Abstracts

Anderson Ferreira da Cunha

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

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

Célia Maria de Almeida Soares

Metabolic changes in *Paracoccidioides* spp. during host infection

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Paracoccidioidomycosis is the most important systemic mycosis in Latin America that is acquired by inhalation of mycelium propagules of dimorphic fungi of the genus *Paracoccidioides*. In the host lung, the fungus transits to the yeast form and can disseminate to different organs and tissues. Successful infection requires a rapid adaptation to the host milieu by changing the expression of gene repertoires. During the infection caused by *Paracoccidioides*, alveolar epithelial cells are the first contact of the fungus with the host. We established a successful model for obtaining fungal cells straight from infected lungs. Characterization of the transcriptome and proteome of fungi derived

from epithelial lung demonstrated a metabolic shift in response to the host environment stress. For instance, the cell wall metabolism was reorganized, and virulence factors were expressed, especially those related to the fungus invasion.

Chengshu Wang

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

Chengshu Wang

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Filamentous fungi including mushrooms frequently and spontaneously degenerate during subsequent culture maintenance on artificial media, which shows the loss or reduction abilities of asexual sporulation, sexuality, fruiting, and production of secondary metabolites, thus leading to economic losses during mass production. To better understand the underlying mechanisms of fungal degeneration, different fungal species were employed for comprehensive analyses. We found the linkage of oxidative stress to culture degeneration, mitochondiral DNA from the sectors undergoes non-enzymatic glycation, and treating healthy mycelia with H_2O_2 significantly increased the frequency of colony sectorization and reproduced the glycation pattern shown in sectors. Taken together with the verifications of cell biology and biochemical data, a comparative mitochondrial proteome analysis revealed that, unlike the healthy wild type, a spontaneous fluffy sector culture of *Aspergillus nidulans* demonstrated the characteristics of mitochondrial dysfunctions. Relative to the wild type, the features of cytochrome *c* release, calcium overload and up-regulation of apoptosis inducing factors evident in sector mitochondria, which suggested a linkage of fungal degeneration to cell apoptosis. However, the sector culture could still be maintained for generations without the signs of growth arrest. Up-regulation of the heat shock protein chaperones, anti-apoptotic factors, and DNA repair proteins in the sector could account for the compromise in cell death. Our studies not only shed new light on the mechanisms of spontaneous degeneration of fungal cultures but will also provide alternative biomarkers to monitor fungal culture degeneration.

Claudina Rodrigues-Pousada

Molecular mechanisms of arsenic detoxification: novel insights from yeast

Claudina Rodrigues-Pousada

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Arsenic is a double-edge sword. On the one hand, it is a powerful carcinogen and, on the other, it is used therapeutically to treat acute promyelocytic leukemia. We have reported that arsenic causes in yeast disruption of iron homeostasis as a consequence of a defective high-affinity iron uptake mediated by Fet3 and Ftr1, whose mRNAs are drastically decreased upon arsenic exposure. We also provide data showing that arsenic disrupts iron uptake in mammals. Response to arsenic stress in *Saccharomyces cerevisiae* is orchestrated by the regulatory protein Yap8, which mediates transcriptional activation of *ACR2* and *ACR3* genes encoding, respectively, an arsenate-reductase and a plasma membrane arsenite efflux transporter. Our study contributes to the knowledge on the molecular mechanisms underlying stress response of yeast to arsenate, because it provides the first genetic and biochemical

evidence that Yap8, through the cysteine residues 132, 137, and 274, is the sensor of presence of arsenate in the cytosol. We show for the first time the essential role of the Mediator complex in transcriptional activation of ACR2 by Yap8. Based on our data, we propose an order-of-function map to recapitulate the sequence of events taking place in cells injured with arsenite. Modification of the sulfhydryl state of these cysteines converts Yap8 in its activated form, triggering the recruitment of the Mediator complex to the ACR2 promoter, through interaction with the tail subunit Med2. The Mediator complex then transfers the regulatory signals conveyed by Yap8 to the core transcriptional machinery, which culminates with TBP occupancy, ACR2 upregulation, and cell adaptation to arsenate stress. Additional co-factors are required for the transcriptional activation of ACR2 by Yap8, particularly the nucleosome remodeling activity of SWI/SNF complex and the SAGA complex.

Deborah Bell-Pedersen

Circadian Clock Regulation of Stress Response Pathways in Neurospora

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My lab studies how the circadian clock functions in organisms to regulate daily rhythms in behavior, physiology, and biochemistry. Using the fungus *Neurospora crassa* as a model system, and extending this work into mammalian cells, we discovered that the clock regulates the activity of conserved MAPK signaling pathways involved in stress responses and the control of cell growth and division. Excitingly, this finding provides a rationale for observations that deregulation of the clock in humans contributes to cancer. In addition, we discovered a link between MAPK activity and translation elongation control through phosphorylation of eEF2. This link, along with evidence for clock control of the activity of the stress-regulated translation initiation factor eIF2α, provides a novel mechanism to control stress- and clock-controlled translation initiation and elongation. We are currently using ribosome profiling to understand how specific mRNAs are selected for translational control following acute stress and over circadian time.

Diego Bonatto

Lactic acid as a stressful molecule for yeast in sourdough and lambic mixed fermentations

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Sourdough, a mixture of cereal flour and water, used to produce artisan sour breads, and lambic, a traditional sour beer originated from Belgium, are both fermentation products characterized by a complex and dynamic microbiota composed mainly of different genera of lactic acid-producing bacteria (e.g., Lactobacillus, Leuconostoc, and Pediococcus sp.) and yeasts (Saccharomyces, Candida, Pichia, and Hansenula sp.). From the perspective of microbial ecology, sourdough and lambic are very stressful ecosystems that contain a high carbohydrate concentration, very low pH, and oxygen limitation, which promote a strong selective force on communities of yeast and lactic acid bacteria (LAB). Although many works have examined the population dynamics of LAB and yeast communities in sourdough bread and lambic beer, little has been explored about nutrient and pH stress in microbiota. The effects of stress in yeasts promoted by high concentration of lactic acid

generated during sourdough/lambic fermentations is virtually unknown. We addressed this question by using a systems biology approach to evaluate the major targets of lactic acid in yeast, as well as the major biochemical pathways that lactic acid can modulate, specifically redox pathways.

Drauzio Eduardo Naretto Rangel

Illuminated fungi during mycelial growth produce conidia with increased stress tolerance

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

Drauzio Eduardo Naretto Rangel

Asphyxiated fungi during mycelial growth produce conidia with increased stress tolerance

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Hypoxia (low oxygen concentrations) and anoxia (a total depletion in the level of oxygen) in filamentous fungi causes significant changes in their metabolism, germination, mycelial growth, and conidial production. However, little is known about the phenotypic effects of hypoxia and anoxia during mycelial growth on the tolerance of the developing fungal conidia to different stress conditions. Conidia of the entomopathogenic fungi *Aschersonia aleyrodis*, *Beauveria bassiana*, *Isaria fumosorosea*, *Lecanicillium aphanocladii*, *Metarhizium anisopliae*, *M. brunneum*, *M. robertsii*, *Simplicillium lanosoniveum*, *Tolypocladium cylindrosporum*, and *T. inflatum* were produced on potato dextrose agar (PDA) medium under continuous hypoxia (Petri dishes sealed tree times with Parafilm®), discontinuous anoxia (the cultures were grown for 24 h under normoxia and transferred to anaerobiosis jars for five days, then transferred back to normoxia for eight days), and compared with conidia produced under normoxia (normal oxygen concentrations), and on minimal medium (MM = Czapek medium without sucrose, a condition that induces high stress tolerance). The conidial tolerance of these fungal species produced under these four different conditions were evaluated in relation to: A) wet-heat 38 or 45 °C depending on the species tolerances, B) to menadione, a potent inducer of reactive oxygen species, C) to osmotic stress caused by potassium chloride, D) to UV radiation, and E) to genotoxic stress caused by 4-nitroquinoline 1-oxide (4NQO). Several fungal species were more stress tolerant when conidia were produced under hypoxic and anoxic conditions as compared with conidia produced in the normoxic condition; for instance, growth under hypoxic condition induced higher conidial tolerance of *A. aleyrodis* to KCl and 4NQO; of *B. bassiana* to menadione, KCl, UV radiation, and 4NQO; of *I. fumosorosea* only to heat; of *L. aphanocladium* to only heat; of *M. anisopliae* to heat, menadione, KCl, and UV radiation; of *M. brunneum* to menadione, KCl, and 4NQO; of *M. robertsii* to menadione, KCl, and UV radiation; of *T. cylindrosporum* only to heat; and of *T. inflatum* to menadione and KCl. Conidia of *S. lanosoniveum* never responded with increased tolerance to any stress conditions. Growth under anoxic condition induced higher conidial tolerance of *A. aleyrodis* to heat, KCl, and 4NQO; of *B. bassiana* to UV radiation, and 4NQO; of *M. anisopliae* to menadione, KCl, and 4NQO; of *M. brunneum* to menadione and KCl; of *M. robertsii* to KCl and UV radiation; and of *T. inflatum* only to KCl. Conidia of *T. cylindrosporum* produced under anoxic condition did not respond to any stress. The fungi *I. fumosorosea*, *L. aphanocladium*, and *S. lanosoniveum* died under anoxic condition and, therefore, did not produce conidia. When conidia were produced under nutritional stress under normoxic condition a much higher tolerance to the majority of stress conditions were found particularly for *Beauveria* and *Metarhizium* species. For example: growth under nutritional stress induced higher conidial tolerance of *B. bassiana* to menadione, KCl, UV radiation, and 4NQO; of *I. fumosorosea* to only KCl; of all *Metarhizium* species to heat, menadione, KCl, and UV radiation; of *T. cylindrosporum* to

menadione and UV radiation; of *T. inflatum* to heat and UV radiation; and of *S. lanosoniveum* to menadione and KCl. Conidia of *L. aphanocladium* produced under nutritional stress never responded with increased tolerance to any stress conditions. *Aschersonia* did not produce conidia on MM. The hypoxic condition did not decrease conidial production in the majority of species; the only reduction was for *S. lanosoniveum* and *A. aleyrodis*. The anoxic condition caused mycelial death of three fungal species, but in the other fungal species, this condition generally did not harm conidial production. The conidial production on MM was negligible. In conclusion, growth under hypoxia and anoxia produced conidia with higher stress tolerance in relation to conidia produced in normoxic condition. The nutritive stress generated by minimal medium, however, induced a much higher stress tolerance.

Elias Hakalehto

Mutual tolerances of fungi and bacteria within stressed microbial communities

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Since the pioneering studies of Alexander Fleming, there has been a temptation to assume that interactions between fungi and bacteria are always or, at least, usually antagonistic. Such microbes are known to deploy toxic substances and stressors in a Sisyphean cycle of biological warfare between microbial populations, which can determine the composition of their communities. However, it is also known that, in many habitats (such as soils), phylogenetically diverse microbes can sustainably coexist, jointly partaking in the exploitation of resources. Within the equilibrium created by the omnipresent antimicrobial activities, competition for resources, co-metabolism of substrates, molecular communication and other phenomena, there is considerable temporal and spatial flux. Fungal mycelia can extend into, or travel from one to another type of, soil microhabitats and can thereby access and release potential substrates and other potential resources; some species of bacteria have the metabolic versatility to degrade relatively recalcitrant substances. We characterized interactions between soil fungi and bacteria *in vitro* under standard conditions, and under habitat-relevant stresses, using a Portable Microbe Enrichment Unit (Finnoflag Oy, Kuopio, Finland). This revealed intimate metabolic interactions between members of these phylogenetic groups, as well as an enhanced stress biology of the diverse taxa. Stressors produced by bacteria, such as *Lactococcus lactis*, were nevertheless found to constrain fungal growth. The dualistic concept of competition-versus-cooperation may be inadequate to explain the complexity and subtly of fungi/bacteria interactions, the implications of which extend throughout microbial stress biology, agricultural biotechnology, ecosystems function, pathogen biology, food spoilage, and other fields.

Elis Christina Araujo Eleutherio

Oxidative Stress and Aging: Learning from Yeast Lessons

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

Éverton Kort Kamp Fernandes

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

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The current study compared the tolerance to heat and UV-B radiation of conidia and blastospores of *Metarhizium anisopliae* s.l. (IP 363), *Metarhizium robertsii* (IP 146), and *Beauveria bassiana* s.l. (IP 361 and GC 307). Suspensions (10^3 propagules mL^{-1}) of conidia were exposed to heat (45 °C) for 0 (control), 60, 120, 240, or 360 min, while blastospores, presumably more sensitive to heat, were exposed for 0 (control), 15, 30, 45, 60, 90, 120, or 150 min. After exposure, an aliquot was inoculated and spread on PDAY medium with chloramphenicol (0.055% v/v) in Petri plates, and incubated for 7 days at 27 °C and RH ≥ 80%. Colonies were then counted to calculate the relative percent of Colony Forming Units (CFU). Conidia of IP *M. anisopliae* 363 were more tolerant to heat than blastospores at 60 and 120 min exposure, whereas blastospores of *B. bassiana* CG 307 were more tolerant than their conidia. ARSEF 324 conidia were more tolerant to heat (79.1%) than conidia of IP 363 (55.5%), IP 146 (1.5%), GC 307 (0%), and IP 361 (0%) at 2 h exposure, as well as

blastospores after 60 min exposure, demonstrating mean percent CFU of 100%, 12.3%, 30.7%, 55%, and 0%, respectively. In addition, propagules of ARSEF 324 were exposed to heat for 0 (control), 1, 2, 4, 6, 8, 16, 24, 32, 40, 48, 56, or 64 h. From 1 to 4 h exposure, conidia and blastospores of ARSEF 324 did not differ in tolerance to heat; however, from 6 to 48 h exposure conidia were more tolerant than blastospores, and at 56 and 64 h exposure, conidia and blastospores were both susceptible to heat. Fungal suspensions were also inoculated and spread on PDAY in Petri plates, and exposed to UV-B radiation for 0 (control), 1.33, 2.67, 4.01, 5.35, 6.69, or 8.03 kJm^{-2}, at 743,75 mW m^{-2} Quaite weighted irradiance. Plates were incubated for 7 days at 27 °C and RH ≥ 80%. No difference in mean relative percent CFU between conidia and blastospores of a same isolate was observed, demonstrating that both propagules were equally susceptible to UV-B. Tolerance of blastospores of IP 146, IP 363, IP 361, CG 307, and ARSEF 324 did not differ among them. In conclusion, variation of response to stress among isolates indicated that blastospores may be promising for biological control of arthropods, and that suitable adjuvants which aim at protecting fungal propagules against abiotic factors are required for both conidia and blastospores. The selection of isolates with marked natural tolerance to heat and UV-B radiation may increase performance of bioproducts.

Gilberto Úbida Leite Braga

Photodynamic inactivation of plant-pathogenic fungi: so what is stopping us?

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Classical approaches to plant-pathogenic fungi control are constrained by the development of resistance and a limited range of new and effective fungicides. Recent increases in consumer awareness of, and legislation for, environmental and human health increasingly demand safer antimicrobials. There is, therefore, an urgent need for innovative strategies for control pre- and post-harvest diseases as well as food-borne pathogens. Antimicrobial photodynamic treatment, known to be effective in a clinical context, has been evaluated in agriculture to control plant pathogenic fungi and bacteria and to eliminate food-borne pathogens on seeds, sprouts, fruits, and vegetables. Here, we review the ecology of naturally-occurring, photodynamic processes including the light-activated antimicrobial activities of some plant metabolites and the fungi-induced photosensitization in plants. The inhibitory mechanisms of both natural and synthetic light activated substances, known as photosensitizers, are discussed in the contexts of microbial stress biology and agricultural biotechnology. A special focus will be given on the photodynamic inactivation of plant-pathogenic fungi.

Gustavo Henrique Goldman

Carbon catabolite repression in filamentous fungi

Gustavo H. Goldman

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Carbon catabolite repression (CCR) is a process that selects the energetically most favorable carbon source in an environment. CCR represses the use of less favorable carbon sources when a better source is available. Glucose is the preferential carbon source for most microorganisms because it is rapidly metabolized, generating quick energy for growth. In the filamentous fungus *Aspergillus nidulans*, CCR is mediated by the transcription factor CreA, a C2H2 finger domain DNA-binding protein. The aim of this work was to investigate the regulation of CreA and characterize its functionally distinct protein domains. CreA depends in part on de novo protein synthesis and is regulated in part by ubiquitination. CreC, the scaffold protein in the CreB-CreC deubiquitination (DUB) complex, is essential for CreA function and stability. Deletion of select protein domains in CreA resulted in persistent nuclear localization and target gene repression. A region in CreA conserved between *Aspergillus* spp. and *Trichoderma reesei* was identified as essential for growth on various carbon, nitrogen, and lipid sources. In addition, a role of CreA in amino acid transport and nitrogen assimilation was observed. Taken together, these results indicate previously unidentified functions of this important transcription factor. These novel functions serve as a basis for additional research in fungal carbon metabolism with the potential aim to improve fungal industrial applications.

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Iran Malava

The contribution of the Cell Wall Integrity Pathway to virulence in *Aspergillus fumigatus*

Iran Malavazi

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Aspergillus fumigatus is an allergen of mammals and an important opportunistic pathogen that causes invasive pulmonary aspergillosis in immunosuppressed individuals. The ability to handle different stress conditions is essential to the survival and virulence of the pathogen inside the host. Therefore, environmental changes are sensed by invading microorganisms and transduced through signaling transduction pathways that lead to cell adaptation strategies. The Cell Wall Integrity (CWI) pathway is a signaling cascade primarily activated in fungal cells under conditions of synthesis and/or remodeling of the cell wall. In *S. cerevisiae*, CWIP is launched by the activation of the Protein Kinase C (*PKC1*), which interplays with a MAP kinase cascade (MAPK) leading to the phosphorylation of the associated *RLM1* transcription factor. We investigated the function of different genes of the *A. fumigatus* CWI pathway. Δ*rlmA* and *pkcA*^{G579R} mutant strains exhibit an altered cell wall organization in addition to defects related to vegetative growth and tolerance to cell wall-perturbing agents. A genetic analysis indicated that *rlmA* is positioned downstream of the *pkcA* and *mpkA* genes in the CWI pathway. As a consequence, *rlmA* loss-of-function leads to the altered expression of genes that encode cell wall-related proteins. RlmA positively regulates the phosphorylation of MpkA. The Δ*rlmA* strain had attenuated virulence in a neutropenic murine model of invasive pulmonary aspergillosis. Our results suggest that RlmA functions as a transcription factor in the *A. fumigatus* CWI pathway, acting downstream of PkcA-MpkA signaling and contributing to the virulence of this fungus.

Jesús Aguirre

ROS sensing and fungal cell differentiation

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We proposed that life's early confrontation with reactive oxygen species (ROS) led cells to evolve not only defense mechanisms, but also the utilization of ROS as signaling molecules to regulate growth and development. Using the fungus *Aspergillus nidulans*, we have shown that transcription factors (TF) SrrA, NapA, and AtfA are individually required to survive oxidative stress and that its inactivation has profound effects on asexual and sexual development. In GFP tagged functional versions of FT NapA and SrrA, as well as transcriptomic and protein interactome experiments, NapA and SrrA interact in the nucleus in the presence of H_2O_2 and during asexual development. Consistent with this, NapA is required for the expression of multiple genes during asexual differentiation, which indicates that ROS are produced during cell differentiation. TF AtfA and the MAPK-activated protein kinase SrkA are regulated by the stress MAPK SakA, which belongs to the Hog1/p38 family and is involved in transducing many different types of environmental stresses. SakA-SrkA nuclear interaction is also observed during normal asexual development in dormant spores. Using SakA and SrkA S-tag pull-downs and purification studies coupled to mass spectrometry, we have shown that SakA interacts with SrkA, the stress MAPK MpkC, the PPT1-type phosphatase AN6892 and other proteins involved in cell-cycle regulation, DNA-damage response, mRNA stability and protein synthesis, mitochondrial function, and other stress-related responses. This indicates that oxidative stress induces DNA damage and mitochondrial fission and that SakA and SrkA mediate cell cycle arrest and regulate mitochondrial function during stress. These results provide new insights about the mechanisms by which SakA and SrkA regulate the remodeling of cell physiology during oxidative stress and development.

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John E. Hallsworth

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

John E. Hallsworth

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

the water activity minimum for germination and growth – of extreme fungal xerophiles to < 0.590 water activity. The findings were considered in context of key questions relating to terrestrial ecosystems, biotechnology, and the astrobiology field.

Jon Takemoto

New Generation Aminoglycoside Fungicides

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

Laura Selbmann

Resistance of Antarctic cryptoendolithic fungi to radiations

Laura Selbmann

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The cryptoendolithic black fungus, *Cryomyces antarcticus*, spreads exclusively in the McMurdo Dry Valleys, Antarctica, which is the best Terrestrial analogue for Mars. Conditions on the surface are incompatible with life, so it lives within porous rocks as its last chance for survival. The almost complete isolation over a timescale of evolutionary significance led to the evolution of a unique, extremely adapted, and resistant genotype. Stress tolerance goes well beyond the conditions, already prohibitive, of its natural environment. These include extreme of temperatures, both low and high, desiccation, vacuum, salinity UV radiation, and even Space conditions. In the frame of the STARLIFE Project, aiming to define the effect of ionizing radiation of astrobiological relevance on different test microorganisms, *C. antarcticus* was irradiated with gamma rays, Helium, Deuteron, and X-rays. The fungus was able to survive even high doses with minimal or no DNA alteration; this bewildering stress tolerance is mainly due to melanins that also confer the astonishing capacity to convert ionizing radiation to metabolic energy. Radioresistance is, therefore, not an exclusive prerogative of prokaryotes such as *Deinococcus radiodurans*.

Luis M. Corrochano

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

Luis M. Corrochano

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

Luis F. Larrondo

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

Luis F. Larrondo

Millennium Nucleus for Fungal Integrative and Synthetic Biology. P. Universidad Católica de Chile, Santiago, **Chile**

Circadian clocks are molecular devices that allow organisms to anticipate daily cyclic challenges, temporally modulating different processes. Thus, plant defense mechanisms against pathogens have been reported to vary daily in *Arabidopsis thaliana*. Although the plant–pathogen interaction is a two-sided story, nothing was known regarding circadian regulation of pathogenic traits. Thus, we characterized a functional circadian clock in the phytopathogenic fungus *Botrytis cinerea*. By using different plant and Botrytis clock-null mutants, we demonstrate that the interaction between this

pathogen and its host varies with the time of day, because the *B. cinerea* circadian clock key in regulating this outcome. In Neurospora, the FREQUENCY (FRQ) protein is the main component of the circadian oscillator, a role that is also conserved for the Botrytis ortholog BcFRQ1. Surprisingly, in this fungus, this protein appears to play extra-circadian roles, as it plays a critical function in asexual/sexual decisions. Nevertheless, developmental phenotypes triggered by the absence of BcFRQ1 can be reverted by nutritional cues, placing this protein at the crossroad between circadian and metabolic regulation. In addition, we have observed how light/dark regulation impact the ability of this fungus to deal with cell-wall stressors, by a mechanism that is still under study.

Márcia Eliana da Silva Ferreira

CO_2 sensing in *Aspergillus fumigatus*

Márcia Eliana da Silva Ferreira

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The concentration of carbon dioxide (CO_2) is 0.033% in the environment and can reach up to 5% in the host (about 150-fold higher). Thus, during the infection process, *Aspergillus fumigatus* must adapt to different CO_2 levels. Carbonic anhydrases (CAs) are ubiquitous enzymes, found in all organisms, that catalyse the reversible hydration of CO_2 to bicarbonate (HCO_3^-), to maintain efficient CO_2/HCO_3^- homeostasis. These enzymes have already been related to CO_2 sensing in other fungi. *A. fumigatus* has four CA-encoding genes, named *cafA-D*. *cafA* and *cafB* are constitutive and strongly expressed, whereas *cafC* and *cafD* are weakly expressed, but CO_2-inducible genes. Only the double mutant Δ*cafA*Δ*cafB* is unable to grow at 0.033% CO_2, and this growth defect can be restored by high CO_2 concentrations (5%). *A. fumigatus* Δ*cafA*, Δ*cafB*, Δ*cafC*, Δ*cafD*, and Δ*cafA*Δ*cafB* mutant strains are fully virulent in a low-dose murine infection, suggesting that the CAs are not required for development and virulence of the *A. fumigatus* in the mammalian host. On the other hand, this fungus modifies the expression of some genes when it is transferred from an atmosphere of 0.033% CO_2 to one of 5% CO_2 (data not published), suggesting the importance of these genes to the virulence of *A. fumigatus*. The *cipC* gene (Afu5g09330) is involved in this adaptation process and is important for the virulence of *A. fumigatus*, making it a target for study of new therapies to treat invasive aspergillosis. Other genes, such as those encoding tyrosinase (Afu3g01070), HMG-CoA synthase (Afu8g07210), oxidoreductase (Afu2g00750), and α 1,3-glucanase (Afu1g03352), also had their expression altered when *A. fumigatus* was transferred to 5% CO_2; however, the importance of these genes to the virulence has not been established yet.

Maria Célia Bertolini

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

Maria Célia Bertolini

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The fungus *Neurospora crassa* has been widely used as a model organism to understand fundamental aspects of eukaryotic biology. The knowledge of its genome sequence has allowed 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 set of mutant strains to identify transcription factors/proteins that either directly or indirectly regulate reserve carbohydrate metabolism in *N. crassa*. Functional studies of some transcription factors have allowed us to characterize signaling pathways not yet characterized in *N. crassa* and have revealed divergent roles when compared to other model organisms. In addition, some transcription factors are involved in different cellular processes, including stress response. In this talk, I will present the results related to the characterization of some transcription factors/proteins that we have studied in the fungus *N. crassa*.

Mario A. Fares

The role of gene duplication in the origin of adaptations to abiotic stress in yeast

Mario A. Fares

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In non-motile organisms, such as the yeast *Saccharomyces cerevisiae*, ability to buffer environmental challenges is fundamental for their survival when environmental perturbations that are potentially lethal impact the cell. What genetic or regulatory mechanisms have evolved to facilitate a rapid response to environmental challenges in *S. cerevisiae* is a central question in evolutionary microbiology. A fundamental trait of *S. cerevisiae* is having 30% of its genome formed by duplicated genes. Gene duplication has been shown to have a major role in the origin of major evolutionary innovations in fungi, plants, and animals. We investigate the role that gene duplication has in the adaptation to a number of environmental conditions that impose a stress to the cell. We find that gene duplication is key to two kinds of responses: i) responses to short-term exposure of *S. cerevisiae* to abiotic stresses, and ii) adaptation of *S. cerevisiae* to long-term exposure to abiotic stresses. Such duplicated genes are involved in metabolic shifts from fermentative to oxidative processes, energy-costly processes, and stress response processes. The cellular processes in which duplicated genes are involved during the long-term adaptation to stress are markedly different from those triggered during short-term exposure of *S. cerevisiae* to stress. The mode of evolution of these duplicated genes provides supports to the sub-functionalization of the gene copies after duplication and their regulatory re-programing in the cells subjected to stress.

Michelle Momany

Heterogeneity and stress response

Michelle Momany

University of Georgia, Fungal Biology Group & Department of Plant Biology, Athens, GA, **USA**

Invasive fungal infections kill more than one million people each year. Many of these infections start with the inhalation of fungal spores from the environment. The environmental conditions during sporulation can vary widely, imposing many different kinds of stress. Germination often occurs in conditions that are very different from those of sporulation, especially in the case of pathogenic fungi whose environmentally produced spores break dormancy and germinate within the lungs of mammalian hosts. We are using the filamentous fungal pathogen of humans *Aspergillus fumigatus* to

investigate the influence of sporulation conditions on germination potential. Our results show that fungal conidia are not homogeneous and that the level of heterogeneity is influenced by stress during sporulation.

Monika Schmoll

Light dependent regulation of carbon and secondary metabolism in *Trichoderma reesei*

Monika Schmoll

Austrian Institute of Technology, Department of Health and Environment, Bioresources Unit, Tulln, **Austria**

Changing light conditions, caused by the rotation of earth resulting in day and night or growth on the surface or within a substrate, result in considerably altered physiological processes in fungi. For the biotechnological workhorse *Trichoderma reesei* (syn. *Hypocrea jecorina*), regulation of glycoside hydrolase gene expression, especially cellulase expression, was shown to be a target of light dependent gene regulation. Investigation of genes regulated in response to light in *T. reesei* and their distribution within the genome revealed several genomic clusters. We investigated the relevance of one light regulated gene cluster in regulation of secondary metabolite production and regulation of enzyme expression. High performance thin layer chromatography of supernatants from strains lacking the genes of this cluster in light and darkness along with transcript analysis and mass spectrometry indicates that secondary metabolite production and enzyme expression are connected processes in *T. reesei*. Moreover, this regulatory connection is different in light and darkness.

Natalia Requena

Striving for a life in harmony in the arbuscular mycorrhizal symbiosis

Natalia Requena

Karlsruhe Institute of Technology, Botanical Institute, Department of Molecular Phytopathology, Karlsruhe, **Germany**

Microorganisms are permanently challenged with hazardous environmental conditions that restrict their potential for survival and reproduction. To overcome this threat, many of them evolutionarily opted for a life in symbiosis. Fungi from the Glomeromycota phylum engaged in a life in mutualistic symbiosis with plant roots more than 450 million years ago. Since then, plants have provided fungi with carbohydrates and in turn have become an improved inorganic fertilization. However, it has only been recently recognized that AM fungi are not just mere colonizers of the root and that symbiosis implies overcoming the default surveillance system of the plant for the adaptation to a life inside the plant. Our discovery of the first AM fungal effector protein SP7 (Kloppholz et al., 2011) challenges the paradigm that AM fungi are naïve colonizers of plants and that the symbiosis is exclusively controlled by the plant. In general AM fungi could use at least three types of effectors to interact with the plant: i) effectors to avoid being recognized; ii) effectors for suppressing defense responses elicited after recognition; and iii) effectors that that allow manipulation of the plant cell metabolism. We have now studied in detail the molecular mechanisms by which SP7 and the members of the SP7-like family entirely rewire the plant cell program. We hypothesized that the conserved domain structure of this group of proteins despite their sequence divergence would point to a conserved biochemical function on different specific plant targets. Results from microarray analyses as well as localization and interaction studies converged to show that this might be indeed the case and that

fungal accommodation involves a plant rewiring exerted on conserved nodes required to cope with abiotic stresses.

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

Nicolás Pedrini

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

Nicolás Pedrini

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

Nilce M. Martinez-Rossi

Adaptation to environmental stress in the dermatophyte Trichophyton rubrum

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Heat shock proteins (Hsps) are molecular chaperones highly conserved among different organisms exerting many cellular functions. In fungi, the functioning of these proteins is implicated in morphogenesis, pathogenicity, stress responses, and drug resistance, among others. Hsp90, particularly, plays a central role in the cell, modulating the activities of regulators and signaling networks. Thus Hsp90 emerges as a molecular target for antifungal therapy. Here, we analyzed the *hsp* genes in the dermatophyte *Trichophyton rubrum*, which is a keratinolytic fungus and the primary cause of skin and nail mycoses in humans. Transcriptional analyses showed that some *hsp* and related genes were modulated according to particular environmental challenges, such as nutrient sources, interaction with cells, and molecules of the host tissue, and drug exposure. Blocking Hsp90 function by chemical inhibition affected the transcription profile of some of these genes, decreased the growth of *T. rubrum* in an ex vivo model of nail infection, and increased the susceptibility of the fungus to some antifungal. Our results suggest involvement of Hsp90 in the regulation of other Hsps, the pathogenicity, and drug susceptibility of *T. rubrum*. Moreover, the synergism observed between the

chemical inhibition of Hsp90 and the effect of some antifungal in reducing the fungal growth raise Hsp90 as the potential target to treat dermatophytosis.

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Reinhard Fischer

Light regulation in *Aspergillus nidulans*

Reinhard Fischer

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Bacteria often use two component systems (TCS) as phosphorylation relays to transmit environmental signals from the cell surface to the inner cell. In comparison, microbial eukaryotes use MAP kinase phosphorylation cascades, although TCS are commonly found in the fungal kingdom. Interestingly, in the case of stress-sensing, fungi use a composite signaling cascade comprised of a TCS plus a downstream MAP kinase cascade to trigger gene expression. Besides osmolarity or oxidative stress, fungi sense many other environmental factors, one of which is light[1,2]. Light controls morphogenetic pathways but also the production of secondary metabolites such as penicillin. In the case of light sensing, a signaling cascade appears to be unnecessary, because light does not stop at the cell surface. However, here we found that phytochrome-dependent light signaling in *Aspergillus nidulans* uses the stress-sensing signaling cascade to transmit the signal from the cytoplasm into nuclei[3]. In a screening for *blind* mutants, the MAP kinase HogA/SakA was identified by whole-genome sequencing. The phytochrome FphA physically interacted with the histidine-containing phosphotransfer protein YpdA and caused light-dependent phosphorylation of the MAP kinase HogA/SakA and its shuttling into nuclei. In the absence of FphA, HogA/SakA still responded to osmotic stress but not to light. The HogA pathway thus integrates several stress factors and can be considered as a hub for environmental signals. Our work shows a link between light sensing and stress sensing in general. A similar link has been discovered for blue-light sensing in *Trichoderma atroviride*[4].

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2 Dasgupta, A., Fuller, K. K., Dunlap, J. C. & Loros, J. J. (2015) Seeing the world differently: variability in the photosensory mechanisms of two model fungi. *Environ. Microbiol.* **18,** 5-20.

3 Yu, Z., Armant, O. & Fischer, R. (2016) Fungi use the SakA/HogA pathway for phytochrome-dependent light signaling. *Nature Microbiol.* **1,** 16019.

4 Esquivel-Naranjo, E. U., Garcia-Esquivel, M., Medina-Castellanos, E., Correa-Perez, V. A., Parra-Arriaga, J. L., Landeros-Jaime, F., Cervantes-Chavez, J. A. & Herrera-Estrella, A. (2016) A *Trichoderma atroviride* stress-activated MAPK pathway integrates stress and light signals. *Mol Microbiol.* **in press**.

Rocco L. Mancinelli

Microbes in Space

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The empty, cold radiation environment of outer space presents an environmental challenge for any form of life. Earth's biosphere has evolved for more than 3 billion years shielded by the protective

blanket of the atmosphere protecting terrestrial life from the hostile outer space environment. Within the last 50 years, space technology has provided tools for transporting terrestrial life beyond this protective shield in order to study, *in situ,* their responses to selected conditions of space. From a biological perspective applicable to organisms ranging from humans to microbes, the two most influential physical changes experienced onboard an orbiting spacecraft are the state of near-weightlessness created by the vehicle's freefall trajectory and the increased radiation exposure incurred as a consequence of being outside Earth's protective atmosphere. Other environmental factors, such as space vacuum, thermal extremes, solar UV radiation and the presence of high-velocity micrometeoroids and orbital debris, are mitigated by spacecraft design in order to provide internal conditions conducive to sustaining life. Microbes have flown in space since the early 1960's. During that time nearly all organisms exposed to the space environment were killed except *Bacillus subtilis* spores. With the development of ESA's BioPan and EXPOSE facilities many more types of organism have been flown and have been shown to survive exposure to the space environment. These organisms now represent a broad range of species among the Bacteria (e.g. *Bacillus* and Cyanobacteria) Archaea (e.g., *Halorubrum*), and Eukarya (e.g., *Trichoderma*). Their mechanism of survival varies and will be discussed. Within a spacecraft such as the International Space Station (ISS) organisms the immediate and primary physical factor they need to contend with is microgravity. Microgravity has been shown to alter microbial growth and metabolism in a variety of ways. Data gathered from exposure to the space environment and exposure to microgravity in the ISS provides a better understanding of the potential implications of forward contamination of extraterrestrial environments during interplanetary missions, and a better understanding of the physiology of the organisms and their stress responses. Understanding how microbes behave in space may facilitate the production of a variety of compounds of interest to the pharmaceutical, biomaterials, agriculture industries, agriculture and more. Examples of potential future experiments and their significance will also be presented.

Roger Finlay

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

Roger Finlay

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

related responses will enable us to manage root and rhizosphere microbial communities to achieve sustainable improvements in plant health and yield.