University of Dundee

The second International Symposium on Fungal Stress

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The Second International Symposium on Fungal Stress: ISFUS

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Abstract

The topic of ‘fungal stress’ is central to many important disciplines, including medical mycology, chronobiology, plant and insect pathology, industrial microbiology, material sciences, and astrobiology. The International Symposium on Fungal Stress (ISFUS) brought together researchers, who study fungal stress in a variety of fields. The second ISFUS was held in May 2017 in Goiania, Goiás, Brazil and hosted by the Instituto de Patologia Tropical e Saúde Pública at the Universidade Federal de Goiás. It was supported by grants from CAPES and FAPEG. Twenty-seven speakers from 15 countries presented their research related to fungal stress biology. The Symposium was divided into seven general topics: 1. Fungal biology in extreme environments; 2. Stress mechanisms and responses in fungi: molecular biology, biochemistry, biophysics, and cellular biology; 3. Fungal photobiology in the context of stress; 4. Role of stress in fungal pathogenesis; 5. Fungal stress and bioremediation; 6. Fungal stress in agriculture and forestry; and 7. Fungal stress in industrial applications. This article provides an overview of the science presented and discussed at ISFUS-2017.

Keywords: agricultural mycology; forest mycology; industrial mycology; medical mycology; fungal stress mechanisms and responses.

Introduction

Research into fungal biology and fungal stress can help solve many issues related to human health, climate change, food security, environmental impacts, etc. (Rangel et al., 2015a; Rangel et al., 2015b). Fungi can be used for bioremediation (Gadd, 2016); to replace synthetic pesticides (Li et al., 2010; Rangel and Correia, 2003; Santi et al., 2011); to produce biofuels

(Alper et al., 2006; Lam et al., 2014), novel antibiotics (Mygind et al., 2005), enzymes (Maheshwari et al., 2000), and useful chemicals (Hagedorn and Kaphammer, 1994). Soil-dwelling fungi may enhance plant health and crop production of in arid environments (Gal-Hemed et al., 2011; Molina-Montenegro et al., 2016), and fungi can help degrade and valorize organic waste materials (Hultberg and Bodin, 2017). Understanding fungal metabolism at biophysical extremes for life can enable effective preservation of foods, documents, and artefacts and has implications for astrobiology in relation to habitability of hostile environments and preventing contamination of other planetary bodies during space exploration (Stevenson et al., 2015a; Stevenson et al., 2015b; Stevenson et al., 2017). On the other hand, fungal pathogens represent a serious threat to crops, animals, and humans (Rangel et al., 2015a). Fungi are also used as models for basic research on the biology of eukaryotic cells (Rangel et al., 2015a). Thus, understanding how fungi deal with stress during growth or while infecting a host will help optimize the use of fungi in biotechnological applications, improve the environment, and fight fungal diseases.

Like all living organisms, fungi must cope with a variety of stresses to survive, including ionizing radiation (Dadachova and Casadevall, 2008; Zhdanova et al., 2000); water activity (Stevenson et al., 2015a); acidic and alkaline environments (Rangel et al., 2015c; Steiman et al., 2004); hypoxic or anoxic stress (Bonaccorsi et al., 2006; Camilo et al., 2008; Hillmann et al., 2015; Rangel et al., 2015c); chaotropicity (Hallsworth et al., 2003); hydrophobicity (Bhaganna et al., 2010); poisons and toxic chemicals (Pennisi, 2004; Pointing, 2001); solar UV radiation (Braga et al., 2001; Braga et al., 2015; Braga et al., 2002); agricultural and industrial pollutants (Pennisi, 2004; Rangel et al., 2010a); biotic stress (Druzhinina et al., 2011; Saxena et al., 2015); nutritive stress (Ferreira et al., 2017; Rangel et al., 2006a); oxidative stress (reactive oxygen

species, ROS) (Azevedo et al., 2014; Eleutherio et al., 2015; Huarte-Bonnet et al., 2015; Rangel, 2011); heat stress (Rangel et al., 2005; Rangel et al., 2010b; Souza et al., 2014); cold activity (Santos et al., 2011); and extreme cold (Chin et al., 2010; Selbmann et al., 2015).

The International Symposium on Fungal Stress (ISFUS) is a unique meeting which brings together mycology community under the common umbrella of "stress", be it environmental or host-related, and promotes unique interaction between researchers and cross-pollination of ideas. Before ISFUS, there had never been a scientific meeting specifically dedicated to the study of fungal stress.

The first ISFUS was held in 2014 in São José dos Campos, in the state of São Paulo, Brazil featured presentations from 33 researchers from 10 countries, including students and young, mid-career, and senior researchers. A good number of discussions, collaborations, and new friendships were also initiated. (Rangel et al., 2015a; Rangel et al., 2015b). Researchers, who had been studying fungal stress for years and knew each other through journal articles were given the opportunity to finally meet. Young scientists were able to connect with more experienced researchers. From the first ISFUS, over 33 collaborative articles have been published, which included those published in a special issue of *Current Genetics* that was devoted to ISFUS 2014 (Rangel et al., 2015a; Rangel et al., 2015b). The first ISFUS was supported by a generous grant from FAPESP (São Paulo Research Foundation).

The first ISFUS was such a success that the organizers decided to host another ISFUS. The second ISFUS occurred in May 2017 in Goiania, Goiás, Brazil. Drauzio E.N. Rangel conceived and was the primary organizer of both symposia. Alene Alder-Rangel, Gilberto U. L. Braga, John E. Hallsworth, and Luis M. Corrochano helped bring the symposia to fruition.

The second ISFUS was supported by grants from CAPES and FAPEG. The Instituto de Patologia Tropical e Saúde Pública at the Universidade Federal de Goiás (UFG) acted as the host institution. Corporate sponsors included Elsevier (Amsterdam, Netherlands - which provided the students awards), Biocontrol (Sertãozinho, SP, Brazil), Koppert Biological Systems (Piracicaba, SP, Brazil), and Alder's English Services (Goiânia, GO, Brazil). The logo of the 2017 symposium (Figure 1) features one of the most-studied ascomycetes, *Aspergillus nidulans*, and illustrates several key stress parameters that fungi cope with to survive. Twenty-seven speakers from 15 countries (Figures 2 and 3) presented talks about their cutting-edge research related to fungal stress. In addition, there were 42 poster presentations. One hundred participants attended ISFUS-2017, with about a third from the UFG, and the rest from other Brazilian and international universities.

The ISFUS-2017 Abstracts Book, which feature abstracts from the presentations and posters, is available in the Electronic Supplementary Material 1 of this article. Articles highlighting the contributions to the conference are published in this *Fungal Biology* special issue entitled “Biology of Fungal Systems under Stress”, which focuses on cellular biology, ecology, environment, agriculture, medical mycology, and biotechnology in the context of fungal stress biology (Chen et al., 2017; Ferreira et al., 2017; Huarte-Bonnet et al., 2017b; Keyhani, 2017; Malo et al., 2017; Muniz et al., 2017; Tonani et al., 2017).

By design, the second ISFUS facilitated interactions between researchers from Brazil and other countries. Researchers were provided platforms to discuss their work in-depth with the diverse yet intimate group. Long lunch breaks and social activities provided many opportunities for all the delegates to interact, share ideas, and promote lasting contacts and collaborations. Assembling active researchers for the purpose of interdisciplinary exchange will inevitably

stimulate ideas for new lines of research across international borders. Several such new international collaborations are already underway due to this ISFUS.

ISFUS 2017: a brief synopsis

The ISFUS-2017 began Monday morning with a welcome talk by Drauzio E. N. Rangel. He encouraged everyone to follow their intuition as inspired by Albert Einstein: “The intellect has little to do on the road to discovery. There comes a leap in consciousness, call it Intuition or what you will, the solution comes to you and you don't know how or why.” Drauzio said: “Intuition comes when you have an open and clear heart like a child to follow where your curiosity leads you, in science and in life.”

Flávia Aparecida de Oliveira (Figure 4), the Director of the Instituto de Patologia Tropical e Saúde Pública (the host institution), welcomed researchers. The president of the Foundation for Research Support of Goiás (FAPEG), Maria Zaira Turchi (Figure 5), praised the work of the organizers of the Symposium saying, “Times are difficult; despite the little financial incentive in Brazil, the determination of faculty professors to make things happen is very beautiful.” Also participating in the opening ceremony were Dr. João Teodoro Pádua (Figure 5), Chief of Staff of the University presidency; Dr. Luis M. Corrochano, Universidad de Sevilla (Figure 5); Dr. John E. Hallsworth, Queen’s University Belfast (Figure 5); and Alene Alder-Rangel, the co-chair (Figure 5).

The Symposium was organized around seven general topics related to fungal stress accordingly to the program found in the Electronic Supplementary Material 2:

1. Fungal biology in extreme environments

The microbial biosphere is limited by thermodynamic and biophysical parameters such as temperature, water activity, chaotropicity, salinity, and ionizing radiation. Fungi survive and, in

the case of extremophiles, retain metabolic activity and grow at the thermodynamic fringes for life on Earth. Therefore, elucidating cellular stress mechanisms and responses and adaptations in fungi are imperative to understand life in the context of the various constraints for Earth ecosystems (Runner and Brewster, 2003; Stevenson et al., 2017; Yakimov et al., 2015).

Ekaterina Dadachova talked about the resistance of melanized fungi to both sparsely and densely ionizing radiation (Casadevall et al., 2017; Pacelli et al., 2017a; Shuryak et al., 2015). Two melanized fungi - a fast-growing *Cryptococcus neoformans* and a slow-growing *Cryomyces antarcticus* - were subjected to densely ionizing deuterons. Melanin protected both fungi; however, *Cryomyces antarcticus* was more resistant to deuterons than *Cryptococcus neoformans*. The irradiated cells were analyzed by a panel of metabolic assays – XTT (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide), MTT (2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide), and ATP (adenosine triphosphate). XTT showed increased activity in melanized strains of both species, while the activity in non-melanized cells either remained stable or decreased. In a follow-up study performed with only *C. neoformans* cells, transmission electron microscopy (TEM) demonstrated the removal of polysaccharide capsules by radiation, in both melanized and non-melanized cells, and considerable damage to the cell wall and organelles was observed in the non-melanized cells.

Laura Selbmann also discussed the resistance of the Antarctic cryptoendolithic fungus *Cryomyces antarcticus* to radiation. This organism is a perfect model for astrobiological studies because it lives in the closest Mars analogue on Earth, the McMurdo Dry Valleys in Antarctica (Selbmann et al., 2015). This fungus has been selected for a number of astrobiological projects: STARLIFE (Moeller et al., 2017); and two funded by the European Space Agency (ESA) and Italian Space Agency (ASI): LIFE and BIOlogy and Mars Experiment, (Onofri et al., 2012;

Onofri et al., 2015); and Lichens and Fungi Experiment, BIOMEX (de Vera et al., 2012). Its resistance was tested in terms of survival and DNA damage in response to different types of space-relevant radiation (i.e. UV) and sparsely (up to 117 kGy) and densely ionizing radiation (up to 1000 Gy). The fungus showed considerable resistance to all the conditions tested, remaining viable up to 55.81 kGy (Pacelli et al., 2017b).

John E. Hallsworth's talk, 'A story of glycerol', detailed the interventions that this polyol can make in the cellular biology and ecology of microbes, such as enhancement of biological control and reduction of temperature and water-activity minima for growth (Hallsworth and Magan, 1995); mechanisms by which glycerol exerts these activities including its ability to enhance macromolecular flexibility (entropically increased disorder) at high concentrations; a method to quantify this entropic activity of solutes (chaotropicity) (Cray et al., 2013); and a long-term research endeavor to try to demonstrate that microbes can retain metabolic activity and maintain growth below the limit for life recognized since the 1960s (i.e. 0.605 water activity) (Stevenson et al., 2015a). This culminated in the discovery that glycerol can facilitate differentiation and germination of xerophilic fungi, most notably *Aspergillus penicillioides*, down to 0.585 water activity (Stevenson et al., 2017). He finished the talk by posing a series of intriguing questions e.g.: 1) does glycerol determine the extent of and failure points for the functional biosphere, and 2) does abiotic glycerol influence habitability of hostile environments (both terrestrial & extraterrestrial)?

2. Stress mechanisms and responses in fungi: molecular biology, biochemistry, biophysics, and cellular biology

Certain fungal species such as *Saccharomyces cerevisiae*, *A. nidulans*, and *Neurospora crassa* have been used by the scientific community for decades as effective eukaryotic models.

Many genetic, molecular, cell biology, biochemical, and biophysical research tools and techniques have been developed and perfected using these model systems. The value of the tools and techniques is evidenced by research that addresses questions about the fundamental processes which drive fungal stress and responses including the perception of the stress, signal transduction, and cellular responses to fungal stresses (Brown and Goldman, 2016; de Nadal and Posas, 2015; Ho and Gasch, 2015; Rangel et al., 2015b).

Gustavo H. Goldman presented studies about regulation of *Aspergillus nidulans* CreA-mediated catabolite repression by Fbx23 and Fbx47, which are F-box subunits of the Skp, Cullin, F-box containing (SCF) ubiquitin ligase complex. Carbon catabolite repression (CCR) is a process that selects the energetically most-favorable carbon source in an environment, by suppressing the use of less-favorable carbon sources when a better one is available (Brown et al., 2014). 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 (Ries et al., 2016). The aim of his work was to investigate the regulation of CreA. 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. Goldman's research group screened a collection of null mutations for F-box encoding genes and identified two of them as important for carbon catabolite repression and derepression. Immunoprecipitation of one of them revealed several potential targets involved in CreA regulation.

Maria Celia Bertolini focused on the *Neurospora crassa* RUV-1 protein, which is identified as a protein involved in heat stress response (Freitas et al., 2008). This protein, together with its

paralogue RUV-2, belongs to the AAA+ ATPase protein family and is annotated as an ATP-dependent DNA helicase. The proteins have been identified as components of several macromolecular complexes, implicated in many cellular processes in different organisms. In *N. crassa*, the *ruv-1* transcript and RUV-1 protein are up-regulated under heat stress; however, *ruv-2* transcript is not regulated under the same condition. In addition, cellular localization analyses showed that both proteins move to the nucleus under heat stress.

3. Fungal photobiology in the context of stress

The entire second day of the Symposium focused on fungal photobiology. Fungi respond to light as environmental signals that modulate several aspects of their biology, including development and metabolism. However, excess light causes biological stress and most fungi respond by synthesizing protective pigments and enzymes for repairing UV-induced DNA damage (Braga et al., 2015; Braga et al., 2006; Fischer et al., 2016; Idnurm et al., 2010; Rangel et al., 2006b; Rangel et al., 2011).

Luis M. Corrochano began the morning explaining how light is the ultimate source of energy for life. However, light is both a signal from the environment and a damaging agent for all organisms. Most fungi respond to light by regulating gene transcription, and a key response to light is the activation of genes for repairing UV-induced DNA damage. In *Phycomyces*, a cryptochrome seems to act as a blue-light regulated DNA repair enzyme (Tagua et al., 2015).

Gerhard Braus stated that light represents a stress signal in fungi and induces different reactions. *A. nidulans* develops in the soil in the absence of light, primarily in closed sexual fruiting bodies linked to a specific secondary metabolism as overwintering structures. In contrast, light promotes and accelerates the formation of conidiophores, which release asexual spores into the air. Various control layers coordinate fungal development, virulence, and secondary

metabolism. They include the control of transcription and histone modification, signal perception and transduction as well as protein localization and stability (Bayram and Braus, 2012; Sarikaya-Bayram et al., 2014; Sarikaya-Bayram et al., 2015).

Monika Schmoll's presentation was about *Trichoderma reesei*, an important producer of plant cell-wall degrading enzymes and heterologous proteins. Therefore, it is of utmost importance to understand the factors influencing the regulation cascade that lead to high efficiency production of enzymes - particularly the previously unconsidered effect of light (Stappler et al., 2016). Regulation of cellulase gene expression is connected with regulation of secondary metabolism in *T. reesei* with differences in light and darkness (Monroy et al., 2017). Screening for cellulose-sensing receptors permitted identification of two G-protein coupled receptors (GPCRs). These GPCRs are essential for chemotropical sensing of the building block glucose and morphological changes on natural substrate surfaces. Additionally, these receptors act as checkpoints for posttranscriptional up-regulation of secreted cellulose degrading enzymes on cellulose and lactose (Stappler et al., 2017). Analysis of the photoreceptor ENV1 revealed an evolutionarily conserved mechanism to integrate stress responses with light response in the Hypocreales (Lokhandwala et al., 2015). This finding highlights the importance of stress responses in diverse interconnected regulatory processes in *T. reesei*, such as light-dependent regulation, enzyme expression, metabolite production, and chemical communication in nature.

Luis Larrondo showed how circadian clocks are molecular devices that allow organisms to anticipate daily cyclic challenges by temporally modulating different processes. Using clock-null mutants of *Botrytis cinerea*, Larrondo's group found that interaction between this phytopathogenic fungus and its host varies with the time of day (Canessa et al., 2013; Hevia et al., 2016; Hevia et al., 2015). In *Neurospora*, the FREQUENCY protein (FRQ) is the main

component of the circadian oscillator (Ruoff et al., 2005), a role that is also conserved for the *Botrytis* ortholog BcFRQ1. This protein also appears to play a critical function in asexual/sexual decisions. Nevertheless, developmental phenotypes triggered by the absence of FRQ can be reversed by nutritional cues.

Kevin Fuller explained that for the saprophyte and opportunistic pathogen *Aspergillus fumigatus*, visible light leads to conspicuous effects on colonial growth, e.g. the induction of mycelial pigments or asexual spores, as well as an induction of genes involved in DNA repair (Fuller et al., 2016; Fuller et al., 2013). Transcriptome analysis revealed that in *A. fumigatus* most regulated genes are repressed by light, including those involved in oxidative phosphorylation, ergosterol biosynthesis, and metal ion homeostasis. The biological significance of these light-repressed categories is more difficult to discern, but likely reflects a difference between metabolic conditions the fungus faces at the soil surface and deeper in the soil/compost. Interestingly, there is a correspondence between genes that are induced under hypoxia (Barker et al., 2012) and those repressed by light, and so the current model is proposed: at the sub-surface (dark), the fungus experiences low-oxygen concentrations and genes involved in hypoxia adaptation are up-regulated by the conserved regulator SrbA (Willger et al., 2008); at the soil surface, where oxygen levels are ambient, photoreceptors (LreA, FphA) down-regulate those hypoxia-adaptive pathways, including ergosterol metabolism and iron homeostasis. As the surface is also a site for optimal dispersal (i.e. open air) and exposure to ultraviolet radiation, genes involved in sporulation and resistance to genotoxic damage are induced by light. Fuller and colleagues are currently dissecting the interplay between canonical light and hypoxia-regulatory pathways as well as probing the conservation of ergosterol biosynthesis and drug sensitivity by light in other fungal pathogens.

Gilberto U. L. Braga explained that antimicrobial photodynamic treatment (APDT) is a promising alternative to conventional antifungal agents that can be used to kill fungi, which cause diseases in animals or plants (de Menezes et al., 2014; Gonzales et al., 2017). APDT, using phenothiazinium photosensitizers, efficiently kills planktonic cells of *Candida* species and conidia of several pathogenic fungi, damages the fungal plasma membrane increasing its permeability and greatly impacting their proteomes (Brancini et al., 2016).

Drauzio E. N. Rangel completed the day by discussing how fungi illuminated during mycelial growth produce conidia with increased stress tolerance. Light is an important stimulus for many fungi and it has been shown to induce production of *Metarhizium robertsii* conidia with increased stress tolerance (Rangel et al., 2015c; Rangel et al., 2011). White light, as well as blue light during mycelial growth, induces higher conidial stress tolerance, higher germination rates, and higher virulence in *Metarhizium robertsii*, but nutritional stress always produces conidia with more intense stress tolerance and virulence than conidia produced under white or blue light (Rangel, 2011; Rangel et al., 2008; Rangel et al., 2006a; Rangel et al., 2012).

4. Role of stress in fungal pathogenesis

Fungal pathogens have evolved numerous mechanisms to escape host defenses such as thermotolerance, toxin production, masking or modulating pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors (PRRs), and overcoming oxidative defenses (Sales-Campos et al., 2013; Stappers and Brown, 2017). Research on fungal pathogenesis is a wide field that encompasses basic research on host-pathogen interactions, cell and molecular biology, and development and aging, as well as applied research in crop protection, food security, public health, and medicine.

Jon Y. Takemoto addressed global challenges for crop production and food security and the critical roles for research into fungal stress and biology (Fisher et al., 2012). He described strategies behind the recent discovery of a new generation of aminoglycoside fungicides aimed to help counter the critical shortage of effective, safe, and environmentally friendly fungicides against crop diseases. K20 is a new membrane-targeting amphiphilic aminoglycoside that is not toxic and a broad-spectrum antifungal, which can be produced at scalable, kilogram levels (Chang and Takemoto, 2014). K20 by itself, or in combination with current crop fungicides (employed at lower than recommended rates), shows promise in combating several crop diseases including the devastating wheat disease, Fusarium Head Blight.

Célia M. A. Soares discussed metabolic changes in *Paracoccidioides* spp. during human host infection. Members of the *Paracoccidioides* complex, the etiologic agents of paracoccidioidomycosis, cause disease in healthy and immunocompromised patients in Latin America. Her team developed a method to harvest *Paracoccidioides brasiliensis* yeast cells from infected murine lung to facilitate *in vivo* transcriptional and proteomic profiling (Lacerda Pigosso et al., 2017). They compared the *in vivo* to *in vitro* and *ex vivo* responses of *Paracoccidioides* spp., as obtained by proteomic analysis (Lima et al., 2014; Parente-Rocha et al., 2015).

Iran Malavazi described the contribution of the cell wall integrity pathway to virulence in *Aspergillus fumigatus*. He showed that besides its role in cell wall reinforcement and remodeling, the cell wall integrity pathway (Rocha et al., 2015) is an important hub for production of fungal secondary metabolites. The fumiquinazoline (Fq) production is regulated by the transcription factor RlmA and the MAP kinase MpkA. In fact, the RlmA transcription factor binds to the promoter region of most of the genes of the Fq cluster genes. The results indicate an

unprecedented connection of the CWI pathway with the biosynthesis of a conidia-born secondary metabolite.

Marcia R. von Zeska Kress related her research about antimicrobial photodynamic inactivation and photodynamic therapy. *Neoscytalidium* spp. and *Fusarium* spp. are filamentous fungi widely distributed in nature that cause non-dermatophyte onychomycosis that have significant resistance to commercial antifungal therapy. APDT with the phenothiazinium photosensitizers methylene blue, toluidine blue, new methylene blue, and the pentacyclic phenothiazinium S137 were able to kill both quiescent and germinated arthroconidia and microconidia of *Neoscytalidium* spp. and *Fusarium* spp., respectively. The photodynamic therapy with phenothiazinium photosensitizers on *Fusarium moniliforme* infection of *Galleria mellonella*, the model for fungal virulence and susceptibility testing, showed that this therapy is a promising alternative to antifungal treatment against this filamentous fungus (de Menezes et al., 2016; Tonani et al., 2017).

Alexandre M. Bailão explained that the black fungi *Fonsecaea pedrosoi* and *Cladophialophora carrionii* are the most common agents of Chromoblastomycosis, a subcutaneous mycosis frequently diagnosed in tropical regions. The virulence strategies used by these fungi are poorly understood. As iron is an essential element, pathogenic fungi have developed molecular mechanisms to obtain the metal during infection. *F. pedrosoi* and *C. carrionii* have genes encoding for reductive and siderophore-mediated iron-uptake systems, and the transcriptional levels of those genes are induced upon iron limitation. Moreover, these pathogens produce ferricrocin as intra- and extracellular siderophores (Silva-Bailão et al., 2017).

5. Fungal stress and bioremediation

Fungi are ubiquitous in polluted habitats, and they exhibit remarkable tolerance to organic

and inorganic contaminants. Some fungi synthesize specific metal-binding peptides or metallothioneins in response to metal pollutants. Fungi also exhibit morphological differentiation in response to toxic stress, such as the formation of hyphal aggregates and cords, melanized cell forms as well as thigmotropism and chemotropism to locate a favorable microenvironment. Metabolic versatility underpins enzyme expression, carbon metabolism, pollutant transport, and production and excretion of metabolites that immobilize oxalates, oxides, phosphates, and carbonates (Gadd, 2007, 2010; Gadd, 2016). The morphological and metabolic versatility of fungi provides several advantages for bioremediation approaches, not the least their capacity to combat and overcome stress in adverse environments.

Rosane M. Peralta was the only speaker about using fungi for bioremediation. The capability of white rot fungi to biodegrade several recalcitrant pollutants has generated a considerable research interest in this area of industrial/environmental microbiology. The ability of white rot fungi to degrade pollutants appears to be related to the capability of producing extracellular non-specific lignin-degrading enzymes, especially peroxidases and laccases, as well as to produce intracellular oxidases generating of H₂O₂ and cytochrome P450 (Coelho-Moreira et al., 2013; Maciel et al., 2013). WRF can be an alternative to reduce the ecological problems caused by the accumulation of these products in nature.

6. Fungal stress in agriculture and forestry

Stress conditions, particularly UV radiation and heat from sunlight are important regulators of fungal communities in agriculture and forest (Bidochka et al., 2001; Ferreira et al., 2017; Wang and Wang, 2017). Many fungi have been developed into commercial biological control agents and are being mass produced to be used in agriculture and forestry (Li et al., 2010) to promote plant growth (Vega et al., 2009), promote plant defense responses (Vega et al., 2009);

and to control plant diseases (Costa et al., 2013; Druzhinina et al., 2011), plant parasitic nematodes (Siddiqui and Mahmood, 1996), aquatic weeds (Cother and Gilbert, 1994), terrestrial weeds (Moraes et al., 2014), and insects (Alston et al., 2005; Faria and Wraight, 2007; Keyser et al., 2017). Different abiotic environmental factors cause stress and consequently harm these important fungi in agricultural systems, highlighting the need to study stress tolerance of fungi used in agriculture and forestry.

Elias Hakalehto's presentation was about competitive interactions between fungi and other microbes in stressed ecosystems. In mixed cultures, fast-growing Gram-positive bacteria play a dominant role. For example, the fungi that pioneer in soil do not compete with bacteria as much for the degradation products as they spread their influence on novel sources of organic raw materials. The bacterial strains, in turn, receive an advantage of the biodegradation by fungi. Therefore, the instances of confrontation between fungi and bacteria are limited to specific conditions only. The effects of the industrially upgraded fungal enzymes have been tested in biorefinery trials for producing carbon and energy sources for undefined mixed cultures fortified with some industrial bacterial strains under controllable gas flow (den Boer et al., 2016; Schwede et al., 2017). The fungi colonize hostile or poor environments with the help of their aerial mycelium; transport nutrients along the growing hyphae; change the surroundings by enzymatic activities; and mobilize nutrients from various sources and niches. Fungi also produce sexual and asexual spores to conquer new areas, and nutrient sources (fungal spores fill the atmosphere). There is evidence about the relatively even distribution of the spores in the layers of the atmosphere detected from samples collected by a jet at altitudes of 300-1000 m (Hakalehto, 2015).

Roger D. Finlay discussed ways in which root-associated fungi mediate stress responses of

plants. Symbiotic mycorrhizal fungi play important roles in reducing abiotic and biotic stress to plants in forestry and agriculture, and can minimize negative effects of soil acidification, Al-toxicity, and base cation leaching (Finlay, 2008). High-throughput DNA sequencing and stable isotope-based studies suggest that ectomycorrhizal fungi may play an important role in fractionation of Mg isotopes and uptake of base cations through weathering of minerals (Fahad et al., 2016). These fungi also influence patterns of stable carbon storage in humified material and secondary minerals. Moreover, these fungi may have global impacts on sequestration or release of atmospheric CO₂ (Clemmensen et al., 2013; Finlay and Clemmensen, 2017).

Chengshu Wang elucidated the cause-effect relationships between oxidative stress and fungal culture degeneration. Filamentous fungi undergo frequent culture degeneration during successive maintenance on artificial media by showing fluffy mycelium growth and colony sectorization (Butt et al., 2006). The degenerate fungal cultures show the loss or reduced abilities to sporulate, perform sexual cycle, fruit, and/or produce secondary metabolites. Molecular and biochemical characterizations reveal that fungal culture degeneration is a sign of cell aging (Wang et al., 2005), and the occurrence of spontaneous oxidative stress, i.e., cellular accumulation of reactive oxygen species, is connected with mitochondrial dysfunction and thereby fungal culture degeneration (Li et al., 2014; Li et al., 2008; Xiong et al., 2013).

Octavio Loera explained how sublethal oxidant states improve production and quality of conidia in entomopathogenic fungi (EF). EF conidia control insect plagues in crop fields where abiotic factors weaken conidia. During production of conidia by *Metarhizium*, *Beauveria*, and *Isaria*, controlled oxidant stress improves the conidial yields, and production of stronger and more infective conidia is possible (Muñiz-Paredes et al., 2017). This implies metabolic adjustments leading to cross protection mechanisms, which could be feasible in large-scale

processes (Miranda-Hernández et al., 2016).

Nicolás Pedrini studies molecular interactions between EF and insects. During insect-cuticle degradation as well as during invasion and proliferation throughout their host, EF secrete a suite of enzymes and secondary metabolites that help them cope with the stressful situation they have to endure to finally achieve a successful infection (Huarte-Bonnet et al., 2017a; Pedrini, 2017; Wang and Wang, 2017). Moreover, insects trigger innate immune reactions to prevent microbial proliferation. By analysis of available transcriptomic and metabolomic data, several components and mechanisms involved in this fungi-insect interaction were reviewed.

Everton K. K. Fernandes presented a study about stress tolerance of EF conidia and blastospores. The study compared the tolerance to heat (45 °C) and UV-B radiation between conidia and blastospores of *Metarhizium* spp. and *Beauveria bassiana* s.l. He discussed the principles for this comparison concerning their use for biological control of arthropods.

7: Fungal stress in industry

Many industrial processes essential for meeting societal needs use fungi that play central roles in those processes. Examples are industrial ethanol production using yeast strains and the brewery industry utilizing *Saccharomyces pastorianus*. Research into the biology of fungi within industrial systems have catalyzed research into other areas such as eukaryotic responses to oxidative stress and effects on aging (Wei et al., 2007; Zhao and Bai, 2009).

Elis C. A. Eleutherio explained how fungi can be used as models to study oxidative stress (da Silva et al., 2012; Fernandes et al., 2007). Evidence shows that oxidative stress is connected to life span (Mannarino et al., 2008). Throughout the world, leading causes of death are age-related diseases, such as cancer and neurodegenerative diseases. Her presentation highlighted the

value of the yeast *S. cerevisiae* as a model to investigate the oxidative stress response and its potential impact on aging and age-related diseases (Brasil et al., 2013; França et al., 2017).

Diego Bonatto's topic was the delicate balance between hybrid genomes and brewery stresses in lager yeasts. *Saccharomyces pastorianus* has been employed in the brewery industry for the fermentation of lager beers, a product consumed worldwide. Despite its industrial importance, little is known about how *S. pastorianus* deals with brewery stress. His group has evaluated the major stress-associated biological mechanisms using transcriptome and systems biology analyses. [ref](#)

Anderson Ferreira da Cunha finished the Symposium with his study of thermotolerance and ethanol-resistance in *Saccharomyces cerevisiae* strains. Several stress factors are involved in the efficiency of production during ethanol fermentation. High ethanol concentrations and high temperatures are the most relevant ones. A good industrial strain must be sufficiently robust to respond well to this environmental stress, without altering its fermentative characteristics during the whole crop season. Since 2010, his group has been sampling different fermentation tanks trying to find yeasts able to grow at temperatures above 40 °C and at concentrations of ethanol above 12%. They have isolated four different thermotolerant and two ethanol-tolerant strains, which produced superior ethanol yield than strains currently used in ethanol plants. These strains showed a high potential of direct application for ethanol production.

Elsevier Student Competition Awards

Elsevier sponsored awards to recognize excellent work by graduate students in the area of fungal stress. For the award competitions, students submitted original research articles formatted for journal publication. Two of these articles are featured in this special issue (Huarte-Bonnet et

al., 2017b; Muniz et al., 2017). The award winners also gave oral presentations at ISFUS. The articles were read and judged by several of the international speakers following the evaluation criteria found in the website <https://isfus.wordpress.com/>.

Four doctoral students received silver Elsevier awards: Mariane Paludetti Zubieta (PhD student in Functional and Molecular Biology at the Universidade Estadual de Campinas). Her article was titled: “Understanding the production of recombinant proteins in *Aspergillus nidulans* by global proteome profiling” (Figure 6). Ronaldo A. Pereira-Junior (PhD student in Tropical Medicine and Public Health, Universidade Federal de Goiás), and his article was titled: “Riboflavin: A supplement that increases the tolerance of *Metarhizium* species against UV-B radiation by over-expressing photolyase, laccase, and polyketide synthase genes” (Figure 7 and 10). Carla Huarte-Bonnet (PhD student in Biotechnology and Molecular Biology, Universidad Nacional de La Plata) from Argentina was the only non-Brazilian award winner. Her article was titled: “Alkane-grown *Beauveria bassiana*: mycelial pellets formation, oxidative stress induction, and cell surface alterations” (Figure 8 and 10) (Huarte-Bonnet et al., 2017b). Elen Regozino Muniz (PhD student in Tropical Medicine and Public Health at the Universidade Federal de Goiás). Her article was titled: “Impact of short-term temperature challenges on the larvicidal activities of the entomopathogenic watermold *Leptolegnia chapmanii* against *Aedes aegyptii*, and development on infected dead larvae” (Figure 9 and 10) (Muniz et al., 2017).

The weekend after the ISFUS-2017 about half of the international speakers, several Brazilian speakers, and a few international participants took an excursion to Pirenópolis, in the state of Goiás. This historic town, about 130 km from Goiânia, attracts many tourists due to its richly preserved history and surrounding mountains. On a guided tour to Pireneus State Park, we experienced the beautiful waterfalls and then hiked up a hill to view the magnificent sunset over

the Pico dos Pireneus. The following day was spent at Vagafofo Wildlife Sanctuary, walking through the riparian forest and tasting a large variety of local foods for brunch. We also enjoyed the history and cuisine of Pirenópolis. The excursion was an excellent opportunity to see more of Brazil and become better acquainted with our colleagues.

Conclusion

This special issue of *Fungal Biology* was inspired by the International Symposium on Fungal Stress. Most of the articles were written by speakers or participants of ISFUS-2017. Individuals who find the subject of fungal stress fascinating and attractive are invited to consider attending the next ISFUS, tentatively planned for 2019.

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FIGURE LEGENDS

Figure 1. Logo of the second International Symposium on Fungal Stress (ISFUS-2017) that was hosted at the Universidade Federal de Goiás in Goiânia, GO, Brazil. This figure shows the some of the stress parameters that fungi are subjected to such as ionizing radiation, acidic and alkaline environments, hypoxic or anoxic conditions, poisons in general such as genotoxic and oxidative products, UV radiation from Sun, pollution from industry and agriculture, salt stress, nutritive stress, and heat from solar radiation and other sources.

Figure 2. Speakers of the second International Symposium on Fungal Stress – ISFUS-2017. Standing from left to right: Elias Hakalehto (Finland), Jon Y. Takemoto (USA), Maria Celia Bertolini (Brazil), John E. Hallsworth (UK), Kevin K. Fuller (USA), Rosane M. Peralta (Brazil), Luis M. Corrochano (Spain), Luis Larrondo (Chile), Roger D. Finlay (Sweden), Laura Selbmann (Italy), Monika Schmoll (Austria), Célia M. A. Soares (Brazil), Elis Eleutherio (Brazil), Ekaterina Dadachova (Canada), Gerhard Braus (Germany), Iran Malavazi (Brazil), Gustavo Goldman (Brazil), Diego Bonatto (Brazil), Anderson F. da Cunha (Brazil), and Chengshu Wang (China). Standing from left to right: Drauzio E. N. Rangel (Brazil), Amanda Rangel (daughter of the organizers), Alene Alder-Rangel (Brazil), Gilberto U. L. Braga (Brazil), Octavio Loera (Mexico), Alexandre M. Bailão (Brazil), and Marcia R. Z. Kress (Brazil).

Figure 3. Speakers of the second International Symposium on Fungal Stress – ISFUS. From left to right in the top row: Kevin Fuller (USA), Gustavo Goldman (Brazil), Chengshu Wang (China), Gerhard Braus (Germany), Rosane M. Peralta (Brazil), Iran Malavazi in the back (Brazil), Elis Eleutherio (Brazil), Célia M. A. Soares in the back (Brazil), Jon Y. Takemoto (USA), Maria Celia Bertolini (Brazil), Marcia R. Z. Kress in the back, not visible (Brazil). Monika Schmoll (Austria), John E. Hallsworth in the back (UK), Elias Hakalehto in the back (Finland), Luis Larrondo (Chile), Luis M. Corrochano (Spain), Roger Finlay (Sweden), Laura Selbmann (Italy), Anderson F. da Cunha in the back (Brazil), Ekaterina Dadachova (Canada), Diego Bonatto (Brazil), and Alexandre M. Bailão (Brazil). From left to right in the lower row: Drauzio E. N. Rangel (Brazil), Gilberto U. L. Braga (Brazil), and Octavio Loera (Mexico).

Figure 4. Opening Ceremony: Flávia Aparecida de Oliveira, the director of IPTSP (the host institution) welcoming researchers and students.

Figure 5. Opening Ceremony: Alene Alder-Rangel, John E. Hallsworth, Luis M. Corrochano, Maria Zaira Turchi, João Teodoro Pádua, Flávia Aparecida de Oliveira, and Drauzio E. N. Rangel.

Figure 6. Elsevier Award given to Mariane Paludetti Zubieta, presented by Drauzio E. N. Rangel (left) and Luis M. Corrochano (right).

Figure 7. Elsevier Award given to Ronaldo A. Pereira-Junior, presented by Drauzio E. N. Rangel (left) and Luis M. Corrochano (right).

1055 Figure 8. Elsevier Award given to Carla Huarte-Bonnet, presented by Drauzio E. N. Rangel (left)
1056 and Luis M. Corrochano (right).

1057
1058 Figure 9. Elsevier Award given to Elen Regozino Muniz, presented by Drauzio E.N. Rangel
1059 (left) and Luis M. Corrochano (right).

1060
1061 Figure 10. Elsevier Award winners Ronaldo A. Pereira-Junior, Carla Huarte-Bonnet, and Elen
1062 Regozino Muniz. From left to right: Alene Alder-Rangel, Dr. João Teodoro Pádua (from the
1063 office of the University President), Flávia Aparecida de Oliveira (Director of IPTSP
1064 Universidade Federal de Goiás), Elen Regozino Muniz (PhD student), Drauzio E. N. Rangel
1065 (professor Universidade Federal de Goiás), Carla Huarte-Bonnet (PhD student), Luis M.
1066 Corrochano (professor Universidad de Sevilla), and Ronaldo A. Pereira-Junior (PhD student).