Chapter 12
Molecular Basis of Stress-Tolerant Genes in Extreme Microorganisms

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Abstract Diversity of abiotic and biotic stress responses is a great threat to agricultural and industrial development. Genetic modifications are one of the most promising methods for the improvement of economically important plants or microbes. However, most genes harboring in ordinary organism cells play little role in stress-resistant biotechnology. Some species thrive in extreme environments, such as the hypersaline marine, the cold Antarctic continent, dry desert, or hot springs, where general eukaryotes such as plants or yeasts can hardly survive, revealing that extreme environmental organisms are promising genetic resources for biotechnology. Extremophilic fungi are excellent models to provide understanding in resistant mechanisms that allow higher organisms to overcome stress; these fungi present valuable genetic resources for isolation of resistance genes to be applied in genetic engineering and biotechnology. As a model fungus, the unicellular yeast *Saccharomyces cerevisiae* exhibits characteristics responses to a variety of stressors; it has led to the discovery of two significant osmotic-resistance pathways: the high-osmolarity glycerol response (HOG1) pathway and calcineurin-dependent pathway. With the increasing number of fungal species being characterized and sequenced, extremophilic fungi are found to be better systems for the isolation of abiotic stress resistance and related genes. So far, a series of environmental stress-related genes have been investigated in diverse fungi, and no doubt these specific resistance genes could be valuable for the improvement of crop tolerance. Interestingly, several ribosomal proteins recently isolated from the extremophilic fungi have been reported to possess moonlighting functions. Collectively, a tremendous number of tolerant genes cloned from extremophilic fungi appeared to be more resistant to

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abiotic stress than their homologs or orthologs cloned from ordinary fungi, though these proteins are highly conserved and exist in a wide variety of organisms. Taking into account the special characteristics/mechanisms of genes from extremophilic fungi in stress responses, the application of these types of genes might be more valuable and reliable for biotechnology.

Keywords Extremophilic fungi · Abiotic stress · Tolerant genes · Tolerant mechanism · Biotechnology · Calcineurin pathway

12.1   Introduction

From the biological and environmental evolution perspective, fungi are one of the earliest eukaryotes to colonize the ancient earth (Gray and Shear 1992; Horodyski and Knauth 1994). Considering the harsh physical environments on ancient earth, to ensure the chances of survival, fungi might be more tolerant or resistant to adverse environmental factors than the latter appeared plants or other organisms. The level of response to environmental factors differs significantly from organism to organism; however, maintenance of metabolic flux and cellular mechanisms relies upon the organisms' ability to keep their functional states stable when they are under extreme stress. This diversifies the microbial survival at specific niche (Ranawat and Rawat 2017). Within the last few decades, a number of fungal species (halophile, xerophiles, or thermophile) that can live in a variety of extreme environments have been isolated. For example, *Eurotium herbariorum* that can survive in 340 g/L total dissolved salts was isolated from the Dead Sea (Kis-Papo et al. 2001; Yan et al. 2005); *Sarcinomyces petricola* strain A95 (a representative strain of rock-inhabiting fungi) was isolated from a marble rock surface near the Philopappos Monument on Musaios Hill of Athens (Gorbushina 2007); a thermophilic fungus *Thermomyces lanuginosus* isolated is able to survive at 62 °C, the highest growth temperature recorded so far (Singh et al. 2003); and in our laboratory, the fungal strain *T. lanuginosus* MY21 isolated was observed to be capable of growing at 65 °C (Meng and Wei 2019, unpublished). The stress-tolerant molecular mechanism and corresponding stress-tolerant genes in extreme microorganisms are also gradually undeviled.

In plants, great achievements have been made in recent years in understanding the abiotic stress responses and molecular mechanisms (Bhatnagar-Mathur et al. 2008; Gupta and Huang 2014; Zhan et al. 2015), but crop-breeding practice of higher resistant varieties remains unsatisfactory due to the lack of abiotic stress resistance germplasms. Wild germplasms are of interests, as wild crops may have retained genetic information before the domestication and artificial selection of modern plants (Lam et al. 2010). Wild germplasms screening, however, is a daunting and time-consuming task; and most likely we will never find certain wild germplasms again because of their extinction in the modern agricultural practices and environmental changes. Considering the survival abilities in the extreme conditions, extremophilic fungi may provide special or different resistance mechanism

compared to plant. The genetic basis of abiotic stress resistance in extremophilic fungi makes it a unique genetic resource to improve abiotic stress resistance of crops. Here we highlight the abiotic stress resistance mechanisms and resistant genes in extremophilic fungi. In addition, application strategies for genetic engineering are also discussed.

12.2 The Stress-Tolerant Mechanism in Extremophilic Fungi

Fungi like other eukaryotic organisms such as plants depend on signaling-receiving and transmitting systems to respond to, survive, and thrive under the imposed adverse conditions. In eukaryote microorganisms, the yeast *S. cerevisiae* is known to have moderate levels of tolerance. It presents a rather poor performance in the presence of salt, drought, extreme temperature, and other stressors (Prista et al. 2002, 2005; Serrano and Gaxiola 1994). Thus, a wild-type *S. cerevisiae* is not the best model organism, neither for salt tolerance nor for sensitivity to salt. However, *S. cerevisiae* was an excellent tool for genetic manipulation (fast growth rate and easy transformation) and has been extensively applied in field of resistance research. The high-osmolarity glycerol (HOG1) pathway which is an essential stress-signaling module has been broadly studied in fungi: from the yeast *S. cerevisiae* to the filamentous fungus *Trichoderma harzianum* (Brewster et al. 1993; Delgado-Jarana et al. 2006). HOG1 is also found in extremophilic fungi. Nevo group testified that *Eurotium herbariorum* HOG1 is highly similar to the homologs from non-extreme fungi such as *Aspergillus nidulans*, *S. cerevisiae*, and *Schizosaccharomyces pombe* (Yan et al. 2005). However, it appears that HOG1 is found exclusively in fungi and no homolog gene has been detected in plants.

The HOG1 regulation system in yeast involves two pathways, the low-osmolarity SHO1 pathway and high-osmolarity SLN1 pathway. The difference between the two pathways lies at the SHO1 and SLN1 sensors, but both pathways ultimately lead to glycerol biosynthesis and the glycerol concentration for osmotic balance (Brewster et al. 1993). Besides salt, HOG1 also responds to a variety of other stressors (Delgado-Jarana et al. 2006), suggesting cross-talking feature in HOG pathways.

Another stress-responsive system required for salt stress tolerance in yeast is calcineurin; this protein phosphatase complex is dependent on calcium ion and calmodulin (Mendoza et al. 1994; Nakamura et al. 1993). Calcineurin is required for the genes' transcription of sodium and calcium ion ATPases and a cell wall β-1,3-glucan synthase through regulating CRZ1/TCN1, the downstream zinc-finger transcription factor (Matheos et al. 1997; Stathopoulos-Gerontides et al. 1999). The salt-responsive calcineurin-CRZ1 pathway is also involved in yeast stress responses (Juvvadi et al. 2014). When CRZ1 was overexpressed in the industrial baker's yeast HS13 strain, tolerance to both salt and freeze was increased (Panadero et al. 2007).

The calcineurin pathway, unlike HOG1, is highly conserved in eukaryotes from yeast to animals, which can be searched in the public nucleic acid sequence repository (http://www.ncbi.nlm.nih.gov/genbank). In plants, the physiology functions of calcineurin have been clarified (Luan et al. 1993; Allen and Sanders 1995), and the osmotic stress resistance is associated with the increased expression of calcineurin pathway genes. In fungi, the calcineurin homologs PsCNA1/PsCNB1 from the wheat rust disease fungus *Puccinia striiformis* have been studied. Results indicated that the calcineurin signaling pathway participates in stripe rust morphogenetic differentiation, especially the formation of haustoria during the early stage of infection and during the production of urediniospores (Zhang et al. 2012). Interestingly, calcineurin may be a multifunctional enzyme, because it was required for not only drug tolerance but also hyphal growth and virulence in *Candida tropicalis* (Chen et al. 2014). In contrast, relatively little is known about the calcineurin pathway in extremophilic fungi.

12.3   The Molecular Basis of Osmoregulation in Extremophilic Fungi

To remain viable, fungi under extreme conditions must regulate and keep essential cellular processes. The fluidity and components of the plasma membrane play important roles in maintaining the cell membrane physiological functions and the adaption to extreme conditions (Turk et al. 2007; Zhang et al. 2015). Plasma-membrane fluidity has been regarded as a typical indicator of fitness for survival in extreme environments (Turk et al. 2007). Unsaturated fatty acids are key compounds in the plasma membrane, and cellular unsaturated fatty acids constitutions are directly controlled by fatty acid desaturases. In *Pichia pastoris* GS115, cellular fatty acids compositions were changed with the increased or decreased expression of desaturases; in addition, deletions of fatty acid desaturases give rise to increased resistance to adverse environmental stress (Zhang et al. 2015). In Turk's study, all tested fungi showed increased plasma-membrane fluidity in response to increased salt concentrations. However, when salinity exceeded their optimal range, the extremophilic fungi (*Hortaea werneckii*, *Cryptococcus liquefaciens*) showed decreased plasma-membrane fluidity, reflecting the limitation of cell membrane remodeling.

Microorganisms have developed two main strategies for osmotic adjustment: influx of K^+ and accumulation of low molecular weight solutes of some organisms, such as hyper-/thermophiles, which utilize a combination of both strategies by accumulating negatively charged compatible solutes and potassium. This mechanism of osmosis adjustment occurs in extremophilic archaeal families *Halobacteriaceae* and *Haloferacaceae* (Gupta et al. 2015, 2016). But this has not been documented in majority of thermophilic microorganisms.

Another strategy relies on accumulation of compatible solutes or osmolytes, which are small organic compounds used for osmotic adjustment that do not interfere with cell function (Brown 1976; Ventosa et al. 1998). Thermophilic and hyperthermophilic organisms generally accumulate very unusual compatible solutes, namely, di*myo*-inositol phosphate, di-mannosyldi*myo*-inositol phosphate, diglycerol phosphate, mannosylglycerate, and mannosylglyceramide, which have not been identified in bacteria or archaea that grow at low and moderate temperatures (Santos and da Costa 2002).

A large variety of microorganisms, from bacteria to filamentous fungi, rely exclusively on the accumulation of compatible solutes for osmoadaptation. Compatible solutes, such as trehalose, glycine betaine, and α-glutamate, are widespread in microorganisms, while others are restricted to a few organisms. Chloride is accumulated in molar concentrations in the cytoplasm of *Halobacillus halophilus* to cope with salt stress (Saum et al. 2013). Polyols are also widespread compatible solute among fungi and algae but are very rare in bacteria and unknown in archaea. Ectoine and hydroxyectoine are examples of compatible solutes found only in bacteria (Santos and da Costa 2002).

The role of compatible solutes, however, goes beyond osmotic adjustment alone, to the protection of cells and cell components from freezing, desiccation, high temperature, and even UV radiations (Welsh 2000; Santos and da Costa 2002; Beblo-Vranesevic et al. 2016). The protective role of trehalose against several stress conditions has been amply demonstrated (Simola et al. 2000), and the accumulation of glycerol, the canonical osmolyte of yeast, has also been correlated with the acquisition of thermotolerance (Siderius et al. 2000).

Many polyols have been reported to contribute fungi to survive at high-salt concentrations or drought conditions. And among these compatible solutes, glycerol and trehalose have been extensively studied. Glycerol is the major product when extremophilic fungi, such as *Aspergillus glaucus*, grow on glucose-contained medium with high concentrations of NaCl (Liu et al. 2015). In the process of glycerol biosynthesis, key enzymes determine the production of intracellular glycerol and therefore impact on osmotic stress tolerance. Glycerol-3-phosphate dehydrogenase encoded by the gene of GPD1 in *S. cerevisiae* is important for yeast survival under osmotic stress (Albertyn et al. 1994). The yeast glycerol-3-phosphatases gpp1p and gpp2p are also essential for glycerol biosynthesis, but their roles in the cellular responses to osmotic, anaerobic, and oxidative stress are different (Pahlman et al. 2001).

To conquer high osmotic stress by biosynthesis of glycerol is inefficient and uneconomical. The active retention and uptake of glycerol are necessary when fungi are at high osmotic environments. Aquaglyceroporins (AQGPs; GlpFs in yeast) transport glycerol along with water, and other uncharged solutes are involved in osmoregulation in myriad species. The two genes encoding AQGPs in the yeast genome, Fps1 (Tamás et al. 1999, 2009; Oliveira et al. 2003) and Yfl054 (Oliveira et al. 2003; Hohmann et al. 2000), are functional glycerol facilitators. Fps1plays a key role in yeast osmoregulation by regulating intracellular glycerol levels during changes in external osmolarity (Luyten et al. 1995; Hohmann et al. 2007;

Ahmadpour et al. 2014), whereas the cellular function of Yfl054 remains uncertain (Oliveira et al. 2003). Recently, the AQGPs of arbuscular mycorrhizal fungus have received a lot of attention. The aquaglyceroporin GintAQPF2 from *Glomus intraradices*, a member of the γ subgroup (Xu et al., 2013), showed high activity when exposed to polyethylene glycol and high capacity to transport water, which is crucial for transformed yeast cells to survive osmotic stress (Li et al. 2013). In the halophilic fungus *Aspergillus glaucus*, the aquaglyceroporin gene *AgGlpF* has been demonstrated to be a water/glycerol channel (Liu et al. 2015). Interestingly, *AgGlpF* functions not only in *S. cerevisiae* and *Neurospora crassa* but also in model plants. When *AgGlpF* was expressed in *Arabidopsis thaliana*, the transgenic lines survived under high osmotic pressure and particularly under drought stress.

Another metabolite associated with osmoregulation is trehalose, the highly stable disaccharide commonly found in nature. Trehalose has multiple functions (Elbein et al. 2003), and is well-known for osmoprotection where correlations between accumulation of trehalose and high resistance to various stresses have been observed (Crowe et al. 1992). However, an unbiased study carried out by Petitjean et al. (2015) casted doubt on this long time-believed that trehalose is an osmoprotectant. By combining the use of mutant strains expressing catalytically inactive variants of Tps1, MAL$^+$ yeast strains were able to accumulate trehalose from an exogenous supply, and the authors found that the stress-protecting role of trehalose in the yeast was largely overestimated: trehalose actually was unable to protect yeast cells from dying; on the contrary, it is the Tps1 protein, the key enzyme for synthesis of trehalose, that played essential roles for yeast survival in response to temperature, oxidative, and desiccation stress (Petitjean et al. 2015).

In thermophiles, thermophilic lipids should be considered as lipids that normally function at high temperature and are termed as "thermophilic lipids" (Koga 2012). The other important factors that help bacterial membrane to withstand high temperature stress are hopanoids. In *Methylobacterium extorquens*, hopanoids interact with glycolipids in bacterial outer membranes to form a highly ordered bilayer in a manner analogous to the interaction of sterols with sphingolipids in eukaryotic plasma membranes; additionally, multidrug transport is impaired in a hopanoid-deficient mutant of the Gram-negative, which introduces a link between membrane order and an energy-dependent, membrane-associated function in prokaryotes (Sáenz et al. 2015). In *Bacillus acidocaldarius*, the production of hopanoids was increased sharply with temperature increase from 60 to 65 °C, therefore suggesting hopanoids play a role in counteracting the increased fluidity of cell membrane (Schaechter 2016). Though we do not know the concrete molecular mechanism of thermophilic lipid metabolic regulation, the research advances may indicate osmo-regulation is not limited to polyols only. To uncover osmoregulation mechanisms, more polyols and moonlighting proteins must be further investigated.

12.4   Stress-Responsive Genes in Extremophilic Fungi

Hyperthermophilic microorganisms thrive in volcano rent water or hot springs. Chaperones play crucial roles in hyperthermophilic life. The chaperones accumulated in these hyperthermophilic microorganisms illustrate that chaperones are important for organism survival and organism must spend a substantial proportion of its metabolic energy for the folding and refolding of its proteins. In hyperthermophiles, *Thermococcus kodakarensis*, *Thermus thermophilus*, and *Sulfolobus* sp. are typical species. The thermotolerance and induction of heat-shock proteins make hyperthermophiles to adapt to hostile environmental conditions (Trent et al. 1997; Cava et al. 2009).

A predominant 55 kDa protein (TF55) was rapidly expressed, while *S. shibatae* B12 cells exposed to 88 °C environment (Trent et al. 1990). TF 55 belongs to the chaperones containing tailless-complex polypeptide family of chaperonins. The HSP60 molecular chaperonins in response to heat shocks, termed as CpkA and CpkB, have been intensively studied in *T. kodakarensis* (Fujiwara et al. 2008). The two genes were expressed in different patterns at different temperatures; but all the results supported the essential role of CpkA and CpkBin cell growth at low and high temperatures. HSP60 proteins have also been identified in methanogens also (Ambily Nath and Loka Bhararthi 2011). Transcriptional analysis of the hyperthermophilic archaea on *Sulfolobus solfataricus* revealed 26 vapBC of family TA (toxin-antitoxin) loci in its genome on raising the growth temperature from 80 to 90 °C (Cooper et al. 2009).

Cold-shock responses have been documented in almost all unicellular organisms from thermophiles such as *Thermus thermophilus* (Mega et al. 2010) and *Thermus* sp. GH5 (Yousefi-Nejad et al. 2011) to mesophiles such as *Caulobacter crescentus* (Balhesteros et al. 2010) to psychrophiles such as *Pseudoalteromonas haloplanktis* (Piette et al. 2012) and *Psychromonas sarctica* (Jung et al. 2010).

The aerobic thermophilic bacterium, *Thermus* sp. GH5, usually lives at temperatures between 70 and 75 °C. Under different cold-shock conditions, several proteins, involved in the degradation of carbon and synthesis of amino acids and nucleotides, were upregulated (Yousefi-Nejad et al. 2011), and accumulation of transaldolase and ribose 5-phosphate isomerase in late cold shock (Minic 2015).

The cold-shock protein of *Thermoanaerobacter tengcongensis* MB4, TteCspC, is involved in the survival of the organism at 50–80 °C. It is a common phenomenon that a single *csp* gene presents among most thermophilic anaerobes. In *T. tengcongensis*, there are more other cold-shock response genes, including DNA replication, recombination, and repair; transcriptional and translational regulation of genes plays effective roles in diverse processes. A number of novel low-temperature specific genes were also identified. Interestingly, the cold-shock response proteins, including TteCspC, function as molecular chaperones (Liu et al. 2014a).

In *Bacillus stearothermophilus* TLS33, eight cold-shock-induced proteins were characterized, but all these proteins have markedly different expression patterns. Interestingly, six of these cold-shock-induced proteins were correlated with the

sigma B protein which plays an important role in signal transduction pathway of bacterial sporulation, suggesting the functional adaptation of this bacterium to environmental cold-shock stress (Topanurak et al. 2005).

As a salt-loving fungus, *Debaryomyces hansenii* has been extensively investigated in recent years. It is able to accumulate high concentrations of sodium without being any damages and also grow well under additional stress factors such as high temperature and extreme pH in the presence of 0.25 M NaCl (Almagro et al. 2000). Through screening *S. cerevisiae* transformants that contain the genomic library prepared from *D. hansenii* (Prista et al. 2002), a series of genes associated with salt tolerance were characterized. The *DhGZF3* gene, which encodes a GATA transcription factor homolog to Dal80 and Gzf3 in *S. cerevisiae*, has been functionally analyzed in *D. hansenii*. In *S. cerevisiae* expression system, the *DhGZF3* gene plays a role in a negative transcription factor (García-Salcedo et al. 2006). Using the cDNA library from the stress-tolerant *Rhodotorula mucilaginosa*, more than 100 *S. cerevisiae* transformants that are tolerant to concentrations of various osmolytes have been screened by Gostinčar and Turk (2012). Among the sequenced clones, 12 genes mediated increased stress tolerance that were upregulated in the *R. mucilaginosa*. Recently, from the *D. hansenii* genome database, Pereira et al. (2014) analyzed nine candidates of polyol/H(+) symporters by heterologous expression in *S. cerevisiae*. Five distinct polyol/H(+) symporters were confirmed, among which two symporters were tested to be specific for uncommon substrates as galactitol and D-(+)-chiro-inositol.

The abiotic stress resistance genes isolated from extremophilic filamentous fungi appear to be more resistant than homologs from ordinary fungi; however, in extremophilic filamentous fungi, the stress tolerance genes are relatively few. *EhHOG*, as mentioned above, is the *E. herbariorum* MAPK kinase gene similar to HOG1 homologs from *A. nidulans*, *S. cerevisiae*, *Schizosaccharomyces pombe*, and most other fungi; but hog1 mutant complemented with EhHOG outperformed the wild type under high salt and freezing-thawing conditions (Yan et al. 2005), indicating the higher genetic fitness of EhHOG in comparison with the corresponding HOG from *S. cerevisiae*. Some genes isolated from the halophilic fungus *A. glaucus* were also found to be more resistant to osmotic stress than the common fungi such as *S. cerevisiae* and *Magnaporthe oryzae*. A yeast expression library containing full-length cDNAs of *A. glaucus* was constructed and used to screen salt resistance transformants in our lab. The ribosomal protein L44 (RPL44), one of the proteins of the large ribosomal subunit 60S, was obtained according to its association with salt resistance. In comparison with the homologous sequence from *M. oryzae*, MoRPL44 in a yeast expression system, the results indicated that yeast cells with overexpressed *AgRPL44* were more resistant to salt, drought, and heavy metals than yeast cells expressing *MoRPL44* at a similar level of stress. In addition, when *AgRPL44* was introduced into *M. oryzae*, the transformants also displayed significantly enhanced tolerance to salt and drought, indicating the unique osmosis resistance ability from the halophilic fungus. Similar results were also obtained in the studies of another ribosomal protein subunit of AgRPS3aE (Liang et al. 2015), the AgglpF (Liu et al. 2015), a 60S protease subunit, and 14 other unknown or predicted

genes including the cell wall-degrading enzymes such as chitinase, cellulase, and glucanase. The common features of all these genes are highly conserved, at least not specific to extremophilic fungi, but they obviously support transgenic cells or organisms surviving under stress conditions, suggesting special mechanisms to be uncovered in future and potential values for genetic engineering.

12.5 Transgenic Application and Concluding Remarks

Plants can benefit from foreign genes, such as CtHSR1 from the halophytic yeast *Candida tropicalis*, transferring the ability to adapt to adverse environments (Martínez et al. 2015). Many genes from diverse fungi have been successfully transferred into plants. *T. harzianum* is commonly used as a mycoparasite fungus for agriculture biological control. On the other hand, *T. harzianum* has moderate levels of tolerance to stressors; it is regarded as an active agent with abilities to induce resistance to abiotic stress in plants and to promote plant growth (Dana et al. 2006; Shoresh et al. 2010). *T. harzianum* provides an excellent genetic pool for cloning multiresistance genes. For example, ThHog1 (Delgado-Jarana et al. 2006), HSP70 (Montero-Barrientos et al. 2008, 2010), and Thkel1 (Hermosa et al. 2011) were successively characterized to be the genes responsible for resistance to salt or other stressors. Interestingly, some genes generally associated with cell wall degradation were shown to be associated with stress tolerance, when they were transferred into plants (Nicolás et al. 2014). This is not dissimilar to the ribosomal protein subunits RPL44 and RPS3aE described above. Considering the moderate levels of tolerance in *T. harzianum*, the homologous genes from extremophilic fungi could be even more resistant. Therefore, it is important and necessary to identify and characterize more genes related to stress resistance regardless of their origin and novelty.

Crops are often exposed to multiple stresses. One gene with multiple actions such as *Trichoderma* hsp70 is no doubt efficient and economic. Transgenic *Arabidopsis* containing hsp70 showed an enhanced tolerance to oxidative, osmotic, and salt stresses (Montero-Barrientos et al. 2010). The highly conserved ribosomal protein subunits like RPL44 and RPS3aE are also promising candidates for creating tolerance-enhanced crops without consideration of their biosafety (Liu et al. 2014b; Liang et al. 2015). These genes are generally in the downstream of resistant pathway and likely to have direct contribution to stress tolerance. Therefore, other physiological traits in transgenic plants may not be seriously affected, even if all these genes are overexpressed.

To a transformed gene, low levels of expression may have no anticipated function; however, high levels of expression probably affect the bioassay or productivity of the plant. Spatiotemporal expression of specific and controllable genes is in need. Inducible promoters are available in stress resistance genetic engineering. In plants, there exist many stress-responsive genes, particularly in sensitive plants (Dey et al. 2015). The resistant gene or genes from an extremophilic fungus driven by a plant-inducible promoter constitute the so-called two-component sensor systems

(de Wit 1992). This strategy will solve the problem of excess cellular materials and energy (ATP) consumption.

References

Ahmadpour D, Geijer C, Tamás MJ, Lindkvist-Petersson K, Hohmann S (2014) Yeast reveals unexpected roles and regulatory features of aquaporins and aquaglyceroporins. Biochim Biophys Acta 1840:1482–1491

Albertyn J, Hohmann S, Thevelein JM, Prior BA (1994) GPD1, which encodes glycerol 3-phosphate dehydrogenase, is essential for growth under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high osmolarity glycerol response pathway. Mol Cell Biol 14:4135–4144

Allen GJ, Sanders D (1995) Calcineurin, a type 2B protein phosphatase, modulates the calcium-permeable slow vacuolar ion channel of stomatal guard cells. Plant Cell 7:1473–1483

Almagro A, Prista C, Castro S, Quintas C, Madeira-Lopes A, Ramos J, Loureiro-Dias MC (2000) Effects of salts on *Debaryomyces hansenii* and *Saccharomyces cerevisiae* under stress conditions. Int J Food Microbiol 56:191–197

Ambily Nath IV, Loka Bhararthi PA (2011) Diversity in transcripts and translational pattern of stress proteins in marine extremophiles. Extremophiles 15(2):129–153

Balhesteros H, Mazzon RR, da Silva CAPT, Lang EAS, Marques MV (2010) CspC and CspD are essential for *Caulobacter crescentus* stationary phase survival. Arch Microbiol 192:747–758

Beblo-Vranesevic K, Koschnitzki D, Rettberg P (2016) Ignicoccus hospitalis: a polyextremophilic microorganism with unusual high radiation tolerance. DGDR 2016

Bhatnagar-Mathur P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep 27(3):411–424

Brewster JL, de Valoir T, Dwyer ND, Winter E, Gustin MC (1993) An osmosensing signal transduction pathway in yeast. Science 259:1760–1763

Brown AD (1976) Microbial water stress. Bacteriol Rev 40:803–846

Cava F, Hidalgo A, Berenguer J (2009) *Thermus thermophilus* as biological model. Extremophiles 13:213–231

Chen YL, Yu SJ, Huang HY, Chang YL, Lehman VN, Silao FG, Bigol UG, Bungay AA, Averette A, Heitman J (2014) Calcineurin controls hyphal growth, virulence, and drug tolerance of Candida tropicalis. Eukaryot Cell 13(7):844–854. https://doi.org/10.1128/EC.00302-13

Cooper CR, Daugherty AJ, Tachdjian S, Blum PH, Kelly RM (2009) Role of vapBC toxin antitoxin loci in the thermal stress response of *Sulfolobus solfataricus*. Biochem Soc Trans 37:123–126

Crowe JH, Hoekstra FA, Crowe LM (1992) Anhydrobiosis. Annu Rev Physiol 54:579–599

Dana MM, Pintor-Toro JA, Cubero B (2006) Transgenic tobacco plants overexpressing chitinases of fungal origin show enhanced resistance to biotic and abiotic stress agents. Plant Physiol 142: 722–730

de Wit PJ (1992) Molecular characterization of gene-for-gene systems in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. Annu Rev Phytopathol 30: 391–418

Delgado-Jarana J, Sousa S, González F, Rey M, Llobell A (2006) ThHog1 controls the hyperosmotic stress response in Trichoderma harzianum. Microbiology 152(Pt 6):1687–1700

Dey N, Sarkar S, Acharya S, Maiti IB (2015) Synthetic promoters in planta. Planta 242(5): 1077–1094. https://doi.org/10.1007/s00425-015-2377-2

Elbein AD, Pan YT, Pastuszak I, Carroll D (2003) New insights on trehalose: a multifunctional molecule. Glycobiology 13:17R–27R

Fujiwara S, Aki R, Yoshida M, Higashibata H, Imanaka T, Fukuda W (2008) Expression profiles and physiological roles of two types of molecular chaperonins from the hyperthermophilic archaeon *Thermococcus kodakarensis*. Appl Environ Microbiol 74(23):7306–7312

García-Salcedo R, Casamayor A, Ruiz A, González A, Prista C, Loureiro-Dias MC, Ramos J, Ariño J (2006) Heterologous expression implicates a GATA factor in regulation of nitrogen metabolic genes and ion homeostasis in the halotolerant yeast *Debaryomyces hansenii*. Eukaryot Cell 5(8): 1388–1398

Gorbushina AA (2007) Life on the rocks. Environ Microbiol 9(7):1613–1631

Gostinčar C, Turk M (2012) Extremotolerant fungi as genetic resources for biotechnology. Bioengineered 3(5):293–297. https://doi.org/10.4161/bioe.20713

Gray J, Shear W (1992) Early life on land. Am Sci 80:444–456

Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genomics 2014:701596. https://doi.org/10.1155/2014/701596

Gupta RS, Naushad S, Baker S (2015) Phylogenomic analyses and molecular signatures for the class Halobacteria and its two major clades: a proposal for division of the class Halobacteria into an emended order Halobacteriales and two new orders, Haloferacales ord. nov. and Natrialbales ord. nov., containing the novel families Haloferacaceae fam. nov. and Natrialbaceae fam. nov. Int J Syst Evol Microbiol 65(Pt 3):1050–1069. https://doi.org/10.1099/ijs.0.070136-0

Gupta RS, Naushad S, Fabros R, Adeolu M (2016) A phylogenomic reappraisal of family-level divisions within the class Halobacteria: proposal to divide the order Halobacteriales into the families Halobacteriaceae, Haloarculaceae fam. nov., and Halococcaceae fam. nov., and the order Haloferacales into the families, Haloferacaceae and Halorubraceae fam nov. Antonie Van Leeuwenhoek 109(4):565–587. https://doi.org/10.1007/s10482-016-0660-2

Hermosa R, Botella L, Keck E, Jiménez JA, Montero-Barrientos M, Arbona V, Gómez-Cadenas A, Monte E, Nicolás C (2011) The overexpression in *Arabidopsis thaliana* of a *Trichoderma harzianum* gene that modulates glucosidase activity, and enhances tolerance to salt and osmotic stresses. J Plant Physiol 168:1295–1302

Hohmann I, Bill RM, Kayingo I, Prior BA (2000) Microbial MIP channels. Trends Microbiol 8:33–38. https://doi.org/10.1016/S0966-842X(99)01645-5

Hohmann S, Krantz M, Nordlander B (2007) Yeast osmoregulation. Methods Enzymol 428:29–45. https://doi.org/10.1016/S0076-6879(07)28002-4

Horodyski RJ, Knauth LP (1994) Life on land in the precambrian. Science 263(5146):494–498

Jung YH, Yi JY, Jung HJ, Lee YK, Lee HK, Naicker MC, Uh JH, Jo IS, Jung EJ, Im H (2010) Overexpression of cold shock protein A of *Psychromonas arctica* KOPRI 22215 confers cold-resistance. Protein J 10(29):136–142

Juvvadi PR, Lamoth F, Steinbach WJ (2014) Calcineurin as a multifunctional regulator: unraveling novel functions in fungal stress responses, hyphal growth, drug resistance, and pathogenesis. Fungal Biol Rev 28(2–3):56–69

Kis-Papo T, Grishkan I, Oren A, Wasser SP, Nevo E (2001) Spatiotemporal diversity of filamentous fungi in the hypersaline Dead Sea. Mycol Res 105:749–756

Koga Y (2012) Thermal adaptation of the archaeal and bacterial lipid membranes. Archaea 2012: 789652. https://doi.org/10.1155/2012/789652

Lam HM, Xu X, Liu X, Chen W, Yang G, Wong FL, Li MW, He W, Qin N, Wang B, Li J, Jian M, Wang J, Shao G, Wang J, Sun SS, Zhang G (2010) Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. Nat Genet 42(12): 1053–1059. https://doi.org/10.1038/ng.715

Li T, Hu YJ, Hao ZP, Li H, Chen BD (2013) Aquaporin genes GintAQPF1and GintAQPF2 from *Glomus intraradices* contribute to plant drought tolerance. Plant Signal Behav 8:e24030. https://doi.org/10.4161/psb.24030

Liang X, Liu Y, Xie L, Liu X, Wei Y, Zhou X, Zhang S (2015) A ribosomal protein AgRPS3aE from halophilic *Aspergillus glaucus* confers salt tolerance in heterologous organisms. Int J Mol Sci 16(2):3058–3070. https://doi.org/10.3390/ijms16023058

Liu B, Zhang Y, Zhang W (2014a) RNA-seq-based analysis of cold shock response in *Thermoanaerobacter tengcongensis,* a bacterium harbouring a single cold shock protein encoding gene. PLoS One 9(3):e93289. https://doi.org/10.1371/journal.pone.0093289

Liu XD, Xie L, Wei Y, Zhou XY, Jia B, Liu J, Zhang SH (2014b) Abiotic stress resistance, a novel moonlighting function of ribosomal protein RPL44 in the halophilic fungus *Aspergillus glaucus*. Appl Environ Microbiol 80(14):4294–4300. https://doi.org/10.1128/AEM.00292-14

Liu XD, Wei Y, Zhou XY, Pei X, Zhang SH (2015) *Aspergillus glaucus* aquaglyceroporin gene *glpF* confers high osmosis tolerance in heterologous organisms. Appl Environ Microbiol 81(19):6926–6937. https://doi.org/10.1128/AEM.02127-15

Luan S, Li W, Rusnak F, Assmann SM, Schreiber SL (1993) Immunosuppressants implicate protein phosphatase regulation of K+ channels in guard cells. Proc Natl Acad Sci U S A 90:2202–2206

Luyten K, Albertyn J, Skibbe WF, Prior BA, Ramos J, Thevelein JM, Hohmann S (1995) Fps1, a yeast member of the MIP family of channel proteins, is a facilitator for glycerol uptake and efflux and is inactive under osmotic stress. EMBO J 14:1360–1371

Martínez F, Arif A, Nebauer SG, Bueso E, Ali R, Montesinos C, Brunaud V, Muñoz-Bertomeu J, Serrano R (2015) A fungal transcription factor gene is expressed in plants from its own promoter and improves drought tolerance. Planta 242(1):39–52. https://doi.org/10.1007/s00425-015-2285-5

Matheos DP, Kingsbury TJ, Ahsan US, Cunningham KW (1997) Tcn1p/Crz1p, a calcineurin-dependent transcription factor that differentially regulates gene expression in *Saccharomyces cerevisiae*. Genes Dev 11:3445–3458

Mega R, Manzoku M, Shinkai A, Nakagawa N, Kuramitsu S, Masui R (2010) Very rapid induction of a cold shock protein by temperature downshift in *Thermus thermophilus*. Biochem Biophys Res Commun 399:336–340

Mendoza I, Rubio F, Rodriguez-Navarro A, Pardo JM (1994) The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. J Biol Chem 269:8792–8796

Minic Z (2015) Proteomic studies of the effects of different stress conditions on central carbon metabolism in microorganisms. J Proteomics Bioinform 8:080–090. https://doi.org/10.4172/jpb.1000355

Montero-Barrientos M, Hermosa R, Nicolás C, Cardoza RE, Gutiérrez S, Monte E (2008) Overexpression of a *Trichoderma*HSP70 gene increases fungal resistance to heat and other abiotic stresses. Fungal Genet Biol 45:1506–1513

Montero-Barrientos M, Hermosa MR, Cardoza RE, Gutiérrez S, Nicolás C, Monte E (2010) Transgenic expression of the *Trichoderma harzianum* hsp70 gene increases *Arabidopsis* resistance to heat and other abiotic stresses. J Plant Physiol 167:659–665

Nakamura TY, Liu Y, Hirata D, Namba H, Harada S, Hirokawa T, Miyakawa T (1993) Protein phosphatase type 2B (calcineurin)-mediated, FK506-sensitive regulation of intracellular ions in yeast is an important determinant for adaptation to high salt stress conditions. EMBO J 12:4063–4071

Nicolás C, Hermosa R, Rubio B, Mukherjee PK, Monte E (2014) *Trichoderma* genes in plants for stress tolerance—status and prospects. Plant Sci 228:71–78

Oliveira R, Lages F, Silva-Graça M, Lucas C (2003) Fps1p channel is the mediator of the major part of glycerol passive diffusion in *Saccharomyces cerevisiae*: artefacts and re-definitions. Biochim Biophys Acta 1613:57–71. https://doi.org/10.1016/S0005-2736(03)00138-X

Pahlman AK, Granath K, Ansell R, Hohmann S, Adler L (2001) The yeast glycerol 3-phosphatases gpp1p and gpp2p are required for glycerol biosynthesis and differentially involved in the cellular responses to osmotic, anaerobic, and oxidative stress. J Biol Chem 276:3555–3563

Panadero J, Hernández-López MJ, Prieto JA, Randez-Gil F (2007) Overexpression of the calcineurin target CRZ1 provides freeze tolerance and enhances the fermentative capacity of baker's yeast. Appl Environ Microbiol 73(15):4824–4831

Pereira I, Madeira A, Prista C, Loureiro-Dias MC, Leandro MJ (2014) Characterization of new polyol/H+ symporters in *Debaryomyces hansenii*. PLoS One 9(2):e88180. https://doi.org/10. 1371/journal.pone.0088180

Petitjean M, Teste MA, François JM, Parrou J, L. (2015) Yeast tolerance to various stresses relies on the Trehalose-6P synthase (Tps1) protein, not on Trehalose. J Biol Chem 290(26): 16177–16190. https://doi.org/10.1074/jbc.M115.653899

Piette F, Leprince P, Feller G (2012) Is there a cold shock response in the Antarctic psychrophile *Pseudoalteromonas haloplanktis*? Extremophiles 16:681–683

Prista C, Soeiro A, Vesely P, Almagro A, Ramos J, Loureiro-Dias MC (2002) Genes from *Debaryomyces hansenii* increase salt tolerance in *Saccharomyces cerevisiae* W303. FEMS Yeast Res 2:151–157

Prista C, Loureiro-Dias MC, Montiel V, García R, Ramos J (2005) Mechanisms underlying the halotolerant way of *Debaryomyces hansenii*. FEMS Yeast Res 5(8):693–701

Ranawat P, Rawat S (2017) Stress response physiology of thermophiles. Arch Microbiol 199(3): 391–414. https://doi.org/10.1007/s00203-016-1331-4

Sáenz JP, Grosser D, Bradley AS, Lagny TJ, Lavrynenko O, Broda M, Simons K (2015) Hopanoids as functional analogues of cholesterol in bacterial membranes. Proc Natl Acad Sci U S A 112(38):11971–11976. https://doi.org/10.1073/pnas.1515607112

Santos H, da Costa MS (2002) Compatible solutes of organisms that live in hot saline environments. Environ Microbiol 4:501–509

Saum SH, Pfeiffer F, Palm P, Rampp M, Schuster SC, Müller, V., Oesterheldt, D. (2013) Chloride and organic osmolytes: a hybrid strategy to cope with elevated salinities by the moderately halophilic, chloride dependent bacterium *Halobacillus halophilus*. Environ Microbiol 15(5): 1619–1633

Schaechter E (2016) Bacterial hopanoids: the lipids that last forever. In: Schaechter E (ed) In the company of microbes: ten years of small things considered, pp 146–149. https://doi.org/10. 1128/9781555819606.ch37

Serrano R, Gaxiola R (1994) Microbial models and salt stress tolerance in plants. Crit Rev Plant Sci 13:121–138

Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43

Siderius M, Van Wuytswinkel O, Reijenga KA, Kelders M, Mager WH (2000) The control of intracellular glycerol in *Saccharomyces cerevisiae* influences osmotic stress response and resistance to increased temperature. Mol Microbiol 36:1381–1390

Simola M, Hanninen AL, Stranius SM, Makarow M (2000) Trehalose is required for conformational repair of heat-denatured proteins in yeast endoplasmic reticulum but not for maintenance of membrane traffic function after severe heat stress. Mol Microbiol 37:42–53

Singh S, Madlala AM, Prior BA (2003) *Thermomyces lanuginosus*: properties of strains and their hemicellulases. FEMS Microbiol Rev 27(1):3–16

Stathopoulos-Gerontides A, Guo JJ, Cyert MS (1999) Yeast calcineurin regulates nuclear localization of the Crz1p transcription factor through dephosphorylation. Genes Dev 13:798–803

Tamás MJ, Luyten FC, Sutherland A, Hernandez J, Albertyn J, Valadi H, Li H, Prior BA, Kilian SG, Ramos J, Gustafsson L, Thevelein JM, Hohmann S (1999) Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. Mol Microbiol 31:1087–1104. https://doi.org/10.1046/j.1365-2958.1999.01248.x

Tamás MJ, Thorsen M, Jacobson T, Vooijs R, Schat H (2009) Extracellular glutathione protects yeast cells from arsenite toxicity. Microbiology 140(Pt 8):1855

Topanurak S, Sinchaikul S, Sookkheo B, Phutrakul S, Chen ST (2005) Functional proteomics and correlated signaling pathway of the thermophilic bacterium *Bacillus stearothermophilus* TLS33 under cold- shock stress. Proteomics 5:4456–4471

Trent JD, Osipiuk J, Pinkau T (1990) Acquired thermotolerance and heat shock in the extremely thermophilic archaebacterium *Sulfolobus* sp. strain B12. J Bacteriol 172(3):1478–1484

Trent JD, Kagawa HK, Yaoi T, Olle E, Zaluzec NJ (1997) Chaperonin filaments: the archaeal cytoskeleton? Proc Natl Acad Sci U S A 94:5383–5388

Turk M, Abramović Z, Plemenitas A, Gunde-Cimerman N (2007) Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. FEMS Yeast Res 7(4):550–557

Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62:504–544

Welsh DT (2000) Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. FEMS Microbiol Rev 24:263–290

Yan J, Song WN, Nevo E (2005) A MAPK gene from Dead Sea fungus confers stress tolerance to lithium salt and freezing-thawing: prospects for saline agriculture. Proc Natl Acad Sci U S A 102(52):18992–18997

Yousefi-Nejad M, Naderi-Manesh H, Khajeh K (2011) Proteomics of early and late cold shock stress on thermophilic bacterium, *Thermus* sp GH5. J Proteome 74:2100–2111

Zhan X, Zhu JK, Lang Z (2015) Increasing freezing tolerance: kinase regulation of ICE1. Dev Cell 32(3):257–258. https://doi.org/10.1016/j.devcel.2015.01.004

Zhang H, Guo J, Voegele RT, Zhang J, Duan Y, Luo H, Kang Z (2012) Functional characterization of calcineurin homologs PsCNA1/PsCNB1 in *Puccinia striiformis* f. sp. *tritici* using a host-induced RNAi system. PLoS One 7(11):e49262. https://doi.org/10.1371/journal.pone.0049262

Zhang M, Liu Z, Yu Q, Mao J, Zhang B, Xing L, Li M (2015) Deletion of genes encoding fatty acid desaturases leads to alterations in stress sensitivity in *Pichia pastoris*. FEMS Yeast Res 15(4): fov020. https://doi.org/10.1093/femsyr/fov020