

Fungal survival under temperature stress: A proteomic perspective

Nurlizah Abu Bakar^{1,2}, Saiful Anuar Karsani³, Siti Aisyah Alias^{Corresp. 1, 2}

¹ Institute of Ocean and Earth Sciences, Universiti Malaya, Kuala Lumpur, Malaysia

² National Antarctic Research Centre, Universiti Malaya, Kuala Lumpur, Malaysia

³ Institute of Biological Sciences, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia

Corresponding Author: Siti Aisyah Alias

Email address: saa@um.edu.my

Background. Increases in knowledge of climate change generally, and its impact on agricultural industries specifically, has led to a greater research effort aimed at improving understanding of the role of fungi in various fields. Fungi play a key role in soil ecosystems as the primary agent of decomposition, recycling of organic nutrients. Fungi also include important pathogens of plants, insects, bacteria, domestic animals and humans, thus highlighting their importance in many contexts. Temperature directly affects fungal growth and protein dynamics, which ultimately will cascade through to affect crop performance. To study changes in the global protein complement of fungi, proteomic approaches have been used to examine links between temperature stress and fungal proteomic profiles.

Survey methodology and objectives. A traditional rather than a systematic review approach was taken to focus on fungal responses to temperature stress elucidated using proteomic approaches. The effects of temperature stress on fungal metabolic pathways and, in particular, heat shock proteins (HSPs) are discussed. The objective of this review is to provide an overview of the effects of temperature stress on fungal proteomes.

Concluding remarks. Elucidating fungal proteomic response under temperature stress is useful in the context of increasing understanding of fungal sensitivity and resilience to the challenges posed by contemporary climate change processes. Although useful, a more thorough work is needed such as combining data from multiple -omics platforms in order to develop deeper understanding of the factor influencing and controlling cell physiology. This information can be beneficial to identify potential biomarkers for monitoring environmental changes in soil, including the agricultural ecosystems vital to human society and economy.

Fungal Survival under Temperature Stress: a Proteomic Perspective

Nurlizah Abu Bakar^{1,2}, Saiful Anuar Karsani³, Siti Aisyah Alias^{1,2}

¹ Institute of Ocean and Earth Sciences, Universiti Malaya, Kuala Lumpur, Malaysia.

² National Antarctic Research Centre, Universiti Malaya, Kuala Lumpur, Malaysia

³ Institute of Biological Sciences, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia.

Corresponding Author:

Siti Aisyah Alias¹

C308, IPS Building, University Malaya, Kuala Lumpur, 50603, Malaysia

Email address: saa@um.edu.my

Abstract

Background. Increases in knowledge of climate change generally, and its impact on agricultural industries specifically, has led to a greater research effort aimed at improving understanding of the role of fungi in various fields. Fungi play a key role in soil ecosystems as the primary agent of decomposition, recycling of organic nutrients. Fungi also include important pathogens of plants, insects, bacteria, domestic animals and humans, thus highlighting their importance in many contexts. Temperature directly affects fungal growth and protein dynamics, which ultimately will cascade through to affect crop performance. To study changes in the global protein complement of fungi, proteomic approaches have been used to examine links between temperature stress and fungal proteomic profiles.

Survey methodology and objectives. A traditional rather than a systematic review approach was taken to focus on fungal responses to temperature stress elucidated using proteomic approaches. The effects of temperature stress on fungal metabolic pathways and, in particular, heat shock proteins (HSPs) are discussed. The objective of this review is to provide an overview of the effects of temperature stress on fungal proteomes.

Concluding remarks. Elucidating fungal proteomic response under temperature stress is useful in the context of increasing understanding of fungal sensitivity and resilience to the challenges posed by contemporary climate change processes. Although useful, a more thorough work is needed such as combining data from multiple -omics platforms in order to develop deeper understanding of the factor influencing and controlling cell physiology. This information can be beneficial to identify potential biomarkers for monitoring environmental changes in soil, including the agricultural ecosystems vital to human society and economy.

Introduction

Global warming and associated environmental changes impact living organisms in various ways. The recent Intergovernmental Panel on Climate Change (IPCC 2018) Special Report: Global

Warming of 1.5°C, predicted that extreme hot day events in mid-latitudes may warm by up to ~3°C with global warming of 1.5°C. It is also predicted that extreme night-time low temperatures at high latitudes warm by ~4.5°C (IPCC 2018). If current trends persist, global ice loss will continue, the temperate regions will experience more frequent extreme high and low temperature events (Francis & Skific 2015), and different parts of the tropics will experience longer drought and wet seasons (Overland et al. 2015). This can potentially lead to the breakdown of ecosystem structure and functions, making them yet more sensitive to future climate changes. There have already been some drastic changes in rainfall patterns and patterns of temperature variation (Kundzewicz et al. 2005), already leading to significant reductions in land surface area suitable for crop cultivation (Lesk et al. 2016). Several reviews have addressed research approaches for the development of improved crops in low-quality soils, and of soil management systems to improve resilience to current climate change challenges (Fan et al. 2012; Rao et al. 2016b; Zhang et al. 2013). Major outcomes of these include the use of better-adapted phenotypes (Rao et al. 2016a; Simova-Stoilova et al. 2016), integrated soil-crop system management (Zhang et al. 2013), and maintenance of functional diversity in the soil fungal community by retaining a capacity for symbiosis-driven recycling of organic nutrient pools (Kyaschenko et al. 2017).

The fundamental importance of including mycological studies in efforts to sustain agricultural industries and underpin economic growth is recognised in the European Union's common research 2014-2020 program, known as *Horizon 2020* (Lange et al. 2012). Fungi are generally the most important agent of primary decomposition and nutrient cycling in soil microbial communities (A'Bear et al. 2014b). In boreal forest management and long-term forest production, the functional diversity of the ectomycorrhizal fungal community plays an important role in facilitating symbiosis-driven recycling of organic nutrient pools (Kyaschenko et al. 2017). The key role of fungi identified in such studies highlights the importance of understanding fungal responses to temperature stress. Active fungi initiate stress tolerance strategies at the molecular level and this can be observed in the physiological changes they undergo. Under temperature stress conditions, the tertiary structure of molecules such as enzymes and other functional proteins can be damaged; they will not function properly and may be degraded. Fungi may produce heat or cold marker molecules such as heat shock proteins and chaperones to assist in the repair of functional structure (Bai et al. 2015; Tiwari et al. 2015). There will be substantial physiological changes that can be understood from their molecular-level responses. Such physiological changes can include the production of anti-freeze proteins coating the cell wall (Bagwell & Ricker 2019), the production of extracellular hydrolases such as chitinolytic enzymes (Fenice 2016), and increased production of heat shock proteins to minimize protein misfolding (Kroll et al. 2014; Miteva-Staleva et al. 2017). Such stress tolerance strategies improve cell and organism survival in stressed and sub-optimal environments (Williams et al. 2011).

Changes in protein contents generate a signature that can be identified and characterized. Potentially, these signatures can help understand organismal responses to stress, and be used as biomarkers for monitoring environmental changes and their effects on the ecosystem. Comparative analyses of fungal proteomic profiles can be used to understand changes in protein abundance that may serve as important biomarkers of temperature stress (Kroll et al. 2014). To date, most studies of fungal proteomic response towards temperature stress have been conducted on pathogenic species. Examples include the black yeast, *Exophiala dermatitidis*, that can cause serious skin infections in humans (Tesei et al. 2015); *Aspergillus flavus*, a producer of lethal aflatoxins (Bai et al. 2015; Delgado et al. 2015); *Aspergillus fumigatus*, a thermo-tolerant human pathogen (Albrecht et al. 2010); *Ustilago maydis*, a basidiomycete that causes corn smut disease (Salmeron-Santiago et al. 2011); and *Metarhizium acridum*, an entomopathogenic species that can be used as an alternative to chemical insecticides (Barros et al. 2010). Several studies have also been carried out on extremophiles such as *Cryomyces antarcticus* in order to understand extreme stress tolerance from the proteomic perspective (Isola et al. 2011; Tesei et al. 2012; Zakharova et al. 2014).

In the last 10 years an increasing number of studies have analysed fungal temperature stress responses using proteomic approaches (Albrecht et al. 2010; Bai et al. 2015; Salmeron-Santiago et al. 2011; Tesei et al. 2015; Zakharova et al. 2014). Some have also used ‘multi-omic’ approaches that combine the simultaneous analysis of different molecules (proteins, mRNA, metabolites) to better understand complex biological responses (Bai et al. 2015; Su et al. 2016). Since the late 1990s, proteomic methodologies and technologies have evolved from an approach that relied on two-dimensional gel electrophoresis to gel-free analyses. The protein extraction method is a fundamentally important stage in any proteomic analysis. Optimisation of protein extraction is key in maximising yield and resolution, followed by the selection of proteomic profiling methods (Bianco & Perrotta 2015; Daim et al. 2015; Isola et al. 2011). Label-free or labelled proteomic approaches, such as isotope and fluorescent labelling, are now used extensively because of their many advantages and provide useful information on differential expression of proteins in complex biological samples (Fricker 2018). However, proteomic remains a very costly field, and interpreting the data obtained requires advanced knowledge of the entire technical process involved, as well as understanding of appropriate statistical principles for estimation and inference (Karpievitch et al. 2010).

Survey methodology and objective

This review was developed based on research article journals published between 2008 and 2020, indexed in the Web of Science Core Collection and Scopus, Elsevier databases. We focus on studies of fungal response towards temperature stress elucidated using proteomic approaches. The effects of temperature stress (high and low) on metabolic pathways and HSPs specifically are a primary focus because, these two components are crucial in energy regulation and protein

turnover, and hence for cell survival. This review provides an overview on the effects of temperature stress, on fungal proteomes and the implications this has for fungal responses within the ecosystem to current climate change challenges. The majority of the identified literature documents studies of filamentous fungi, although there are exceptions studying other groups, in particular the microcolonial rock-inhabiting fungus *C. antarcticus* and the black yeast *E. dermatitidis*.

High temperature stress

In general, definitions of high temperature stress have primarily been made based on the optimum growth temperature (T_{opt}) and maximum growth temperature (T_{max}) (Su et al. 2016; Tesei et al. 2012). However, this is not always the case. For example, in the study of pathogenic fungi, experimental temperature selection is often based on the host's body temperature (Tesei et al. 2015). Temperature can also be selected based on the induction or optimisation of mycotoxin production (Bai et al. 2015; Salmeron-Santiago et al. 2011). Deviations from the optimum temperature are expected to trigger a response at the molecular level, with the fungal proteome altering to prevent damage to the cell. Another important parameter in high temperature stress studies is the total duration or interval of heat exposure. Shorter exposures of seconds or minutes can lead to different proteomic expression compared to longer, or repeat, exposures (Albrecht et al. 2010; Tesei et al. 2015). This is because native proteins take time to undergo the processes of denaturing, unfolding or refolding, or eventually to be degraded under high temperature conditions (Aragno 1981). There are two important stages in understanding the impact of high temperature stress on cells: the onset of any stress response, and its upper thermal limit. Together, these often give an indication of specific temperature stress response pathways. With an increase in environmental temperature, a higher metabolic rate is generally anticipated in cells. Thus, heat response pathways will be initiated to utilize the extra energy that is being produced from an increase in metabolic rate, and proteomic profiling can be used to understand the physiological changes taking place (Richter et al. 2010). However, on average, cells are only able to tolerate an increase in temperature of a few degrees above T_{opt} (Aragno 1981). Further increase in temperature beyond this limit causes irreversible damage and ultimately leads to cell death.

Table 1 summarises significant findings on fungal proteome profiling in response to high temperature stress, including the identification of proteins and suggestion of pathways that are potentially related to the identified proteins. A recent integrated -omics study of *Mrakia psychrophila* provided insight into the adaptation mechanisms of psychrophilic fungi (Su et al. 2016). At 4°C and 20°C, *M. psychrophila* produced an increased amount of major facilitator superfamily (MFS) proteins that are involved mainly in energy metabolism, compared to the level at 12°C (optimal growth temperature range 12°C-15°C). However, heat shock proteins were upregulated only at 20°C, which likely leads to the activation of the unfolded protein

response (UPR). Consistent with this, at 20°C, there was a negative correlation between the protein level change and transcript level change. The proteome abundances of another psychrophilic fungus, *Friedmanniomyces endolithicus* (optimal growth temperature range 10°C-15°C), assessed using a classical 2D gel electrophoresis (non-comparative) approach, showed a reduction from 284 (at 28°C) to 224 (comparison between 15°C and 28°C) protein spots (Tesei et al. 2012). The authors hypothesized that, with exposure to high temperature, the basic set of proteins necessary to survive is relatively stable without the help of various chaperones. It is also likely that other non-protein protective metabolites and molecules are involved in the response pathways (Keller 2019).

For human pathogenic fungi, their pathogenic characteristics become significant at body temperature. These have been explored in many species, such as *Penicillium marneffe* and *A. flavus*. Gauthier (2017) reviewed the molecular strategies used by thermally dimorphic fungi, focusing on their ability to adapt to core body temperature (37°C) and transition to yeast morphology for virulence capabilities. These fungi used various strategies, such as upregulation of virulence factors, to promote cell adhesion to host, lysis of macrophages, avert cytokine responses, and impair host immunity. *Penicillium marneffe* is the only dimorphic species in its genus and forms its secondary cellular development, which is a uninucleate yeast, at 37°C (Chandler et al. 2008). Increased levels of RanA expression, a GTP-binding nuclear protein that plays a role in nucleocytoplasmic transport, suggest that there is an additional signalling mechanism involved during phase transition in *P. marneffe*. Comparative work on extracellular proteomes of *P. marneffe* in yeast and mycelial phases showed upregulation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and heat shock protein 60 (HSP60), respectively, that may play an important role in cell-host adherence (Lau et al. 2013). Bai et al. (2015) used an integrated transcriptomic and proteomic approach to study the response towards temperature changes in *A. flavus*, a species whose growth optimum is 37°C, but that produces highest levels of mycotoxin is at 28°C. The data obtained showed that a subset of 664 proteins involved in translation-related pathways, metabolic pathways and biosynthesis of secondary metabolites were differentially expressed at the two temperatures. At 28 °C, the expression pattern of proteins and transcripts related to aflatoxin biosynthesis were upregulated, and the authors suggested that change in the *aflR* transcript level was a better marker for the activation of aflatoxin biosynthesis. However, they also noted that there was a low correlation between overall transcript level and protein concentration in *A. flavus*, suggesting that the post-transcription modification processes may play a critical role in the regulation of the final protein expression level.

The plant pathogen, *U. maydis* is a well-studied causative agent for corn smut disease, but there are also reports of *Ustilago* spp. infections in humans (McNeil & Palazzi 2012; Teo & Tay 2006). Under various stress conditions, fungi often accumulate trehalose and initiate trehalose biosynthesis pathways (Al-Bader et al. 2010; Nwaka & Holzer 1997). In a study of the effect of

temperature stress on *U. maydis*, increased production of 11 proteins, commonly up-regulated in response to osmotic and sorbitol stress, was observed (Salmeron-Santiago et al. 2011). However, there were no changes in the trehalose concentration, thus suggesting that the up-regulated proteins are common proteins in the general stress response of *U. maydis*, non-specific to trehalose metabolism pathways. A more recent review (Perez-Nadales et al. 2014) highlighted the overall mechanisms of pathogenesis and some unifying themes among various fungal model organisms, emphasising the importance of conserved signalling pathways such as the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A and mitogen-activated protein (MAP) kinases, and the central roles of secondary metabolic pathways in a very wide range of pathogenic fungi. However, comparison of the effect of temperature on the pathogenicity of fungal model organisms was not a focus of that study.

Mesophilic fungi, which have T_{opt} of 25-30°C, are generally able to tolerate a wider range of temperatures and associated stress, having a relatively wider range of temperature-dependent growth curves (Dix & Webster 1995). In a non-comparative experiment on three mesophilic fungi (*Exophiala jeanselmei*, *Coniosporium perforans* and *Penicillium chrysogenum*), high temperature exposure led to variation in their proteomic expression patterns (Tesei et al. 2012). Exposure to 40°C increased the number of expressed proteins detected in *P. chrysogenum* but decreased those of *E. jeanselmei* and *C. perforans*. - A comparison was also made between species at 40°C by selecting *P. chrysogenum* as the reference strain for mesophilic black fungi, which revealed 50 and 62 common protein spots in *E. jeanselmei* and *C. perforans*, respectively. The study suggested that there was a lack of a heat-shock protein response in *E. jeanselmei* and *C. perforans*. Furthermore, the disappearance of spots from the same pI and molecular mass range (respectively 5-7 and 30-90 kDa) suggested that both strains probably downregulated similar sets of proteins. Tesei et al. (2012) hypothesized that either the basic set of proteins that is important in high temperature exposure is stable without the help of heat-shock proteins or that other non-protein protective metabolites are involved. It is important to understand the limitations of such non-comparative experiments conducted through gel-based methods, where technical variations between replicates are expected. Quantification and identification of protein spots can be a challenge if differences in staining occur between two gels with no normalization. An integrated analysis of transcriptomic and proteomic responses of *A. fumigatus* exposed to high temperature stress improved understanding of the thermotolerant characteristics responsible for the pathogenicity of this fungus (Albrecht et al. 2010). The study identified 91 differentially regulated protein spots representing 64 different proteins using two-dimensional difference gel electrophoresis (2D-DIGE) and tandem mass spectrometry (MS/MS) identification methods. These included a number of previously undescribed putative targets for the heat shock regulator Hsf1, providing evidence for Hsf1-dependent regulation of mannitol biosynthesis, translation, cytoskeletal dynamics, and cell division in *A. fumigatus*. Albrecht et al. (2010) also demonstrated a negative correlation between protein expression and gene transcription levels in *A. fumigatus* when exposed to supra-optimal temperatures (from 30°C to 48°C). The synthesis of most

proteins was delayed by 60 to 90 minutes (medium delay) and up to 105 minutes (strong delay), after gene transcription by exposure to supra-optimal temperatures.

High temperature exposure causes specific stress response mechanisms to be activated that can be further explored using fungal proteomic profiles. Most fungi respond to high temperature stress by increasing the production of heat-shock proteins and chaperones and initiating alternative metabolic pathways (Albrecht et al. 2010; Bai et al. 2015; Su et al. 2016). However, some species show no significant changes in their proteome profiles, suggesting that the basic set of proteins needed for survival are highly thermotolerant (Tesei et al. 2015; Tesei et al. 2012). It has also been hypothesized that this is explained by the production of secondary metabolites and other non-protein protective metabolites that cannot be determined through proteomic approaches (Zhang et al. 2016). In addition, high temperature stress may induce a negative correlation between gene transcription and protein production in fungal cells due to post-translational modification and the initiation of protein degradation pathways, such as the ubiquitin-mediated protein degradation pathway and spliceosome-mediated decay (SMD) pathway (Albrecht et al. 2010; Bai et al. 2015).

Low temperature stress

The study of low temperature stress resistance in microorganisms and other organisms has led to many beneficial findings. Proteins, such as cold-adapted enzymes, have been applied in many biotechnology industries such as in the manufacture of food and beverages, detergents, textiles, and in industrial molecular biology (Sarmiento et al. 2015). Research on cold-adapted proteins often involves psychrophilic or psychrotolerant microorganisms from the polar regions or deep oceans (Cavicchioli et al. 2000). Psychrophiles produce cold-adapted proteins with unique characteristics that have weak protein interactions, low thermal stability and increased specific activity, in order to achieve higher protein activity and flexibility at low temperatures (Reed et al. 2013). Protein structures are modified with subtle changes in the amino acid composition, thus remaining functional under extremely cold conditions. Wang et al. (2017) reviewed fungal adaptation to cold stress, providing an overview of life history strategies and highlighting the importance of cold-adapted fungi in the discovery of novel secondary metabolites and enzymes. Studies on fungal proteome profiles in response to low temperature stress are summarised in Table 2.

In a study of black rock-inhabiting fungi exposed to low temperature stress, their proteomic profiles generally showed an increase in total number of spots, except for *P. chrysogenum* (Tesei et al. 2012). *Friedmanniomyces endolithicus* and *C. perforans* proteome profiles exhibited changes in high molecular mass protein spots (70 – 170 kDa), while that of *E. jeanselmei* showed pattern changes at slightly lower molecular mass (25 – 100 kDa). *Penicillium chrysogenum* exhibited a decrease in total protein spots, interpreted as being due to lower metabolic activity

under cold stress. *Exophiala dermatitidis*, a mesophilic fungus that is pathogenic in humans, was experimentally exposed to low temperature in order to understand the relationship between its thermotolerance properties and pathogenicity (Tesei et al. 2015). The strain was exposed to 1°C (low temperature stress) for one week and comparison was then made with proteome profiles obtained at 37°C (optimum temperature for growth) and 45°C (high temperature stress). Using 2D-DIGE and a nano-scale liquid-chromatography electrospray-ionisation, tandem mass spectrometry (nLC-ESI-MS/MS), the study showed an average of 1700 protein spots detected in *E. dermatitidis*. Exposure to low temperature stress led to a reduction in proteins associated with metabolic activity, mostly relating to general carbon metabolism. A large set of proteins involved in energy metabolism pathways were down-regulated, such as malate synthase, malate dehydrogenase, acetyl-coenzyme A synthetase and glyceraldehyde-3-phosphate dehydrogenase. Decreased levels of proteins involved in the response to heat stress such as Hsp70s, elongation factor 1 α and Hsp30 might also be related to the reduction in metabolic rate and associated reduced energy consumption. Despite utilising alternative metabolic pathways, *E. dermatitidis* undergoes downregulation of metabolic pathways under exposure to non-optimal temperatures, resulted in a much slower growth rate. Comparison of the proteome profiles of *E. dermatitidis* under high and low temperature stress demonstrated the contribution to its success as a pathogen made by regulating the expression of basic thermotolerance proteins and modulating the production of proteins in major metabolic pathways.

Mrakia psychrophila, another psychrophilic yeast, showed a slightly different response to low temperature stress compared to *E. dermatitidis*, but more similar to other cold-adapted fungi such as *Flammulina velutipes* (Liu et al. 2017) and *Mortierella isabellina* (Hu et al. 2016). The responses included desaturation of fatty acids and accumulation of glycerol (Su et al. 2016). At 4°C, two proteins were upregulated (glutamine synthetase, GLNA and MFS transporter protein, MPSY), and four proteins were downregulated (citrate synthase, succinyl co-A ligase, pyruvate decarboxylase and ribosomal protein). Comparative transcriptomic analysis showed that genes involved in ribosome production and energy metabolism were also upregulated at 4°C. Proteomic analysis indicated that protein levels were positively correlated with transcription levels in some pathways under low temperature stress, suggesting the upregulation of chaperones and energy metabolism pathways. A study of the white rot fungus, *F. velutipes*, showed a similar change in protein profile in response to short-term and long-term exposure to low temperature (Liu et al. 2017). Of 63 differentially expressed proteins, 31 were upregulated, 24 of which were involved in energy metabolic pathways, amino acid biosynthesis and metabolism, signalling pathways, transport and translation. Four upregulated proteins were involved in energy metabolic pathways such as starch and sucrose metabolism (catalase, glucose-6-phosphate isomerase, trehalase and beta-glucosidase). However, catalase was upregulated only in the short term after exposure to low temperature, and its levels returned to normal after longer exposure to low temperature. Eleven differentially expressed proteins were involved in the biosynthesis of nine amino acids, indicating a role played in cold stress response by modifying nitrogen-containing

molecule storage. Signalling molecules and processes involved in protein degradation were also upregulated, thus suggesting the importance of these molecules in controlling *F. velutipes* mycelium growth and fruiting body formation under cold stress. Proteomic profiling of *M. isabellina* (a soil fungus involved in many biotechnological applications) under low temperature stress identified 44 differentially expressed proteins under cold stress (Hu et al. 2016). These proteins were mainly involved in the regulation of ATP synthesis (ATP synthase subunit beta, ATP synthase d subunit), glycolytic pathways (fructose-bisphosphate aldolase), protein modification and electron transport (cytochrome c oxidase polypeptide 5B). The responses identified in *M. isabellina* also supported protein degradation pathways playing an important role in cold stress responses in fungi. Proteins such as HSPs and transitional endoplasmic reticulum ATPase (TER ATPase), that are essential in proteasome pathways, were also upregulated under cold stress.

Integrating the findings of these different studies has led to deeper understanding of cold-adaptive mechanisms in fungi. They respond to low temperature stress by modifying their energy metabolic pathways, increasing the production of chaperones to minimise misfolded protein production, and regulating the synthesis of amino acids that are important in nitrogen storage. In contrast with studies of high temperature stress, the overall response of many fungal species indicates that low temperature does not cause irreversible damage to cells, with responses acting by modifying molecular content rather than wholesale transformation of complex protein networks. The huge amount of information that can be generated in fungal proteomic studies examining low temperature stress can be utilised for more specific approaches, for example in process optimization and purification of cold-adapted biomolecules. Future studies on cold adaptation mechanisms in fungi will benefit from a focus on identifying common proteins that act as cold stress biomarkers and exploiting the advantages of cold-adapted proteins in biotechnological applications.

Effects of temperature stress in fungal proteomes

Fungi respond to temperature stress through regulation of various proteins, that can be visualised using proteomic profiling techniques within the limitations noted above. Changes in fungal proteomes exposed to various temperature stresses (Tables 1, 2) demonstrate that many cellular functions are affected, such as the TCA cycle and energy production and metabolism, oxidative phosphorylation, regulation of mitotic activities, cell cycle and division, transcriptional and translational stages of biosynthetic pathways, cell wall and cytoskeleton reorganisation, transport protein remodelling, defence against oxidative and other stresses, and cellular signalling mechanisms. Undeniably, these proteins and their roles in cellular functions are highly significant, with the complex cellular protein network maintained through protein homeostasis (Mühlhofer et al. 2019; O' Neill et al. 2020). It is extremely challenging to identify any specific set of proteins as being the most affected by or vulnerable to temperature stress, or being used as

potential temperature stress biomarkers. Metabolic pathways and HSPs are considered a primary focus and elaborated below. These two components are crucial in energy regulation and protein turnover, and hence for cell survival (Yan et al. 2020). More widely, biosynthesis and regulation of metabolic pathways and HSPs are of importance in many applied fields such as agriculture, food biotechnology and medicine (Ene et al. 2014; Lamoth et al. 2016; Sarmiento et al. 2015).

Effect of temperature stress on metabolic pathways

Fungal adaptation towards temperature stress involves alterations in the utilisation of many metabolic pathways in trade-offs to compensate for the amount of energy needed or the extra energy to be utilised in delivering the stress response. As temperature increases, the rate of metabolism (of a non-endothermic organism) increases as a purely physical consequence, and then rapidly declines at higher temperatures as the metabolic systems start to fail – eventually leading to cell death. Metabolic regulation is one strategy employed by organisms to adapt to temperature stress, which is controlled by factors such as concentration of substrates, products or allosteric effectors (Clarke 2018; Suarez & Moyes 2012). Understanding thermal adaptation in fungi requires an overall understanding on the effects of temperature on pathway flux, including such things as the roles and limitations of enzymes involved, concentration of substrates and products, and other protein and non-protein components (Schulte 2015). Advances in proteomic technologies have made it possible to determine many of the proteins involved in the complex network of metabolic pathways, as low as in zeptomole-scale mixtures (Swaminathan et al. 2018), but it remains challenging to accurately identify and allocate all protein molecules involved into their various pathways (Timp & Timp 2020). However, many studies have developed visual diagrams of complex protein-protein interaction networks, and it is accepted that temperature stress causes quantifiable changes in these metabolic pathways compared to non-stress conditions (Bai et al. 2015; Kostadinova et al. 2011).

Glycolysis is an important metabolic pathway in most organisms, including fungi. Many fungi produce enzymes to break down complex polysaccharides such as cellulose, hemicellulose, pectin and starch to produce glucose (Krishnan et al. 2016; Xiong et al. 2017). Many studies have confirmed that fungi produced a range of extracellular hydrolases to help them utilise different carbon and nitrogen sources under exposure to temperature stress (Krishnan et al. 2017; Tajuddin et al. 2018). The production of these enzymes responds to changing environmental temperature, in order to maintain metabolic requirements and protein turnover. As noted earlier, trehalose production is also important for fungi under temperature stress. Trehalose is known to be important in the acquisition of thermotolerance and desiccation tolerance in many fungal species (and much more widely across multiple groups of biota) (Everatt et al. 2015; Gancedo & Flores 2004; Liu et al. 2019; Tereshina 2005). In *Saccharomyces cerevisiae*, trehalose and intracellular water stabilise the membrane structure and other intracellular networks under temperature stress conditions (Piper 1993). Various studies document increased activity of

trehalases, such as the trehalose synthase and fructose-1,6-biphosphatase, in response to heat stress (Bonini et al. 1995; Cai et al. 2009). In addition, enzymes such as cAMP-dependent protein kinase and plasma membrane ATPase also play significant roles in fungal thermotolerance determination (Jurick Ii et al. 2004; Piper 1993). Enolase, an enzyme that converts 2-phosphoglyceric acid to phosphoenolpyruvic acid in glycolysis, is also crucial in the heat stress response of many fungal species and yeasts (Ji et al. 2016). It has been suggested that enolase (ENO1) is closely involved in heat stress responses, and ENO1 of yeasts and streptococcal strains isolated from rats has high thermal stability (Cuéllar-Cruz et al. 2013; Iida & Yahara 1985; Kustrzeba-Wójcicka & Golczak 2000).

Effect of temperature stress on heat shock proteins (HSPs)

In the event of heat stress, HSPs are produced in cells to protect proteins from aggregation, unfold or refold aggregated proteins, or target them for the degradation pathway. Although initially named following discovery in heat stress experiments, HSPs are a part of general stress responses, produced when cells and organisms are exposed to multiple types of stressors. Most HSPs are molecular chaperones produced constitutively or induced upon cell stress, which can be triggered by a temperature change of just a few degrees (Richter et al. 2010). Heat shock proteins are primarily categorised and named according to their molecular mass, which varies from 10 to 100 kDa. Their multifunctional properties have made them an important and reliable target biomarker in various fields such as crop management, plant and microbe adaptation towards environmental stress, and cancer related studies (Jee 2016). HSPs are known to play a role in temperature-stressed environments (Tiwari et al. 2015), but understanding the complexity of the HSP network as a defence mechanism in the cellular environment requires much further research. The cellular functions of the various HSPs that are involved as part of the heat shock response network in fungi, using *S. cerevisiae* as a model, has been discussed in depth (Verghese et al. 2012). Evidence suggests that HSPs are involved in cell cycle arrest (Vergés et al. 2007), metabolic reprogramming (Elliott et al. 1996), modulating cell wall and membrane dynamics (Shaner et al. 2008; Truman et al. 2007; Winkler et al. 2002) and protein aggregation (Nathan et al. 1997).

Heat shock protein 60 (HSP60) is a highly conserved molecule in many organisms and has been found to respond to various stress conditions in fungi. This HSP is upregulated in response to increased temperature in species such as *A. fumigatus*, *Aspergillus terreus*, *P. chrysogenum*, *Scedosporium apiospermum*, *Trichophyton mentagrophytes*, *Candida albicans* and *S. cerevisiae* (Raggam et al. 2011). HSP60 has also been proposed to play a role in the assembly of precursor polypeptides into oligomeric complexes following incorporation into the mitochondrial matrix (Patriarca & Maresca 1990). Galello et al. (2014) recently described the interaction of HSP60 and Ira2 with Bcy1, a regulatory subunit of protein kinase A (PKA) in *S. cerevisiae*. Using an MS-based proteomic approach, this study demonstrated that HSP60 localized the entire PKA-

Ras complex to mitochondria under the regulation of the cAMP-PKA-signalling pathway. There are also reports of HSP70 having a role in enhancing fungal resistance to heat stress and other abiotic stresses (2010; Montero-Barrientos et al. 2008). The overexpression of HSP0 genes in *Trichoderma harzianum* T34 resulted in an increase in biomass and enhanced tolerance to other abiotic stresses after heat shock treatment (Montero-Barrientos et al. 2008). Subsequently, the function of the HSP70 gene from *T. harzianum* T34 was further studied in the transgenic plant, *Arabidopsis thaliana*, showing that the transgenic plants exhibited higher tolerance towards heat stress (Montero-Barrientos et al. 2010). HSP90, one of the most ubiquitous chaperones in yeasts (Nathan et al. 1997), was found to interact with calcineurin (Imai & Yahara 2000) and respond to heat stress indirectly through facilitating the activation of the MAPK complex (Truman et al. 2007). More recent work has shown that HSP90 plays a central role in heat stress responses in *Fusarium graminearum*, in addition to its crucial roles in fungal vegetative growth, reproduction and virulence (Bui et al. 2016). In *A. fumigatus*, HSP90 has been implicated in drug resistance, as disruption of the HSP90 circuitry leads to activation of antifungal activity in caspofungin (Lamoth et al. 2016).

Concluding remarks

The importance of fungi and of mycological studies in the development of the bioeconomy and in environmental monitoring has been accepted and highlighted by researchers worldwide. Excellent reviews are available documenting fungal response towards abiotic stress and how fungi can affect the tolerance of other organisms, especially plants, towards multiple stressors (A'Bear et al. 2014a; Coleman-Derr & Tringe 2014; East 2013; McCotter et al. 2016; Rangel et al. 2018). Many studies have set out to understand fungal heat stress response mechanisms in order to help develop tools for crop monitoring and production as well as producing heat-tolerance species that are better able to response to climate change challenges (Jagadish et al. 2010; Simova-Stoilova et al. 2016). Research on fungal molecular responses towards temperature stress has been widely explored using different omics approaches. Studies on fungal genomes, transcriptomes, secretomes and proteomes have provided new knowledge and understanding of these responses (Albrecht et al. 2010; Bai et al. 2015; Cologna et al. 2018; Kroll et al. 2014). The outcomes of these studies will need to be fully exploited to maximise the potential of fungi both in biotechnological industries and in natural environment under current climate change scenarios (Classen et al. 2015; Lange et al. 2012; Rangel et al. 2018). As is clear from this review, there is currently limited available literature documenting the use of proteomics in studying the effects of temperature stress on fungal proteins, and most reports to date have focused on pathogenic fungi. Despite the tremendous promise of proteomics, technical limitations that affect sensitivity and resolution continue to limit its practical utility. In almost all studies considered here, detection was limited to medium and high abundance proteins, the majority of which were related to metabolism and chaperones. With currently available data from proteomics, it remains difficult to provide comprehensive descriptions of the biological

changes that can be observed. At present this means our ability to identify specific biomarkers for any given change is very limited, as many confounding factors can influence the production of these more abundant proteins.

These issues can be overcome in future. More thorough interrogation of the proteome, including integration within multi-omics platforms, will allow for deeper insights into the mechanisms underlying changing cell physiology. If potential biomarkers are to be identified, proteomic analyses must go beyond medium and high abundance proteins. Several strategies can be employed to achieve this. Specific sub-proteomes of proteins and their associated pathways already identified as changing in initial analyses can be further explored. A targeted proteomics approach, for example looking at specific sub-fractions of the cell (cell wall, specific organelles, etc.) may be beneficial. This will allow for the identification of low abundance proteins that may include, for instance, signaling proteins with specific roles in heat stress response. Further information can also be obtained by conducting investigations using multi-omics platforms applied to the same samples simultaneously, to provide both complementary and confirmatory information.

Acknowledgements

The authors acknowledge the Malaysian Ministry of Higher Education (MOHE) through their funding programme Higher Centre of Excellence (HiCoE) (grant number IOES-2014G), and Universiti Malaya Research Programme (UMRP) (grant number RP026A-18SUS), and postgraduate sponsorship from the Majlis Amanah Rakyat Malaysia (MARA Scholarship Programme). We also thank Prof. Peter Convey from the British Antarctic Survey (BAS), United Kingdom, for commenting on the manuscript.

References

- A'Bear AD, Jones TH, and Boddy L. 2014a. Potential impacts of climate change on interactions among saprotrophic cord-forming fungal mycelia and grazing soil invertebrates. *Fungal Ecology* 10:34-43. 10.1016/j.funeco.2013.01.009:
- A'Bear AD, Jones TH, and Boddy L. 2014b. Size matters: What have we learnt from microcosm studies of decomposer fungus–invertebrate interactions? *Soil Biology and Biochemistry* 78:274-283. 10.1016/j.soilbio.2014.08.009:
- Al-Bader N, Vanier G, Liu H, Gravelat FN, Urb M, Hoareau CM, Campoli P, Chabot J, Filler SG, and Sheppard DC. 2010. Role of trehalose biosynthesis in *Aspergillus fumigatus* development, stress response, and virulence. *Infection and Immunity* 78:3007-3018. 10.1128/IAI.00813-09:
- Albrecht D, Guthke R, Brakhage AA, and Kniemeyer O. 2010. Integrative analysis of the heat shock response in *Aspergillus fumigatus*. *BMC Genomics* 11:32. 10.1186/1471-2164-11-32:

519 Aragno M. 1981. Responses of Microorganisms to Temperature. In: Lange OL, Nobel PS, Osmond CB,
520 and Ziegler H, eds. *Physiological Plant Ecology I: Responses to the Physical Environment*.
521 Berlin, Heidelberg: Springer Berlin Heidelberg, 339-369.

522 Bagwell SN, and Ricker JV. 2019. Antifreeze proteins: effective adaptations of organisms for low
523 temperature survival. *BIOS* 90:158-170, 113. 10.1893/BIOS-D-17-00007:

524 Bai Y, Wang S, Zhong H, Yang Q, Zhang F, Zhuang Z, Yuan J, Nie X, and Wang S. 2015. Integrative
525 analyses reveal transcriptome-proteome correlation in biological pathways and secondary
526 metabolism clusters in *A. flavus* in response to temperature. *Scientific Reports* 5:14582.
527 10.1038/srep14582:

528 Barros BH, da Silva SH, dos Reis Marques Edos R, Rosa JC, Yatsuda AP, Roberts DW, and Braga GU.
529 2010. A proteomic approach to identifying proteins differentially expressed in conidia and
530 mycelium of the entomopathogenic fungus *Metarhizium acridum*. *Fungal Biology* 114:572-579.
531 10.1016/j.funbio.2010.04.007:

532 Bianco L, and Perrotta G. 2015. Methodologies and perspectives of proteomics applied to filamentous
533 fungi: from sample preparation to secretome analysis. *International Journal of Molecular*
534 *Sciences* 16:5803-5829. 10.3390/ijms16035803:

535 Bonini BM, Neves MJ, Jorge JA, and Terenzi HF. 1995. Effects of temperature shifts on the metabolism
536 of trehalose in *Neurospora crassa* wild type and a trehalase-deficient (tre) mutant. Evidence
537 against the participation of periplasmic trehalase in the catabolism of intracellular trehalose.
538 *Biochimica et Biophysica Acta (BBA) - General Subjects* 1245:339-347. 10.1016/0304-
539 4165(95)00098-4:

540 Bui D-C, Lee Y, Lim JY, Fu M, Kim J-C, Choi GJ, Son H, and Lee Y-W. 2016. Heat shock protein 90 is
541 required for sexual and asexual development, virulence, and heat shock response in *Fusarium*
542 *graminearum*. *Scientific Reports* 6:28154. 10.1038/srep28154:

543 Cai Z, Peng G, Cao Y, Liu Y, Jin K, and Xia Y. 2009. Trehalose-6-phosphate synthase 1 from
544 *Metarhizium anisopliae*: clone, expression and properties of the recombinant. *Journal of*
545 *Bioscience and Bioengineering* 107:499-505. 10.1016/j.jbiosc.2009.01.007:

546 Cavicchioli R, Thomas T, and Curmi PM. 2000. Cold stress response in Archaea. *Extremophiles* 4:321-
547 331.

548 Chandler JM, Treece ER, Trenary HR, Brenneman JL, Flickner TJ, Frommelt JL, Oo ZM, Patterson MM,
549 Rundle WT, Valle OV, Kim TD, Walker GR, and Cooper CR, Jr. 2008. Protein profiling of the
550 dimorphic, pathogenic fungus, *Penicillium marneffe*. *Proteome Science* 6:17. 10.1186/1477-
551 5956-6-17:

552 Clarke A. 2018. *Principles of thermal ecology : temperature, energy and life*: Oxford : Oxford University
553 Press.

554 Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JAM, Cregger MA, Moorhead LC, and
555 Patterson CM. 2015. Direct and indirect effects of climate change on soil microbial and soil
556 microbial-plant interactions: What lies ahead? *Ecosphere* 6:1-21. 10.1890/ES15-00217.1:

557 Coleman-Derr D, and Tringe SG. 2014. Building the crops of tomorrow: advantages of symbiont-based
558 approaches to improving abiotic stress tolerance. *Frontiers in Microbiology* 5:283.
559 10.3389/fmicb.2014.00283:

560 Cologna NdMd, Gómez-Mendoza DP, Zanoelo FF, Giannesi GC, Guimarães NCdA, Moreira LRdS,
561 Filho EXF, and Ricart CAO. 2018. Exploring Trichoderma and Aspergillus secretomes:
562 Proteomics approaches for the identification of enzymes of biotechnological interest. *Enzyme and*
563 *Microbial Technology* 109:1-10. 10.1016/j.enzmictec.2017.08.007:

- Cuéllar-Cruz M, Gutiérrez-Sánchez G, López-Romero E, Ruiz-Baca E, Villagómez-Castro JC, and Rodríguez-Sifuentes L. 2013. Identification of *Candida albicans* heat shock proteins and *Candida glabrata* and *Candida krusei* enolases involved in the response to oxidative stress. *Central European Journal of Biology* 8:337-345. 10.2478/s11535-013-0138-9;
- Daim LD, Ooi TE, Yusof HM, Majid NA, and Karsani SA. 2015. Optimization of Protein Extraction and Two-Dimensional Electrophoresis Protocols for Oil Palm Leaf. *The Protein Journal* 34:304-312. 10.1007/s10930-015-9626-x;
- Delgado J, Owens RA, Doyle S, Asensio MA, and Nunez F. 2015. Impact of the antifungal protein PgAFP from *Penicillium chrysogenum* on the protein profile in *Aspergillus flavus*. *Applied Microbiology and Biotechnology* 99:8701-8715. 10.1007/s00253-015-6731-x;
- Dix NJ, and Webster J. 1995. Fungi of Extreme Environments. In: Dix NJ, and Webster J, eds. *Fungal Ecology*. Dordrecht: Springer Netherlands, 322-340.
- East R. 2013. Microbiome: Soil science comes to life. *Nature* 501:S18. 10.1038/501S18a;
- Elliott B, Haltiwanger RS, and Fletcher B. 1996. Synergy between trehalose and Hsp104 for thermotolerance in *Saccharomyces cerevisiae*. *Genetics* 144:923-933.
- Ene IV, Brunke S, Brown AJP, and Hube B. 2014. Metabolism in fungal pathogenesis. *Cold Spring Harbor perspectives in medicine* 4:a019695-a019695. 10.1101/cshperspect.a019695;
- Everatt MJ, Convey P, Bale JS, Worland MR, and Hayward SA. 2015. Responses of invertebrates to temperature and water stress: A polar perspective. *Journal of Thermal Biology* 54:118-132. 10.1016/j.jtherbio.2014.05.004;
- Fan M, Shen J, Yuan L, Jiang R, Chen X, Davies WJ, and Zhang F. 2012. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. *Journal of Experimental Botany* 63:13-24. 10.1093/jxb/err248;
- Fenice M. 2016. The Psychrotolerant Antarctic Fungus *Lecanicillium muscarium* CCFEE 5003: A Powerful Producer of Cold-Tolerant Chitinolytic Enzymes. *Molecules* 21. 10.3390/molecules21040447;
- Francis J, and Skific N. 2015. Evidence linking rapid Arctic warming to mid-latitude weather patterns. *Philosophical Transactions of the Royal Society A* 373:20140170.
- Fricker L. 2018. Quantitative Peptidomics: General Considerations. In: Schrader M, and Fricker L, eds. *Peptidomics: Methods and Strategies*. New York, NY: Springer New York, 121-140.
- Galello F, Moreno S, and Rossi S. 2014. Interacting proteins of protein kinase A regulatory subunit in *Saccharomyces cerevisiae*. *Journal of Proteomics* 109:261-275. 10.1016/j.jprot.2014.07.008;
- Gancedo C, and Flores C-L. 2004. The importance of a functional trehalose biosynthetic pathway for the life of yeasts and fungi. *FEMS Yeast Research* 4:351-359. 10.1016/S1567-1356(03)00222-8;
- Gauthier GM. 2017. Fungal Dimorphism and Virulence: Molecular Mechanisms for Temperature Adaptation, Immune Evasion, and In Vivo Survival. *Mediators of Inflammation* 2017:8491383. 10.1155/2017/8491383;
- Hu B, Luo M, Ji X, Lin L, Wei Y, and Zhang Q. 2016. Proteomic analysis of *Mortierella isabellina* M6-22 during cold stress. *Archives of Microbiology* 198:869-876. 10.1007/s00203-016-1238-0;
- Iida H, and Yahara I. 1985. Yeast heat-shock protein of Mr 48,000 is an isoprotein of enolase. *Nature* 315:688-690. 10.1038/315688a0;
- Imai J, and Yahara I. 2000. Role of HSP90 in salt stress tolerance via stabilization and regulation of calcineurin. *Molecular and Cellular Biology* 20:9262-9270.

- IPCC. 2018. Summary for Policymakers. In: Global Warming of 15°C An IPCC Special Report on the impacts of global warming of 15°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Masson-Delmotte V, P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield editor.: World Meteorological Organization. Geneva, Switzerland.
- Isola D, Marzban G, Selbmann L, Onofri S, Laimer M, and Sterflinger K. 2011. Sample preparation and 2-DE procedure for protein expression profiling of black microcolonial fungi. *Fungal Biology* 115:971-977. 10.1016/j.funbio.2011.03.001:
- Jagadish SVK, Muthurajan R, Oane R, Wheeler TR, Heuer S, Bennett J, and Craufurd PQ. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *Journal of Experimental Botany* 61:143-156. 10.1093/jxb/erp289:
- Jee H. 2016. Size dependent classification of heat shock proteins: a mini-review. *Journal of exercise rehabilitation* 12:255-259. 10.12965/jer.1632642.321:
- Ji H, Wang J, Guo J, Li Y, Lian S, Guo W, Yang H, Kong F, Zhen L, Guo L, and Liu Y. 2016. Progress in the biological function of alpha-enolase. *Animal Nutrition* 2:12-17. 10.1016/j.aninu.2016.02.005:
- Jurick Ii WM, Dickman MB, and Rollins JA. 2004. Characterization and functional analysis of a cAMP-dependent protein kinase A catalytic subunit gene (*pka1*) in *Sclerotinia sclerotiorum*. *Physiological and Molecular Plant Pathology* 64:155-163. 10.1016/j.pmpp.2004.07.004:
- Karpievitch YV, Polpitiya AD, Anderson GA, Smith RD, and Dabney AR. 2010. Liquid Chromatography Mass Spectrometry-Based Proteomics: Biological and Technological Aspects. *Annals of Applied Statistics* 4:1797-1823. 10.1214/10-AOAS341:
- Keller NP. 2019. Fungal secondary metabolism: regulation, function and drug discovery. *Nature reviews Microbiology* 17:167-180. 10.1038/s41579-018-0121-1:
- Kostadinova N, Vassilev S, Spasova B, and Angelova M. 2011. Cold stress in Antarctic fungi targets enzymes of the glycolytic pathway and tricarboxylic acid cycle. *Biotechnology and Biotechnological Equipment* 25:50-57. 10.5504/BBEQ.2011.0122:
- Krishnan A, Convey P, Gonzalez-Rocha G, and Alias SA. 2016. Production of extracellular hydrolase enzymes by fungi from King George Island. *Polar Biology* 39:65-76. 10.1007/s00300-014-1606-7:
- Krishnan A, Convey P, Gonzalez M, Smykla J, and Alias SA. 2017. Effects of temperature on extracellular hydrolase enzymes from soil microfungi. *Polar Biology*. 10.1007/s00300-017-2215-z:
- Kroll K, Pahtz V, and Kniemeyer O. 2014. Elucidating the fungal stress response by proteomics. *J Proteomics* 97:151-163. 10.1016/j.jprot.2013.06.001:
- Kundzewicz ZW, Ulbrich U, brücher T, Graczyk D, Krüger A, Leckebusch GC, Menzel L, Pińskwar I, Radziejewski M, and Szwed M. 2005. Summer Floods in Central Europe – Climate Change Track? *Natural Hazards* 36:165-189. 10.1007/s11069-004-4547-6:
- Kustrzeba-Wójcicka I, and Golczak M. 2000. Enolase from *Candida albicans* — purification and characterization. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 126:109-120. 10.1016/S0305-0491(00)00169-3:

650 Kyaschenko J, Clemmensen KE, Hagenbo A, Karlton E, and Lindahl BD. 2017. Shift in fungal
651 communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris*
652 stands. *The Isme Journal* 11:863. 10.1038/ismej.2016.184:

653 Lamothe F, Juvvadi PR, and Steinbach WJ. 2016. Heat shock protein 90 (Hsp90): A novel antifungal
654 target against *Aspergillus fumigatus*. *Critical Reviews in Microbiology* 42:310-321.
655 10.3109/1040841X.2014.947239:

656 Lange L, Bech L, Busk PK, Grell MN, Huang Y, Lange M, Linde T, Pilgaard B, Roth D, and Tong X.
657 2012. The importance of fungi and of mycology for a global development of the bioeconomy.
658 *IMA Fungus* 3:87-92. 10.5598/imafungus.2012.03.01.09:

659 Lau SK, Tse H Fau - Chan JSY, Chan Js Fau - Zhou AC, Zhou Ac Fau - Curreem SOT, Curreem So Fau -
660 Lau CCY, Lau Cc Fau - Yuen K-Y, Yuen Ky Fau - Woo PCY, and Woo PC. 2013. Proteome
661 profiling of the dimorphic fungus *Penicillium marneffei* extracellular proteins and identification
662 of glyceraldehyde-3-phosphate dehydrogenase as an important adhesion factor for conidial
663 attachment. *The FEBS Journal* 280:1742-4658. 10.1111/febs.12566:

664 Lesk C, Rowhani P, and Ramankutty N. 2016. Influence of extreme weather disasters on global crop
665 production. *Nature* 529:84. 10.1038/nature16467:

666 Liu J-Y, Men J-l, Chang M-c, Feng C-p, and Yuan L-G. 2017. iTRAQ-based quantitative proteome
667 revealed metabolic changes of *Flammulina velutipes* mycelia in response to cold stress. *Journal*
668 *of Proteomics* 156:75-84. 10.1016/j.jprot.2017.01.009:

669 Liu X-m, Wu X-l, Gao W, Qu J-b, Chen Q, Huang C-y, and Zhang J-x. 2019. Protective roles of trehalose
670 in *Pleurotus pulmonarius* during heat stress response. *Journal of Integrative Agriculture* 18:428-
671 437. 10.1016/S2095-3119(18)62010-6:

672 McCotter SW, Horianopoulos LC, and Kronstad JW. 2016. Regulation of the fungal secretome. *Current*
673 *genetics* 62:533-545. 10.1007/s00294-016-0578-2:

674 McNeil JC, and Palazzi DL. 2012. Ustilago as a Cause of Fungal Peritonitis: Case Report and Review of
675 the Literature. *Journal of the Pediatric Infectious Diseases Society* 1:337-339.
676 10.1093/jpids/pis043:

677 Miteva-Staleva JG, Krumova ET, Vassilev SV, and Angelova MB. 2017. Cold-stress response during the
678 stationary-growth phase of Antarctic and temperate-climate *Penicillium* strains. *Microbiology*
679 163:1042-1051. 10.1099/mic.0.000486:

680 Montero-Barrientos M, Hermosa R, Cardoza RE, Gutiérrez S, Nicolás C, and Monte E. 2010. Transgenic
681 expression of the *Trichoderma harzianum* hsp70 gene increases Arabidopsis resistance to heat
682 and other abiotic stresses. *Journal of Plant Physiology* 167:659-665.
683 10.1016/j.jplph.2009.11.012:

684 Montero-Barrientos M, Hermosa R, Nicolás C, Cardoza RE, Gutiérrez S, and Monte E. 2008.
685 Overexpression of a *Trichoderma* HSP70 gene increases fungal resistance to heat and other
686 abiotic stresses. *Fungal Genetics and Biology* 45:1506-1513. 10.1016/j.fgb.2008.09.003:

687 Mühlhofer M, Berchtold E, Stratil CG, Csaba G, Kunold E, Bach NC, Sieber SA, Haslbeck M, Zimmer
688 R, and Buchner J. 2019. The Heat Shock Response in Yeast Maintains Protein Homeostasis by
689 Chaperoning and Replenishing Proteins. *Cell Reports* 29:4593-4607.e4598.
690 10.1016/j.celrep.2019.11.109:

691 Nathan DF, Vos MH, and Lindquist S. 1997. In vivo functions of the *Saccharomyces cerevisiae*
692 Hsp90 chaperone. *Proceedings of the National Academy of Sciences* 94:12949.
693 10.1073/pnas.94.24.12949:

694 Nwaka S, and Holzer H. 1997. Molecular Biology of Trehalose and the Trehalases in the Yeast
695 *Saccharomyces cerevisiae*. In: Moldave K, ed. *Progress in Nucleic Acid Research and Molecular*
696 *Biology*: Academic Press, 197-237.

697 O' Neill JS, Hoyle NP, Robertson JB, Edgar RS, Beale AD, Peak-Chew SY, Day J, Costa ASH, Frezza C,
698 and Causton HC. 2020. Eukaryotic cell biology is temporally coordinated to support the energetic
699 demands of protein homeostasis. *Nature Communications* 11:4706. 10.1038/s41467-020-18330-
700 x:

701 Overland J, Francis JA, Hall R, Hanna E, Kim S-J, and Vihma T. 2015. The melting Arctic and
702 midlatitude weather patterns: are they connected? *Journal of Climate* 28:7917-7932.
703 10.1175/JCLI-D-14-00822.1:

704 Patriarca EJ, and Maresca B. 1990. Acquired thermotolerance following heat shock protein synthesis
705 prevents impairment of mitochondrial ATPase activity at elevated temperatures in
706 *Saccharomyces cerevisiae*. *Experimental Cell Research* 190:57-64. 10.1016/0014-
707 4827(90)90143-X:

708 Perez-Nadales E, Almeida Nogueira MF, Baldin C, Castanheira S, El Ghalid M, Grund E, Lengeler K,
709 Marchegiani E, Mehrotra PV, Moretti M, Naik V, Osés-Ruiz M, Oskarsson T, Schäfer K,
710 Wasserstrom L, Brakhage AA, Gow NAR, Kahmann R, Lebrun M-H, Perez-Martin J, Di Pietro
711 A, Talbot NJ, Toquin V, Walther A, and Wendland J. 2014. Fungal model systems and the
712 elucidation of pathogenicity determinants. *Fungal Genetics and Biology* 70:42-67.
713 10.1016/j.fgb.2014.06.011:

714 Piper PW. 1993. Molecular events associated with acquisition of heat tolerance by the yeast
715 *Saccharomyces cerevisiae*. *FEMS Microbiology Reviews* 11:339-355.

716 Raggam RB, Salzer HJ, Marth E, Heiling B, Paulitsch AH, and Buzina W. 2011. Molecular detection and
717 characterisation of fungal heat shock protein 60. *Mycoses* 54:e394-399. 10.1111/j.1439-
718 0507.2010.01933.x:

719 Rangel DEN, Finlay RD, Hallsworth JE, Dadachova E, and Gadd GM. 2018. Fungal strategies for dealing
720 with environment- and agriculture-induced stresses. *Fungal Biology*.
721 10.1016/j.funbio.2018.02.002:

722 Rao IM, Beebe SE, Polania J, Grajales M, Cajiao C, Ricaurte J, García R, and Rivera M. 2016a.
723 Evidence for genotypic differences among elite lines of common bean in the ability to remobilize
724 photosynthate to increase yield under drought. *The Journal of Agricultural Science* 155:857-875.
725 10.1017/S0021859616000915:

726 Rao IM, Miles JW, Beebe SE, and Horst WJ. 2016b. Root adaptations to soils with low fertility and
727 aluminium toxicity. *Annals of Botany* 118:593-605. 10.1093/aob/mcw073:

728 Reed CJ, Lewis H, Trejo E, Winston V, and Evilia C. 2013. Protein adaptations in archaeal
729 extremophiles. *Archaea* 2013:373275. 10.1155/2013/373275:

730 Richter K, Haslbeck M, and Buchner J. 2010. The Heat Shock Response: Life on the Verge of Death.
731 *Molecular Cell* 40:253-266. 10.1016/j.molcel.2010.10.006:

732 Salmeron-Santiago KG, Pardo JP, Flores-Herrera O, Mendoza-Hernandez G, Miranda-Arango M, and
733 Guerra-Sanchez G. 2011. Response to osmotic stress and temperature of the fungus *Ustilago*
734 *maydis*. *Archives in Microbiology* 193:701-709. 10.1007/s00203-011-0706-9:

735 Sarmiento F, Peralta R, and Blamey JM. 2015. Cold and Hot Extremozymes: Industrial Relevance and
736 Current Trends. *Frontiers in Bioengineering and Biotechnology* 3:148.
737 10.3389/fbioe.2015.00148:

- 738 Schulte PM. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic
739 understanding of the responses of ectotherms to a changing environment. *The Journal of*
740 *Experimental Biology* 218:1856. 10.1242/jeb.118851:
- 741 Shaner L, Gibney PA, and Morano KA. 2008. The Hsp110 protein chaperone Sse1 is required for yeast
742 cell wall integrity and morphogenesis. *Current genetics* 54:1-11. 10.1007/s00294-008-0193-y:
- 743 Simova-Stoilova L, Vassileva V, and Feller U. 2016. Selection and Breeding of Suitable Crop Genotypes
744 for Drought and Heat Periods in a Changing Climate: Which Morphological and Physiological
745 Properties Should Be Considered? *Agriculture-Basel* 6:19. 10.3390/agriculture6020026:
- 746 Su Y, Jiang X, Wu W, Wang M, Hamid MI, Xiang M, and Liu X. 2016. Genomic, Transcriptomic, and
747 Proteomic Analysis Provide Insights Into the Cold Adaptation Mechanism of the Obligate
748 Psychrophilic Fungus *Mrakia psychrophila*. *G3: Genes|Genomes|Genetics* 6:3603-3613.
749 10.1534/g3.116.033308:
- 750 Suarez RK, and Moyes CD. 2012. Metabolism in the age of ‘omes’. *The Journal of Experimental Biology*
751 215:2351. 10.1242/jeb.059725:
- 752 Swaminathan J, Boulgakov AA, Hernandez ET, Bardo AM, Bachman JL, Marotta J, Johnson AM,
753 Anslyn EV, and Marcotte EM. 2018. Highly parallel single-molecule identification of proteins in
754 zeptomole-scale mixtures. *Nature Biotechnology* 36:1076-1082. 10.1038/nbt.4278:
- 755 Tajuddin N, Rizman-Idid M, Convey P, and Alias Siti A. 2018. Thermal adaptation in a marine-derived
756 tropical strain of *Fusarium equiseti* and polar strains of *Pseudogymnoascus* spp. under different
757 nutrient sources. In: *Botanica Marina*. p 9.
- 758 Teo LH, and Tay YK. 2006. *Ustilago* species infection in humans. *British Journal of Dermatology*
759 155:1096-1097. 10.1111/j.1365-2133.2006.07515.x:
- 760 Tereshina VM. 2005. Thermotolerance in Fungi: The Role of Heat Shock Proteins and Trehalose.
761 *Microbiology* 74:247-257. 10.1007/s11021-005-0059-y:
- 762 Tesei D, Marzban G, Marchetti-Deschmann M, Tafer H, Arcalis E, and Sterflinger K. 2015. Proteome of
763 tolerance fine-tuning in the human pathogen black yeast *Exophiala dermatitidis*. *Journal of*
764 *Proteomics* 128:39-57. 10.1016/j.jprot.2015.07.007:
- 765 Tesei D, Marzban G, Zakharova K, Isola D, Selbmann L, and Sterflinger K. 2012. Alteration of protein
766 patterns in black rock inhabiting fungi as a response to different temperatures. *Fungal Biology*
767 116:932-940. 10.1016/j.funbio.2012.06.004:
- 768 Timp W, and Timp G. 2020. Beyond mass spectrometry, the next step in proteomics. *Science Advances*
769 6:eaax8978. 10.1126/sciadv.aax8978:
- 770 Tiwari S, Thakur R, and Shankar J. 2015. Role of Heat-Shock Proteins in Cellular Function and in the
771 Biology of Fungi. *Biotechnology Research International* 2015.
- 772 Truman AW, Millson SH, Nuttall JM, Mollapour M, Prodromou C, and Piper PW. 2007. In the Yeast
773 Heat Shock Response, Hsf1-Directed Induction of Hsp90 Facilitates the Activation of the Slt2
774 (Mpk1) Mitogen-Activated Protein Kinase Required for Cell Integrity. *Eukaryotic Cell* 6:744.
775 10.1128/EC.00009-07:
- 776 Vergés E, Colomina N, Gari E, Gallego C, and Aldea M. 2007. Cyclin Cln3 Is Retained at the ER and
777 Released by the J Chaperone Ydj1 in Late G1 to Trigger Cell Cycle Entry. *Molecular Cell*
778 26:649-662. 10.1016/j.molcel.2007.04.023:
- 779 Verghese J, Abrams J, Wang Y, and Morano KA. 2012. Biology of the Heat Shock Response and Protein
780 Chaperones: Budding Yeast as a Model System. *Microbiology and Molecular Biology Reviews*
781 76:115. 10.1128/MMBR.05018-11:

- Williams TJ, Lauro FM, Ertan H, Burg DW, Poljak A, Raftery MJ, and Cavicchioli R. 2011. Defining the response of a microorganism to temperatures that span its complete growth temperature range (-2 degrees C to 28 degrees C) using multiplex quantitative proteomics. *Environmental Microbiology* 13:2186-2203. 10.1111/j.1462-2920.2011.02467.x:
- Winkler A, Arkind C, Mattison CP, Burkholder A, Knoche K, and Ota I. 2002. Heat Stress Activates the Yeast High-Osmolarity Glycerol Mitogen-Activated Protein Kinase Pathway, and Protein Tyrosine Phosphatases Are Essential under Heat Stress. *Eukaryotic Cell* 1:163. 10.1128/EC.1.2.163-173.2002:
- Xiong Y, Wu VW, Lubbe A, Qin L, Deng S, Kennedy M, Bauer D, Singan VR, Barry K, Northen TR, Grigoriev IV, and Glass NL. 2017. A fungal transcription factor essential for starch degradation affects integration of carbon and nitrogen metabolism. *PLoS genetics* 13:e1006737-e1006737. 10.1371/journal.pgen.1006737:
- Yan Z, Zhao M, Wu X, and Zhang J. 2020. Metabolic Response of *Pleurotus ostreatus* to Continuous Heat Stress. *Frontiers in Microbiology* 10:3148-3148. 10.3389/fmicb.2019.03148:
- Zakharova K, Sterflinger K, Razzazi-Fazeli E, Noebauer K, and Marzban G. 2014. Global proteomics of the extremophile black fungus *Cryomyces antarcticus* using 2D-Electrophoresis. *Natural Science* 2014.
- Zhang F, Chen X, and Vitousek P. 2013. An experiment for the world. *Nature* 497:33. 10.1038/497033a:
- Zhang X, Ren A, Li M-J, Cao P-F, Chen T-X, Zhang G, Shi L, Jiang A-L, and Zhao M-W. 2016. Heat Stress Modulates Mycelium Growth, Heat Shock Protein Expression, Ganoderic Acid Biosynthesis, and Hyphal Branching of *Ganoderma lucidum* via Cytosolic Ca²⁺. *Applied and Environmental Microbiology* 82:4112. 10.1128/AEM.01036-16:

Table 1(on next page)

Summary of fungal proteomic profiling analysis when exposed to high temperature stress (work published from 2008-2020).

T_{exp} – Temperature of exposure, T_{opt} – Temperature of optimal growth

Proteomic profiling approaches.

⁽¹⁾ – Gel- free methods

⁽²⁾ – Gel-based method

* – Non-conclusive remarks (no proteins identification involved)

1 Table 1: Summary of fungal proteomic profiling analysis when exposed to high temperature stress (work published from 2008-2020)

2

T_{exp} (°C)	T_{opt} (°C)	Time	Species	# no. of proteins		Proteins identified	Classification/Pathways involved	Refs.
20°C	12°C	1 month	<i>Mrakia psychrophila</i> ⁽¹⁾	1673	Down	Citrate synthase, DLST, LSC2 MTCP1 CLD1	TCA cycle Oxidative phosphorylation Glycerophospholipid metabolism	(Su et al. 2016)
					Up	DNAJ MPSY4181, MPSY2821	Heat shock proteins MFS transporters	
28°C	15°C	7 days	<i>Friedmanniomyces endolithicus</i> ⁽²⁾	284	Down	Reduced no. of protein spots (141)	*Reduction in total number of protein spots, indicating a lack of a heat shock response	(Tesei et al. 2012)
37°C	25°C	1 day	<i>Penicillium marneffei</i> ⁽²⁾	270	Up	Hsp 30, Hsp 70, antigenic mitochondrial protein HSP60 hypothetical protein (activator of HSP90 ATPase)	Heat shock proteins	(Chandler et al. 2008)
						succinyl-CoA synthetase alpha subunit, beta subunit of ATP synthase, NAD- dependent formate dehydrogenase adenylate kinase, phosphoglycerate kinase Ran GTPase spi1 Cdc48p UDP-N-acetylglucosamine pyrophosphorylase	Energy production and metabolism Regulate mitotic activities Cell cycle and division Synthesis of N- acetylglucosamine (monomeric component of cell-wall chitin)	
37°C	28°C	1.5 days	<i>Aspergillus flavus</i> ⁽¹⁾	3886	Down	2-heptaprenyl-1,4- naphthoquinone methyltransferase,	Translation and biosynthetic pathways	(Bai et al. 2015)

						esterase/lipase, predicted O-methyltransferase, aminotransferase GliI-like, and aflE		
37°C	28°C	2 hours	<i>Ustilago maydis</i> ⁽²⁾	-	Up	Enolase, Phosphoglycerate kinase Mitochondrial HSP 70, HSP 70, HSP 60 β-succinyl CoA synthetase Glutathione S-transferase V/A- ATPase-A Oxidoreductase (AKR's)	Carbohydrate metabolism Protein folding Amino acid metabolism Redox regulation Ion homeostasis Other	(Salmeron-Santiago et al. 2011)
40°C	28°C	7 days	<i>Exophiala jeanselmei</i>	174	Down	Reduced no. of protein spots (208)	*Spots from the same pI (5-7) and molecular weight range (30-90 kDa) were extinct after heat stress, suggesting that both probably downregulated similar sets of proteins.	(Tesei et al. 2012)
			<i>Coniosporium perforans</i> ⁽²⁾	255	Down	Reduced no. of protein spots (70)		
			<i>Penicillium chrysogenum</i> ⁽²⁾	601	Up	Increased no. of protein spots (220)		
40°C	28°C	48 hours	<i>Pleurotus ostreatus</i> ⁽¹⁾	61	Up	Calmodulin 2-Methylcitrate synthase mitochondrial, Type 1 phosphatases regulator YPI1 Putative aryl-alcohol dehydrogenase AAD6, Phenylalanine ammonia-lyase (fragment), Peptidyl-prolyl cis-trans isomerase ATP-dependent RNA helicase ded1, Elongation factor 1-alpha, Enolase 2, Fructose-2,6-bisphosphatase HSP 60 mitochondrial, HSP	Developmental process Organelle part Catalytic activity Binding proteins Heat shock proteins	(Zou et al. 2018)

					SSA2, HSP82, hsp98				
					70	Down	Subtilisin-like protease CPC735_066880 ATP-dependent RNA helicase DHH1, Myosin-1, Serine/threonine-protein kinase ppk8, Tubulin beta chain	Catalytic activity Binding proteins	
48°C	30°C	0-4 minute s, Time interval (heat shock)	<i>Aspergillus fumigatus</i> ⁽¹⁾	1886	Up	HSP 70 chaperone HSP88 Tubulin alpha-1 subunit, actin-bundling protein Sac6 Nuclear movement protein NudC Allergen Asp F3 Transketolase TktA, hexokinase Kxk, Adenosylhomocysteinase, Nitrite reductase NiiA Cdc48	Heat shock protein Cell wall and cytoskeleton Transport protein Defence against oxidative and nitrosative stress Carbohydrate and nitrogen metabolism Cell cycle and division	(Albrecht et al. 2010)	
					Down	Mitochondrial co-chaperone GrpE CRAL/TRIO domain protein Cytochrome c oxidase polypeptide vib	Chaperone Transport protein Energy generation		
50°C	30°C	24 hours	<i>Aspergillus niger</i> 3.316 ⁽¹⁾	481	Up	phosphatidylinositol 4- kinase type II subunit alpha, β-galactosidase, carboxypeptidase β-1,6-glucanase, extracellular α-glucosidase rhamnogalacturonate lyase A	Cellular signalling Carbohydrate metabolism Cell wall organisation	(Deng et al. 2020)	

4 Legends and abbrev.
 5 T_{exp} – Temperature of exposure, T_{opt} – Temperature of optimal growth
 6 Proteomic profiling approaches.
 7 ⁽¹⁾ – Gel- free methods
 8 ⁽²⁾ – Gel-based method
 9 * – Non-conclusive remarks (no proteins identification involved)
 10

Table 2 (on next page)

Studies on fungal proteomic profiling in response to low temperature stress (published from 2008-2020).

* - Non-conclusive remarks (no proteins identification involved)

** - 45°C is not the optimal growth temperature for *E. dermatitidis*. However, it is one of the experimental temperature stress (high temperature stress)

1 Table 2: Studies on fungal proteomic profiling in response to low temperature stress (published from 2008-2020)

T_{exp} (°C)	T_{opt} (°C)	Time	Species	# no. of proteins		Proteins identified	Pathways/functions involved	Refs.
1°C	15°C	1 week	<i>F. endolithicus</i>	466	-	Increased no. of protein spots (41)	*Increased in high molecular weight spots (range from 70 to 170 kDa), suggesting production of cold-acclimation proteins	(Tesei et al. 2012)
1°C	28°C	1 week	<i>E. jeanselmei</i>	387	-	Increased no. of protein spots (5)	*Changes in expression patterns (spots with MW of 25 – 100 kDa)	
			<i>C. perforans</i>	494		Increased no. of protein spots (169)	*exhibited high molecular weight spots (range from 70 to 170 kDa), suggesting	
			<i>P. chrysogenum</i>	358		Decreased no. of protein spots (23)	*production of cold-acclimation proteins slight decreased indicates downregulation of metabolic activity	
1°C	37°C	1 week	<i>E. dermatitidis</i>	1700	Up	14-3-3 family proteins Minor allergen Alt a7 Nucleoside diphosphate kinase Glyceraldehyde-3-phosphate dehydrogenase	Signalling proteins Small allergen molecules ATP production pathway Glycolytic pathway	(Tesei et al. 2015)
					Down	Acetyl-coenzyme A synthetase Alcohol oxidase Aldehyde dehydrogenase Phosphoenol pyruvate carboxykinase [ATP] Malate synthase, glyoxysomal	Acetate metabolism Alcohol metabolism Aldehyde metabolism Gluconeogenesis Pyruvate metabolism	
1°C	45°C**	1 week	<i>E. dermatitidis</i>	1700	Up	14-3-3 family proteins Acetyl-coenzyme A synthetase Minor allergen Alt a7 Nucleoside diphosphate kinase Glyceraldehyde-3-phosphate dehydrogenase Hsp30	Signalling proteins Acetate metabolism Small allergen molecules ATP production pathway Glycolytic pathway Heat shock proteins	
					Down	Alcohol oxidase	Alcohol metabolism	

						Aldehyde dehydrogenase Phosphoenol pyruvate carboxykinase [ATP] Beta-lactamase Hsp70-like protein	Aldehyde metabolism Gluconeogenesis Antibiotic resistance proteins Heat shock proteins	
4°C	12°C	NA	<i>M. psychrophila</i>	1673	Up	GLNA MPSY protein	Amino acid metabolism MFS transporter	(Su et al. 2016)
					Down	citrate synthase and LSC2 proteins PDC	TCA cycle Glycolysis	
12-15°C	23-25°C	2 weeks	<i>Flammulina velutipes</i>	1198	Up	Trehalase (Fragment) Proteasome subunit β type	Trehalose metabolism Proteosome pathway	(Liu et al. 2017)
					Down	60s ribosomal protein Acetylmethionine aminotransferase Glutathione-disulfide reductase Adenylosuccinate synthetase 1	Heat shock proteins Urea cycle Oxidative stress pathway Purine biosynthesis	
12-15°C	23-25°C	3 days	<i>Flammulina velutipes</i>	1198	Up	Heat shock cognate 70 Catalase	Heat shock proteins Catalytic enzymes	
					Down	Mitochondrial cytochrome T-complex protein eta subunit (Tc1-eta) Serine hydroxymethyltransferase Transketolase Methylmalonate-semialdehyde dehydrogenase	Electron transport chain Chaperonins Serine metabolism Pentose phosphate pathway Amino acid metabolism	
15°C	30°C	24 hours	<i>Mortierella isabellina</i> <i>M6-22</i>	1800	Up	fructose-bisphosphate aldolase cytochrome c oxidase polypeptide 5B ATP synthase subunit β, ATP synthase δ subunit E3 ubiquitinprotein ligase BRE1, and histone	Glycolytic pathway Electron transport ATP production Ubiquitin–proteasome pathway	(Hu et al. 2016)

acetyltransferase GCN5

- 2 * – Non-conclusive remarks (no proteins identification involved)
- 3 ** - 45°C is not the optimal growth temperature for *E. dermatitidis*. However, it is one of the experimental temperature stress (high
- 4 temperature stress)