Abstracts of Presentations

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Species of the filamentous-ascomycetes genus *Trichoderma* are among the most commonly isolated saprotrophic fungi. They are frequently found in soil and growing on wood, bark, other fungi and innumerable other substrates, demonstrating their high opportunistic potential and their adaptability to various ecological conditions. The main mechanism for survival and dispersal of *Trichoderma* is through the production of conidia (asexual spores) that has evolved with several sophisticated molecular mechanisms to respond to environmental cues that trigger conidiation. This developmental process is induced not only by nutrient depletion, but also by light and mechanical injury. In this sense, although light responses in fungi are reasonably well understood, information on their response to injury is extremely limited. The response to injury is of particular interest because we have recently discovered the existence of a mechanism in *T. atroviride* conserved in plants and animals. We have used biochemical and functional genomics approaches to study injury-induced conidiation in *Trichoderma*. High-throughput RNAseq allowed us to identify genes responsive to this stimulus. Interestingly, functional classification of injury responsive genes suggested the involvement of reactive oxygen species, increases in intracellular calcium and the activation of calcium signaling pathways; as well as, the participation of lipids and activation of the cell cycle. Indeed, this mechanism involves the production of reactive oxygen species (ROS) by the NADPH oxidase complex, since Nox1 and NoxR mutants are affected in conidiation in response to damage. Based on these data, we have proposed a model for the signaling cascades that participate in this process involving MAPKs. Accordingly, mutants in two of the three MAPK genes present in the *T. atroviride* genome present major defects in conidiation induced by injury. Given the observed transcriptional response to injury and the existence of the highly conserved mechanism of regulation of gene expression based on RNAi in *Trichoderma*, we decided to analyze the role of the components of the RNAi machinery in this process. Analysis of gene replacement mutants in all components of the machinery revealed that Dcr2 and Rdr3 play a major role in conidiation induced by injury. Transcriptome analyses of the Δdcr2 mutant in response to injury revealed major differences in transcription of genes involved in activation of the cell cycle, as well as in mRNA levels of genes encoding ROS scavenging proteins.
Looking back upon 50 years of research.

Anita Panek

Retiree from Departamento Bioquímica, Instituto de Química, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

From the role of a mere storage carbohydrate, trehalose was lifted to the rank of a cell membrane protector and protein stabilizer, then as free radicals came into focus, trehalose came to be considered as a general preserver of cellular integrity during severe environmental stresses.

Metarhizium: An Outdoor Survival Guide

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Metarhizium spp. fungi are being developed as biofertilizers and as agents for the biocontrol of many insect pests including those that vector human disease. Their potential stems from a versatile genetic tool-kit, which has evolved to endure the myriad environmental stresses they encounter in the soil, on plant roots and in insects. Understanding and harnessing the evolvability of Metarhizium spp. is essential to the successful implementation of natural and genetically modified fungi in the field. Research has already elucidated many of the strategies these fungi employ to persist in an ever-changing world. As we continue to uncover the complex mechanisms Metarhizium spp. use to overcome short and long term environmental stresses, we are applying current knowledge to advance efforts to control mosquito populations in a semi-field trial in Burkina Faso and to mitigate potential risks in the application of Metarhizium spp. as plant growth-promoting fertilizers. This work provides lessons and a guide for the exploration and use of fungi to address relevant world problems.

The implications of increased UV-B radiation on microbial control of insects

Donald W. Roberts
Although not universally accepted in the popular press, there is a very large body of scientific and anecdotal evidence that supports the concept that atmospheric changes on earth have occurred in recent years, and that their negative effects are increasing over time. In addition to precise documentations by scientific teams, global warming is evidenced by obvious diminution of polar and high-altitude ice; and by somewhat erratic but significant rises in ocean and land surface temperatures. Concurrently, and of great importance to our meeting on the effects of stress on fungi; UV-B irradiance is still high. Biological significance of UV levels depends on wavelength, with the biologically more active shorter wavelengths (i.e., UV-B) being most important. Reduction of CFC release worldwide has stabilized ozone layer depletion, which is beneficial because the ozone layer filters out most UV-B emitted by the sun. Nevertheless, warming of earth and ocean surfaces from CO2, methane, etc., is expected to increase another greenhouse gas, viz., water vapor. This will reduce stratosphere temperature, and thereby allow further ozone-layer depletion since this reaction, with its associated increase in UV-B irradiation of the earth (including fungi), is enhanced at low temperatures. The causes of fungal growth inhibition following UV-B exposure are myriad, and include DNA and cellular enzyme damage. Several speakers at this meeting will discuss this in detail. Naturally occurring epizootics of entomopathogenic fungi can be very devastating to insect populations; but, in many cases, mortalities are insufficient to afford plant protection. Brazil is the world leader in biological control of insects using entomopathogenic fungi. Ultraviolet radiation and the heat from the sun are the two main factors that reduce fungal viability in the field. Most species of entomopathogenic fungi are very susceptible to UV-B irradiation e.g., M. robertsii, M. anisopliae, Beauveria bassiana, Verticillium lecanii, Aphanocladium album, and Isaria fumosorosea. An exception is M. acridum, a species found in the deserts areas of Africa, Australia, and Mexico, which is somewhat tolerant to UV-B, and exceptionally tolerant to heat. There are a number of research areas that need to be explored before the promising potential entomopathogenic fungi for microbial control is realized. Among these are studies on the effect of environment on (a) viability, (b) disease induction, and (c) discovery of formulation additives that preserve viability and enhance virulence in the field. It is clear that significant levels of UV-B irradiation will be present on Earth for at least the next several decades and will impair insect biological control with entomopathogenic fungi. Therefore, for effective field use in the future, there is a serious need for studies on how to protect insect fungi from UV exposure by a) increasing their UV-B tolerance or b) physically protecting the fungi from exposure. Three approaches in the Roberts’ lab, in recent years, to address and/or obviate difficulties with UV-B have been to, a) seek new wild type isolates from nature in the hope of finding new fungi with high tolerance to UV-B; b) test adjuvants to prepare formulations to protect conidia from UV-B in the field; and c) investigate the potential of using insect pathogenic fungi in their recently discovered role as endophytes, since the plant tissue will afford considerable UV-B protection.
Phenotypic plasticity in stress tolerance of insect-pathogenic fungi

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The conidial tolerance of entomopathogenic fungi to heat and UV-B radiation is greatly influenced by alterations of the growth-environment. Physical, chemical, and nutritional conditions during mycelial growth can induce great variability in conidial tolerance. For instance, conidia of *Metarhizium robertsii* produced on insect cadavers of Lepidoptera and Coleoptera had the lowest conidial tolerance to UV-B radiation; conidia produced on potato dextrose agar medium amended with yeast extract (PDAY) had medium UV-B tolerance; and conidia produced on minimal medium (MM) had the highest UV-B tolerance. It was also found that conidia produced on MM were more heat tolerant than conidia produced on PDAY. Mycelial growth under high osmolarity and alkalinity medium also induced higher conidial tolerance to heat and UV-B radiation. Conidia produced on PDAY medium supplemented with NaCl or KCl induced higher heat tolerance and UV-B tolerance than conidia produced only on PDAY. Conidia produced on potato dextrose broth amended with yeast extract (PDBY) adjusted to pH 8.04 and pH 9.45 also had higher UV-B tolerance, but not heat tolerance. Osmolarity and alkalinity, however, induced a stress condition in the fungus that reduced conidial production. Physical conditions such as growth under illumination also induced both higher UV-B and heat tolerance but did not decreased conidial production. In conclusion, growth under certain nutritive or physical conditions induced higher tolerance to UV-B radiation and heat but in some cases the conidial yield was greatly reduced.

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Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin

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Life on Earth has always existed in the flux of ionizing radiation. However, fungi seem to interact with the ionizing radiation differently from other inhabitants of the Earth. Recent data show that melanized fungal species like those from Chernobyl’s reactor respond to ionizing radiation with enhanced growth. Fungi colonize space stations and adapt morphologically to extreme conditions. Radiation exposure causes upregulation of many key genes, and an inducible microhomology-mediated recombination pathway could be a potential mechanism of adaptive evolution in eukaryotes. The discovery of melanized organisms in high radiation environments, the space stations, Antarctic mountains, and in the reactor cooling water combined with phenomenon of ‘radiotropism’ raises the tantalizing possibility that melanins have functions analogous to other energy harvesting pigments such as chlorophylls.

**Elis C. A. Eleutherio**

**Regulation of the Saccharomyces cerevisiae trehalose synthase complex in response to heat stress**

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Trehalose, a non-reducing disaccharide formed by two glucose molecules linked by a 1α-1α bond, accumulates upon heat, cold or osmotic stress. This sugar stabilizes and protects membranes and proteins in several organisms, increasing their tolerance to adverse conditions. The most usual pathway of trehalose synthesis, found in the yeast Saccharomyces cerevisiae and also in many fungi, eubacteria, archaea, insects, and plants, involves two enzymes: trehalose-6-phosphate synthase (Tps1), which catalyzes the synthesis of trehalose-6-phosphate (T6P), and trehalose-phosphatase (Tps2), which dephosphorylates T6P to trehalose. The complex of synthesis in yeast also includes two other proteins, Tsl1 and Tps3, which seem to have regulatory functions. In this work, it was observed that absence of Tsl1 totally abolished the increase in Tps1 activity and accumulation of trehalose in response to a heat stress at 40°C/ 1h, whereas deficiency of Tps3 only reduced Tps1 activity and trehalose. In silico analyses showed that both Tps3 and Tsl1 contain putative phosphorylation sites for cAMP-dependent Protein Kinase (PKA), suggesting that Tps1 activity may be regulated by phosphorylation. Corroborating this idea, Tps1 in extracts of heat stressed cells was inhibited in vitro by ATP. Mg in the presence of cAMP. In contrast, cAMP-dependent phosphorylation had
little or no inhibiting effect on Tps1 activity in extracts of tps3 cells, indicating that the complex of trahalose synthesis is phosphorylated in Tps3. Furthermore, we observed that tps3 mutant strain accumulates a higher proportion of T6P in response to a heat shock. Contrary to WT strain, Tps2 activity was not induced in tps3 mutant. We also observed that T6P inhibits Tps1 activity. Taken together these results suggest Tps3 is an activator of Tps2. To perform this task, Tps3 must be non-phosphorylated. To stop readily trehalose synthesis during recovery from stress, Tps3 is phosphorylated by PKA, decreasing Tps2 activity and, consequently, increasing T6P levels which, in turn, inhibits Tps1. These results not only confirm the previous suggested roles for Tsl1 and Tps3, but also provide evidence for the mechanism by which Tps3 regulates the trehalose-synthase complex in exponentially growing heat shocked yeast cells.

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**Sex in Phycomyces, a way out of stress**

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Sex in the heterothallic Mucorales is a complex developmental process that results from the interaction of vegetative mycelia of strains of opposite sex. The zygospores were shown to be sexual structures at the beginning of the twentieth century. When placed for several months on a watery surface, those of Phycomyces blakesleeanus germinate and produce recombination products, the germspores, that return to the vegetative cycle. The zygospores of many Mucorales never germinated in the laboratory. This is particularly disappointing in the cases of Mucor circinelloides, a research organism with well developed molecular genetics, and Blakeslea trispora, used in industry. Observations on Phycomyces zygospores suggest a leap forward in the hope for a change of conditions. Thus, the formation is favored by cultivation on poor media at lower temperatures than those optimal for growth and is induced by acetic acid and other small carboxylic acids and by unknown substances from potatoes. The recombination process initially imposes a strong selection and then introduces abundant and varied mutations of all sorts, again an evolutionary adaptation to changing environments. The sexual process starts with an exchange of pheromones between mycelia of opposite sex growing near each other. These signals are apocarotenoids; other apocarotenoids stimulate carotene production, oxygen consumption and other processes and thus create an autocatalytic crescendo in sexual activities. The young zygospore contains thousands of nuclei of both sexes, but they disappear suddenly and usually a single diploid nucleus is formed. The reduction process involves repeated mitoses with nearly random chromosome losses. Each
zygospore eventually forms a germ sporangium with thousands of multinucleate germ spores. The nuclei in each germ spore are haploid and genetically identical and grow into vegetative mycelia. These are large coenocytes and may become heterokaryotic through spontaneous mutations. A few germ spores are aneuploid and grow into complex heterokaryons. Heterokaryosis is maintained through the vegetative spores, because these are multinucleate and pack random samples of the nuclei in the mycelium. New results on the ecology of Phycomyces explain the often perplexing observations in the laboratory as evolutionary adaptations required by its habitat, its role in nature and its dispersion mechanisms.

Characterization of Metarhizium species and varieties based on molecular analysis, heat tolerance and cold activity

Éverton Kort Kamp Fernandes

Identification of Metarhizium spp. currently relies primarily on DNA-based methods; but heat and cold exposures can be used as rapid tools to tentatively identify some important Metarhizium species complexes. Conidial suspension of Metarhizium spp. isolates from a wide range of sources exposed to wet heat (45 ± 0.2°C) for 8 h were divided clearly into two groups: 1) all isolates of Metarhizium from anisopliae [M. anisopliae sensu lato (Masl)] and flavoviride (Mf) complex with virtually zero conidial relative germination (RG); and 2) M. acridum isolates with high heat tolerance (ca. 70–100% RG). Conidia plated on PDAY medium and incubated at 5 °C for 15 days, however, demonstrated that RGs for Masl and Mac isolates were virtually zero, whereas the two Mf were highly cold active (100% RG). Conversely, no correlation was observed between molecular clusters of 53 Brazilian isolates of Beauveria bassiana sensu lato (s.l.) and their tolerance to heat or UV. High variability in conidial thermostolerance was found among the B. bassiana s.l. isolates after exposure to 45 °C for 2 h, as evidenced by low (0–20%), medium (20–60%), or high germination (60–80%). At low temperatures (5 °C), most of the B. bassiana s.l. isolates germinated well (ca. 100%). An attempt to correlate the latitude of origin with thermostolerance or cold activity indicated that B. bassiana s.l. isolates from higher latitudes were more cold-active than isolates from nearer the equator, but there was not a similar correlation for heat. Also, there was high variability in tolerance to UV-B radiation (7.04 kJ m⁻²) among the Brazilian B. bassiana isolates, ranging from virtually zero tolerance to almost 80% tolerance. In addition, bioassays with all 53 Brazilian B. bassiana isolates correlated with reports on their natural UVB tolerance, thermostolerance and cold activity singled out a few (five) with high potential for controlling the cattle tick Rhipicephalus microplus under field conditions. Ticks are controlled mostly by application of chemical products; but these acaricides have several negative side effects, including toxicity to animals, environmental contamination, and induction of chemical resistance in some tick populations. Laboratory studies clearly demonstrate that these
fungi can cause high mortality in all developmental stages of several tick species, and also reduce oviposition of infected engorged females. Tick mortality following application of fungi in the field, however, often is less than that suggested by laboratory tests, possibly due to many negative biotic and climatic factors, especially heat and solar radiation. To increase efficacy of fungal agents for biological control of ticks under natural conditions, several points need consideration, but particularly: 1) selection of effective isolates (viz., high virulence; and tolerance to high temperature, ultraviolet radiation and desiccation), and 2) investigation on advances in formulation of conidia that increase their persistence in the field.

**Francesc Posas**

**Control of gene expression and cell cycle progression by the Hog1 SAPK in response to stress**

Francesc Posas

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Exposure of cells to increases in extracellular osmolarity results in the activation of the Hog1 stress-activated protein kinase. Activation of these MAP kinases is required to generate a set of osmoadaptive responses essential to survive under high osmolarity. Adaptation to osmostress requires the induction of a large number of genes, which indicates the necessity to regulate several aspects of the cell physiology. Induction of gene expression is highly dependent on the presence of the MAP kinase, which suggests a key role for the HOG signaling pathway in the regulation of gene expression in response to osmostress. The MAPK also controls cell cycle. Here, the MAPK is able to modulate cell cycle delay in different phases which highlight the relevance of cell cycle control in response to stress.

**Gertien J. Smits**

**The simplest signal: protons as second messengers controlling cell division rate and more**

Gertien Smits, Rick Orij, Anna Zakrzewska, Azmat Ullah, Stanley Brul

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All organisms have evolved to cope with changes in environmental conditions, ensuring the optimal combination of proliferation and survival. Using the yeast deletion collection to study the acquisition of tolerance towards lethal conditions upon pre-exposure to mild stresses, we found an inverse correlation between mutant growth rate and stress survival. Stress resistance and acquired stress tolerance in S. cerevisiae are governed by a combination of stress-specific and general processes. The reduction of growth rate, irrespective of the cause of this reduction, leads to redistribution of resources toward stress tolerance functions, thus preparing the cells for impending change. In a different screen, we recently identified a novel aspect of this growth control, namely intracellular pH (pHi). The proton is the most coupled metabolite in the metabolic network, but its concentration is highly dynamic and responds to environmental change. pHi control requires not only vacuolar proton pumps, mitochondrial function, and many growth related functions. We show that pHi quantitatively controls yeast growth rate, likely as a second messenger: This control can be alleviated by single gene deletions, revealing that protons function as a signal controlling growth.

**Gilberto U.L. Braga**

**Antifungal photodynamic treatment as an alternative to control plant and human pathogenic fungi**

Gilberto U.L. Braga

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Increasing tolerance to currently used fungicides has stimulated the development of new strategies to control pathogenic fungi. Antifungal photodynamic treatment (APDT) is an alternative and promising antifungal discovery platform that can be used to control localized mycoses in plant and animal hosts or to kill fungi in the environment. The approach is based on the use of a photosensitizer (PS) that binds to the surface or preferentially accumulates in the target fungal cell. Subsequent exposure of the PS to light of an appropriate wavelength starts a photochemical process that produces several reactive oxygen species (ROS), such as peroxides and singlet oxygen, leading to non-specific oxidative damage and causing the subsequent death of the fungal cells without significant harm to the host. In comparison with currently used fungicides, the multiple and variable targets of reactive oxygen species reduce the chance of selecting tolerant microorganisms. An additional advantage of APDT is that it is able to kill both metabolically active and dormant or quiescent structures (such as conidia), unlike many conventional fungicides that kill only metabolically active cells. In this talk the state-of-
the-art antifungal photodynamic treatment to control plant and human pathogenic fungi will be presented and discussed.

Gustavo H. Goldman

Aspergillus nidulans signal transduction mechanisms for cellulase production and glucose sensing

Gustavo H. Goldman

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Despite recent advances in the understanding of lignocellulolytic enzyme regulation, less is known about how different carbon sources are sensed and the signaling cascades that result in the adaptation of cellular metabolism and hydrolase secretion. Therefore, the role played by non-essential protein kinases (NPK) and phosphatases (NPP) in the sensing of carbon and/or energetic status was investigated in the model filamentous fungus Aspergillus nidulans. Eleven NPKs and seven NPPs were identified as being involved in cellulase, and in some cases also hemicellulase, production in A. nidulans. The regulation of CreA-mediated carbon catabolite repression (CCR) in the parental strain was determined by fluorescence microscopy, utilising a CreA::GFP fusion protein. The sensing of phosphorylated glucose, via the RAS signalling pathway induced CreA repression, while carbon starvation resulted in derepression. Growth on cellulose represented carbon starvation and derepressing conditions. The involvement of the identified NPKs in the regulation of cellulose-induced responses and CreA derepression was assessed by genome-wide transcriptomics (GEO accession 47810). CreA::GFP localisation and the restoration of endocellulase activity via the introduction of the ΔcreA mutation, was assessed in the NPK-deficient backgrounds. The absence of either the schA or snfA kinase dramatically reduced cellulose-induced transcriptional responses, including the expression of hydrolytic enzymes and transporters. The mechanism by which these two NPKs controlled gene transcription was identified, as the NPK-deficient mutants were not able to unlock CreA-mediated carbon catabolite repression under derepressing conditions, such as carbon starvation or growth on cellulose. Collectively, this study identified multiple kinases and phosphatases involved in the sensing of carbon and/or energetic status, while demonstrating the overlapping, synergistic roles of schA and snfA in the regulation of CreA derepression and hydrolytic enzyme production in A. nidulans. The importance of a carbon starvation-induced signal for CreA derepression, permitting transcriptional activator binding, appeared paramount for hydrolase secretion.

Jan Dijksterhuis

Fungi and the Indoor Challenge
Fungi are present in every cubic meter of air inside human dwellings. This can be as survival structures as spores. In addition, fungal material as non-viable fungal cells and fragments of cells are present. The presence of these fungi is related to human health as a potential source of antigens causing allergy. Alternatively, defacement of indoor surfaces caused by fungal growth is an ongoing concern. Indoor fungal growth is expected to change the composition of the population of fungal species. Next generation sequencing studies suggest that the diversity of the mycobiota indoors is much higher than known from traditional detection methods. If fungal spores are able to deposit and conditions become conducive for growth, they germinate and form a fungal mycelium. This is not an easy task; nutrients are generally low and water availability is transient as it may drop below the levels that support fungal growth. Due to these restrictions, indoor fungal growth mostly occurs in another time scale than observed in the laboratory. However, fungi do grow in, most probably, a large proportion of human dwellings. This contribution reflects on the fungal indoor population, growth on surfaces, and the vital role of water.

Javier Avalos

**Biological roles of carotenoids in fungi**

Javier Avalos

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Carotenoids are terpenoid pigments widespread in nature, produced by bacteria, fungi, algae and plants. They are also found in animals, which usually obtain them through the diet. Carotenoids in plants provide striking yellow, orange or red colors to fruits and flowers, and play important metabolic and physiological functions, especially relevant in photosynthesis. However, their functions in non-photosynthetic bacteria, yeast and filamentous fungi, is less understood. The chemical heterogeneity of fungal carotenoids suggests a considerable functional diversification. However, they apparently do not play important physiological roles in the fungal cells, as indicated by their absence in some species and the normal growth exhibited by albino mutants in others. This communication summarizes the current knowledge on the functional basis for carotenoid production in fungi. Chemical analyses of carotenoids are available in many fungal species, but genetic and biochemical studies of their carotenoid biosynthetic pathways have been limited to few model systems, producing β-carotene (Mucorales, as Phycomyces, Blakeslea and Mucor), astaxanthin (Xanthophyllomyces) or neurosporaxanthin (Neurospora and Fusarium). The conjugated polyene chain of
Carotenoids provide chemical reactivity against oxidizing agents and free radicals that could be damaging for essential cell components. This reactivity makes the carotenoids efficient scavengers of reactive oxygen species. Accordingly, an increasing amount of observations supports protective roles of carotenoids against oxidative stress in different fungal species. The microorganisms have efficient enzymatic systems to deal with the generation of reactive oxygen species by basal metabolism, but the carotenoids seem to play a secondary protective role, possibly helping to alleviate the damaging effects on cell membranes, where they are presumably accumulated. In addition, the carotenoids are intermediary products in the biosynthesis of physiologically active apocarotenoids or derived compounds. This is the case of retinal, obtained from the symmetrical oxidative cleavage of β-carotene. Retinal is the light-absorbing prosthetic group of the rhodopsins, membrane-bound photoreceptors present also in many fungal species. In Mucorales, β-carotene is an intermediary in the synthesis of trisporoids, apocarotenoid derivatives that include the sexual hormones the trisporic acids, and they are also presumably used in the synthesis of sporopollenin polymers. In conclusion, fungi have adapted their ability to produce carotenoids for different non-essential functions, related with stress tolerance or with the synthesis of physiologically active by-products.

Jay C. Dunlap

Regulatory networks in fungi governing global responses to changes in light and time

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Most fungi are highly responsive to their immediate environment and use these responses to trigger pathways to cope with fungal stress. Sophisticated light sensors respond acutely to changes in the photic environment, and circadian clocks allow anticipation of repeating environmental changes. Neurospora has proven to be a tractable model for understanding the networks underlying these responses. In this organism, blue light is detected by FAD stably bound by the transcription factor WC-1, eliciting photochemistry that drives a conformational change in the complex of WC-1 and WC-2 (WCC) resulting in activation of gene expression from promoters bound by the WCC. Changes in light intensity are detected by a second photoreceptive protein, VVD, that again uses FAD as a chromophore (Chen, Dunlap & Loros, FGB 47, 922-9, 2010). The circadian system allows anticipation of recurring environmental changes, and comprises a negative feedback loop wherein the WCC, in the dark, drives expression of frq. FRQ stably interacts with casein kinase 1 and with FRH (a putative RNA helicase that does not function enzymatically in the clock), and after phosphorylation-mediated delays, the complex downregulates the WCC (Baker, Loros,
Light and stress: the photobiological system in *Neurospora crassa*

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Light is an indispensable energy source for life and also serves as influential environmental information of daily and seasonal time to organisms across kingdoms. In fungi environmental light induces stress resistance. Within the fungi, molecular mechanisms of light responsiveness are best understood in the model system *Neurospora crassa*. Six percent of the Neurospora genome is light inducible at the level of gene expression, controlled largely by the LOV domain transcriptional activator White Collar-1 (WC-1) in complex with White Collar-2 (WC-2). This acute response to light results in cascades of transcriptional activators and repressors that eventually control biological outputs including development and general metabolism. An additional LOV containing photoreceptor, Vivid (VVD) permits the organism to repress the initial light response and then respond to increasing light intensities, a process called photoadaptation, and also facilitates accurate entrainment of circadian rhythms. We are currently examining molecular interactions between VVD and WC-1 to understand this complex regulation of light regulated processes.

Polygenic analysis of multiple stress tolerance traits in *Saccharomyces cerevisiae* by pooled-segregant whole-genome sequence analysis

Johan M. Thevelein
Most traits of industrial importance in yeast and other industrial microorganisms are polygenic traits, i.e. traits determined by multiple genes acting together. Most quantitative traits for instance are polygenic. Genetic analysis of polygenic traits has been an important challenge for many years. Screening of S. cerevisiae strain collections has revealed a wide diversity for these complex traits, with natural strains often being superior for a specific desirable trait compared to industrial yeast strains.

We have developed pooled-segregant whole-genome sequence analysis to map all QTLs (Quantitative Trait Loci) determining a complex trait in a yeast strain that is superior for a trait of interest compared to an industrial target yeast strain. Multiple rounds of random inbreeding with the first-generation segregants is used to downscale the size of the QTLs, reducing the number of candidate causative genes in the centre of the QTL. Reciprocal hemizygosity analysis and allele exchange are used to identify and confirm the causative genes in the QTLs. We have applied this technology platform to several stress tolerance traits of yeast that are of prime importance in first- and second-generation industrial bioethanol production. The genetic basis of ethanol tolerance of cell proliferation and maximal ethanol accumulation capacity was determined and specific mutant genes identified for both properties. Higher ethanol tolerance has a major effect on yield and productivity in bioethanol production, because it improves the fermentation rate, the attenuation of the sugar, the maximal final ethanol titer, reduces the liquid volumes in the plant and lowers sensitivity to other stress factors. Mutant genes underlying higher thermotolerance and acetic acid tolerance have also been identified. The superior alleles found in the different strains are used to improve the performance of industrial yeast strains for first and second-generation bioethanol production.

John E. Hallsworth

Chaotropicity acts as a determinant of biotic windows, competitive interactions, and ecological success in fungi

John E. Hallsworth

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Water gives life to, regulates, and can ultimately prevent metabolism fungal cells due to dependence of the cellular system on water: macromolecular interactions amongst other factors. All substances dissolved in water reduce water activity, impact on hydrophobic forces within and between macromolecular systems and/or metabolites, and can disorder macromolecules and macromolecular assemblages (via their chaotropic activity) at the level of tertiary/quaternary structure. We have developed an assay to determine and quantify chaotropicity for chemically diverse substances that can be universally applied to ions, sugars, alcohols, aromatics, etc. For phylogenetically diverse yeasts and fungi
we found that chaotropicity can determine the extent of growth windows at low water activity, low temperature, and in the presence of organic solvents such as ethanol and hydrophobic substances such as benzene and toluene. Fungal cells are able to utilize chaotropic solutes when required to expand their growth windows and/or utilize stabilizing substances (kosmotropes, including some compatible solutes) to counter the chaotropic activity of stressful environments. Many antimicrobial substances produced by yeasts and fungi exert their inhibitory or lethal activities via their chaotropicity (e.g. ethanol, butanol, acetone, and urea) or via their chaotropicty-mediated action as hydrophobic stressors (e.g. isoamyl acetate, ethyl octanoate, pentane, ethyl acetate, and 2-phenylethanol). Via a number of metabolic- and genome-level adaptations of the stress biology of some yeasts and fungi, including *Saccharomyces cerevisiae* and a number of *Aspergillus* species, are consistently able to dominate their respective habitats. Ultimately chaotropicity can determine the ecological distribution and success of individual fungal strains and species.

Kevin K. Fuller

**The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light**

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For a variety of fungi, light serves as an important spatial and temporal cue for regulating developmental and metabolic programs. We have recently shown that the mold pathogen *Aspergillus fumigatus* regulates broad aspects of its physiology in response to both blue and red portions of the visible spectrum. Included in the *A. fumigatus* photoresponse is a reduction in conidial germination kinetics, increased hyphal pigmentation, enhanced resistance to acute ultra-violet and oxidative stresses, and increased susceptibility to cell wall perturbation. Thus far, we have identified important roles for a blue light receptor, LreA, and a red-light sensing phytochrome, FphA, in mediating the *A. fumigatus* photoresponse, as deletion of one or both of these genes leads to defects in light-controlled pigmentation, germination and cell wall homeostasis. However, the light-mediated resistance to oxidative and UV-stress persist in the *lreA/fphA* double deletion mutant, suggesting that additional photoreceptors are operative in *A. fumigatus*. Microarray and qRT-PCR analyses have revealed broad transcriptional changes that support the observed phenotypes, including the light-induction of DNA repair genes (e.g. photolyase) and the light-repression repression of cell wall-associated genes. Included among the light-repressed genes were several involved in the synthesis of ergosterol, including the lanosterol 14-alpha demethylase. These transcriptional responses indeed correlated with a light-enhanced susceptibility of *A. fumigatus* to sterol-targeting antifungals, namely voriconazole, the drug of choice for treating invasive aspergillosis. Intriguingly, this drug susceptibility phenotype was ablated in the *lreA/fphA* mutant, suggesting that exploitation of photoreceptor pathways may augment the treatment of *A. fumigatus* infection. Taken together, these data
demonstrate the importance of the photic environment on the physiology of *A. fumigatus* and provide a foundation for future studies into an under-unexplored area of this important pathogen.

**Luis M. Corrochano**

**Light in the fungal world: a stress, a signal, or both?**

Luis M. Corrochano, Carmen Ruger-Herreros, Alejandro Miralles-Durán, M. del Mar Gil-Sánchez, and Eva M. Luque

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Light can be used as a signal from the environment to increase reproductive success, but it can be harmful due to damage to DNA. 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. This may be relevant in nature, as we have observed that the capacity of wild-type strains of *Neurospora* to accumulate carotenoids after exposure to light correlates with the latitude of the site where the strains were collected. Zygomycete fungi have multiple wc genes. In *Phycomyces blakesleeanus* one of them encodes a photoreceptor, MadA, that is required for all responses to light. In *Mucor circinelloides*, on the contrary, each Wc-1 protein plays a specific role in photoreception. A comparison of the set of photoreceptor genes in the genomes of selected fungi gives clues on the origin of fungal vision and its evolution across the fungal kingdom. The presence of multiple wc-1 genes is only observed in Zygomycete fungi, but other fungal genomes have a very complete set of additional photoreceptor genes that should allow light reception in a wide range of wavelengths, from the near UV to red light. Different evolutionary strategies have helped fungi to acquire the proteins that allow the perception of light.

**Marcia Regina von Zeska Kress**

**Non dermatophyte fungi and its profiles under different stress conditions.**

Marcia Regina von Zeska Kress
Mycoses on the surface of the human body are a common infection and have become an important public health topic once they cause invasive infections in patients with cancer and/or immunosuppression live longer. *Neoscytalidium dimidiatum*, *N. hyalinum*, *Fusarium* sp., and *Aspergillus fumigatus* are non dermatophytes responsible for superficial infection mimicking dermatophytosis and called non dermatophytes. The treatment outcome with the commonly used antifungal, for the treatment of superficial infections caused by dermatophytes and yeasts, has the feature of low frequency eradication of the infection caused by non dermatophytes fungi. Thus, the comprehension of the molecular aspects of these responses of these fungi to different stress conditions may help to establish new therapeutic strategies. Here, the focus is the characterization of *Neoscytalidium dimidiatum*, *N. hyalinum*, *Fusarium* sp., and *Aspergillus fumigatus* under different stress condition. Clinical isolates of *N. dimidiatum* and *N. hyalinum* have shown different allelic profiles by multilocus sequence typing analysis and peculiar characteristics on *in vitro* antifungal susceptibility testing methods. Additionally, studies on antimicrobial photodynamic therapy are providing an interesting perspective to control *Fusarium* and *Neoscytalidium* species infections. Finally, the transcriptomic responses of *A. fumigatus* to the presence of 1,3-B-glucan synthase inhibitor, anidulafungine, revealed different mRNA expression level of genes involved in a variety of cell function such as metabolism, cell rescue, defense and virulence, cellular transport, transcription, development, and cell cycle and DNA processing. *masA*, a gene that encodes a protein with unknown function and a signal peptide, is among the highly expressed genes in the presence of anidulafungine and was also found as highly expressed in a transcriptome analysis of *A. fumigatus* exposed to voriconazole, an antifungal agent that blocks the ergosterol biosynthesis pathway by inhibiting the enzyme 14-α-demethylase. In addition to high expression of *masA* under stress on the fungal cell wall (caspofungin, anidulafungin and congo red) and cell membrane (amphotericin B and voriconazole), *masA* is also highly expressed in lipids biosynthesis (myriocine, phytosphingosine, cerulenina and lovastatin), oxidative stress (menadione), and DNA synthesis (5-flucytosine).

**Identification of transcription factors/proteins regulating stress response in Neurospora crassa**

Maria Célia Bertolini
The fungus *Neurospora crassa* has been widely used as a model organism for the understanding of fundamental aspects of eukaryotic biology. The knowledge of its genome sequence has allowed the identification of the proteins required for gene regulation, such as the transcriptional regulatory proteins. The availability of a set of deletion strains, each carrying a deletion in a specific ORF encoding a transcription factor, allows the screening for genes linked to a particular phenotype. We have been using this mutant strains set to identify transcription factors/proteins that either directly or indirectly regulate glycogen metabolism in *N. crassa*. Transcription factors play a key role in transcription regulation as they recognize and directly bind to defined sites in promoter regions of target genes, and thus modulate differential expression. Using different strategies we have identified some transcription factors/proteins that are involved in gene expression regulation under heat stress, when the cells are transferred from normal growth temperature (30 ºC) to heat stress (45 ºC). In addition, some proteins were also involved in the regulation of different types of stress response. In this talk, I will be presenting the results related to the identification and characterization of some transcription factors/proteins that we have been studying in the fungus *N. crassa*.

Water stress of fungi on indoor surfaces

Mirjam Bekker & Karel A. van Laarhoven

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Indoor fungal growth causes discoloration of surfaces, but also poses a threat to the health of inhabitants. It is generally accepted that indoor fungal growth is mainly limited by the presence of moisture, – often expressed as ‘relative humidity’ (RH) –. The relation between moisture and proliferation of indoor fungi has mostly been studied under laboratory conditions on idealized culture media such as agar. Such conditions, however, are different from a realistic indoor situation. Firstly, the use of agar media usually implies steady state conditions, whereas indoor moisture conditions fluctuate around the threshold for minimal growth. Secondly, the osmotic state of pore water in indoor materials is fundamentally different from the osmotic state in agar. Whereas the availability of water in agar – often expressed as ‘water activity’ ($a_w$) – is controlled by the addition of osmolytes, the $a_w$ of pore water in indoor surfaces is coupled to the indoor RH. The aim of our research is to unravel the ways in which the dynamic indoor climate and the nature of a substrate’s material-water interactions influence fungal growth. This must lead to better strategies to prevent indoor mould. Quantification of fungal growth on indoor substrates is a challenge, because growth on porous substrates is sparse and substrates are non-transparent. We developed macroscopic and microscopic video techniques to quantify fungal growth on a porous substrate in real
time and under controlled temperature and humidity conditions. In our experiments, the indoor fungus *Penicillium rubens*, formerly known as *P. chrysogenum*, was used as model organism. Gypsum was used as a model building material. It was found that, on gypsum, hyphal growth rates of *P. rubens* decrease not only with decreasing $a_w$, but also with decreasing moisture content ($\theta$). We confirmed that the effects of $a_w$ and $\theta$ are distinct, and that the critical $a_w$ that is minimally required for growth on gypsum depends on $\theta$. Further, we investigated the recovery of *P. rubens* on gypsum after a period of desiccation. The results suggest that the recovery is caused by both germination of remaining conidia and regrowth of other parts of the mycelium.

**Naresh Magan**

*Environmental stress impacts on secondary metabolite gene clusters, growth and metabolite production - a systems approach.*

Naresh Magan

*Applied Mycology Group, Cranfield Soil and AgriFood Institute, Cranfield University, Bedford, England, U.K.*

Plant stress inevitably leads to increased susceptibility to fungal infection, pre- and post-harvest, and potential for contamination by mycotoxins (Magan et al., 2011). There has been significant interest in the impact that environmental stress has on the ecology of mycotoxigenic fungi and their mycotoxin production. The genes involved in mycotoxin production are clustered together and include key regulatory and structural genes critical to the biosynthetic pathways. We have utilised a unique mycotoxin-related microarray to understand the relationship between interacting conditions of water availability x temperature on relative gene expression and related this to growth and mycotoxin production in Fusarium graminearum and F. culmorum (deoxynivalenol), Aspergillus flavus (aflatoxin) and Fusarium verticillioides (fumonisins). These data sets have been integrated for the first time using a mixed growth model and linking this to expression of key genes in the toxin biosynthetic pathways (6 TRI, 10 Afl and 9 FUM genes) to develop predictive models (Heydt-Schmidt et al., 2011; Abdel-Hadi et al., 2012; Medina et al., 2013). By examining the relationship between key regulatory and structural genes it is possible to obtain a better understanding of their role under interacting environmental conditions using ternary diagrams to examine relative kinetics of related gene expression under interacting environmental stresses. Recently these studies have been extended to examine the effect of water x temperature x elevated CO2 to simulate climate change scenarios. Studies of A. flavus growth, q-PCR of key genes and aflatoxin production suggest that some regulatory and structural genes may be stimulated resulting in elevated aflatoxin production. This suggests that interacting environmental stresses may have differential effects at a molecular, cell and colony level.

**References**

Nemat O Keyhani

Signaling, stress response, and virulence: insights from entomopathogenic fungi

Nemat O Keyhani

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Fungal pathogens of insects, such as Beauveria bassiana, are important natural regulators of insect populations in diverse ecosystems. B. bassiana can also grow as a saprophyte as well as form intimate, endophytic relationships with certain plants. Whether engaged in saprophytic, plant associated, or pathogenic growth, stress responses play central roles in the ability of the fungus to adapt to various environmental conditions. Characterization of the three major mitogen-activated-protein kinase (MAPK) pathways; Fus3/Kss1, Hog1, and Slt2 suggest overlapping as well as unique contributions of these signaling pathways to B. bassiana development, stress response, and virulence. In addition, at least one G-protein coupled receptor, linking carbon sensing to specific B. bassiana developmental stages, and the regulator of G-protein signaling (RGS) protein have been implicated in stress response and virulence. Furthermore, a number of downstream transcription factors (CreA and Msn2), that interlink carbon and nitrogen signaling, stress response, and virulence have been characterized. Finally, the roles of antioxidant defense systems (catalases and super oxide dismutases) and compatible solute forming enzymes (mannitol dehydrogenase) in mediating stress response and virulence have been probed. Although still incomplete, a model is emerging in which there is a high likelihood of interactions between specific proteins in each of the discrete signaling and transcriptional activation pathways that help shape the fungal response to carbon/nitrogen availability, environmental and host derived stress, and the ability of the fungus to parasitize insect targets. Identifying such instances of crosstalk and integrating interactions with plants into the picture are emerging frontiers in research on entomopathogenic fungi.
How stressed are entomopathogenic fungi during growth on insect cuticular hydrocarbons?

Nicolás Pedrini

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Unlike other insect-pathogenic microorganisms that must be ingested to initiate the disease (virus, bacteria, nematodes and protozoa), entomopathogenic fungi normally invade by penetrating through the host cuticle. Although the major bulk components of the insect cuticle are protein and chitin, the outermost epicuticular surface layer comprises a complex mixture of non polar lipids, mainly composed by very long chain hydrocarbons. Entomopathogenic fungi are able to degrade insect epicuticular hydrocarbons, incorporating them into cellular components. However, a reduced growth and lower conidiation yields have been observed in alkane-grown fungi compared to control growth on rich media without hydrocarbon added. This and other facts suggest that fungi might be exposed to stress conditions during cuticular hydrocarbon assimilation. In this work, several biomarkers -with emphasis in antioxidant response and oxidative stress levels- will be evaluated in alkane-grown B. bassiana and their role in this process will be discussed.

Contribution of proteomics to the understanding of the Aspergillus fumigatus stress response

Olaf Kniemeyer

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The fungus Aspergillus fumigatus is a ubiquitous mould with a saprophytic lifestyle. So it is typically found in soil and decaying organic matter. However, A. fumigatus may also cause a broad spectrum of diseases in humans, ranging from allergic or locally restricted infections to invasive mycoses. Systemic A. fumigatus infections occur usually only in patients who are severely immunocompromised. These are characterized by high mortality due to difficult diagnosis and a very limited number of available antifungal therapeutics. So there is a need for increased understanding of the A. fumigatus infection process to facilitate the development of novel therapeutic strategies. Both during the course of infection and at its natural habitat, Aspergillus fumigatus has to cope with several kinds of abiotic stress including depletion of nutrients, reactive oxygen species and low oxygen levels (hypoxia). To identify novel hypoxia-adapting
pathways in A. fumigatus we have characterized the changes of the proteome in response to short (within 24 hours) and long periods (7-10 days) of hypoxia. Cultivation under hypoxic and normoxic condition was performed by using an oxygen-controlled fermenter. Changes of the proteome were characterized by 2D-fluorescence difference gel electrophoresis (DIGE). Our data suggest a robust response to hypoxia with a significant impact on key primary metabolic pathways such as glycolysis, the TCA cycle and nitrogen metabolism. Interestingly, respiratory proteins, NO-detoxifying enzymes and some secondary metabolite biosynthesis gene clusters were up-regulated during hypoxia. To get a deeper knowledge about the specific role of metabolic pathways in adaptation to hypoxia, we have started to characterize candidate genes for their role in hypoxia by generating deletion mutants. First data will be presented and discussed.

Pedro Moradas Ferreira

Oxidative stress responses in *Saccharomyces cerevisiae*: role of sphingolipid signaling

Pedro Moradas-Ferreira and Vítor Costa

*Instituto de Biologia Molecular e Celular and Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.*

In yeast, as in higher eukaryotes, reactive oxygen species (ROS) are produced as normal by-products of cellular metabolism. Under physiological conditions, the cell defense mechanisms are able to destroy ROS or repair oxidative damages. This balance is disturbed when cells are exposed to diverse environmental stress conditions, as well as during aging, and the accumulation of cellular damages may lead to cell death. The yeast *S. cerevisiae* has been used as a eukaryotic model system to characterize the molecular mechanisms of oxidative stress adaptive response and its role in cell longevity. The adaptive response to oxidative stress involves ROS sensing and a network of signaling proteins that regulate changes in gene expression. Sphingolipids have been identified as signaling molecules involved in the regulation of stress responses, including oxidative stress, cell cycle arrest, proliferation, apoptosis and aging. Our recent studies have shown that Isc1p, the yeast orthologue of mammalian neutral sphingomyelinase-2 (nSMase2) that acts on yeast inositol phosphosphingolipids, plays a key role in oxidative stress resistance and chronological lifespan, modulating redox homeostasis, iron levels and cell death by caspase-dependent apoptosis. The shortened chronological lifespan of isc1Δ cells is associated with an increase in the levels of dihydro-C26-ceramide and phyto-C26-Ceramide species. Consistent with ceramide activating protein phosphatases, loss of the ceramide-activated protein phosphatase Sit4p suppresses the shortened chronological lifespan, oxidative stress sensitivity and mitochondrial dysfunctions of isc1Δ cells. The activation of the HOG (High Osmolarity Glycerol) pathway is also deleterious for isc1Δ cells since ceramide signaling increases the phosphorylation of the Hog1p mitogen activated protein kinase and the disruption of HOG1 attenuates the phenotypes of Isc1p-deficient cells. These results support the involvement of ceramide as a key signaling molecule in the regulation of oxidative stress response and aging.
Acknowledgements: we thank FEDER (Fundo Europeu de Desenvolvimento Regional) through the program “Programa Operacional Fatores de Competitividade-COMPETE” and FCT (Fundação para a Ciência e Tecnologia) for financial support through the projects NORTE-07-0124-FEDER-000001 and FCOMP-01-0124-FEDER-028210.

Raymond J. St. Leger

Stress is the rule rather than the exception for Metarhizium

Raymond J. St. Leger

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The insect pathogenic plant root symbiont Metarhizium experiences many situations that restrict its growth whether living in host insects or on plant roots. These include a range of physical, chemical, and biological effects involving UV and extremes of temperature, pH, nutrient availability, toxic metals and other pollutants, and insect host defenses such as production of active oxygen species. Aside from virulence, the major impediment to reliable pest control with Metarhizium is its sensitivity to UV and temperature extremes. However, increased levels of stress tolerance can be engineered into Metarhizium quite simply by reprogramming the expression of single downstream endogenous genes. For example, overexpression of RNA binding proteins resulted in Metarhizium with increased tolerance to cold stress, overexpression of photolyase increased tolerance to UV, and increased expression of heat shock protein 25 also resulted in improved tolerance to several stress conditions, including heat, and osmotic pressure. Conversely, disruption of these genes greatly reduced persistence, and could provide genetic containment for genetically engineered hypervirulent strains.

Roger D. Finlay

Stress responses in interactions of plant roots with symbiotic mycorrhizal fungi and bacterial antagonists of fungal pathogens

Roger D. Finlay

Department of Forest Mycology & Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden

Plant roots are exposed to an enormous diversity of different microorganisms with different trophic strategies and need to respond appropriately to both beneficial symbionts, as well as potential pathogens. The fungi and bacteria interacting with plant roots also interact with each other in ways that are still only superficially understood. Biotic and abiotic stress plays a central role in these interactions. In my lecture I will
discuss the ways in which different types of mycorrhizal fungal symbionts reduce the nutritional and non-nutritional stress on their plant hosts, but also the ways in which the fungi themselves respond to different sorts of abiotic stress. Many of the fungal pathogens causing plant diseases also experience biotic stress caused by bacterial antagonists that may have potential as biological control agents. These interactions have been investigated in two projects using a mixture of genomic and transcriptomic approaches following genome sequencing of Serratia isolates in collaboration with the US Joint Genome Institute (JGI). Responses of antagonistic bacteria to the plant pathogen *Rhizoctonia solani* and responses of the fungus to the bacteria have been monitored using similar high-throughput sequencing techniques. The results of these studies will be discussed in relation to phenotypic and genetic traits underlying effective fungal symbionts and plant pathogens, as well as those of bacterial antagonists.

**Stefan Hohmann**

**Integrative analysis of yeast osmoregulation**

Stefan Hohmann

*Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden*

In response to hyperosmotic stress yeast cells accumulate glycerol to counteract the loss of volume and molecular crowding in the compressed cells. Glycerol accumulation is controlled by Hog1 at multiple levels: expression of genes encoding enzymes for glycerol production or transporters for glycerol uptake; direct control of metabolic enzymes; the activity of the Fps1 glycerol export channel. I will present the different roles and mechanisms of these different control levels and provide illustration of how those control mechanisms operate under different conditions.
BOOK OF ABSTRACTS
Different intensities of visible light during mycelial growth induces differently the conidial tolerance to stress in *Metarhizium robertsii*

Luciana P. Dias¹,² and Drauzio E. N. Rangel²

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The threshold of illumination during mycelial growth was studied on the influence of the conidial tolerance of the entomopathogenic fungus *Metarhizium robertsii* (ARSEF 2575) to the oxidative agent menadione. The fungus was grown at 26 °C for 14 days in five treatments: 1) growth on minimal medium (MM) in the dark; 2) growth on potato dextrose agar (PDA) in the dark inside the Panasonic incubator; 3) growth on PDA medium under continuous visible light in the Panasonic incubator; 4) growth on PDA medium in the dark inside the Marconi incubator; 5) growth on PDA medium under continuous visible light inside the Marconi incubator. For the Panasonic incubator, four intensities of light were studied with 1, 3, 4, and 5 lumens. The germination of conidia produced under these treatments was subsequently evaluated on PDA medium supplemented with menadione at the concentrations 0.10 and 0.15 mM. For control, conidia were germinated on PDA medium. The germination was evaluated counting at least 300 conidia after 24 h at 26 °C. Each treatment was repeated four times with a new batch of conidia produced for each repetition. Conidia produced on minimal medium were more tolerant to menadione, followed by conidia produced under visible light inside the Marconi incubator. Conidia produced inside the Panasonic incubator at 4 and 5 lumens were more tolerant to menadione, but less tolerant than conidia produced under light in the Marconi incubator. Conidia produced in the Panasonic incubator at 1 and 3 lumens showed somewhat increased tolerance compared to control in the dark, but they were less tolerant than conidia produced in the Marconi incubator.

Keywords: Visible light; menadione; entomopathogenic fungi; stress tolerance; oxidative stress

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**STUDENT COMPETITION**

QSUN® an equipment for evaluating the effect of realistic solar UV radiation on fungal pathogens of insects

Luciana P. Dias¹,², Claudinéia A. S. Araújo¹, Breno Pupin¹, Drauzio E. N. Rangel¹
The low survival of entomopathogenic fungi when used for insect control in agriculture is due to ultraviolet radiation and heat from the solar irradiation. In this study, conidia of Beauveria bassiana (ARSEF 252), Metarhizium brunneum (ARSEF 1187), Metarhizium robertsii (ARSEF 2575), Tolypocladium cylindrosporum (ARSEF 3392), Isaria fumosorosea (ARSEF 3889), Tolypocladium inflatum (ARSEF 4877), Metarhizium anisopliae (ARSEF 5749), Lecanicillium aphanocladii (ARSEF 6433), Simplicillium lanosoniveum (ARSEF 6651) and Aschersonia aleyrodis (ARSEF 10276) were produced on potato dextrose agar and (PDA). The spore suspension was inoculated onto PDA medium supplemented with benomyl (0.003%) and exposed to UV radiation in the QSUN Xenon Test Chamber XE3, which produces a radiation realistically compared with the natural irradiance of the sun. Based on LT50, the isolates ARSEF 1187, 2575, 3392, 3889, and 5749 were the most tolerant. The isolates ARSEF 4877, 6433, 6651, and 10276 have moderate tolerance. The isolate ARSEF 252 was the least tolerant. The QSUN Xenon Test Chamber XE3 is well used in the pharmacological industry, but it was never considered to be used to test microorganisms that are used in biological control of insects. Therefore, the equipment provided an important tool for evaluating the microbial agents for insect control.

Keywords: Entomopathogenic fungi; ultraviolet radiation, response to stress.

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STUDENT COMPETITION

ISFUS-0003C

Different oxygen concentrations during the mycelial growth induce increased tolerance of conidia to potassium chloride in entomopathogenic fungi

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Oxygen is important for the survival of a large proportion of eukaryotic organisms. The lack of oxygen can occur with a very high frequency, depending on the mode of life of the organism. Furthermore, it is known that the mechanisms of adaptation to hypoxia are variable and that this condition is a key factor in the virulence of pathogenic fungi. During the course of infection by fungus, pathogenic fungi endure several adverse factors from the host insect, such as osmotic stress within the body of the host. The study aimed to evaluate the influence of different oxygen concentrations during the mycelial growth on the conidial tolerance of ten species of entomopathogenic fungi to osmotic stress caused by potassium chloride (KCl). The experiments were done as follows: A) Control: mycelial growth on Petri dishes with PDA medium under normal oxygen conditions (normoxic condition); B) Hypoxic condition: cultures on Petri dishes with PDA medium were sealed with Parafilm® three times and maintained for 14 days; C) Anoxic condition: Petri dishes with mycelial growth were transferred to anaerobic jar on the second day of growth for five days, and then on the fifth day, the plates were transferred under aerobic conditions; D) Petri dishes with minimal medium (MM) under normoxic condition. The cultures were incubated at 26 ± 1 °C for 14 days. Germination of conidia was assessed on PDA medium supplemented with KCl concentrations from 0.9 to 2.4 M. Mycelial growth under hypoxia induced the increased conidial tolerance to osmotic stress for the isolates ARSEF 252, 1187, 2575, 4877, 5749, and 10276. Mycelial growth under anoxic stress induced increased tolerance to osmotic stress for the isolates ARSEF 252, 1187, 2575, 4877, 5749, and 10276. Mycelial growth under nutritional stress (MM) induced increased conidial tolerance to osmotic stress for the isolates ARSEF 252, 1187, 2575, 3889, and 5749. The conidial tolerance to osmotic stress, therefore, was improved for several isolates when growth was subjected to hypoxic, anoxic, and nutritive stress (e.g. ARSEF 252, 1187, 2575, and 5749). However, the tolerance level among hypoxic, anoxic, and nutritive stress was not always similar.

Keywords: Oxygen concentration; potassium chloride; entomopathogenic fungi; osmotic stress

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STUDENT COMPETITION

**ISFUS-0003D**

**Visible light during the mycelial growth induces increased tolerance of conidia to potassium chloride in entomopathogenic fungi**

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Light is one of many signs that fungi use to perceive and interact with the environment, providing critical information about their habitat. During secondary metabolism, the most commonly reported effect of light is the change in fungal spore either asexual or as sexual stimulation/sexual repression on sporulation. Fungi not only perceive different qualities of light, but also perceive different light intensities. Mycelial growth under light condition may improve conidial stress tolerance, in this study we evaluate the influence of visible light on the tolerance of conidia of ten species of entomopathogenic fungi to osmotic stress caused by potassium chloride (KCl). The fungi were grown as follow: 1) on potato dextrose agar medium (PDA) grown in the dark (control), 2) on PDA grown under continuous visible light (400-700 nm) during the entire incubation period, or 3) under nutritive stress (= Czapek medium without sucrose) in the dark. The growth under light was carried out under fluorescent Sylvania 15 W at a distance of 30 cm Petri dishes. The cultures were incubated at 26 ± 1 °C for 14 days. Germination of conidia was assessed on PDA or PDA medium supplemented with KCl concentrations from 0.9 to 2.4 M. Mycelial growth under constant light induced significantly increased tolerance to osmotic stress conidia of the isolates, ARSEF 252, 1187, 2575, 3889, and 10276. Mycelial growth under nutritional stress (MM) significantly induced increased conidial tolerance to osmotic stress in five isolates, ARSEF 252, 1187, 2575, 3889, and 5749. In conclusion, mycelial growth under light improved tolerance to salt stress in five isolates; however, the level of tolerance was not always similar to the tolerance of conidia produced under nutritive stress. Conidia produced on nutritive stress always had the highest tolerance.

Keywords: Visible light; potassium chloride; entomopathogenic fungi; osmotic stress

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STUDENT COMPETITION

ISFUS-0013

Intraspecific and interspecific variation in osmotolerance of entomopathogenic fungi

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Entomopathogenic fungi must be capable of cell division under multiple stresses imposed during the various stages of the lifecycle, some of which take place on the insect surface or within the hemolymph. These include the energy-expensive synthesis and retention of compatible solutes to maintain osmotic pressure. The windows for osmotolerance of 24 isolates of entomopathogenic fungi were determined by assessing conidial germination over a range of KCl concentrations. Germination was evaluated on potato dextrose agar (PDA; control) or PDA+KCl using 31 concentrations of KCl from 100 to 3000 mM (after 24 h; 26 °C). *Trichothecium roseum* was the most osmotolerant (≤ 3000 mM KCl), followed by *Lecanicillium aphanocladii*, *Simplicillium lanosoniveum*, and *Isaria fumosorosea*. Several fungal species showed moderate osmotolerance (≤1700 mM) including *Metarhizium robertsii* (for some isolates), *Metarhizium brunneum*, *Metarhizium anisopliae*, *Tolypocladium inflatum*, *Tolypocladium cylindrosporum*, and *Fusarium coccophilum*. Some isolates showed modest levels of osmotolerance (≤ 1400 mM), including one isolate of *M. robertsii*, one of *M. anisopliae*, two of *M. acridum*, and *Beauveria bassiana*. *Aschersonia aleyrodis* and one isolate of *M. brunneum* were relatively intolerant to osmotic stress (≤ 1000 mM KCl). These findings indicate high levels of inter- and intraspecific variability in osmotolerance for insect-pathogenic fungi. Eighty percent of *Trichothecium roseum* conidia germinated at 2000 mM KCl (equivalent to 0.928 water activity), with a LC50 at 2300 mM, and some germination at < 0.890 water activity (on 3000 mM KCl). This suggests that *T. roseum* is highly xerotolerant and may therefore be unique amongst the entomopathogenic fungi.

Keywords: entomopathogenic fungi, osmotic stress, conidial germination, osmotolerance, xerotolerant.

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**STUDENT COMPETITION**

**ISFUS-0016**

Conidial thermotolerance of *Metarhizium anisopliae* s.l. IP 46 and *Metarhizium robertsii* ARSEF 2575 suspended in different vehicles

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Heat stress limits efficacy of entomopathogenic fungi in arthropod biocontrol programs; formulation of conidia, however, may increase
the tolerance against abiotic factors, especially high temperatures. The current study evaluated the thermotolerance of conidia of *Metarhizium anisopliae* s.l. IP 46 and *Metarhizium robertsii* ARSEF 2575 suspended in water, oil or oil-in-water emulsions. The fungal strains were cultured on PDAY medium in the dark at 27 ± 1 °C; 15 days later, conidia were harvested with a microbiological loop, placed in polystyrene Petri plates, and held for 5 days at 5 ± 1 °C in a desiccator with activated silica gel. Dried conidia were suspended in water (Tween 80, 0.01%), water plus a strong surfactant from General Chemicals (GC, 0.05%), mineral oil-in-water emulsion 5%, 10% or 15%, or in pure mineral or canola oil. Then, conidial suspensions were exposed to 45 ± 0.2 °C for 4 h. Water was heated up to 35 °C before preparation of conidial suspension in order to avoid imbibitional damage to the dried conidia. In addition, conidia of *M. anisopliae* s.l. IP 46, suspended in pure mineral or canola oil, were exposed to 45 ± 0.2 °C for 8, 16, 24, 32, 40 or 48 h. Germination of conidia was assessed 48 h after inoculation onto PDAY and incubation at 27 ± 1 °C in the dark. Relative germination (RG) was calculated in relation to non-heated controls. The present study revealed that conidia of IP 46 or ARSEF 2575 suspended in water solution (Tween 80 0.01% or GC 0.05%) were more susceptible to heat (45 ± 0.2 °C for 4 h) than conidia suspended in pure mineral oil. Conversely, thermotolerance of IP 46 or ARSEF 2575 conidia suspended in mineral oil-in-water emulsions did not differ significantly from conidia suspended in water (Tween 80, 0.01%). Mean RG for ARSEF 2575 conidia suspended in water (GC 0.05%) was 42.5%, whereas RG for conidia suspended in mineral oil was 88.7%. In addition, mean RG of IP 46 conidia suspended in canola oil and exposed to heat (45 ± 0.2 °C) for 8, 16, 24 or 32 h did not differ significantly; RG was, respectively, 83%, 71.1%, 54.4% or 47.2%. Heat exposures for 40 or 48 h, however, had significantly reduced RG, 31.3% or 31.0%, respectively. Also, conidia of IP 46 suspended in mineral oil revealed high RG for long heat treatment periods: 91.8% (8 h), 86.4% (16 h), 74.7% (24 h) or 62.8% (32 h). Heat exposures for 40 h or 48 h had mean RG equal to 48.9% and 51.3%, respectively, and differed statistically from conidia exposed for 8 h. In conclusion, the current study showed that tolerance of *M. anisopliae* s.l. IP 46 and *M. robertsii* ARSEF 2575 to heat was significantly increased when conidia were formulated in pure oil.

Keys-words: Entomopathogenic fungi, thermotolerance, formulation, emulsion.

Acknowledgements: We sincerely thank CNPq and CAPES financial support.

**STUDENT COMPETITION**

**ISFUS-0017**

Antimicrobial photodynamic treatment of the pathogenic fungi *Fusarium moniliforme*, *F. oxysporum* and *F. solani* with four phenothiazinium photosensitzers

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Fusarium species are worldwide fungi widely distributed in soil, plants and different organic substrates, and have been increasingly associated with humans. Fusariosis represent the second most frequent mould causing superficial infection and locally invasive or disseminated infections in immunosuppressed patients associated with high morbidity and mortality rates. From the clinical point of view, control of progression of fusariosis by single-agent antifungal therapy is problematic, leading to a high mortality rate in patients, especially those with immunocompromised. Increasing tolerance to currently used fungicides is a major problem in clinical area and has stimulated the development of alternative strategies to control pathogenic fungi. Antimicrobial photodynamic treatment (APDT) is an alternative and promising antifungal discovery platform that can be used to control superficial and localized invasive mycoses. APDT is based on the use of a photosensitizer (PS) that accumulates in the target fungal cell. Exposure of the PS to light of an appropriate wavelength starts a photochemical process that produces reactive oxygen species (singlet oxygen) leading to non-specific oxidative damage causing the death of the fungal cell without significant harm to the human cells. In comparison with currently used fungicides, the multiple and variable targets of reactive oxygen species reduce the chance of selecting tolerant microorganisms. In the present study, we evaluated the effect of APDT on microconidia of Fusarium moniliforme, F. oxysporum and F. solani with four different phenothiazinium photosensitizer, methylene blue (MB), toluidine blue O (TBO), new methylene blue N (NMBN) and a novel pentacyclic phenothiazinium S137. Initially, the APDT efficacy was determined based on the determination of the minimal inhibitory concentration (MIC) of each PS. For this, 96-well flat-bottomed microtiter plates were used with 0.5 to 200 µM of each PS and 4x10^4 microconidia ml^-1. The plates were exposed to light fluences of 10, 15 and 20 J cm^-2 using a LED array with an emission peak at 635 nm as a light source and the MICs were determined after 48, 72, and 96 hours by visual inspection. Based on the results of APDT efficacy, the optimized conditions for APDT with each PS on the survival of microconidia were determined. 4x10^6 microconidia ml^-1 were incubated with 50-75 µM of MB, 75 µM of TBO, 5-12.5 µM of NMBN and 10 µM of S137 and light fluences of 10 and 15 J cm^-2. The MIC for NMBN were 5, 10 and 12.5 µM for F. oxysporum, F. moniliforme and F. solani, respectively and for S137 were 10 µM for the three fungal species when a light fluence of 15 J cm^-2 was applied. APDT with NMBN and S137 showed the lowest MIC. APDT with NMBN resulted in a reduction of approximately 5 logs in the survival of the microconidia for all three species, and with S137 resulted in a reduction of 3 logs to F. moniliforme and 5 logs to F. oxysporum and F. solani when the light fluence of 15 J cm^-2 was applied. Our results open the interesting perspective of using APDT to control Fusarium species infections.

Keywords: Antimicrobial photodynamic treatment, phenothiazinium photosensitizers, Fusarium moniliforme, Fusarium oxysporum and Fusarium solani.

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**STUDENT COMPETITION**

**ISFUS-0018**

**masA, a gene which encodes a signal peptide is highly expressed under different fungal stress conditions in *Aspergillus fumigatus***

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*Aspergillus fumigatus* is an opportunistic saprophytic filamentous fungi that plays an essential role in the environmental recycling of carbon and nitrogen. In this organism is considered a human pathogen responsible for causing diseases in immunocompromised patients. To better understand the diseases caused by this fungus, it is important to seek knowledge of the composition and architecture of the biosynthetic mechanism of the fungal cell wall and the genes expression modulation during treatment with antifungal drugs. The fungal cell wall is in continuous contact with the host environment and acts as a filter and a reservoir of molecules such as antigens and enzymes actively working for a fungal infection. MAS1 (magnaporte appressoria specific) was initially identified in a differential hybridization analysis of an appressoria cDNA library constructed from Magnaporthe grisea. This gene has also been identified among genes regulated by PMK1 (mitogen-activated protein kinase - MAPKs) expressed specifically during the formation of appressoria in M. grisea. Mas1 gene encodes a protein with unknown function and a signal peptide. The orthologue gene in *A. fumigatus*, masA, was found as highly expressed in a transcriptome analysis of *A. fumigatus* exposed to voriconazole, an antifungal agent that blocks the ergosterol biosynthesis pathway by inhibiting the enzyme 14α-demethylase. Here, we identified masA in a large-scale analysis of gene expression in *A. fumigatus* using microarray hybridization approach. Mycelia of the fungus were incubated with anidulafungin, an echinocandin class member inhibitor of 1,3-β-glucan synthase, and 920 genes differentially expressed were identified. To validate the expression of some of these genes during exposure to anidulafungin, we analyzed eight genes by quantitative PCR, including masA, showing higher expression in the presence of anidulafungin. Additionally, in a quantitative PCR approach, the expression of masA was highly increased in the presence of drugs which affects the fungal cell wall (caspofungin, anidulafungin and congo red), cellular membrane (amphotericin B and voriconazole), lipids biosynthesis (myriocine, phytosphingosine, cerulenina and lovastatin), oxidative stress (menadione) and DNA synthesis (5-flucytosine). The protein MasA::GFP (green fluorescence protein) is localized in the cytoplasm of *A. fumigatus* without stress conditions. masA deleted strain is viable and display no changes in virulence
pattern in an immunosuppressed murine model. Thus, considering the high expression of masA under different stress conditions, it is suggested that this protein has a role in stress response. However, additional studies are necessary to elucidate the specific function of this gene.

Keywords: Aspergillus fumigatus, qPCR, microarray, signal peptide, cell wall, cell membrane, oxidative stress.

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Aspergillus fumigatus is the main causative agent of invasive aspergillosis (IA), a disease that primarily affects immunocompromised patients and has a high mortality rate. In the search for new therapeutic targets to control the severe symptoms of this invasive disease, we found that when A. fumigatus faces an increased CO2 concentration, which occurs during the infection process, the fungus increases the transcription of a cipC-like gene. CipC is a small protein with unknown function that is absent in mammalian cells and could be associated with fungus pathogenesis. In this study, the cipC gene was disrupted and characterized in A. fumigatus to verify its biological function and relevance in the pathogenicity of this fungus. We demonstrated that although cipC is not essential for this fungus, its deletion induced changes in the sensitivity of the fungus to certain stressors and a decrease in the virulence of A. fumigatus. Because cipC is not present in mammalian cells and is important for A. fumigatus virulence, cipC is a promising candidate as a therapeutic target in the control of invasive aspergillosis.

Keywords: Aspergillus fumigatus, cipC, virulence.

Proteomic analysis reveals that Cryptococcus gattii modulates its primary metabolism in response to hypoxia

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The yeast *Cryptococcus gattii* is one of the etiologic agents of cryptococcosis, a disease that can lead to fatal infection of the central nervous system in humans. A common immune response to cryptococcal infections is the formation of cryptococcomas, which is assumed to expose the pathogen to low oxygen levels (0.5-5 %). The aim of this study is to analyze the effect of such conditions on the phenotype and global protein expression of *C. gattii*. The R265 strain of *C. gattii* was grown in liquid YNB 37ºC with reduced O₂ levels (1%). Cells were collected at three different periods of exposure to low oxygen concentration (0h-T0, 24h-T24 and 48h-T48) and analyzed for its growth, as well capsule and melanin production. In addition, total proteins from each condition were submitted to separation by 1D SDS-PAGE followed by mass spectrometry in order to verify changes in the overall protein expression of yeast under low oxygen levels. The proteins identified by LC-MS/MS were classified according to biological processes, molecular functions and cellular components. Only proteins identifications with at least 2 unique peptides and present in at least two biological replicates were considered true identifications. A total of 130 proteins could be identified, with several presenting differential expression in specific conditions (43 proteins only detected in T0, 14 proteins only detected in T24 and 13 proteins only detected in T48). The functional profiling of such proteins reveals that cellular respiration, tricarboxylic acid cycle, oxidative phosphorylation pathway and glycolysis/gluconeogenesis related proteins have an increased expression according to exposure to low oxygen concentrations. In addition, fungal growth and melanization were influenced by oxygen levels. In conclusion, *C. gattii* adapt to low oxygen levels by the modulation of primary metabolism.

Keyword: *Cryptococcus gattii*, hypoxia, proteins, proteomic analysis, oxygen

**ISFUS-0021**

**Thermotolerance of conidia and blastospores of *Metarhizium* spp. from Center West Brazil**

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Most commercialized biological products based on fungi have conidia as active ingredient, although some studies have reported several advantages of blastospores over conidia. Conversely, studies have indicated that blastospores are possibly more susceptible to environmental abiotic factors than conidia, and, therefore, blastospores would have limited efficacy in the field. The current study evaluated the thermotolerance of blastospores and conidia of three *Metarhizium* spp. lineages: IP 363 (*Metarhizium anisopliae* s.l.), IP 146 (*Metarhizium robertsii*), both from the Brazilian Center West region, and ARSEF 324 (*Metarhizium acridum*), included as a standard thermotolerant fungus. Fungi grew in modified Adamék medium, with or without agar, for obtaining conidia or blastospores, respectively. Fungal suspensions (10⁴ propagules/mL) were exposed to 45 ± 0.2 ºC, in a water bath, for different time periods: 0, 60, 120, 240 or 360 minutes for conidia, and 0, 15, 30, 45, 60, 90, 120 or 150
minutes for blastospores. After heat exposure, 30 µL of suspension (approximately 300 propagules) were spread onto the surface of PDAY medium supplemented with chloramphenicol (0.05% w/v), in Petri plates, and incubated at 27 ± 1 °C in the dark. The number of CFU was assessed 7 days after sample inoculation, and the relative percent culturability calculated. Six repetitions were performed in different days. The data were analyzed by two-way factorial ANOVA followed by Student-Newman-Keuls test. IP 146 showed the lowest thermotolerance for conidia (F_{6,24} = 4.6; p = 0.003) and blastospores (F_{12,42}=15.4; p = 0.00001), reporting 1.5% relative culturability when conidia were exposed to heat for 240 min. On the other hand, IP 363 reached 55.5% relative culturability and ARSEF 324, 79.1%, for conidia exposed to heat for 240 min. IP 363 and ARSEF 324 did not differ for blastospores exposed to heat for 30 min, and relative culturability reached 92% and 90.8%, respectively, whereas IP 146 had 60.7%. No significant difference was observed for culturability of ARSEF 324 conidia (F_{3,8} = 3.3; p = 0.08) or blastospores (F_{6,14} = 1.3; p = 0.31) for all heat exposure periods investigated; even after the longest periods of heat exposure (360 min for conidia or 150 min for blastospores), culturability was superior to 80%. As expected, ARSEF 324 was the most thermotolerant fungus; conidia and blastospores presented significantly high relative culturability in comparison to the propagules of IP 363 and IP 146 exposed to heat for 60 min (F_{2,12} = 7.7; p = 0.007) or 120 min (F_{2,12} = 199.3; p = 0.000). Besides the marked susceptibility of blastospores of IP 363 and IP 146 to heat, it is important to consider that blastospores, in general, develop faster than conidia; and, this advantage might balance the efficacy of blastospores in comparison to conidia in biological control programs.

Key words: thermotolerance, *Metarhizium*, conidia, blastospores.

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STUDENT COMPETITION

**ISFUS-0029**

**Control of gene expression and cell cycle progression by the Hog1 SAPK in response to stress**

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Exposure of cells to increases in extracellular osmolarity results in the activation of the Hog1 stress-activated protein kinase. Activation of these MAP kinases is required to generate a set of osmoadaptive responses essential to survive under high osmolarity. Adaptation to osmostress requires the induction of a large number of genes, which indicates the necessity to regulate several aspects of the cell physiology. Induction of gene expression is highly dependent on the presence of the MAP kinase, which suggests a key role for the HOG...
signaling pathway in the regulation of gene expression in response to osmostress. Activation of Hog1 is important to regulate several steps in mRNA biogenesis from initiation of transcription to elongation, mRNA stability and export. In addition to gene expression, the MAPK also controls cell cycle. Here, the MAPK is able to modulate cell cycle delay in different phases of the cell cycle by acting on central core components of the cell cycle machinery. This tight control of cell cycle progression highlights the relevance of cell cycle control in response to stress. Remarkably, there are several examples that show that the control of cell cycle and the regulation of gene induction are strongly coordinated. For instance, induction of the expression of a lncRNA in Cdc28 serves to control cell cycle reentry after stress. Moreover, control of DNA replication is required to permit stress-responsive gene induction during S-phase. Thus, suggesting there are strong links between the regulation of gene expression and cell cycle control.

Key words; osmostress, SAPK, Hog1, cell cycle, gene expression

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ISFUS-0048

Effect of carotenoid content on sensitivity of
Fusarium fujikuroi to oxidative stress

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Oxidative stress is caused by reactive oxygen species (ROS) generated by normal cell metabolism, such as superoxide anion, hydrogen peroxide (H₂O₂), and hydroxyl radicals. Carotenoids are known for their protective properties against oxidative stress (Edge et al. 1997) and different reports have provided examples of a protective role also in fungi. Exposure to hydrogen peroxide results in enhanced production of β-carotene in Blakeslea trispora, astaxanthin in Xanthophyllomyces dendrorhous and neurosporaxanthin in Neurospora crassa and Fusarium aquaeductuum (Jeong et al. 1999, Iigusa et al. 2005, Liu et al. 2006). Supporting results have been also obtained in non-carotenogenic species, as Saccharomyces cerevisiae. Introduction of the genes needed to produce astaxanthin in this yeast resulted in higher resistance to hydrogen peroxide (Ulkibe et al. 2009). Fusarium fujikuroi is a model system in the study of neurosporaxanthin biosynthesis and its regulation (Avalos et al. 2014). We have investigated the protective role of this xanthophyll against oxidative stress in this fungus. For this purpose, sensitivity to hydrogen
peroxide was assayed on strains with different carotenoid contents: a wild type, two carotenoid-overproducing mutants and an albino mutant. The results showed a correlation between carotenoid levels and hydrogen peroxide resistance. The study was extended to albino mutants isolated from one of the carotenoid overproducers, carrying a mutation in the regulatory gene carS. Expression analyses led to classify these new albino strains in two types: those reverting the transcriptional deregulation produced by the carS mutation (regulatory mutants), and those predictably affected in the genes for early enzymes of the biosynthetic pathway, carRA and carB (structural mutants). Unexpectedly, exposure to hydrogen peroxide of conidia of a structural albino mutant resulted in a positive selection for revertant colonies, containing neurosporaxanthin, providing further support to a protective role of this xanthophyll against oxidative stress.

Keywords: Fusarium fujikuroi, xanthophyll, carotenoid, oxidative stress, gene carS

Comparison of survival of Cladosporium and Penicillium species during different developmental stages under low water activity

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Fungi can grow under widespread environmental conditions. Many different fungi are found in varying food products and indoor environments. Indoor fungi are present in a considerable part of the European dwellings and cause cosmetic and structural damage. The presence of fungi poses a potential threat to human health as a result of continuous exposure as they are able to form allergens and mycotoxins. Fungal growth does not exist without the presence and availability of water. Not much is known on the response of fungi to humidity dynamics during different stages of their development. Relative humidity (r. h.) and water activity (a_w) are used in many studies for the amount of water available for the fungus. A r.h. of 80% (0.8 a_w) or higher is associated with fungal growth. On average the r.h.is below 50% in normal buildings. In order to study the fungal response to humidity dynamics, polycarbonate membranes containing two fungal species, Cladosporium halotolerans and Penicillium rubens, were placed in vessels with fixed relative humidity of 85%, 75%, 58% and 33% and transferred again to high humidity conditions after a week. The different developmental stages of C. halotolerans and P. rubens before and after periods of lower humidity are determined by using Cryo Scanning Electron Microscopy (CryoSEM). A different response to humidity dynamics was seen between several developmental stages and both fungi used.
More in depth research will be done on the specific cellular response of the fungi to humidity dynamics.

Keywords: indoor fungi; relative humidity; water activity; Cladosporium; Penicillium

**ISFUS-0053B**

**Novel trehalose-based oligosaccharides from extreme stress-tolerant ascospores of Neosartorya fischeri (Aspergillus fischeri)**

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Ascospores of *Neosartorya*, *Byssochlamys* and *Talaromyces* can be regarded as the most stress-resistant eukaryotic cells. For example, they can survive exposure at temperatures as high as 85 °C for 100 min or more. Here we describe the identification and characterization of novel trehalose-based oligosaccharides (TOS) as compatible solutes that are accumulated to high levels in ascospores of the fungus *Neosartorya fischeri*. These compounds are also found in other members belonging to the genus *Neosartorya* and occur in other genera within the order Eurotiales that also include *Byssochlamys* and *Talaromyces*. These oligosaccharides consist of a trehalose backbone with one, two or three glucose molecules attached via an α-1,6 linkage. The tetra- and pentasaccharide, dubbed neosartose and fischerose, respectively, have not been reported in nature before. *Neosartorya fischeri* ascospores that contain TOS and trehalose are more viscous and more resistant to the combined stress of heat and desiccation than the ascospores of *T. macrosporus* that contain predominantly trehalose. TOS glasses have a higher glass transition temperature (T_g) than trehalose, and they form a more stable glass with crystallizing molecules, such as mannitol. Our data indicate that TOS are important for prolonged stabilization of cells against stress.

Keywords: ascospores; compatible solutes; stress resistance; trehalose; glassy state

**ISFUS-0053C**

**Two-stage maturation of extreme heat-resistant ascospores of Neosartorya fischeri (Aspergillus**
fischeri) involves reduction of bulk water and accumulation of trehalose and trehalose-based oligosaccharides

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Neosartorya fischeri ascospores survive stresses such as high temperature (85 °C) and drought (<0.5 % RH). In this study, acquisition of stress resistance during maturation of N. fischeri ascospores was related to accumulation of compatible solutes, the presence of bulk water, and redox stability. Ascospores of 11-day-old cultures were killed by a 2 min treatment at 85 °C, while spores of 15-50 day-old cultures survived this treatment. Spores of 50-day-old cultures even resisted a 50 min treatment at 85 °C. Individual ascospores isolated from 11- and 15-day-old cultures contained 3.9 pg (454 mM) and 12.1 pg (1027 mM) compatible solutes, respectively. This amount increased to 15.4 pg (1051 mM) in ascospores of 50-day-old cultures. The composition of the compatible solutes in the ascospores changed during growth of the culture. Glycerol levels had disappeared in ascospores of 15-day-old-cultures, while mannitol levels decreased after day 20. In contrast, the relative amount of trehalose and trehalose-based oligosaccharides increased until 50 days of culturing. Bulk water, as measured by electron spin resonance (ESR) spectroscopy, was much higher in spores of 11-day-old cultures when compared to spores of 15- to 50-day-old cultures. Ascospore maturation also coincided with increased redox stability. This stability gradually increased during maturation. Dry heat storage of 3 days at 60 °C didn’t affect the spin probe immobility or the redox stability of the polar cytoplasmic environment of dried ascospores. However, the redox stability of the more hydrophobic cytoplasmic environment (possibly in the proximity of lipid membranes) did decrease due to dry heat storage. Taken together, this study distinguishes two maturation stages of ascospores. The first stage is accompanied by a reduction of bulk water in the spores, the second stage is characterized by an increase of trehalose and TOS. Redox stability build up was observed during both stages.

Keywords: ascospores; compatible solutes; stress resistance; maturation; viscosity

ISFUS-0059

Involvement of the Neurospora crassa transcription factor encoded by ORF NCU01629 in the regulation of oxidative stress and apoptosis
Oxidation is a fundamental part of the aerobic metabolism of microorganisms. Reactive Oxygen Species (ROS) are produced naturally during the course of aerobic metabolism, however, under environmental adversities, its excess promotes damaging effects such as oxidation of membrane lipids and damage to proteins and DNA. The fungus *Neurospora crassa* has been widely used as a model organism for the study some aspects of the biology in eukaryotes. The knowledge of its genome sequence has allowed starting doing functional genomics and, thus, attributes function to hypothetical-annotated proteins. In this work, we investigated the functional role of the ORF NCU01629 product, a transcription factor belonging to the zinc-finger family with no functional homologues. In vitro analysis of the protein-DNA interactions allowed the identification of its DNA-binding motif as well as the genes likely regulated by this transcription factor. These genes were functionally categorized by FunCat, Gene Ontology and Balst2Go. The results revealed a high involvement of the transcription factor in cellular events related to different stresses oxidative as well as apoptosis. Analyses of the radial growth of the NCU01629KO in petri dishes containing different stressing conditions, such as osmotic, heat and oxidative stress were performed. The mutant strain showed similar growth to the wild-type strain under conditions that induces osmotic (0.1-1.5 M NaCl and sorbitol 1-1.5 M), pH (4.2 and 7.8) and heat (45 °C) stresses. However, the mutant strain growth was strongly influenced under exposition to different ROS inducing agents, such as paraquat (10 μM), menadione (50 μM) and H2O2 (2 mM) and when treated with farnesol (10 μM). The mutant strain showed a lower radial growth in the presence of paraquat and increased resistance when exposed to H2O2 and menadione and irregular growth in the presence of farnesol. Expression of ROS-associated genes (cat-1-cat-2, cat-3, gst-1, gst-2, sod and nox) and apoptotic genes (bax, metascaspases-1A, and p53-like) was analyzed by qPCR. Results showed that the transcription factor is involved in the regulation of the response to oxidative stress, controlling the expression all genes. The gene encoding glutathione S-transferase omega 1 (gst-om), an unpredicted regulation target of the NCU01629 product, was used as negative control. This gene showed not be regulated by the transcription factor in the qPCR analysis. In order to evaluate the ROS production after exposition to different ROS-inducers agents (paraquat, menadione and H2O2), analyses were performed by fluorescence microscopy using the 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) reagent. The preliminary results using H2O2 showed that the mutant strain exhibited a diffuse fluorescence while the wild-type strain showed regular fluorescent dots distributed along the hyphae.

Keywords: *Neurospora crassa*, Transcription factor, ROS, Programmed Cell Death.

Acknowledgments: FAPESP and CNPq for grants and fellowships.
Extracellular pH has an important role in cell biology as it regulates gene expression and consequently influences a variety of processes, such as growth, differentiation, development and pathogenicity. The regulation of gene expression by pH has been extensively studied in Aspergillus nidulans and in several yeasts, including Saccharomyces cerevisiae. In A. nidulans, the central regulator PacC is activated by two successive proteolytic cleavage steps: the first being pH-dependent and activated by the pal genes cascade while the second is proteasome-mediated and pH-independent. PacC recognizes the core consensus sequence 5'-GCCARG-3' present in the promoters of pH-regulated genes. The influence of pH in the regulation of the glycogen metabolism was previously reported in Neurospora crassa. The N. crassa genome has the six A. nidulans Pal homologues and many PacC motifs were identified in the promoters of genes involved in the glycogen metabolism. Here we performed the characterization of the N. crassa pal genes and their participation in the glycogen metabolism regulation by pH through the activation of PACC. The pal mutant strains showed high melanin production and normal growth at pH 5.8. However, they were unable to grow at alkaline pH (7.8) confirming that the N. crassa pal genes are involved in the pH-signaling pathway. The expression of the palA, palB, palF, and palI genes was clearly influenced by pH 7.8 and showed to be regulated by PACC in the same pH. All these gene promoters possess a PACC motif and ChIP-qPCR analyses demonstrated that they were bound by the transcription factor under alkaline pH confirming their regulation by PACC. According to our results, PACC may act as activator of the palA, palB and palI genes. The glycogen accumulation was quantified in all pal mutant strains and compared to the pacCKO and wild-type strain. As previously demonstrated, the pacC mutant strain accumulated higher level of glycogen than the wild-type strain in pH 5.8 and 7.8. However, the levels accumulated by both strains in alkaline pH were lower than pH 5.8. In a similar way, all pal mutant strains, except palI, accumulated more glycogen than the wild-type strain in both pH. All glycogenic gene expression was either up- or down-regulated by alkaline pH in the wild-type strain. In addition, all genes, with the exception of gbn, were regulated by PACC in pH 5.8, indicating that PACC regulates these genes in a pH-independent manner. However, PACC only bound in vivo to the glycogenic gene promoters in pH 7.8. These results indicate that the N. crassa pal genes play a role in the pH-signaling pathway, leading to the PACC activation and regulation of glycogen metabolism by PACC.

Keywords: Neurospora crassa; pal genes; PACC transcription factor; pH-signaling; glycogen
Acknowledgments: FAPESP and CNPq

STUDENT COMPETITION

ISFUS-0067

Effect of heat stress and oil formulation on conidial germination of *Metarhizium anisopliae* on tick cuticle

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The current study evaluated the effect of heat on conidial germination of *Metarhizium anisopliae* IP 119 when suspended in pure mineral oil (Impex®) or in water (Tween 80, 0.01%) and applied to the cuticle of *Rhipicephalus sanguineus* (*in vivo*). Percent conidial germination *in vivo* was compared to the germination of heated conidia inoculated onto artificial medium (*in vitro*). Conidial suspensions of *M. anisopliae* in water or oil, adjusted to 10⁸ conidia ml⁻¹, were exposed for 0 h (control) or 4 h to 45 ± 0.5°C in a water bath, and then, inoculated onto the dorsal surface of *R. sanguineus* engorged females. Ticks were incubated at 27 ± 1°C and RH > 80% for 0, 12, 18, 24, 36, 48 or 72 h. In parallel, aliquots of the aqueous conidial suspensions (control or heated) were inoculated onto PDAY in Petri plates and incubated at 27 ± 1°C and RH > 80% for the same time periods described above. After each incubation time, ticks were fixed, dissected, and a fragment of the dorsal cuticle was removed. Cuticles that received conidia suspended in water, exposed or not to heat, were processed for fluorescence microscopy and scanning electron microscopy (SEM) for evaluation of conidial germination, whereas cuticles that received conidia suspended in oil were processed for SEM only. Conidial germination on PDAY plates was assessed using a phase-contrast microscope. A minimum of 300 conidia per cuticle or plate was evaluated for calculation of relative germination. The conidial germination on the tick cuticle was delayed in comparison to the germination in artificial culture medium. When conidia were exposed to heat, a high percent germination was observed in PDAY medium (61.5%) in comparison to the germination on the tick cuticle (13%), 72 h after inoculation. When conidia were suspended in water or oil, not exposed to heat, and applied to the cuticle, appressoria were observed at 36 h. However, when exposed to heat stress, appressoria were visualized only in conidia suspended in oil. On the tick cuticle, the percent germination of conidia suspended in mineral oil and exposed to heat was high in comparison to the percent germination of conidia suspended in water, at all incubation times investigated. At 36 h of incubation, the mean percent germination of conidia suspended in oil reached 16.3%, whereas conidia suspended in water reached only 2.2%. Thus, the results showed a significant delay in germination for conidia suspended in water and exposed to 45°C in comparison to conidia suspended in mineral oil. Therefore, the results indicated that...
the negative effect of heat on conidial germination was more expressive when conidia were suspended in water and applied to the arthropod cuticle than it would be predicted by the in vitro thermotolerance tests. Also, conidia suffered less interference from heat exposures when suspended in oil. In conclusion, mineral oil protects conidia against the heat effects and enhances the fungal germination on the cuticle of R. sanguineus.

Keywords: Rhipicephalus sanguineus, formulation, entomopathogenic fungi.

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STUDENT COMPETITION

ISFUS-0069

Hyphae on real materials: the influence of the amount of water on the hyphal growth rate of Penicillium rubens on gypsum

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The primary cause for indoor mould growth is generally understood to be the presence of moisture. Specifically, it is the pore water in porous building materials that can be used by fungi. Research on growth on porous substrates has mainly focussed on the role of ambient relative humidity (RH). This is due to its coupling to the water activity (a_w) of the pore water, known for its influence on growth. The role of a substrate’s porous properties, which influence the coupling between RH, a_w, and other parameters such as moisture content (θ), has received less attention. Further, most studies approached the quantification of fungal growth macroscopically, as microscopy is difficult on these rough substrates. The aim of this research was to find the relations between the extension rate of hyphae and respectively the a_w and θ in a porous substrate. We constructed a video microscopy setup that monitors hyphae on gypsum substrates in a chamber with well-defined moisture conditions. Gypsum samples were either equilibrated with a dynamically controlled RH or soaked with an aqueous glycerol solution. This way, it was possible to prepare substrates with different amounts of pore water at equal water activities. Penicillium rubens, a typical indoor fungus formerly known as P. chrysogenum, was used as test organism. The results suggest that hyphal growth rate decreases when a_w or θ are decreased. Further, it was seen that the minimal a_w required for growth depends on θ. One explanation for the influence of θ is its relation to the spatial distribution of water. A sparser network of water could limit a hypha’s access to both water and nutrients. Another factor that could play a role is the varying availability of osmolytes that allow the fungus to deal with osmotic stress. The research is ongoing and currently focuses on the immediate hyphal response to changing moisture conditions.
Keywords: Building Materials; Water Activity; Moisture Content; Hyphal Growth Speed; Video Microscopy

STUDENT COMPETITION

ISFUS-0070

Evaluating the light-induced stress tolerance in *Metarhizium acridum* by proteomics

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Visible light is an important stimulus controlling many aspects of fungal biology. In the entomopathogen *Metarhizium acridum* light is responsible for increasing conidial tolerance against environmental stress. For instance, the light-induced increase in conidial tolerance for ultraviolet radiation can be as high as twofold, which is relevant considering that *M. acridum* has a potential use on the biological control of agricultural pests. Therefore, in an attempt to elucidate the molecular mechanism governing this process, we analyzed the conidial proteome of *M. acridum* grown under Light (12:12h photoperiod for 14 days) and Dark (complete darkness) conditions. Total protein was extracted from conidia of both treatments, purified, quantified, and the same amount of protein from each condition was trypsin-digested and separately injected into a SCX-HPLC (Strong Cation Exchange – High Performance Liquid Chromatography). Collected fractions were then individually injected into a RP-UPLC (Reverse Phase – Ultra Performance Liquid Chromatography) directly coupled to an ESI-Q-TOF MS/MS (Electrospray Ionization – Quadrupole – Time-Of-Flight Mass Spectrometry/Mass Spectrometry). Three independent experiments were performed, each with a technical duplicate injection. Raw data was analyzed using Mascot Distiller (Matrix Science) and Scaffold (Proteome Software). Differences in conidial proteome between Light and Dark treatments were evaluated using precursor ion intensity as a label-free quantitative method. Overall, we identified 355 proteins belonging to conidia (99% protein threshold, 1.0% FDR peptide threshold, 2 peptides). Of these, only four proteins accumulated differently in conidia of Light and Dark conditions (*P* < 0.05 in Mann-Whitney Test). Three proteins were up-regulated under Light and only one was up-regulated in the Dark. One of the proteins up-regulated under Light (HHE domain containing protein, MAC_07529) is homologous to the *cetJ* gene product found in *Aspergillus nidulans*. *cetJ* is a conidiation-associated gene whose transcript accumulates in conidia in response to light. It is worth noting, however, that – as opposed to what happens in *A. nidulans* – there is no evident induction of conidiogenesis by light in *M. acridum*. The other two up-regulated proteins under Light are actin (MAC_05223) and cell cycle control protein (MAC_01024), an ATP-dependent RNA helicase whose specific function is unknown. In the Dark treatment, the only up-regulated protein found was ribitol kinase (MAC_00029), a protein involved in the pentose phosphate pathway. We expect that the results obtained here will
open new possibilities for the study of the light-induced stress tolerance in *M. acridum*. To our knowledge, this is the most complete work on the conidial proteome of *Metarhizium* spp. Keywords: *Metarhizium*, stress, tolerance, proteomics, light

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**ISFUS-0071**

Isolation of the antibacterial agent viridiol from the mangrove endophytic fungus *Hypocrea virens*

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Mangrove forests are among the most productive and diverse ecosystems in the world. The great biotechnological potential of mangrove plants can be related to the endophytic fungal community that is mutualistically associated with the plants. Endophytic fungi are considered a source of novel activities, compounds and biotechnological processes with great and underexplored potential. Fungi from marine environments grow in a habitat with unique conditions, which can contribute to the activation of metabolic pathways for the synthesis of different unknown molecules, and the production of these compounds may support the adaptation of the fungi in the marine ecosystem. The great biodiversity found in mangrove forests shows the importance of research involving these areas, including studies regarding new compounds derived from the endophytic fungi that inhabit these ecosystems. Viridiol was isolated from the mangrove endophytic fungus *Hypocrea virens*, which was obtained from branches of *Avicennia nitida*. The structure of the compound was elucidated by spectroscopic methods, mainly 1D and 2D NMR spectroscopy and mass spectrometry. In bioassays, viridiol showed antimicrobial activity against *Escherichia coli*. The antibiotic-producing strain was identified using internal transcribed spacer (ITS) sequence data. This is the first report of an antimicrobial activity by viridiol against a medically important pathogen. Keywords: Endophytic fungi, *Hypocrea virens*, NMR spectroscopy, *Trichodema*.

**ISFUS-0073**

Growth of *Penicillium rubens* after desiccation

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Indoor fungal growth affects human health and is therefore an important societal problem. To prevent or limit fungal growth it is important to understand how fungi are able to survive the stress conditions of the indoor environment, such as a low average humidity and extreme fluctuations in humidity. To quantify growth of indoor fungi, the “Fungal Observatory Climate controlled aUtomized Set-up” (FOCUS) was developed. Using the FOCUS, fungal growth can be measured accurately as a function of time under controlled temperature and humidity conditions. In the FOCUS surface discoloration of samples is captured in digital images. Discoloration of the surface is a result of pigmentation of conidia that are visible to the naked eye, and used as an indirect measure of growth. In these experiments gypsum (CaSO\(_4\) \(\cdot\) 2 H\(_2\)O) was used as representative of a common building material, and *Penicillium rubens* was used as model organism for indoor fungi. Using cryo-scanning electron microscopy (cryo-SEM), surface images were made to verify growth and sporulation of *P. rubens* on the gypsum samples. The effect of the developmental stage of *P. rubens* prior to a desiccation period on surface discoloration was tested. And the effect of the duration of the desiccation period was tested on surface discoloration. We show that the time to visible sporulation of *P. rubens* on gypsum exposed to continuous high relative humidity (RH) of 97% is 82 hours. When *P. rubens* is exposed to a 48 hour desiccation period, the total time to sporulation increased. However, the time after the desiccation period to sporulation remained 82 hours, irrespective of the developmental stage of *P. rubens* prior to desiccation. It is as if after a long desiccation period the development of *P. rubens* was ‘reset’. This suggests that regrowth after a 48 h desiccation period is caused by remaining conidia. The time to visible sporulation after a short period of desiccation, 1 hour up to 24 hours, is shorter than sporulation after a 48 hour period of desiccation. The growth of *P. rubens* therefore does not seem to be completely reset after such a short desiccation period. Because the time to sporulation after a short period of desiccation is shorter than expected based on remaining conidia, this suggests that next to conidia, also other structures of the mycelium can resume growth after desiccation.

**Keywords:** desiccation, fungal growth, *Penicillium chrysogenum*, *Penicillium rubens*, indoor

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**Photodynamic therapy of clinical isolates of *Neoscytalidium dimidiatum* and *N. hyalinum* with phenothiazinium photosensitizers**

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*Neoscytalidium dimidiatum* is a saprophytic fungus found in soil and vegetation of tropical climate. The habitat of *N. hyalinum* is unknown, and is considered a hyaline variant of *N. dimidiatum*. 
Infections by *N. dimidiatum* and *N. hyalinum* affect immunocompromised and immunocompetent patients, specially causing dermatomycoses and onychomycosis. Considering the increasing resistance to antifungal agents against opportunistic fungi is necessary an alternative treatment to this type of infection. Antimicrobial Photodynamic therapy (APT) is based on the use of a photosensitizer (PS) that accumulates in the target fungal cell. The exposure of the PS to light of an appropriate wavelength starts a photochemical process that produces reactive oxygen species leading to non-specific oxidative damage causing the death of the fungal cell without significant harm to the human cells. So, the aim of this study was to evaluate the effectiveness of APT using four phenothiazinium photosensitizers (PS) against both, *N. dimidiatum* and *N. hyalinum* isolated of skin and nails infection. The APT test was done with methylene blue (MB), toluidine blue O (TBO), new methylene blue N (NMBN) and a novel pentacyclic phenothiazinium S137. Initially, the minimum fungicidal concentration (MFC) was determined to each PS by using 96-well flat-bottomed microtiter plates with 0.5 to 200 µM of each PS and 4×10⁵ arthroconidia ml⁻¹. The plates were exposed to light fluences of 5, 10 and 20 J cm⁻² using a LED array (emission peak 635 nm) and the MFCs were determined after 48, 72, and 96 hours by visual inspection. Based on the results of MFC experiments, the optimized conditions for APT with each PS on the surveillance of arthroconidia were determined. Then, 2×10⁷ arthroconidia ml⁻¹ of two *N. dimidiatum* and one *N. hyalinum* were incubated with 200 µM of MB, TBO, NMBN and 25 µM of S137 for two isolates of *N. dimidiatum*, and 25 µM of MB and NMB, and 10 µM of TBO and S137 for *N. hyalinum*, with light fluences of 5, 10 and 20 J cm⁻². The range MFC for NMBN, MB, TBO and S137 were 10-200 µM, 25-200µM, 10-200µM, 10-37.5 µM, respectively for clinical isolates of *N. dimidiatum*, and 2.5-25 µM, 5-25 µM, 2.5-12.5 µM, 2.5-10 µM respectively for clinical isolates of *N. hyalinum*, when a light fluence of 20 J cm⁻² was applied. APT with MB and TBO resulted in a gradual reduction of growth with the increase of light fluence, about reduction of 1 log with 5 J cm⁻², 3 logs with 10 J cm⁻² and 5 logs with 20 J cm⁻² for *N. dimidiatum*, and 3 logs with 5J cm⁻² and 6 logs to 10 and 20 J cm⁻². NMBN and S137 resulted in a reduction of approximately 6 logs in the surveillance of the arthroconidia for all clinical isolates analyzed, for both species with light fluence of 20 J cm⁻². These results indicate that *N. hyalinum* were more susceptible for APT than *N. dimidiatum*, and APT could be an interesting treatment of skin and nail infections due *N. dimidiatum* and *N. hyalinum*. Keywords: Antimicrobial photodynamic treatment, phenothiazinium photosensitizers, *Neoscytalidium dimidiatum*, *Neoscytalidium hyalinum*.

**ISFUS-0076**

Enhancing UV-B tolerance of *Isaria fumosorosea* and *Beauveria bassiana* conidia by adjuvants

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The ultraviolet radiation (UV-B) component of sunlight influences the efficacy of entomopathogenic fungi in the field. The incorporation of adjuvants (surfactants, emulsifiers and mineral oils) in mycoinsecticide formulations can increase the protection to UV-B radiation, prolong the persistence of conidia and increase the efficacy against target pests. In this study, the protective effect to UV-B radiation of 11 adjuvants on *Isaria fumosorosea* ESALQ-1296 and *Beauveria bassiana* ESALQ-PL63 conidia was evaluated. The fungi were grown on Potato Dextrose Agar medium (PDA) at 25°C with a 14 hours photophase for 10 days. Conidia were harvested from the surface by scraping with metallic spatula and fungal suspensions were prepared at concentration of 1 x 10^6 conidia/mL with each adjuvant. Aliquots of each suspension (150 μl) were inoculated in Rodac® dishes containing 5 mL of PDA culture medium with 5 g/L of Pentabiotico® and 10 µL/L of Derosal® and immediately transferred to a wooden chamber with four UV-B 313EL lamps (Q-lab Cleveland, OH) with a stable level of irradiance (1312.4 mW/m^2 and 5.20 KJ/h) with primarily UV-B (peak at 313 nm) and minimal UV-A radiation output. All dishes were covered with a 0.13 mm-thick cellulose diacetate film, which blocked radiation below 290 nm, and dishes were exposed to UV-B radiation for 1, 2, 3, 4, 6, 7 and 8 hours (total irradiance: 5.20, 10.4, 15.6, 20.8, 31.2, 36.4 and 41.6 KJ/m^2, respectively). Control plates were not exposure to UV-B radiation and all treatments were repeated at least four times. After irradiation, the dishes were incubated at 26 ± 1 °C with a 14 h photophase for 24 hours (control) or 48 hours (irradiated plates). The percentages of germination were assessed by observing at least 200 conidia under optical microscope (400 X). *I. fumosorosea* conidia without addition of adjuvants were more tolerant than *B. bassiana* conidia, obtained mean germination of 18.9 ± 7.3% and 12.6 ± 4.9% after 8 and 4 hours exposure to UV-B radiation, respectively. The incorporation of Iharol 0.5% (a mineral oil) in the conidia suspension enhanced survival of conidia of both fungi, where germination mean achieved 33.9 ± 10.2% in *I. fumosorosea* (after 8 hours to exposure) and 59.3 ± 9.2% in *B. bassiana* (after 4 hours to exposure). However, the surfactants Silwet L-77 0.025% and Argenfrut 1% presented the lowest germination mean in *B. bassiana* suspension (13 ± 6.1% and 21.9 ± 7.3%, respectively) and Du Fol 0.2% and Iharagen-S 0.01% in *I. fumosorosea* suspension (1.1 ± 1% and 0.1 ± 0.08%, respectively).

In conclusion, the addition of the mineral oil Iharol in entomopathogenic fungi formulations could enhance the efficacy of mycoinsecticides in field.

Keywords: microbial control, entomopathogenic fungi, UV-B radiation, mycoinsecticides.

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**ISFUS-0077**

**Black Yeast Fungi under toluene saturated atmosphere**

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Recent studies reported the presence of melanized fungi of the Chaetothyriales order (known as black yeasts) in environments rich on oil derived hydrocarbons, as biofilters for toluene treatment, soil contaminated with oil and gasoline, wood treated with phenolic preservatives and wooden railway treated with creosote. Chaetothyrialean black yeasts clearly tend to assimilate volatile aromatic compounds as the sole carbon source and energy. Extremophilic character of this group suggest their ability to survive at stressing levels of BTEX. This study aimed to evaluate the toluene-tolerance of black fungi isolated from hydrocarbon contaminated soil. The isolated strains were grown on 2% malt agar for 7 days. Later they were inoculated in test tubes containing 3 ml of mineral medium. Each tube had previously your weight obtained in an analytical balance. After inoculation were covered with aluminum foil, which was perforated at the time of incubation to allow diffusion of the atmosphere inside the tube and incubated on a desiccator, where was created an atmosphere rich in toluene as the only available carbon source. The strains that showed higher biomass production were inoculated at a concentration of 20 µl of toluene for 15 days. The decay of hydrocarbon was followed by GC-FID from Shimadzu GC-2014 model, and after 10 days from inoculation, showed that a strain of *Exophiala dermatitidis* was able to tolerate and assimilate the toluene inside the flasks. The result indicates that besides its tolerance, the black yeast has potential for studies on biorremediation of polluted soils.

Keywords: Hydrocarbon; stress tolerance; biodegradation; Chaetothyriales; soil

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**STUDENT COMPETITION**

**ISFUS-0080**

**Methods to enhance entomopathogenic fungi tolerance to UV-B**

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Attaining the full promise of entomopathogenic fungi in arthropod-pest control depends not only on a) the virulence of the fungal isolate, and b) host susceptibility; but also c) tolerance to adverse environmental conditions such as low humidity, high temperatures...
and intense ultraviolet radiation (UV-A and UV-B), and d) fungal persistence in the environment. Two strategies were studied to enhance fungal tolerance to or protection from UV-B damage: i) the addition of protective adjuvants in fungal aqueous suspensions; and ii) *Metarhizium* endophytic colonization, particularly by foliar application of conidia. Relative germination percentage of 20 *Metarhizium* spp. isolates formulated in 10% mineral oil or not were compared. Similar tests with two isolates were done using the commercial available pesticide adjuvant NALCOTROL® (polyvinyl polymer) and with one isolate using Coats Agrialoe® (aloe vera gel adjuvant). The irradiation was provided by two UV-B fluorescent lamps and a cellulose diacetate filter was used to exclude UV-C and short wavelength UV-B radiation. Conidia on PDAY plates (amended with 0.002% benomyl) were exposed to UV-B for 1.5 h (4.07 kJ m⁻²) and incubated at 28 °C for 24 hours. Control conidia were treated similarly but without UV-B exposure. Germination was observed successfully without oil removal. The success in counting conidia in oil was largely due to the methyl blue dye, which stains the fungi and not the bubbles in the formulation that can be easily mis-identified as fungal spores. Mineral oil-based formulations clearly protected conidia from deleterious UV-B effects. NALCOTROL®-based formulations were less effective and Coats Agrialoe® formulations were not effective. The second approach to protecting *Metarhizium* from UV-B effects is based on recent reports of this fungus and its association with plants through rhizosphere competence and endophytic growth in roots. These studies reported invasion of *Metarhizium* into plants through the roots, but not after foliar application (method most commonly used in the biological control of insects using entomopathogenic fungi). Accordingly, we analyzed 20 plants for fungal endophytic colonization. *Metarhizium brunneum* ARSEF 1095 conidia were applied onto leaves; After 14 days, plant tissues (roots, stems and leaves) were surface sterilized (NaClO/alcohol) and inoculated on CTC selective medium to attempt re-isolation of the fungus. Endophytic colonization of cowpea leaves with *M. brunneum* was confirmed by successful cultures of the fungus from surface-sterilized leaves; although, the fungus could not be re-isolated from roots or stems of foliar-inoculated plants.

**Keywords:** Adjuvants, formulations, *Metarhizium*, fungal endophytes

**Acknowledgments:** This research was supported in part by grants from the United States Department of Agriculture (USDA)/ Animal Plant Health and Inspection Service (APHIS). We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) from Brazil for providing scholarships for PSG. VRFB is a CNPq researcher.

**ISFUS-0084 Benzene tolerance of filamentous fungi**

Fernando S. Nobre¹, Ayumi A. Otsuka², Adilson Sartoratto¹, Derlene Attili-Angelis¹,² and Fabiana F. Garboggini¹

¹ Microbial Resource Division, CPQBA, Universidade Estadual de Campinas, Paulínia, SP, 13148-218, Brazil. ² Instituto de
Contamination caused by toxic compounds is a problem of global concern, not only by the magnitude of the impact generated in the environment, but also for damaging human health from carcinogenic to teratogenic effects. Fuel contaminants like monoaromatic hydrocarbons are the constituents that have higher solubility in water and therefore their contamination may reach vast areas, jeopardizing all living organisms. Studies have demonstrated the benzene tolerance of fungal species, highlighting their potential to degrade these substances under conditions of stress. Therefore, many fungal species may have an important role in bioremediation techniques under unfavorable conditions for the majority of microorganisms. We studied the tolerance of fungal strains isolated from soil contaminated areas and the initial analysis used benzene 0.2% as the sole carbon source. Among the 300 isolates tested so far, 57 showed a positive result when inoculated in culture medium with benzene, incubated at 28°C, in triplicates. This growth was observed by the formation of a colorless halo in contrast to a redox indicator used to observe the benzene degradation. The diameters of halos were measured every 3 days in individual plates. Among all isolates tested, one strain showed the largest halo until the moment and filling the entire plate on the 9th day of growth compared to the negative control without the addition of benzene. These preliminary tests demonstrated the fungal ability to tolerate the stress caused by the toxicity of benzene at 0.2%.

Keywords: Hydrocarbon; stress tolerance; biodegradation; benzene; contamination

Acknowledgments: We sincerely thank PETROBRAS for providing the soil samples, and the Foundation for development of Unicamp – FUNCAMP.

**STUDENT COMPETITION**

**ISFUS-0086**

**Abundance and stress tolerance of soil fungal community from native forests, reforestations, and degraded areas**

Paulo C. Ferreira and Drauzio E. N. Rangel

*I Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP 12244-000, Brazil.

Address for correspondence: pcferreira@yahoo.com.br

Microorganisms are essential to the functionality of the soil, particularly in organic matter decomposition and nutrient cycling regulating plant productivity, and shaping the soil structure. The soil microbial community can be used as an indicator of the condition of these ecosystems how it will influence the recovery of degraded areas. This study aimed to quantify seasonally the fungal abundance of two native forest, five reforestation areas with Atlantic rain forest native species, and a sand mining degraded area. In addition, the tolerance of these fungal communities was evaluated to UV-B and heat, and cold activity. The most conserved Atlantic forest fragment,
called Forest 2, provided larger fungal abundance, followed by the oldest reforestation areas. The degraded area, however, had the smallest fungal abundance. Higher tolerance to UV-B radiation and heat was found in the fungal communities of the degraded area and the 2009 reforestation where the incidence of the solar radiation is more intense and the fungal communities are more adapted to these abiotic factors. The cold activity of fungal communities of all studied areas was similar. Therefore, the fungal biodiversity of the Atlantic native forest remnants and completely degraded soils by sand mining where reforestation is being done was of great importance for the understanding its role in environmental recovery of these areas.

Key words: soil fungi; UV-B tolerance; heat tolerance; cold activity; reforestation; native forest; degraded soil.

We sincerely thank the National Council for Scientific and Technological Development (CNPq) of Brazil for DENR grant support # 473104/2008-3, # 478899/2010-6, and 452880/2011-4 and the State of São Paulo Research Foundation (FAPESP) #2010/06374-1. We are also thankful for fellowships from FAPESP 2014/13573 for P.C.F.

STUDENT COMPETITION

ISFUS-0087

The APSES transcription factor StuA is involved in secondary metabolism, oxidative stress response, morphogenesis and virulence in the dermatophyte *Trichophyton rubrum*

Elza A. S. Lang¹, Nalu T. A. Peres¹, Vanderci M. Oliveira¹, Antonio Rossi¹ and Nilce M. Martinez-Rossi¹

Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, SP, 14049-900, Brazil.

Address for correspondence: elzalang@usp.br

The dermatophyte *Trichophyton rubrum* is a human fungal pathogen and the main causative agent of clinical cases of skin and nail mycoses, called dermatophytoses. The ability to degrade and use keratin as a nutrient source is considered one of the main virulence attributes of this fungus, which infects keratinized tissues. We identified a gene transcriptionally modulated in *T. rubrum* during growth in human nail. This gene encodes StuA, a protein belonging to the APSES family of transcription factors, characterized by the presence of the APSES domain, exclusive of the fungi kingdom. The APSES regulators have been studied in several pathogenic and non pathogenic fungal species, including yeasts and filamentous fungi, and shown to be implicated in a wide range of processes. Here, we report the functional characterization of *stuA* of *T. rubrum*. A null mutant was generated by the deletion of *stuA*, by gene targeting. During the growth on solid media, the mutant strain presented shortened aerial hyphae and diminished pigmentation. Microscopy analyses of the mutant strain revealed defects in morphology, with hypertrophy of conidia and hyphae. Compared to the wild type strain, *stuA* mutant exhibited increased germination rate and mycelia...
were more resistant to hydrogen peroxide in complex medium. However, the mutant strain was impaired to grow in an \textit{ex vivo} model of nail infection. Our results suggest the involvement of \textit{stuA} in secondary metabolism, oxidative stress response, morphogenesis and virulence of \textit{T. rubrum}.

Keywords: APSES transcription factor, Trichophyton rubrum, dermatophyte, \textit{stuA}

Acknowledgments: This work was supported by grants from the Brazilian funding agencies FAPESP, CNPq, CAPES, and FAEPA.

\textbf{ISFUS-0089}

\textbf{PacC contributes to osmotic stress tolerance in \textit{Trichophyton interdigitale}}

Nalu T. A. Peres\textsuperscript{1}, Larissa G. Silva\textsuperscript{1}, Vanderci M. Oliveira\textsuperscript{1}, Pablo R. Sanches\textsuperscript{1}, Antonio Rossi\textsuperscript{1} and Nilce M. Martinez-Rossi\textsuperscript{1}

\textit{Department of Genetics, Ribeirão Preto Medical School, University of São Paulo.}

Address for correspondence: nalu@usp.br

The transcription factor PacC is a component of the pH signaling pathway, which has also been implicated in cell wall remodeling, production of secondary metabolites, metal toxicity, and salt stress. In dermatophytes, pathogenic fungi responsible for the majority of nails and skin infections, PacC regulates genes in both acidic and alkaline pH, post translational modifications, such as glycosylation, and keratinolytic activity. In this work, we evaluated the involvement of PacC in osmotic tolerance of the anthropophilic dermatophyte \textit{Trichophyton interdigitale}, and the regulation of HOG (High Osmolarity Glycerol) pathway-related genes by PacC. The \textit{pacC} mutant strain gene was sensitivity to osmotic stress and tolerant to cell wall disturbances, in comparison to the wild type. Given the interplay of different signaling pathways, we analyzed 1000 bp upstream the ORF (Open Read Frame) of the genes composing the HOG pathway searching for the putative PacC DNA binding consensus. This \textit{in silico} analysis revealed that several genes of this pathway present the PacC DNA binding sequence, suggesting a regulation by this transcription factor. However, gene expression analysis showed that the HOG pathway-related genes were not upregulated in response to osmotic stress in \textit{T. interdigitale}, in the conditions tested. Moreover, no differences in the expression profile of these genes were observed between the wild type and \textit{pacC-1} mutant strains. We also evaluated the expression of the gene coding for the Na\textsuperscript{+} ATPase ENA1, involved in the efflux of excessive sodium from the cell, showing that this gene was not regulated by osmotic stress or PacC in the conditions tested. These results indicate that PacC is involved in \textit{T. interdigitale} tolerance to osmotic stress, by regulating genes other than the HOG pathway, and that in this dermatophyte, the genes composing this pathway are not modulated during osmotic challenge. Given the regulation of glycosylation-related genes by PacC in this dermatophyte, it is possible that the activity of some enzymes involved in osmotic stress might be indirectly regulated by PacC, in a post transcriptional manner. Moreover, cell wall remodeling may also account to the \textit{pacC-1} strain sensitivity to osmotic stress, once this strain presented...
tolerance to congo red, a cell wall interfering agent. Nevertheless, other pathways may be related to tolerance to osmotic stress in dermatophytes, a process that should be further evaluated.

Key words: dermatophytes, PacC, osmotic stress.

Financial Support: FAPESP, CNPq, FAEPA.

Assessment of the degradation of synthetic textile effluent by Aspergillus niger AN 400 in sequencing batch reactor

Alyce H. B. Sousa¹, Carolina O. Marinho¹, Aurenivia M. M. Cavalcante¹, Kelly A. R. Pessoa¹, Gustavo E. Santos¹, Carlos R. P. Wanderley¹ and Luiz C. N. Silva¹

¹ Instituto Federal de Educação Ciência e Tecnologia do Ceará-Fortaleza, CE- Brazil.

Address for correspondence: alycehelida@gmail.com

Textile industries have largely contributed to environmental contamination due to the large production of waste with low levels of degradation including dyes, from the steps of dyeing effluents being discharged with intense coloration. All wastewater generated during the stage of textile processing are encaminhas for equalization tanks. The treatment of these effluents has been one of the most important categories of control of water pollution, mainly by the intensity of color and the high concentration of organic contaminants. Brazil is one of the largest textile and apparel producers in the world, being the fifth in the textile segment and the fourth in cooking. The use of fungi in biological reactors constitutes an alternative and innovative technology being used successfully for removal of recalcitrant compounds, such as dyes. The use of fungi for the treatment of persistent relates to high production of extracellular enzymes whose actions make them more amenable to biodegradation recalcitrant compounds. The species of Aspergillus are known for their ability to use dyes as substrate, transforming them into non-toxic compounds or low toxicity, which occurs by the production of extracellular enzymes, which makes it accessible to organopoluente assimilation. The Aspergillus Niger AN400 species was used for this study. Studied the ability of this fungus to degrade a synthetic effluent from the post dyeing a textile step, monitoring the efficiency in relation to the degradation of the dye, organic carbonaceous matter by adding glucose as the nitrogen and cossubstrato materials (nitrate nitrite and ammonia). The reactor was operated at 5 cycles in sequential regimen with retention time (RT) for 48h. The average Indigo Carmine dye removal was 71.47%, and 96.70% Maximum. The average removal efficiencies of COD and soluble crude were 25.43% and 19.23%, respectively. Fungal activity was monitored by the amount of nitrogenous bases, with mean removal of nitrate, nitrite and ammonia were 25.24%, 68.16% and 77.98%, respectively. The average pH was 3.56 compatible with ideal for the growth of fungi of the genus Aspergillus. Monitoring the growth of fungal species by microbiological analysis was performed. Therefore, we highlight the feasibility of the work in the degradation of textile effluents.

Keywords: Dye, degradation, Aspergillus.
STUDENT COMPETITION

ISFUS-0093

Study for removal of dye Indigo Carmine and organic matter in continuous flow reactor with up Aspergillus Niger AN 400

Carolina O. Marinho¹, Alyce H. B. Sousa¹, Luana P. Rodrigues¹, Kelly R. A. Pessoa¹, Gloria M. M. S. Sampaio¹ and Carlos R. P. Wanderley¹

¹ Programa de Pós-Graduação em Tecnologia e Gestão Ambiental – PGTGA do Instituto Federal do Ceará – IFCE, CE, 60040-215, Brasil

Address for correspondence: marinhocarolina@gmail.com

The textile sector has a prominent position in the economy of most developed countries and is also the main activity of developing many so called emerging countries. However, at the same time the textile industry contributes to the development, also contributes to the degradation of water sources to generate large volumes of waste liquids having contaminants, especially synthetic dyes, which are difficult to degrade and cause great environmental impact. Biological treatments have been employed in the removal of dyes from textile effluent promoting the reduction of the organic load present. The advantages of this type of treatment are lower sludge production than other chemical and physical systems, low operating costs and be environmentally friendly. The potential of fungi to remove textile dyes in biological reactors, characterized a technology that is becoming increasingly widespread. This potential can be explained by the fact that micro-organisms manage to identify the dyes, consuming them as nutrients, starting the process of absorption, the transformation of the dye into simpler compounds that are absorbed through the filaments of the fungus being taken. Therefore, the present work studied the removal of textile dye indigo carmine by Aspergillus niger AN400 using continuous and upflow reactor. The variables were monitored dye, organic matter, in terms of Chemical Oxygen Demand (COD) total and soluble, and hydrogenic potential (pH). The reactor was fed with synthetic wastewater textile - simulating the effluent coming from the washing of fabrics in textile processing step - added glucose (0.5 g/L) solution Vischiniac (1 ml/L - micronutrients) and macronutrients as (NH₄)₂SO₄, NaNO₃, KH₂PO₄, MgSO₄, among others, constitute the phase I. In phase II was removed macronutrients remaining glucose (0.5g/L) and micronutrient solution. The reactor was operated for 125 days under Hydraulic Detention Time (HRT) of 12 hours. The highest percentage of the COD removal total and soluble, in phase I, were 88.2% and 88.4%, respectively, since in the absence of macronutrients, the highest rates were 49.4% and 55.3%, respectively. The phase I had higher mean percentage of removal of COD total (74.82%), that phase II (39%) because the macronutrients are essential for fungal metabolism in the synthesis of cellular macromolecules cell interest. Regarding the removal of the dye Indigo Carmine, in phase I, removals reached 99.9%, being the average removals for phase I and II, respectively, 98.8% and 23.1%. The samples were recorded in the pH within the range 3.8 to 6.3, proving the ideal range for fungal metabolism.
Keywords: textile sector; glucose; macronutrients; fungi; metabolism.

STUDENT COMPETITION
<table>
<thead>
<tr>
<th>Time</th>
<th>Sunday October 26</th>
<th>Monday October 27</th>
<th>Tuesday October 28</th>
<th>Wednesday October 29</th>
<th>Thursday October 30</th>
<th>Friday October 31</th>
</tr>
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<tbody>
<tr>
<td>09:00 - 09:40</td>
<td>Luis M. Corrochano</td>
<td>Stefan Holmström</td>
<td>Raymond J. St. Leger</td>
<td>Gilberto U.L. Braga</td>
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<td>10:20 - 10:40</td>
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<tr>
<td>10:40 - 11:20</td>
<td>Jennifer Loom</td>
<td>Frances Pisan</td>
<td>John E. Halloworth</td>
<td>Nicolás Pedrini</td>
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<tr>
<td>11:20 - 12:00</td>
<td>Kevin K. Fuller</td>
<td>Johan M. Thevelein</td>
<td>Jan Dijkstraus</td>
<td>Drauzio E.N. Rangel</td>
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<tr>
<td>12:00 - 14:00</td>
<td>Lunch</td>
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<tr>
<td>14:00 - 14:40</td>
<td>Gertien Smits</td>
<td>Naresh Magan</td>
<td>Enric Gera-Olmedo</td>
<td>Marco R.V.Z. Kress</td>
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<tr>
<td>14:40 - 15:20</td>
<td>Alfredo Herrera Estrella</td>
<td>Pedro Moradas Ferreira</td>
<td>Javier Avalos</td>
<td>Bekker &amp; van Laarhoven</td>
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<tr>
<td>15:20 - 16:00</td>
<td>Coffee Break</td>
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<tr>
<td>16:00 - 16:40</td>
<td>Opening Ceremony</td>
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<tr>
<td>16:40 - 17:20</td>
<td>Tribute to Anita Panek</td>
<td>Maria Celia Bertoloni</td>
<td>Nemat O. Keyhani</td>
<td>Roger Finlay</td>
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<tr>
<td>17:20 - 18:00</td>
<td>Anita Panek</td>
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</table>

October 30 – Thursday morning – CEPLADE auditorium - Chair: Nemat O. Keyhani

Other aspects of the stress response in fungi (continued)

<table>
<thead>
<tr>
<th>Time</th>
<th>Speakers Meeting</th>
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</thead>
<tbody>
<tr>
<td>09:00 - 09:40</td>
<td>Gilberto Ubida Leite Braga - Antifungal photodynamic treatment as an alternative to control plant and human pathogenic fungi - Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.</td>
</tr>
<tr>
<td>09:40 - 10:20</td>
<td>Everton Kort Kamp Fernandes - Characterization of Metarhizium species and varieties based on molecular analysis, heat tolerance and cold activity - Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Brazil.</td>
</tr>
<tr>
<td>10:20 - 10:40</td>
<td>Zeiss Conference</td>
</tr>
<tr>
<td>11:20 12:00</td>
<td>Drauzio Eduardo Naretto Rangel – Phenotypic plasticity in stress tolerance of insect-pathogenic fungi - Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP, Brazil.</td>
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</tbody>
</table>

October 30 - Thursday afternoon – CEPLADE auditorium - Chair: Nicolás Pedrini

Other aspects of the stress response in fungi (continued)

<table>
<thead>
<tr>
<th>Time</th>
<th>Speakers Meeting</th>
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<tbody>
<tr>
<td>14:00 - 14:40</td>
<td>Márcia Regina von Zeska Kress - Non deratophyte fungi and its profiles under different stress conditions - Departamento de Análises Clínicas, Tocxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, SP, Brazil.</td>
</tr>
<tr>
<td>15:20 16:00</td>
<td>Coffee Break and Poster Section</td>
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<tr>
<td>16:00 - 16:40</td>
<td>Zeiss conference</td>
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<tr>
<td>16:40 - 17:20</td>
<td>Donald W. Roberts - The implications of increased UV-B radiation on microbial control of insects - Department of Biology, Utah State University, Logan, UT, USA.</td>
</tr>
<tr>
<td>17:20 - 18:00</td>
<td>Closing Ceremony</td>
</tr>
</tbody>
</table>

Symposium Organizers: Drauzio Eduardo Naretto Rangel and Alene Estelle Alder-Rangel
Picture Aspergillus nidulans ATCC 10074: Marco Antonio de Oliveira
Art “Stressed Aspergillus”: Drauzio Eduardo Naretto Rangel
October 28 – Tuesday afternoon – CEPLADE auditorium - Chair: Everett Kort Kamp Fernan.

Fungal responses to stress: gene regulation and cellular responses (continued)

14:00-14:40 Naresh Magan - Environmental stress impacts on secondary metabolite gene clusters, growth and metabolite production - a systems approach - Applied Mycology Group, Cranfield Soil and AgriFood Institute, Cranfield University, Bedford, U.K.


15:20-16:00 Coffee Break and Poster Section

16:00-16:40 Olaf Kniemeyer - Contribution of proteomics to the understanding of the Aspergillus fumigatus stress response - Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knoll-Institute, Jena, Germany.

16:40-17:20 Nemat O. Keyhani - Signaling, stress response, and virulence: insights from entomopathogenic fungi - Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, USA.

October 29 – Wednesday morning – CEPLADE auditorium – Chair: Kevin K. Fuller

Fungal responses to stress: gene regulation and cellular responses (continued)

09:00-09:40 Raymond J. St. Leger - Stress is the rule rather than the exception for Metarhizium - Department of Entomology, University of Maryland, College Park, MD, USA.

10:00-10:40 Short Coffee Break

10:40-11:20 John E. Hallsworth - Chaotropicity acts as a determinant of biotic windows, competitive interactions, and ecological success in fungi - School of Biological Sciences, MBC, Queen's University, Belfast, UK.

11:20-12:00 Jan Dijkstra - Fungi and the Indoor Challenge - Department of Applied and Industrial Mycology, CBI-KN-4F Fungal Biodiversity Centre, Utrecht, The Netherlands.

October 29 – Wednesday afternoon – CEPLADE auditorium – Chair: John E. Hallsworth

Other aspects of the stress response in fungi

14:00-14:40 Enrique Cerda-Olmedo - Sex in Phycomyces, a way out of stress - Departamento de Genética, Universidad de Sevilla, Sevilla, Spain.


15:20-16:00 Coffee Break and Poster Section

16:00-16:40 Brian R. Lovett - Metarhizium: An Outdoor Survival Guide - Department of Entomology, University of Maryland, College Park, MD, USA.

16:40-17:20 Roger D. Finlay - Stress responses in interactions of plant roots with symbiotic mycorrhizal fungi and bacterial antagonists of fungal pathogens - Department of Forest Mycology & Plant Pathology, Upsala BioCenter, Swedish University of Agricultural Sciences, Upsala, Sweden.

October 26 – Sunday

09:00-16:00 Registration and mounting of the posters

13:00-14:30 University Visit - Signing the visitor book at the Rangel’s Laboratory (speakers only).

14:30-15:30 Meeting with all speakers for scientific collaboration, Brazilian faculty and researchers that are attending the meeting are also invited. Auditorium at Institute of Research and Development (IP&D).

16:00-16:40 Drauzio Eduardo Naretto Rangel and Alene E. Alder-Rangel - Opening Ceremony - CEPLADE auditorium

16:40-17:20 Elis Cristina Araujo Eleutherio - Special Tribute to Anita Panek - Departamento de Bioquímica, Instituto de Química, Federal Universidade do Rio de Janeiro, Rio de Janeiro, Brazil.

19:20-18:00 Anita Panek - Looking back upon 50 years of research. Retires from Departamento de Bioquímica, Instituto de Química, Federal Universidade do Rio de Janeiro, Rio de Janeiro, Brazil.

October 27 - Monday morning – CEPLADE auditorium - Chair: Drauzio Eduardo Naretto

Fungal responses to stress: sensory reception

09:00-09:40 Luis M. Corrochano - Light in the fungal world: a stress, a signal or both? - Departamento de Genética, Universidad de Sevilla, Sevilla, Spain.

09:40-10:20 Jay C. Dunlap - Regulatory networks in fungi governing global responses to changes in light and time - Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH, USA.

10:20-10:40 Short Coffee Break

10:40-11:20 Jennifer Loros - Light and stress: the photobiological system in Neurospora crassa - Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH, USA.

11:20-12:00 Kevin K. Fuller - The fungal pathogen Aspergillus fumigatus regulates growth, metabolism, and stress resistance in response to light - Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH, USA.

October 27 – Monday afternoon – CEPLADE auditorium - Chair: Gilberto Ubida Leite Braga

Fungal responses to stress: sensory reception (continued)

14:00-14:40 Gertien Smits - The simplest signal: protons as second messengers controlling cell division rate and more - Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands.

14:40-15:20 Alfredo Herrera-Estrella - Mechanism of response to physical injury in Trichoderma - Center for Research and Advanced Studies of the National Polytechnical Institute, Chivistán, México.

15:20-16:00 Coffee Break and Poster Section

16:00-16:40 Gustavo Henrique Goldman - Systemic global analysis of genes encoding phosphatases in Aspergillus fumigatus reveals novel virulence determinants - Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.

16:40-17:20 Maria Célia Bertolini - Identification of transcription factors/proteins regulating stress response in Neurospora crassa - Departamento de Biologia e Tecnologia Química, Instituto de Química, Universidade Estadual Paulista, Araraquara, SP, Brazil.

October 28 – Tuesday morning – CEPLADE auditorium - Chair: Luis M. Corrochano

Fungal responses to stress: gene regulation and cellular responses

09:00-09:40 Stefan Hohmann - Integrative analysis of yeast osmoregulation - Department of Cell and Molecular Biology, University of Gothenburg, Gothenburg, Sweden.

09:40-10:20 Elis Cristina Araujo Eleutherio - Regulation of the Saccharomyces cerevisiae trehalose synthase complex in response to heat stress - Departamento Bioquímica, Instituto de Química, Federal Universidade do Rio de Janeiro, Rio de Janeiro, Brazil.