these families their sequences were aligned with MAFFT and submitted to IQ-Tree, as Maximum Likelihood method has a more feasible computational time than Bayesian analysis. Individual phylogenies were reconstructed for each family, in addition to a general phylogeny of the 9 families to validade their homology assignment.

To understand their evolutionary history, from the phylogenies inferred for the gene families, we searched for natural selection marks through nucleotide substitution rates along the DNA sequences of genes of xylose fermenting species and analyzes of expansion and retraction of these genes families. The search for substitution rates is being conducted through the HyPhy program. After determining these rates the results will be analyzed and each family will be functionally annotated through PANNZER and Interpro.

Through the understanding of how evolution has acted on the AKR superfamily, it will be possible to prospect enzymes capable of acting in the fermentation path

with potential for use in industrial applications, helping to solve existing bottlenecks and thus maximizing the productive process of 2G ethanol.

## OPTIMIZATION OF MYCOGENIC SILVER NANOPARTICLES SYNTHESIS AND THEIR ANTIFUNGAL ACTIVITY

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Silver nanoparticles (AgNP) applications have been gaining special attention in the last years due to the unique characteristics that improve their application in different industrial areas. Different methods of AgNP synthesis are being widely described in the literature, however, chemical and physical syntheses are always considered hazardous, costly and can promote interference in biomedical functions. Bacteria, fungi, actinomycetes, yeasts, algae and plants are the main organisms that have been described as AgNP producer. AgNP biosynthesis using fungi is considered promising, mainly due to the capability of this organisms grow faster, get good tolerance and easy metal bioaccumulation and, produce some enzymes that are directly involved in AgNP stabilization and capping presenting unique characteristics for these biological nanomaterials. In this context, the present study was designed to optimize the production of mycogenic AgNP using Penicillium citrinum IB-CLP11, Penicillium sclerotigenum IB-CLP17, Aspergillus niger IB-CLP20 and Penicillium polonicum IB-CLP22. The following parameters were analysed: AgNO3 (0.25-2.0 mM), biomass (50.0-150.0 g•L-1), temperature (0-80 °C), pH (1.0-8.0) and shaking (0200 rpm). Antifungal activities were assessed by minimum inhibitory concentration (MIC) using Aspergillus níger IPT295, Penicillium funiculosum IPT423, Fusarium oxysporum IPT330, Aspergillus fumigatus IPT728, Aspergillus parasitus IPT729 e Trichophyton mentagrophytes IPT311. All selected strains showed capability to biosynthesize good and stable AgNP at 1,0 mM AgNO3 concentration. Best biomass value of for induction of AgNP biosynthesis ranged from between 75.0 and 150.0 g•L-1. The

agitation of 150 rpm allowed the best AgNP formation with smaller size and lower rate of aggregation.The ideal temperature for the formation of AgNPswas found between 25-35 °C. And, the AgNPs produced at pH 4.5 presented better stability. The pathogenic fungi, A. niger and P. funiculosum were the most sensitive, presenting MICs of 20 to 40 µg•mL-1, together with F.oxysporum with a MIC in the range of 30 to 75 µg•mL-1. The pathogenic fungi A. fumigatus, A. parasitus and T. mentagrophytes present higher MICs in the range of 75 to 125 µg•mL-1. In view of the obtained results, an optimization of the biosynthesis process of AgNPs with antifungal action will allow for their stable production, with reduced cost and easy scale-up.

## OPTIMIZATION RHODOTORULA GLUTINIS CULTIVATION CONDITIONS FOR SIGLE-CELL-OIL PRODUCTION FROM GLYCEROL

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The search of technologies that aim the use of industrial waste and by-products from established processes is a strategic point for the development of more efficient and sustainable industries. In this context, the alternative search for the use of glycerol, the main biodiesel production by-product, have an important role in the design of lipid-based biorefineries. Glycerol can be used in biotechnological processes as a substrate to obtain various products, among them microbial oils. Thus, the present study aimed to evaluate the influence of culture conditions on lipids production by the yeast Rhodotorula glutinis NRRLY-12905. The experiments were carried out according to surface methodology, using face-centered 24 full factorial design. In this experimental design were evaluated the effects of substrate concentration (40 to 200 g/L), carbon/nitrogen ratio (20:1 to 100:1), pH (5 to 7) and inoculum concentration (1 to 5 g/L) on the production of lipids. The assays were conducted in 250 mL Erlenmeyer flasks, containing 50 mL of culture medium, incubated in an orbital shaker at 200 rpm, at 30 ° C for 240 h. The culture medium was composed by MgSO4.7H2O (1 g/L), KH2PO4 (20 g/L), and glycerol, yeast extract and (NH4)2HPO4 (according to the experimental

design). Within the evaluated region, the substrate concentration, pH and carbon/nitrogen ratio (C/N) showed statistically significant effects on lipid production. Among these variables, the substrate concentration and pH presented a quadratic behavior, with a maximum lipid accumulation of approximately 140 g/L and pH 6.5, respectively. The effect of the C/N ratio showed a positive behavior in the accumulation of lipids, that is, the increase in the C/N ratio led to an increase in the accumulation of lipids by yeast. Statistical analysis indicated that maximum lipid production by yeast R. glutinis was predicted for cultures with initial glycerol concentration of 140 g/L, C/N ratio of 100 and pH of 6.5. Cultures performed under these conditions presented a cell concentration of about 30 ± 1 g/L after 200 h of cultivation, reaching accumulation of lipids of approximately 15 ± 3 g/L in the same period. In general, the study indicated that lipid production by yeast R. glutinis was favored in culture under high concentrations of glycerol and restrictions of nitrogen sources. The fatty acid profile showed that microbial oil is constituted mainly of oleic acid (31 to 36%), stearic acid (18 to 25%), linoleic acid (18 to 20%) and palmitic acid (20 to 23%). The fatty acids of eighteen and sixteen carbons correspond to 95% of the fatty acid profile of microbial oil. These results contribute to establishing operational conditions that maximize singlecell oil production from glycerol by Rhodotorula glutinis, i.e. an alternative source as renewable raw material for lipid-based biorefineries.

## PHYSIOLOGY OF SACCHAROMYCES CEREVISIAE IN THE PRESENCE OF P-COUMARIC ACID

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During industrial fermentations, yeasts face a variety of stress factors, including a high concentration of ethanol and salts, high temperatures and pH. Additional obstacles arise in the second-generation ethanol production process, where lignocellulosic residues are the substrates for fermentation. The physicalchemical pretreatment steps generate various microbial inhibitors that severely affect yeast

growth and physiology and compromise fermentation efficiency. Lignocellulosic-derived inhibitors are formed during the pretreatment of biomass and depend mainly on the type of biomass used and the process conditions. Many studies have been performed on the formation of inhibitors during the pretreatment of sugarcane bagasse. Knowledge on their formation is very beneficial when the decomposed lignocellulose is used in a fermentation process. These inhibitors can result in problems further downstream since they can inhibit the growth and performance of microorganisms during fermentation. The understanding of the physiological effects of major inhibitory compounds on the performance of S. cerevisiae is essential for the effective implementation of strategies that promote the increase of its robustness and improve fermentation performance. In this sense, we investigated the effect of one major phenolic compound, p-coumaric acid (pCA), on important quantitative physiological parameters of the industrial strain S. cerevisiae SA-1. The dataset presented so far indicated important physiological changes in glucose-limited chemostat cultivations in the presence of 7 mM pCA as compared to the control condition (without pCA addition). We observed an increase in consumption and production rates, such as for glucose (26%), CO2 (12%) and ethanol (53%). On the other hand, we observed a decrease in biomass yield (22%), and in the glycerol production rate (19%). In anaerobic glucose-limited chemostat cultures of the S. cerevisiae strains, carbon is mainly diverted to ethanol and CO2, and minor amounts of glycerol, lactic and acetic acids, with a concomitant formation of yeast biomass. In relation to p-coumaric condition, the ethanol yield of SA-1 control was 21% lower. Acetate was not detected in both industrial strains. The dataset presented so far indicates important physiological differences of industrial strains, such as S. cerevisiae SA-1, in the presence of an important inhibitory compound of sugarcane-based hydrolysates from lignocellulosic biomass. Transcriptomic data in the presence of this inhibitor will contribute further to evaluate these differences.

## SCREENING OF MARINE FUNGI ISOLATED FROM ARAÇÁ BAY SEDIMENTS FOR SILVER NANOPARTICLES BIOSYNTHESIS

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The marine environment has a large fungal diversity that promotes important ecological roles and has an unexplored biotechnological potential. In recent years, marine fungi (Mf) have been described for their capability to biosynthesise metallic nanomaterials. The main features of these organisms are: resistance to extreme conditions, rapid growth, and easy scale-up. In this context, 12 strains of Mf from the Culture Collection of Biosciences from Institute of São Paulo State University (IB-CLP06, IB-CLP08, IB-CLP11, IBCLP12, IB-CLP13, IB-CLP15, IB-CLP16, IB-CLP17, IB-CLP20, IB-CLP22, IBCLP-30, IB-CLP40) were evaluated as potential producers of silver nanoparticles (AgNP). The strains were grown in Malt Extract Broth, at 30 °C, 150 rpm, for 72 hours. At the end of this period, the biomasses were separated from the filtrate and washed in sterile distilled water. Subsequently, 5.0 g of biomass were added to 50 mL of sterile distilled water. This suspension was then incubated at 30ºC and 150 rpm, for 72 hours. In the last biosynthesis step, a new filtration was made. The biomasses were discarded and AgNO3 solution was added to each filtrate. The selection of Mf with AgNP biosynthesis capacity occurred by analysis of the results' set, obtained for the nanomaterials characterisation, from UV-visible analysis, nanoparticle size distribution analysis, Dynamic Light Scattering (DLS), Polydispersity index (PDI), Zeta Potential (Pz), Transmission Electron Microscopy (TEM), and stability assays during 6 months.

Among the 12 strains evaluated, 10 presented AgNP biosynthesis capacity. All AgNPs obtained showed a Plasmid Resonance Band, detected by UV-visible, in the spectrum at wavelength ranging from 410-450 nm, indicating AgNP formation. Following the size considerations, measured by DLS and TEM, the strains IB-CLP06, IB-CLP16, IB-CLP30, IB-CLP40 were excluded from further analysis, since the AgNP size was higher than 100 nm. Similarly, nanoparticles with PDI higher than 0.3 (e.g. the ones from strain IB-CLP13), were disregarded. Finally, considering Pz, which in theory sets as suitable values the ones in modulus of 30 mV or more (since the higher the particle surface charge, the higher the stability of the particle), the IB-CLP15 strain did not attend this requirement, being therefore

also excluded. Thus, only the AgNP produced by the Penicillium citrinum IB-CLP11, Penicillium sclerotigenum IB-CLP17, Aspergillus niger IB-CLP20, and Penicillium polonicum IB-CLP22 strains showed promising morphological and stability results. Considering these results, future work will be the evaluation of antimicrobial capacity and optimisation of the synthesis process.

## STRESS-INDUCED PRODUCTION OF PIGMENTED SECONDARY METABOLITES IN AMAZONIAN FUNGUS PENICILLIUM SCLEROTIORUM LM 5679

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A number of synthetic dyes adversely impact environmental and human health. Fungi however, are an alternative source of pigments that are produced as secondary metabolites and can be used as dyes (many of which are innocuous). We recently isolated and characterized a fungus from Amazonian soils - Penicillium sclerotiorum LM 5679 and carried out the current study to assess its potential for pigment production. Studies in other species suggest that secondary metabolite production can be upregulated under cellular stress, so we cultured LM 5679 under a number of mechanistically distinct stresses: pH, temperature, glycerol concentration (water activity), and NaCl concentration (osmolarity). We also varied inoculum size, light and aeration (agitation). All media were based on Czapech Dox broth or modified Czepech Dox broth (modified by change of pH or supplementation with glycerol or NaCl). Control flasks contained medium adjusted to pH 5 and were inoculated (104 cells/mL) and incubated at room temperature (25 ± 2°C) for 14 days in the dark, without shaking. The experimental treatments were: pH (3; 6; 9); temperature (5; 35 and 45 oC); glycerol

(0.5; 1; and 3 M), NaCl (3, 6; and 12 % w/v), inoculum size (102, 103, 106 cell/mL); light exposure and orbital agitation (50, 100; and 150 rpm). Biomass production was quantified at 15 days according to dry weight (g/L) and gross production of pigments was evaluated at 15 days by spectrophotometry (350 nm). Biomass was highest under the following conditions pH 3 and 9; 25oC; without glycerol; moderate to high NaCl (6 and 12 % w/v); small inoculum size (1x103); exposure to light; and shaking (50 to 150 rpm). Pigment production was optimal under the following conditions: pH 5; 25oC; without glycerol or NaCl; inoculum size of 104 and 106 cell/mL; darkness; and without orbital agitation. We discuss the ecological implications of these findings, and the potential to use stress to optimize pigment production for industrial bioprocesses.

# 4. FUNGAL BIOLOGY IN EXTREME ENVIRONMENTS

## AIRBORNE FUNGAL SPORES ASSEMBLAGES FROM ITAPUÃ STATE PARK, SOUTH OF BRAZIL.

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Fungal spores are biological particles (palynomorphs) between 2 and 50 µm in diameter and are usually present in the air, influenced by the environmental context of their place of origin (climate and vegetation). The air currents carry palynomorphs on large surfaces and local rainfall occurs in the form of pollen rain. This work aims to present qualitative and quantitative data on the occurrence of fungal spores in a pollen rain sample at Itapuã State Park, located in the metropolitan region of Porto Alegre, State of Rio Grande do Sul, in order to characterize the conditions of its occurrence, considering both aspects related to the dispersion, determined by the climate conditions and the types of vegetation cover around the collectors, between forest areas and the human-modified field. The park is a conservation unit and shelters natural environments (Atlantic forest, rocky fields, coastal forest, "vassoural," mixed grassland, moist, bathed and juncal plains.) of the metropolitan region of Porto Alegre. The predominant climate is humid subtropical. The collection was taken using an Oldfield artificial collector along atransect with 20 points, but only 10 points remained until the end of the collection, which is the aim of the analyses. The qualitative analysis was performed using an optical microscope (Nikon E200) at 400X and 1000X magnification, taking into account morphological criteria (shape, number of cells, septa, ornamentation and size). The count considered the minimum number of 200 morphotypes per slide (corresponding to each collection point). In addition, the spatial distribution of the occurrence of morphotypes in relation to the collection points was performed by multivariate exploratory analysis, consisting of PCA (Principal component analysis) and NNA (Neural network analysis). Moreover, 8645 morphotypes were identified. The amerospores (unicellular) were dominant, between 32.1% and 75.8%. The fragmospores (septate) occurred between 40.4%

and 8.4 and the didimospores (two cells) occurred between 42.3% and 10.8%. Other groups, including staurospores, are less significant, below 5%. The PCA analysis identified different morphotypes. A group of forest areas (LPE9-amerospore, LPE 36didimospore) and two groups of the human-modified field (LPE7-amerospore, LPE35-dymospore) and (LPE22-amerospore; LPE55-fragmospore). The NNA indicated the preferential spatial distribution of the morphotypes: amersopores and didimospores occurred in both areas; fragmospores, staurospore /others preferably in the human-modified field and dictiospores in the forest area. Based on the morphological comparison only, 28 morphotypes were related at the level of families and genera: Dacampiaceae (genus Munkovalsaria - didimospore), Halosphaeriaceae (genus Cirrenalia - helicospore), Tetraplosphaeriaceae (genus Tetraploa - estaurospore) Meliolaceae (genus Meliola - fragmospore), Montagnulaceae (genus Paraphaeosphaeria - didimospore), Pleosporaceae (genus Alternaria - fragmospore, Curvularia fragmospore, Pithomyces - dictiospore), Sordariaceae (genus Sordaria - amerospore), Xylariaceae (genus Rosellinia - amerospore), as well as genera Insertae Sedis (Spegazzinia - dictiospore and Torula -fragmospore). The Pleosporales order joined a greater number of families occurring in the studied pollen rain (Meliolaceae, Montagnulaceae, Pleosporaceae and Tetraplosphaeriaceae). The fragmospores (Meliola, Alternaria, Curvularia) and staurospore (Tetraploa) can be characteristic markers of human-modified field. Dictiospore (Pithomyces) can be characteristic markers of the forest area.

## FUNGAL SPORES FROM POLLEN RAIN SAMPLES: IPIRANGA STATE PARK, SP, BRAZIL

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Fungal spores can be found as components in the atmosphere, as well as with other biological particles such as pollen grains and plant spores, called palynomorphs. The variability, dispersion and transport of these particles occur through air currents depending on environmental conditions, such as the climate (temperature and humidity) and the vegetation cover of a geographic region, therefore their occurrence is associated to environmental factors. Due to this,

palynomorphs are widely used in paleoenvironmental and environmental reconstitution studies and aerobiology for air quality. This study aims to present preliminary data on the occurrence and dispersion of fungal spores based on analysing pollen rain in the Ipiranga State Park during an annual cycle from 20/09/08 to 20/03/09 (spring to summer) and from 20/03/09 to 25/09/09 (autumn to winter). The collection was carried out using artificial collectors of the Oldfield type between human-modified field (Points 1 and 3) and in forest areas (Points 2 and 4). Samples were processed using the traditional acetolysis method and then assembling slides. The morphotype description was based on specialised literature considering morphological criteria (number of cells, septa, colour, ornamentation and dimensions), using a microscope (Nixon E200), 400X and 100X magnification. For the count, a minimum number of 100 spores per slide was used. In the spring-summer period, in P1 were found: 71 amerospores, 48 didimospores, 54 fragmospores and 4 dicitiospores. In P2, 120 amerospores, 74 didimospores, 124 fragmospores and 14 dicitiospores were counted. In the autumn-winter period, in the P1 48 amerospores, 44 didimospores, 33 fragmospores and 10 dicitiospores were counted. Although the analyzes are in the initial phase, it is possible to verify some differences in the occurrence of spores in relation to environmental conditions. The partial results point to a decrease in the morphotypes in the autumn-winter period compared to the spring-summer period in P1. Regarding the vegetation cover type, it was observed that there was a greater occurrence of spores in P2, inserted in forest area, than in P1 located in human-modified field. The increase in the occurrence of fungal spores is shown in the forest area and in the springsummer period (wetter period), revealing conditions of higher humidity. In addition, in the P1 (human-modified field), amerospores dominated in both seasons, whereas in the spring-summer period the fragmospores were dominant in P2 (forest area). At the end of the analyses, it is expected to show the pattern of occurrence and distribution of fungal spore morphotypes better in relation to environmental conditions

## ISOLATION OF FUNGI FROM MANGROVE AND HYPERSALINE TIDAL FLATS AREAS

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Mangroves (MG) are forest ecosystems located at the interface between land and sea. Hypersaline tidal flats (HTF) are transitional ecosystems, that commonly border mangrove forests. Both areas perform important functions related to the maintenance of coastal biodiversity as well as support socioeconomic and cultural activities in local communities. Recent investigations have increased our knowledge on the extremophilic microorganisms from mangrove and hypersaline tidal flats areas. In order to preserve, restore, and better understand these ecosystems, researchers have been studying their microbiology, yet few surveys have focused on fungal communities. Fungi from these extremophilic environments represent a new source of several biomolecules. In this context, this study isolated extremophilic fungi from MG and HTF areas. These samples were obtained from JuréiaItatins Ecological Station, Sao Paulo, Brazil, in summer and winter seasons. Twenty gram sediment aliquots were placed in sterile flasks containing 180 mL of sterile saline solution with Tween 80. Then, the samples underwent manual agitation for 10 minutes. Once the samples were stirred, only the supernatant was used for the microbiological analyses. Next, serial dilution was carried out and 50 µL of sample material was inoculated in the center of Petri plates containing malt extract agar (MEA) medium. The samples were incubated at 30 °C for 7 to 14 days. After the incubation period, the growth of filamentous fungi colonies on the plates was determined based on the total number of mold colonies on each incubated plate. After that, each fungal colony was isolated and macroscopic and microscopic characterized. A total of 58 microorganisms were isolated. The greatest fungal genera occurred in MG, with the mainly presence of Aspergillus, Penicillium and Fusarium. Whereas, Aspergillus, Penicillium and Trichoderma fungal genera were found in HTF. The results showed that frequently isolated fungi belong to Aspergillus and Penicillium genera. Based on research

results, it can be considered that MG and HTF are important sources of new biomolecules. This research needs to be continued as an attempt of new drug discovery, which can be useful in health and environmental fields.

## MYCELIAL DEVELOPMENT RESPONSE OF TRICHODERMA CULTIVATED IN CULTURE MEDIUM WITH DIFFERENT CONCENTRATIONS OF TREATED DAIRY EFFLUENT

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Filamentous fungi of the Trichoderma genus are found in a wide range of ecosystems, and may favor cultures in resistance to pathogens, physiological responses and resistance to saline environments. The treated dairy effluent has as main characteristic of high saline and nitrogen concentration, besides lower concentrations of other nutrients, which makes it interesting for reuse in agriculture. Three Trichoderma species were used in this study: Trichoderma atroviride, Trichoderma asperellum and Trichoderma harzianum. These strains were evaluated for their resistance to saline environments in in vitro tests. In order to do so, the strains were cultured in potato agar dextrose (BDA) medium with increasing concentrations of treated dairy effluent, diluted in distilled water, which were: 0; 25; 50; 75 and 100%. These concentrations generated mean salinity levels of 0.95; 2.24; 3.54; 4.79 and 6.03 dS m-1. The experimental design was performed in duplicate, in a completely randomized factorial (3x5), with three repetitions, each one consisting of a Petri dish. Petri dishes and culture media of each treatment were autoclaved for 20 minutes at 1 atm. The inoculum of the Trichoderma strains on the plates was made from 5 mm disks of BDA culture medium containing the active fungus transferred in a sterile way to the Petri dishes. The plates were maintained in a BOD incubator at 27 °C and 12 hours photoperiod. The mycelial growth was evaluated every 24 hours after the inoculation, measuring the colony diameter with a digital caliper. Measurements were made for three days, until the first Petri dish was completely taken by the fungal colony. Then the mycelial growth was calculated by the formula of the

mycelial growth rate index (MGRI): MGRI = S [(D-Db) / N], where D is the mean diameter of the colony on day N, Db is the diameter of the colony from the previous day and N is the number of days after inoculation. The results of the statistical analysis showed that the Trichoderma asperellum and Trichoderma harzianum strains had no significant difference between them in the different effluent concentrations tested. Trichoderma atroviride presented lower MGRI in almost all the effluent concentrations, except of the treatment 100% effluent, in which there was no statistical difference between the studied strains. The tree strains had linear decrease of the MGRI as the effluent concentration in culture medium increased, presenting $(R)^2$ correlation coefficients upper to 89%. The results obtained show that the studied strains of Trichoderma are capable of developing in environment salinized by treated dairy effluent.

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# 5. IONIZING RADIATION, HEAT, AND OTHER STRESSES IN FUNGAL BIOLOGY

## EFFECT OF COLD ATMOSPHERIC PRESSURE PLASMA ON CANDIDA ALBICANS AND AGGREGATIBACTER ACTINOMYCETEMCOMITANS DUAL SPECIES BIOFILMS

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Candida albicans is a dimorphic fungus, a human commensal microorganism found in the gastrointestinal tract, vagina and oral cavity. It is an opportunistic species that affects immunocompromised patients and can be responsible for life-threatening systemic disease. Mortality rates associated with systemic candidosis are reported to be 30% higher than those associated with bacterial infections. Aggregatibacter actinomycetemcomitans is a non-motile, Gram negative coccobacillus, which can be found as a commensal species in the oral cavity. In addition to periodontal diseases, pathogens are directly linked to cases of endocarditis, premature delivery, low birth weight, and loss of organ transplants. A significant proportion of human infections are biofilm associated, wherein the formation of mixed-species biofilms could perpetuate a protective environment. The aim of the study was to investigate the behavior of dual species biofilms formed by Candida albicans ATCC 18804 and Aggregatibacter actinomycetemcomitans ATCC 29523 under stress produced by Low Temperature Atmospheric Pressure Plasma - LTAPP. The LTPP system was flushed with helium (99.5% purity) and the gas flow rate was adjusted in 2.0 SLM, with the parameters of 32 kHz and 1.0W and voltage amplitude of 13.0 kV. The distance between nozzle exit and bottom of the well was kept fixed to 1.5 cm. Biofilms were formed from fresh cultures of 24 hours in 96 wells microplates. Plates were incubated at 37°C for 24 hours, under aerobiosis, in tryptic soy broth after a pre adhesion of 90 minutes. After, biofilms were exposed to plasma plume

for 1.0, 2.5, 5.0 and 7.5 minutes. Then, biofilms were washed with 200 µL sterile saline solution. Resultant suspensions were serial diluted and plated on Sabouraud dextrose agar supplemented with chloramphenicol and tryptone soy agar to determine the colony forming units of C. albicans and A. actinomycetemcomitans, respectively. Control group was not exposed to plasma plume. The experiment was performed in triplicate. LTAPP exposure for 2.5 min did not reduce microbial counts. After 5 minutes of exposure, reductions in C. albicans (6.25 x 104) and A. actinomycetemcomitans (7.75 x 106) counts in relation to control (1.75 x 108 and 1.32 x 105, respectively) were detected. Reductions were also detected after 7.5 min of exposure. We concluded that ROS and other reactive species of LTAPP inhibited mixed biofilms of C. albicans and A. actinomycetemcomitans, reducing the number of viable cells from 5 minutes of exposure.

Keywords: cold plasma, reactive species, fungi, bacteria.

## SAKA AND MPKC STRESS MAPKS SHOW OPPOSITE AND COMMON FUNCTIONS DURING STRESS RESPONSES AND DEVELOPMENT IN ASPERGILLUS NIDULANS

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Stress activated MAP kinases (SAPKs) of the Hog1/Sty1/p38 family are specialized in transducing stress signals. In contrast to what is seen in animal cells, very few fungal species contain more than one SAPK. Aspergillus nidulans and other Aspergilli contain two SAPKs called SakA/HogA and MpkC. We have shown that SakA is essential for conidia to maintain their viability and to survive high H2O2 concentrations. H2O2 induces SakA nuclear accumulation and its interaction with transcription factor AtfA. Although SakA and MpkC show physical interaction, little is known about MpkC functions. Here we show that ?mpkC mutants are not sensitive to oxidative stress but in fact MpkC inactivation partially restores the oxidative stress resistance of ?sakA mutants. ?mpkC mutants display about 2-fold increase in the production of fully viable conidia. The inactivation of the SakA upstream MAPKK PbsB or the simultaneous elimination of sakA and mpkC result in virtually identical phenotypes, including decreased radial growth, a

drastic reduction of conidiation and a sharp, progressive loss of conidial viability. SakA and to a minor extent MpkC also regulate cell-wall integrity. Given the roles of MpkC in conidation and oxidative stress sensitivity, we used a functional MpkC::GFP fusion to determine MpkC nuclear localization as an in vivo indicator of MpkC activation during asexual development and stress. MpkC is mostly localized in the cytoplasm of intact conidia, accumulates in nuclei during the first 2 hours of germination and then becomes progressively excluded from nuclei in growing hyphae. In the conidiophore, MpkC nuclear accumulation increases in vesicles, metulae and phialides and decreases in older conidia. Oxidative and osmotic stresses induce MpkC nuclear accumulation in both germinating conidia and hyphae. In all these cases, MpkC nuclear accumulation is largely dependent on the MAPKK PbsB. Our results indicate that SakA and MpkC play major, distinct and sometimes opposing roles in conidiation and conidiospore physiology, as well as common roles in response to stress. We propose that two SAPKs are necessary to delay (MpkC) or fully stop (SakA) mitosis during conidiogenesis and the terminal differentiation of conidia, in the highly prolific phialoconidiation process characteristic of the Aspergilli.

# 6. FUNGAL STRESS IN AGRICULTURE: INCLUDING BIOLOGICAL CONTROL OF INSECT PESTS

## ANTAGONISTIC ACTIVITY OF BACTERIA FROM QUADRILÁTERO FERRÍFERO, MINAS GERAIS, TO COLLETROTRICHUM LINDEMUTHIANUM

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Anthracnose is caused by the fungus Colletotrichum lindemuthianum that is commonly controlled by fungicides. However, recent studies have demonstrated progressive resistance of this pathogen induced by the frequent use of these compounds. This phenomenon evidences the importance of using biological controllers for this phytopathogen. In this context, this study aimed to investigate 18 bacterial isolates obtained from Quadrilátero Ferrífero in Minas Gerais state, plants as potential biocontrolers of C. lindemuthianum. These isolates were grown for 24 hours in liquid LB medium under agitation of 180 rpm. The optical densities were adjusted to 0.5 and the isolates were then confronted with C. lindemuthianum previously grown for 10 days on PDA in the dark at 26 °C ± 2 °C by the following assays: direct inhibition, indirect inhibition by volatile compounds, inhibition mediated by non-volatile compounds; and inhibition after thermostability assay. For direct inhibition assays, the bacterial isolates were transferred to the center of petri plates containing PDA forming a square with 3 cm edge. Next, a mycelial disc (? 6 mm) was placed at the central portion of the bacterial square. To analyze the antagonist potential of volatile compounds by indirect inhibition, plates with three septa were used, the mycelial discs were inoculated into one of the septa, 10 µl of the isolates in another septa and one remained empty. The plates were conditioned with the lid upwards. To test inhibition by indirect non-volatile

compounds, 200 µl of sterile strains were inoculated on cellophane previously positioned above the PDA medium in Petri plates. After growth, the cellophane containing bacterial colonies was removed and mycelial disc was added on the surface of the culture medium. For the thermostability assay, 200 µl of the isolates were subjected to heat treatment 80 °C for 5 min and then inoculated in the cellophane in the same conditions described above. For all assays the plates were sealed and placed in BOD in the dark at 26 °C ± 2 °C for 10 days and mycelial growth was measured with a digital pachymeter at the end of this period using a control as reference. Of the 18 isolates investigated, five were distinguished by inhibiting between 75 and 90% of C. lindemuthianum growth. Isolated 10 showed the greatest antagonistic potential to this pathogen by direct inhibition method. Moreover, this bacterial isolate demonstrated thermostability of the inhibitory compound inducing a 90% reduction of the phytopathogen mycelial growth. In addition, four other isolates showed inhibition between 85 and 90% by direct contact, but also showed thermosensitive except for one isolate, in which the bacteria showed thermostability, but not the inhibitory compound. In all cases, the antagonism mediated by volatile compounds were insignificant (7 and 12%). It was verified that environmental isolates from a geosystem that lack studies could be efficient controllers of C. lindemuthianum growth in vitro, allowing to establish a proof of concept for biological control tests of this phytopathogen in the field. The identification of the isolates was preserved for the purpose of intellectual property protection.

## BIODIVERSITY AS RESOURCE FOR THE DEVELOPMENT OF MICROBIAL-BASED INSECTICIDES IN SUSTAINABLE AGRICULTURE AND FORESTRY

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Entomopathogenic fungi are globally distributed and can be found in different agro-ecosystems. Several investigations underline the complexity of function and effect of specific microorganisms in agrosystems. Today several national and international programs exist in subjects such as agriculture, but when it comes to biological control, there are no specific working groups that bring together

researchers from Brazil and Germany. For that, the upcoming bilateral project Bio-Entosource (Biodiversity as resource for the development of microbial-based insecticides in sustainable agriculture and forestry) aims to assess the biodiversity of entomopathogenic fungi in two different climatic zones (Germany and Brazil) in one model crop (soybean), apple and in eucalyptus as a resource for the development of microbial control strategies for sustainable agriculture and forestry to promote the use of microbial biodiversity for diverse germplasm to enable a better assessment of species potential. A special focus in this project is laid on the natural and functional biodiversity of entomopahtogenic fungi. For that, the occurrence of entomopathogenic fungi in both environments (annual and perennial crop) and cropping systems (organic and integrated) will be investigated bilateral. To proof the biological activity of isolated entomopathogenic fungi and to generate more knowledge on like e.g. host specificity and virulence factors they will be tested against pest and beneficial insects and plant pathogens. To forecast consequences of climatic changes and to optimize the development of new sustainable biocontrol agent strains will be compared under different environmental conditions by modulating different environmental conditions under standardized laboratory conditions to understand adaptation mechanisms. After new entomopathogenic fungi were isolated, characterized and knowledge about specific survival strategies was be generated, the utilization of natural resources will be proven to determine the market potential of the selected strains in Brazil and/or Europe. Within Longterm bilateral partnerships the development of new bio-based strategies will be completed by institutional cooperation.

## BIOGENIC IRON NANOPARTICLE BASED ON TRICHODERMA HARZIANUM: SYNTHESIS, CHARACTERIZATION AND TOXICITY

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The Trichoderma harzianum is a mycoparasite fungus used in biological control due to its feature of promoting the plants growth. However, this fungus is sensitive to abiotic and biotic factors, such as, pH, humidity and ultraviolet radiation (UV), which may lead to a decrease in its efficacy. The biogenic metal nanoparticle synthesis may be an alternative to these problems, as these nanoparticles can provide protection for this fungus. Biogenic nanoparticles of iron in general are compatible with the organisms, presenting lower toxicity, which allows their application in the environment. Biogenic synthesis allows synergy between the chosen metal and the potential of the metabolites of the reducing organism. The aim of this work was to synthesize a biogenic iron nanoparticle (using FeCl3 as the precursor) based on T. harzianum, to perform its characterization using dynamic light scattering (DLS), microeletrophoresis and nanoparticle tracking analysis (NTA), to evaluate the biological activity against Sclerotinia sclerotiorum (white mold), the development kinetics of T. harzianum in the presence of the iron nanoparticles with and without UV exposition, its phytotoxicity through the germination assay using beans, lentil and tomato seeds. The MTT test and trypan blue test were used to evaluate cytotoxicity. The biogenic NPs had 207.3 nm of diameter, the polydispersity index was 0.45, zeta potential of 13.47mV, the concentration was 1.9x10e10 NPs/mL. Regarding the activity, the nanoparticles presented high control power of the phytopathogen Sclerotinia sclerotiorum. The growth kinetics assay of T. harzianum in the presence of biogenic nanoparticles showed that the nanoparticles accelerate the growth of Trichoderma. The nanoparticles did not interfere in the germination of the seeds and presented no cytotoxicity. Regarding the results of this study, it was possible to conclude that the nanoparticles had a great potential for the control of phytopathogens, without presenting toxicity to non-target organisms, allowing their use in agriculture.

## DISCOVERING NEW INHIBITORS OF THE ALTERNATIVE OXIDASE ENZYME (AOX) FROM MONILIOPHTHORA PERNICIOSA, CACAO'S PATHOGEN, USING EXTRACTS FROM NEONECTRIA DITISSIMA-STUD

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The basidiomycete fungus Moniliophthora perniciosa is a pathogen that is responsible for one of the most devastating plant diseases in cacao trees in Brazil and Latin America, the Witches' Broom Disease. M. perniciosa has an important mitochondrial enzyme called Alternative Oxidase (AOX) that is a crucial survival factor and is an important mechanism for fungal resistance to antifungal agents during disease development - especially the biotrophic phase. The ascomycete fungus Neonectria ditissima (syn Nectria Galligena) is known to produce natural compounds that inhibit the AOX from Trypanosoma brucei, such as Collectochlorin B and Ascofuranone. In this study, we aim to evaluate extracts from N. ditissima, in order to detect their potential effect as inhibitors of AOX from M. perniciosa. For that, N. ditissima was grown in 1) malt extract medium; 2) meat extract medium and 3) potato dextrose medium (BD). Initial results from extraction and sample preparation were obtained.The liquid-liquid extraction from all the culture media tested and the solid-liquid extraction from the fungal mycelia were not efficient as a source of AOX inhibitors. This was evaluated in biological assays using the model Pichia pastoris, which has an endogenous AOX. Therefore, a new sample preparation and extraction method was tested. Freeze-drying the N. ditissima mycelium grown in malt extract medium was extracted with hexane and subsequently with methanol resulted in biologically active extracts against P. pastoris. Inhibiting the main electron pathway with Azoxystrobin (a known inhibitor of complex III) allowed us to evaluate the specific inhibition of P. pastoris AOX. For that, extracts were dissolved in sterile DMSO to the final concentration of 40 mg/mL. P. pastoris was grown in liquid YPG (1% yeast extract, 2% peptone extract and 1% glycerol) in rotatory shaker at 30ºC, in the presence of each extract (final concentration of 0.4 mg/mL) combined or not with azoxystrobin (0.5 mg/mL). Five microlitres of the saturated cell culture were transferred to 150 µL of fresh YPG medium added with each extract. Those cultures were maintained under agitation and the growth of P. pastoris was spectrophotometrically accompanied through

absorbance at 600 nm every 15 minutes during 72 hours. The raw data was processed using MatLab, and the growth curve from each culture was obtained. The freeze-dried extract restrained growth of P. pastoris using azoxystrobin as a inhibitor of the main electron pathway, resulted in specific inhibition of P. pastoris AOX, indicating that the new extraction method was successful. The further chemical and biological characterization of those extracts will provide valuable information and aid the development of a natural source of antifungal control agent against M. perniciosa.

## ENCAPSULATION OF TRICHODERMA HARZIANUM IN POLYMERIC MICROPARTICLES BY SPRAY-DRYING

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The Trichoderma harzianum can be used to attack pathogenic plant fungi of different species (Botrytis, Rhizoctonia, Sclerotinia, Pythium, Phytophthora and Fusarium). The mechanisms involved in Trichoderma spp. control of fungal pathogens are mycoparasitism, nutrient competition, rhizosphere competence, cell-wall degrading enzymes production, as well as induced defense responses in plants. This fungus is common used as biological control products, able to compete commercially with chemical products, that has caused a number of environmental problems. However, these biological control products showed low stability and low shelf-life. In this way, microencapsulation have been used by several researchers to provide in living cells with a physical barrier against the external factors from the environment. So, the main objective of this study was to optimize the preparation conditions of microparticles containing the fungus through the spray-drying. Thus, different matrices for fungus encapsulation were tested. For this, detailed research was carried out in literature in order to determine the best polymeric materials. The spray-dryer process parameters as flow rate of drying air, outlet temperature, as well as, polymers and fungus concentration were changed. The experiments were performed in a spray drying LM MSD 1.0

(Labmaq) and the particles droplets were produced through a pneumatic nozzle (0.7 mm). The results showed that changes in temperature as well as drying air flow rate significantly influenced the recovery yield of formulations. For the microparticles based on the alginate polymer in the concentration of 2%, the yield increased by 8%, after increasing the drying air flow rate. Gum arabic was also tested in the experiments at a concentration of 20% achieving yield greater than 38%. Initial results showed that the formulation containing Trichoderma harzianum (5%), gum arabic (20%), outlet temperature of 70 °C and drying air flow at 1.60 m3/min resulted in the best yields. SEM analyzes showed that the majority of the particles had spherical morphology, with sizes ranging from 5 - 40 µm.By the way, studies are being carried out to determine the viability of formulations produced by spraydrying as well as the potential applications of this system in crop protection.

## ENCAPSULATION OF TRICHODERMA HARZIANUM TO INCREASE ITS EFFECTIVENESS IN BIOLOGICAL CONTROL

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For many years, the control of pests and diseases in agriculture has been achieved using chemicals. However, excessive use of these has caused a number of environmental problems. An alternative to this would be the use of biological control, which is considered an efficient practice in pest control. The aim of this study was to encapsulate T. harzianum in alginate microparticles as well as characterize the system using physical and chemical methods and also evaluate the inhibitory potential of this system against S. sclerotiorum. In addition, the phytotoxicity in tomatoes and beans were investigated. The microparticles were prepared by ionic gelation methodology and the characterization was done by measuring the size distribution and morphology of the particles with fungi by scanning electron microscopy (SEM). The inhibitory potential was performed by in vitro antagonism assays, where the microparticles and the pathogen were placed on opposite sides of the Petri dish and incubated at 27 °C. The phytotoxic assay was performed with beans and tomatoes, evaluating shoot and root

measurements. As results, the alginate microparticles showed a mean size of 2000 µm and spherical morphology. The surface of the particles changed with the amount of fungi used. In vitro antagonism studies showed that microparticles containing the fungus were able to provide greater control of S. sclerotiorum, presenting classification 2 (scale of 1 to 5, according to Bell et al., (In vitro antagonism of Trichoderma species against six fungal plant pathogens. Phytopathology 72:379–382, 1982) and there was no sclerotia formation. In addition, phytotoxicity studies did not show significant differences in root and shoot measurements indicating no phytotoxicity in the tested cultures. As conclusions, the results showed that T. harzianum was successfully encapsulated and that the system was able to increase the bioavailability of the fungus, hence contributing to increased effectiveness of biological control.

## NATURAL OCCURRENCE OF ENTOMOPATHOGENIC FUNGI IN APPLE ORCHARDS IN GERMANY

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In the project “Biological control as ecosystem service in integrated and organic pome fruit cultivation” we investigated the occurrence of entomopathogenic fungi (EPF) and evaluated their controlling function as natural antagonists. Therefore, soil samples were collected three times a year in spring, summer and autumn from 2016-2018. The sampling took place in three of the main apple growing regions in Germany. These regions are located in the North (Altes Land), the Center (Kraichgau) and the South (Lake Constance) of Germany. The samples were collected in integrated as well as in organic managed orchards and additionally in orchards with very few or without pest management or plant protection.
Using the Galleria bait method by Zimmermann (1986) and a modified version with Tenebrio molitor, the soil samples were examined for the occurrence of entomopathogenic fungi of the genera Beauveria, Isaria and Metarhizium. Furthermore, different tests were conducted with three of the isolated

entomopathogenic fungi to describe their attributes. In tests on agar plates, we examined the influence of three chemical-synthetic fungicides, one copper and one sulfur based fungicide and one herbicide on entomopathogenic fungi. All of these pesticides are commonly used in apple orchards and therefore a negative impact on the occurrence of entomopathogenic fungi cannot be excluded. Additional bioassays were conducted with three fungal strains to test their virulence against pest insects of apple. The results show a considerable regional difference in the occurrence of entomopathogenic fungi. In southern Germany the genus Metarhizium was dominating whereas in northern Germany mainly the genus Beauveria was isolated. Furthermore, no major seasonal effects were found. All of the chemical-synthetic fungicides had a negative impact on the tested entomopathogenic fungi, whereas the copper and sulfur based fungicide differed. No fungal growth was monitored when the Glyphosate-based herbicide was added to the agar medium. The impact on pest insects of apple orchards indicates that there is an ecosystem service of EPF in apple orchards.

## GROWTH TEMPERATURE AND UV RADIATION STRESS RESPONSES IN METARHIZIUM ANISOPLIAE STRAIN CPMA1502

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The entomopathogenic fungi Metarhizium anisopliae CPMa1502 was isolated by Cenipalma in Barrancabermeja, Santander (Colombia) from an infected fruit scraper insect of oil palm (Demotispa neivai) (Bondar) (Coleoptera: Chrysomelidae). Ecophysiological characterization (temperature and UVB radiation) was carried out with fungi conidia harvested from Petri dishes with MAYP (YM with potato extract) medium at the laboratory level. Conidia were exposed to four temperatures (20, 25, 30, and 35 °C) and UVB radiation for several exposure times between 2 to 60 min (302 nm, radiation of 0.28 kW/m2). The response variables were cell viability, conidia germination, conidial vigor, and radial growth rate for the two types of experiments. Under the four temperatures conditions, cell viability of the fungi was not affected significantly, showing percentages of vigor and germination above 96%. The radial growth rates at each temperature were not significantly different. Concurrently, cell viability and radial growth rate were not disturbed by the different exposure times of UVB radiation. Nonetheless, the germ tube formation decreased as the exposure time increased, especially with one hour of exposure (equivalent to 8 hours of solar radiation in field in the Barrancabermeja region). Under these conditions, the vigor was 66.02% and germination 79.49%. Thus, M. anisopliae CPMa1502 showed tolerance to high environmental temperatures (30-35 °C) and to long exposure times of UV radiation, conditions characteristic in Latin America tropical regions. Therefore, maybe its field application and efficacy as a biological control agent of D. neivai would not be affected by the environmental conditions in the areas where the oil palm is grown in Colombia.

# EFFECT OF ABIOTIC FACTORS ON VIABILITY AND CHARACTERIZATION OF METARHIZIUM RILEYI NM017

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Noctuid insects, Chloridea virescens and Helicoverpa zea, are the main pests in crops like soybeans, corn, and cotton, because they cause severe damage. These insects are widely distributed on the American continent, causing losses up to 50% of yearly production. Agrosavia isolated a strain of Metarhizium rileyi Nm017, and after experiments under laboratory conditions demonstrated an efficacy of 75.8% on Chloridea virescens and 92.5% on Helicoverpa zea. To evaluate the tolerance of M. rileyi Nm017 to abiotic factors, the conidia were exposed to different stress conditions of pH, temperature, and UVB radiation (UPC lamp, 3UV-38, 0.28 kW/m2 radiation). The response variables were conidia vigor, conidia germination, and sporulation (conidia/cm2). Neither medium variations in pH between 4 to 9, and nor temperature between 20 to 35 °C affected conidia viability. Nevertheless, under UVB radiation conditions that simulate two hours of exposure to solar radiation in the Orinoquia area (Colombian Llanos Orientales), the conidia vigor and germination percentages (<80%) were reduced, and sporulation increased to approximately twice that of the control (without exposure to UVB radiation).

Finally, M. rileyi Nm017 production was evaluated in YM (malt and yeast extract) and SMAY medium (Sabouraud Maltosa Yeast). M. riley Nm017 conidia from YM had a relative hydrophobicity of 92.8% and activity of protease 43.9 IU/mg and lipase 8.3 IU/mg dried conidia. In contrast, conidia from SMAY had a relative hydrophobicity of 83.7%, and a protease and lipase activity of 43.9 IU/mg and 2.8 IU/mg dried conidia, respectively.

## EFFECT OF SUBSTRATE COMPOSITION AND DRYING PROCESS ON METARHIZIUM RILEYI NM017'S CONIDIA QUALITY FOR CONTROL OF HELICOVERPA ZEA

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The drying process is an important step during the production process of biopesticides. Nonetheless, this process causes a stress condition associated to loss of water; resulting a decrease of enzymatic activity, viability, and virulence in case of entomopathogenic fungi. Thus, suitable conditions must be implemented through the entire process to improve desiccation tolerance and keep stable cells. Therefore, the aim of this work was to evaluate several medium compositions in solid-state fermentation, and determine the effect on viability, induction of enzymatic activity, and virulence of dried conidia. The mass production of M. rileyi fungi was carried out in five (T1-T5) substrates supplemented with different sources of nitrogen and micronutrients (vitamins and minerals), with T1 as a control medium based on rice. M. rileyi was incubated at 25±2 °C (40±10% Relative humidity) for 7 days, later, the substrate in trays was dried by convection at 25±2 °C, without air recirculation, until the moisture content decreased below 7%. Samples were collected before and after drying process. Results suggest that drying process had a negative effect on enzymatic activity, which was reduced in all substrates (37-47%). Nonetheless, the higher chitinase activity after drying was achieved in substrates T2 and T5 (3.9 and 4.2 IU/g dried substrate, respectively). The conidia harvested from all substrates before drying process caused a mortality of 90% on second instar of H. zea, and after drying, the virulence of conidia significantly decreased with efficacies of 20 and 60%. Thus, the culture medium composition influenced stability and virulence of conidia after drying process.

# 7. STRESS IN FUNGAL PATHOGENESIS

## ANTIFUNGAL ACTIVITY OF OZONIZED SUNFLOWER OIL IN DERMATOPHYTOSIS

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Dermatophytosis is a zoonotic skin disease caused by fungus, called dermatophytes, which is common in domestic animals. The infection is more common in hot and humid regions, as tropical and subtropical clima in dermatophytosis tes and it presents high incidence in these regions. The antifungal resistance is well-known worldwide and the search for alternative antimicrobial alternatives is welcome. In this context ozone may appear as an interesting approach and the combination of ozone with a natural oil may present a feasible alternative since the half-life of ozone is higher in oil medium. The objective of this study was to compare the antifungal efficacy of natural sunflower oil and its ozonized form in different concentrations in wild strains obtained from dogs diagnosed with dermatophytosis and in their ATCC analogous, and to establish the minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs). To identify the microorganisms on the skin, the lesions were scraped with a sterile scalpel from the edges and samples from 42 lesions were obtained. After microbiological identification, two types of fungi were identified (Microsporum canis and Trichophyton mentagrophytes), thereafter samples were obtained to a cell concentration of approximately 106 CFU/mL. Samples of M. canis (ATCC 36299) and T. mentagrophytes (ATCC 9533) were obtained in the same concentration. All samples were treated with fresh and ozonated sunflower oil. In order of determine the sunflower oil concentration in natura and ozonized for MIC and MFC determination, 10 different concentrations (0, 0.4, 0.8, 1.7, 3.2, 6.25, 12.5, 25, 50 and 100%) were tested. The biopsies

lesions showed that 54% of the dogs presented M. canis, 33% T. mentagrophytes and 13% presented both fungal species. On the analyses of ozonized oil the MICs and MFCs for wild type strains were 100% higher than the values obtained for ATCC 36299 and ATCC 9533 strains (wild M. canis – MIC= 12.5% and MFC=25%, ATCC strain MIC=6.25% and MFC=12.5%) and (wild T .mentagrophytes – MIC=6.25% and MFC=12.5% and ATCC strain MIC=3.12% and MFC=6.2%). However, for the pure sunflower oil the MICs and MFCs for strains (ATCC 36299) and wild M. canis are respectively 12.5% and 50% and 50% and 100%. Interestingly for the strains of T. mentagrophytes there was no difference between wild and ATCC strains neither in MIC nor in MFC (MIC 25% and 50% MFC). Therefore, ozonated sunflower oil showed higher antifungal efficacy compared to the oil in natura in all tested samples. According to our results the combination of a natural oil and ozone may present an efficient alternative to treat domestic animals infected by these fungi.

## ANTIFUNGAL AND ANTI-BIOFILM ACTIVITY OF DESIGNED DERIVATIVES FROM KYOTORPHIN

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The kyotorphin is an endogenous analgesic neuropeptide, L-Tyr-L-Arg (KTP), isolated in 1979 from bovine brain. Previous studies shows that some KTP derivatives possess anti-inflammatory and antimicrobial activity. Kyotorphin derivatives, designated KTP-NH2, KTP-NH2-DL, IbKTP, IbKTP-NH2, MetKTPDL, MetKTP-LD, were designed and synthesized to improve lipophilicity and resistance to enzymatic degradation. In this work, the selected presented KTP derivatives were tested to improve their antimicrobial and antibiofilm activity. The

antifungal screening of the KTP derivatives were ascertained in the representative strains of Candida species including the C. albicans ATCC 10231, C. krusei ATCC 6258, and six clinical isolates (C. dubliniensis 19-S, C. glabrata 217-S, C. lusitaniae 14-S, C. novergensis 51-S, C. parapsilosis 63, C. tropicalis 140-S) from the oral cavity of HIV-positive patients. KTP derivatives were synthesized by standard solution or solid-phase peptide synthesis and purified using RP-HPLC, which resulted in >95 % purity, and were and fully characterized by H-NMR. In our KTP screeening IbKTP-NH2 presented significant antifungal activity against Candida strains, with minimum inhibitory concentrations (MIC) calculated were between 500 to 1000 µM. The derivative IbKTP-NH2 also demonstrated antibiofilm activity against the tested biofilm of Candida clinical isolates, revealed by microtiter broth test and scanning electron microscopy (SEM). The absence of toxic activity and survival after infection was also assessed by derivatives injections using Galleria melonella as experimental infection model. Results reveal that IbKTP-NH2 beyond their known activities, also exhibit noteworthy antimicrobial activity against multidrugresistant bacteria and fungi with no toxicity. Moreover, also was observed an increase in the hemocytes cell of G. melonella infected with C. albicans and survival of species after IbKTP-NH2 treatment. We suggest that the derivative in question, in addition to the physical activity in the membranes observed through morphological changes in the, can provoke a cellular immune response and therefore could be designed for the biomedical application.

## APPLICATION OF PROBIOTICS FORMULATIONS TO PREVENT ORAL CANDIDIASIS: IN VITRO AND IN VIVO STUDY

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Probiotic encapsulation technology is an emerging field that has the challenges of maintaining the viability of probiotic cells in commercial products, as well as providing controlled release that result in the inhibition of pathogens, such as Candida spp. Candidiasis is a major fungal infection of the oral cavity and the increased antifungal resistance has led to the search for alternative antimicrobial treatments. In this context, the objective of this work was to develop probiotic formulations by encapsulating Lactobacillus paracasei 28.4 in gellan gum, aiming its application in the control of oral candidosis. After preparation of the probiotic formulations at various concentrations of gellan gum (0.5-1% w / v), cell viability monitoring under different storage conditions (4°C and room temperature) and release systems (PBS or saliva) for 7 days was evaluated. The ability of formulations to inhibit C. albicans was analyzed in vitro and in the oral candida model in mice. After analyzing the data (ANOVA and Tukey's test), it was verified that L. paracasei 28.4 remained viable in all gellan gum formulations, showing a 1.1-1.4-fold decrease of L. paracasei 28.4 viability. In addition, there was greater release of Lactobacillus when the formulations were in contact with artificial saliva (2.53x106 CFU/mL) in relation to PBS (5.33x103 CFU/mL, $p<0.001$). All formulations were able to inhibit in vitro the growth, biofilm and filamentation of C. albicans. In the in vivo study, only the formulation of 1% can promote colonization of L. paracasei 28.4 in the oral cavity of the mice. Consequently, only this concentration was tested in oral candidiasis, reducing approximately 3 logs of C. albicans CFU/mL when compared with the control group (C. albicans). Therefore, gellan gum presents potential as a biomaterial for probiotics encapsulation in the development of products for oral candidosis control, and the formulation of 1% w/v showed promising results, since this formulation was able to promote the colonization of L. paracasei 28.4 in the mice oral cavity, reducing C. albicans in oral infection.

## 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-), to maintain efficient CO2/HCO3- homeostasis. CO2 sensing and metabolism via CAs play important roles in the proliferation, survival and differentiation of diverse pathogenic fungi infecting human hosts. A. fumigatus has four CAencoding 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 ?cafA?cafB 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 ?cafA?cafB 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 it 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 virulence of A. fumigatus. The cipC gene (Afu5g09330) is involved in this adaptation process and is important for the virulence of A. fumigatus, making it a target for study of new therapies to treat invasive aspergillosis. Other genes, such as those encoding tyrosinase (Afu3g01070), HMG-CoA synthase (Afu8g07210), sugar transporter family protein (Afu7g05550), aldo-keto reductase putative (Afu4g11260), flavin-binding monooxygenase (Afu3g15050), oxidoreductase (Afu2g00750), and alpha 1,3-glucanase/mutanase (Afu1g03352), also had their expression altered when A. fumigatus was transferred to 5% CO2; however, the importance of these genes to the virulence has not been established yet.

## COOPERATION BETWEEN PROTEIN KINASE A (PKA) AND HIGH OSMOLARITY GLYCEROL RESPONSE (HOG) PATHWAYS IS AFFECTING CELL WALL CARBOHYDRATE MOBILIZATION IN ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is an opportunistic human pathogen causing allergic reactions or systemic infections such as invasive pulmonary aspergillosis especially in immunocompromised patients. The fungal cell wall is the main component responsible for recognition by immune system, due to the specific composition of polysaccharide carbohydrates exposed on the surface of the fungal cell wall called pathogen-associated molecular patterns (PAMPs). Key enzymes in the fungal cell wall biosynthesis are a good target for fungal drug development. This report elucidates the cooperation between HOG and PKA pathways and the mobilization of carbohydrates from storage for fungal cell wall biosynthesis. We suggest that the reduced mobilization of simple sugars causes defects in the structure of the fungal cell wall. Aspergillus fumigatus mitogenactivated protein kinases (MAPKs) are involved in maintaining the normal morphology of the cell wall and providing resistance against cell wall-damaging agents. Upon cell wall stress, cell wall-related sugars need to be synthesized from carbohydrate storage compounds. Here we show that this process is dependent of cAMP-dependent protein kinase A (PKA) activity and regulated by high osmolarity glycerol response (HOG) MAPKs SakA and MpkC. These protein kinases are necessary for normal accumulation/degradation of trehalose and glycogen, and the lack of these genes reduces glucose uptake and glycogen synthesis. The reduced glycogen synthesis was observed for SakA and MpkC mutants, which also displayed alterations in carbohydrates exposition on the cell wall. Carbohydrate mobilization is controlled by SakA interaction with PkaC1 and PkaR, suggesting a putative mechanism where the PkaR regulatory subunit leaves the complex and releases the SakA/PkaC1 complex for activation of enzymes involved in carbohydrate mobilization. In summary, we propose that SakA and MpkC are important for the modulation of PKA activity, therefore regulating the availability and mobilization of monosaccharides for fungal cell wall biosynthesis during cell wall damage and osmotic stress response. This work reveals the interconnection between HogA and PKA pathway for carbohydrate mobilization for cell wall construction.

# SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIS) ANTIFUNGAL ACTION AND SYNERGISTIC EFFECT WITH AMPHOTERICIN B AGAINST CRYPTOCOCCUS NEOFORMANS.

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Cryptococcus neoformans are yeasts that mainly affect immunocompromised patients and can cause meningoencephalitis depending on the immune status of the host. The conventional treatments available are facing challenges due to microorganism antifungal resistance, high toxicity and pharmacokinetic limitations. Thus, the objective of this study was to evaluate the antifungal effects of Selective Serotonin Reuptake Inhibitors (SSRIs), fluoxetine hydrochloride (CF) and paroxetine hydrochloride (CP) isolated and combined with amphotericin B (AmB) against C. neoformans. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined by broth microdilution according to European Committee for Antimicrobial Susceptibility Testing (EUCAST). Subsequently, the synergistic activity of SSRIs drugs combined with AmB was evaluated by the chessboard assay. The effect of the MIC concentration (1 x MIC); 2 folds the MIC concentration (2 x MIC) and 2 folds the synergistic concentration were evaluated in biofilms, quantifying the biomass by violet crystal and the effect in biofilm viability by colony forming units per milliliter. In addition, the effects of the drugs in sub-MIC concentration and synergistic combination were evaluated in C. neoformans capsule. The MIC concentration of CF and CP were 9.6 and 41µg/mL, respectively and MFC concentration were the same as MIC for both drugs. The combination of CF and CP with AmB resulted synergistic concentrations able to reduce until 16 folds MIC of isolated compounds and 8 folds of AmB MIC concentration. CF at MIC concentration and 2 x MIC were able to reduce 28% and 22% of biofilm biomass respectively. Biofilm treated with the combination CF + AmB was able to reduce UFC/mL in 10%. The treatment with

CP at 1 x MIC and 2 x MIC reduced biofilm biomass in 36% and 32% respectively. Synergistic combination CP + AmB were able to reduce the biofilm viability in 11%. In addition, we observed that drugs and combinations significantly decreased the size of the yeast capsules. CF and CP in sub-MIC concentration were responsible for the reduction of 65% and 63% of capsule size and the synergistic combination of CF + AmB resulted in 72% of reduction. Therefore, the evaluated drugs presented antifungal potential against C. neoformans and could be considered alternatives for the treatment of this pathogen.

## STUDY OF THE REGULATORY ELEMENTS THAT CONTROL THE UPTAKE OF AMINO ACIDS IN CRYPTOCOCCUS NEOFORMANS

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Opportunistic fungal infections caused by fungi such as Cryptococcus neoformans are a major public health problem. Fungal meningitis caused by this yeast, mainly in immunocompromised individuals, results in a high fatality rate. Lethality is mainly related to the few antifungal drugs, instability of the fungal therapies and the constant emergence of resistant strains. Therefore, novel antifungal therapies are required. Our research group, at Microbial Interactions Laboratory (LIMic), focuses on amino acid biosynthesis and uptake pathways as putative drug targets. Among the nutrient uptake pathways, we have studied the transcription factor Cys3 in C. neoformans, which seems to be an important regulator of sulfur uptake and sulfur amino acid biosynthesis. CYS3 deletion causes cysteine and methionine auxotroph, several growth defects and avirulence in Galleria mellonella animal model. In addition, immunoprecipitation followed by mass spectrometry and yeast two hybrid assay indicate that Cys3 is part of a protein complex involving two protein phosphatases, the calcineurin complex and Gpp2. In this work, GPP2 was deleted from H99 strain and phenotypic analysis showed that (i) gpp2? has a general reduced growth under nutritional deprivation (SD); (ii) is more sensitivity to saline (NaCl), osmotic (KCl) and alkaline stress (pH8) than

wild type; (iii) is sensitive to membrane and cell wall aggressive agents (SDS and Congo Red), respectively; (iv) has reduced urease, phospholipase and melanin activity and (v) has virulence attenuation in the invertebrate animal model G. mellonella. All together these data suggest that GPP2 is very important for survival under stress and virulence in C. neoformans. Currently, the global expression profile and the sub cellular localization of the gpp2? mutant are under investigation by RNAseq and by fluorescent microscopy, respectively. Its putative protein partners are being identified by the yeast two-hybrid assay. These studies may clarify the role of Gpp2 in metabolism, development and pathogenesis.

## THE ASPERGILLUS FUMIGATUS TRANSCRIPTION FACTOR RGLT IS CRUCIAL FOR GLIOTOXIN BIOSYNTHESIS, RESISTANCE TO OXIDATIVE STRESS AND VIRULENCE

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Aspergillus fumigatus is an opportunistic fungal pathogen that causes aspergillosis in immunocompromised individuals, a term collectively referring to a spectrum of lung diseases, whose severity depends on the type of underlying disturbance in the immune system. A. fumigatus expresses and secretes an array of immune-evasive and –modulatory determinants, including secondary metabolites (SMs), that contribute to enhancing fungal fitness and growth within the mammalian host. Gliotoxin (GT) is a SM that induces defects in the function and recruitment of neutrophils, a type of white blood cell that is crucial in fighting A. fumigatus infections. One of the strategies employed by neutrophils, aiming at getting rid of

invading A. fumigatus, is through the production of reactive oxygen species (ROS) that induce oxidative stress to the fungal cells. The aim of this work was to uncover previously uncharacterised transcription factors (TFs) that are crucial for conferring resistance against oxidative stress in order to further elucidate A. fumigatus oxidative stress resistance mechanisms. An A. fumigatus transcription factor (TF) deletion library was therefore screened for growth on allyl alcohol (AA)-induced oxidative stress, which led to the identification of one strain that was highly sensitive to increasing concentrations of AA. This strain also had significant growth defects in the presence of other oxidative stress-inducing compounds. RNA-sequencing in the presence of AA showed a significant down-regulation of gliT, encoding an enzyme required for GT biosynthesis and self-protection, in the TF deletion strain. Subsequently this TF was named RglT (regulator of gliotoxin T) and binding in the gliT promoter region was confirmed by ChIP-seq. Furthermore, GT secretion was abolished in the ?rglT strain, with this strain secreting only the bis-thiomethylated form of GT. In addition, the ?rglT strain lost all ability to protect itself from exogenously added GT. Lastly, RglT was shown to be essential for A. fumigatus virulence in a neutropenic and immunocompetent murine model of invasive aspergillosis, with the ?rglT strain soliciting a greater recruitment of white blood cells than when compared to the wild-type strain. In conclusion, this work identified a TF that directly regulates gliT, mediates resistance against GT and other oxidative stresses, and that is crucial for in vivo virulence.

# 8. STRESS IN POPULATIONS, FUNGAL COMMUNITIES, AND SYMBIOTIC INTERACTIONS

## A COMMON MYCORRHIZAL NETWORK MAINTAINS THE SOIL FERTILITY OF A SITE OF CAMPO RUSPESTRE OVER A QUARTZITE SUBSTRATE IN THE "SERRA DA CALÇADA" REGION (NOVA LIMA – MG)

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The "Serra da Calçada" , located in the municipality of Nova Lima in Minas Gerais State/Brazil (20°06'09.8"S S, 43°59'23.3"W), is formed by a rocky quartzitic outcrop belonging to the "Espinhaço" mountain range which is situated between 1462 and 1478 m above the sea level. This region hosts several headwaters and a native vegetation with high degree of biodiversity and endemism. However, these sites have been degraded by radical sports such as motocross and off-road racings, damaging the vegetation, the rocky outcrop and the headwaters that supply neighboring cities. Aiming at subsidize the restoration of one of these degraded sites, we proposed to study a preserved site located nearby in order to understand the natural patterns of plant distribution, soil fertility and their inter-relations. For that, we assessed the occupancy and abundance of plant families, soil chemical fertility, root mycorrhizal colonization and leaf N content across three blocks sizing 50m x 10m each. The most prevalent plant families were: Asteraceae, Fabaceae, Malpighiaceae, Melastomataceae, Orchidaceae and Poaceae. Soil was predominantly sandy with high levels of organic matter, P, N-NH4 and the latter showed a homogenous distribution across the site. In contrast, N-NO3 was found low and associated with organic matter pools. The leaf N content showed also an equitable distribution across plant families, except for the Fabaceae plants that presented a higher leaf N content likely due to their N-fixing ability. Such homogenous distribution of both soil and leaf N may be explained by

the harmonious arbuscular mycorrhizal fungi (AMF) root colonization (30-40%) found in all the studied plant families since AMF are able to transfer N among plants and organic matter patches via their extra-radicular mycelium. Besides, plants from Orchidaceae family were also found colonized by Orchid Mycorrhizal Fungi (OMF) belonging to the Basidiomycota phylum, which are known as greater organic matter decomposers. Therefore, the presence of the double infection by AMF and OMF in Orchidaceae suggests that this family plays a key role in the establishment of a common mycorrhizal network (CMN) likely involved in N transfer among organic matter, OMF, AMF and all the plants. In this way, this study suggests that such a CMN formed among mycorrhizal and plant species in the Campo rupestre of the Serra da Calçada plays a major role for the harmonious N fertility found in this site. Thus, we recommend the maintenance of arbuscular mycorrhizal associations in future rehabilitation efforts of degraded campo rupestre sites.

## ISOLATION OF PSYCHROTOLERANT, PSYCHROPHILIC AND XEROPHILIC FUNGI OF SOIL SAMPLES FROM AMAZON

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Psychrophilic microbes are abundant in cold environments, and xerophilic species are commonplace in low water-activity habitats. Here, we consider whether psychrophilic and xerophilic fungi might also occur in soils of the hot, humid Amazonian rainforest. The soil samples were collected from primary rainforest belonging to the grounds of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus-AM-Brazil (03°05' 38.29" S; 59° 59' 16.92" W) in March 2019. Soil samples were collected from a depth of 100 cm (claydominated subsoil) and from the surface (humus-rich topsoil), serially diluted in water, and used to inoculate nutrient media. For psychrotolerant and psychrophilic fungi, inoculations were carried out on potato dextrose agar supplemented with chloramphenicol (100

mg/L); incubations were then carried out at 5, 10, 15 and 25°C. For xerophilic fungi, inoculations were carried out on potato dextrose agar supplemented with chloramphenicol (100 mg/L) either without glycerol (0.998 water activity) or with glycerol at 3, 4.5 or 6 M (0.872, 0.811 and 0.745 water activity); and then incubated at 25°C. Petri plates were inspected daily for 14-day to check for fungal isolates. The number of colonyforming units (CFU) per gram were determined. From surface soil samples, one isolate was obtained at 5oC (33 CFU/g), 11 morphologically distinct isolates at 10oC (1x103 CFU/g), 18 morphologically distinct at 15oC (2x104 CFU/g), and 20 morphologically distinct at 25oC (5x104 CFU/g). From the 100-cm-deep soil samples, no isolates were obtained at 5oC, two morphologically distinct isolates at 10oC (5x102 CFU/g), eight morphologically distinct isolates at 15oC (2x103 CFU/g), and 10 morphologically distinct isolates at 25oC (1x104 CFU/g). From surface soil samples, no isolates were obtained at 6 M glycerol, seven morphologically distinct isolates at 4.5 M glycerol (300 CFU/g), 10 morphologically distinct isolates at 3 M glycerol (1x104 CFU/g), and 20 morphologically distinct isolates on media without glycerol (5x104 CFU/g). From the 100-cm-deep soil samples, no isolates were obtained at 6 M glycerol, two morphologically distinct isolates at 4.5 M glycerol (133 CFU/g), three morphologically distinct isolates at 3 M glycerol (200 CFU/g), and 10 morphologically distinct isolates on media without glycerol (1x104 CFU/g). These plates are still being incubated, so it is unclear as yet whether isolates will ultimately grow on the most-stressful xerophile (6 M glycerol) medium. Nevertheless, xerophiles are defined as those isolates able to grow at =0.850 (Pitt, 1975), so those already growing at 4.5 M glycerol are true xerophiles. Studies are now underway on the growth kinetics of these isolates to establish the extent of their psychro- and xerotolerance. Paradoxically, in these Amazonian soils that do not experience temperatures below 20ºC and are not known to dry out, psychrotolerant/philic and xerotolerant/philic fungi are present. As might be expected, however, these are more abundant in the biodiverse and saprotroph-rich surface soil.

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# SERENDIPITY IN THE WRESTLE BETWEEN TRICHODERMA AND METARHIZIUM

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The fungal species Trichoderma are frequently found in soil antagonizing plantpathogenic fungi as well as parasitizing plant-pathogenic nematodes. Metarhizium species are insect-pathogenic fungi that are used throughout world to control agricultural insect pests. In this study, we evaluated the effects of biotic stress of Trichoderma atroviride on Metarhizium robertsii. The tolerance of M. robertsii conidia produced under biotic stress were tested to osmotic stress (KCl), oxidative stress (menadione), UV radiation, and heat stress. A 72 h grown in the dark M. roberstii mycelial plug with 5 mm diam were adhered on four potato dextrose agar medium (PDA) 2 cm from the edge of the Petri dish. Then, four experiments with Trichoderma were done using a 5 mm diam mycelial plug grown in the dark for 24 h and adhered on the same PDA medium 2 cm from the edge of the Petri dish and 5 cm away from the Metarhizium plug. The treatments were: 1) Trichoderma inoculated at the same time with Metarhizium (A0). 2) Trichoderma inoculated two days after the inoculation of Metarhizium (A2); 3) Trichoderma inoculated four days after Metarhizium was inoculated (A4); 4) Trichoderma inoculated 6 days after Metarhizium was inoculated (A6); 5) M. robertsii grown alone on PDA medium (PDA - Control); and 6) M. robertsii grown alone on minimal medium (Czapek medium without sucrose) (MM). Conidia produced on MM were the most tolerant to all stress conditions. For osmotic stress, conidia produced in treatment A4 were the most tolerant, followed by conidia produced in treatment A6. Both A4 and A6 were more tolerant than conidia produced in the control treatment. For oxidative stress, conidia produced in both treatments A4 and A6 were similarly tolerant and more tolerant than conidia produced in the control. For thermal stress, conidia produced in treatments A4, A6, and control (PDA) were similarly heattolerant. For UV-B stress, conidia produced in treatments A4 and A6 were equally tolerant, but more tolerant than conidia produced in the control. For all stress conditions, conidia from the treatments A0 and A2 were not viable. The

germination speed of conidia produced in all treatments, A0, A2, A4, and A6 was also tested. Conidia produced on MM germinated faster than the other treatments. Conidia produced in the treatment A4 were the second fastest, followed by conidia produced in the treatment A6. Both treatment A4 and A6 conidia germinated faster than conidia produced in the control treatment. Conidia produced in the treatments A0 and A2 did not germinated until 24 hours. In conclusion, growth of M. robertsii produced under biotic stress (i.e. A4, and A6) also produced conidia more tolerant to osmotic stress, oxidative stress, and UV-B radiation. Although conidia of M. robertsii produced in the treatments A0 and A2 were not viable, the colonies of Metarhizium were able to form a mycelial barrage to stave off opposing fungal mycelia of Trichoderma. Therefore, Trichoderma was not able to run over Metarhizium colony like it does most other plant pathogens.

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