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REVIEW

Diversity in transcripts and translational pattern of stress proteins in marine extremophiles

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Abstract Extremophiles occur in a diverse range of habitats, from the frigid waters of Antarctic to the superheated plumes of hydrothermal vents. Their in-depth study could provide important insights into the biochemical, ecological and evolutionary aspects of marine microbes. The cellular machinery of such extreme-lovers could be highly flexible to cope with such harsh environments. Extreme conditions of temperature, pressure, salinity, pH, oxidative stress, radiation, etc., above the physiological tolerance level can disrupt the natural conformation of proteins in the cell. The induction of stress proteins (heat/cold shock proteins/salt stress proteins/pressure-induced proteins) plays a vital role in the acclimatization of extremophiles. The present review focuses on the in vitro studies conducted on the transcripts and translational pattern of stress proteins in extremophiles. Though some proteins are unique, a commonality in stress resistance mechanism has been observed, for example, the universal occurrence of HSP60, 70 and the expression of metabolic and DNA repair proteins. The review highlights that among all the stressful conditions, salt/osmotic stress evokes the expression of highest number of transcripts/proteins while psychrophilic condition the least.

Keywords Thermophiles · Psychrophiles · Halophiles · Piezophiles · pH and radiation-resistant microbes · Stress proteins · In vitro studies

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Introduction

The oceanic life has undergone an extraordinary capacity to deal with extreme physical, chemical and environmental parameters. Extremophiles demand the most extreme conditions to survive and thrive. Ecological limiting factors (nutrient availability, temperature, desiccation, salinity, pressure, pH, radiation, etc.) above the physiological tolerance levels destroy the native conformation of proteins thereby making them lose their function. To cope with such extremely difficult conditions, the biomolecular adaptations should be unique and wide ranging. The increased quest for understanding the adaptation strategy of microbes in extreme environments could reveal many unanswered questions in nature. A comparison between non-extremophilic protein and the extremophilic analogue of the same protein showed that the latter showed changes in protein residues and frequencies which impart either more flexibility or stability to the analogue under challenging conditions (Pakchung et al. 2006).

Stress proteins or molecular chaperones are defined as a functional class of unrelated families of proteins that mediate the correct non-covalent assembly of other polypeptide-containing structures, but are not the components of these assembled structures when the latter are carrying out their physiological roles. They are present in the cell under normal conditions, but at very low concentrations. When a cell undergoes stress above their physiological tolerance level, stress proteins are produced in greater quantities. They help the defective-shaped proteins to acquire proper configuration and perform their biological functions.

Several reviews on heat shock proteins (HSPs), their folding mechanisms, chaperone activities, induction by

various stressors, have been published. Regarding the stress proteins from extremophiles, those of Laksanalamai and Robb (2003), Serrano et al. (2003), Cavicchioli et al. (2000), Trent (1996) need special mention.

The present paper has attempted to review the transcriptional and translational pattern of stress proteins in marine extremophiles in vitro. Even though there are a number of stress factors, the scope of the present review focuses on extreme variations in heat, cold, salinity, pH, pressure and radiation. Since the natural habitat of extremophiles resembles the one which prevailed in the primitive ocean ("hot dilute soup"), the in vitro studies of the stress-tolerant mechanisms are relevant as they could yield valuable clues regarding the involvement of specific protein domains (functional part of a protein) or crucial amino acids in providing such a super survival power. Since stress proteins are the most conserved macromolecules on earth, its study in extremophiles could give important insights into the ecological and evolutionary relatedness at the molecular and organismal level. It would give further new perspectives into the biomolecular stability of such organisms over millions of years.

Based on the type of stress parameter extremophily can be mainly categorized into thermophily, psychrophily, halophily, piezophily, acidophily, alkaliphily and radioresistance.

Thermophily

Thermophiles are those organisms that grow optimally at temperatures above 50°C and hyperthermophiles prefer a growth temperature of above 80°C (Trent 2000). Thermophiles have been a focus of study because of the following three reasons: (1) the complex and stable biochemical machinery under extreme heat, (2) they represent the ancestral forms of life on primordial earth and (3) the small size of the genome (2.5 Mbp) (Cava et al. 2009). Despite the enormous amount of macromolecular data (genes and proteins) generated through biotechnological and bioinformatics applications, their evolution and survival at temperatures, at which a normal organism cannot even initiate growth, remain hotly debated (Brown and Lupas 1998). Within hydrothermal environments, a native habitat of hyperthermophiles, temperatures are prone to spatial and temporal variations due to tidal flexing of the earth's crust which causes diurnal fluctuations (Schultz et al. 1996), dynamic fluid flow patterns, and steep temperature gradients. Therefore, hyperthermophiles must employ thermal stress mechanisms to withstand the super-optimal temperatures encountered from these variations.

Thermal stress response

Acquired thermotolerance and heat shock response

When microbes are subjected to super-optimal growth temperatures, the viability of cells decreases with increasing temperature (Neidhardt et al. 1990). However, if a mild hyperthermal stress temperature is applied prior to exposure to a more lethal temperature, then the number of viable cells will remain significantly higher for a longer period of time before they begin to die. This kinetic display of enhanced tolerance to super-optimal temperatures, i.e., the 'acquired thermotolerance' is attributed to the expression of heat shock response (Parsell and Lindquist 1993). Research on thermotolerance and induction of heat shock proteins in hyperthermophiles suggests that these extremophiles could provide new perspectives in such areas since they are members of a separate phylogenetic domain and have evolved to live under hostile environmental conditions (Trent 1996); because of the extremely high temperature ranges of these microbes, a few of them are considered as best laboratory models. They are *Sulfolobus solfataricus, S. acidocaldarius, Pyrococcus furiosus, P. abyssi, Thermococcus kodakarensis, Thermotoga neapolitana* and *Thermus thermophilus* (Cava et al. 2009). The present paper reviews protein at transcript and expression levels in *Sulfolobus*, *Pyrococcus* and *Thermococcus* genera in detail.

Archaea

Euryarchaea

Genus Haloferax Two heat-responsive *cct* genes from the archaeon, *Haloferax volcanii* was investigated at the transcriptional level. The *cct1* and *cct2* genes encoding proteins of 560 and 557 residues were identified in *H. volcanii* and subsequently sequenced. The members of Cct family have been identified only in the domains archaea and eukarya (Kuo et al. 1997). A gene with homology to the bacterial HSP70 is suspected to be present in *H. volcanii* (Daniels et al. 1986).

Genus Methanococcus In methanogens, heat shock response has been reported in *Methanococcus voltae*, in response to a temperature rise from 30°C (normal growth temperature) to 40–50°C (Hebert et al. 1991). In this species, the proteome analysis showed 11 HSPs ranging in molecular mass from 18 to 89 kDa. Both HSP60 and 70 have been identified in other methanogens also. Southern hybridizations indicated that an *hsp70*-related gene is present in *M. barkeri* (Bardwell and Craig 1984) and an *hsp70* gene was later cloned and sequenced from *M. mazei*

(Macario et al. 1991). Immunoassays suggest the presence of HSP60-related proteins in *M. barkeri* (Thole et al. 1988) and a partial sequence of an *hsp60*-related gene were found in *M. jannaschii*.

Crenarchaeota

Genus Sulfolobus Most work on thermal stress response has been focused on the order *Sulfolobales* in the kingdom Crenarchaeota. They provide an interesting model for thermal studies because many of these chemolithotrophic organisms grow aerobically and have optimal (T_{opt}) and maximal (T_{max}) growth temperatures below 100°C (Han and Kelly 1997).

Global transcriptional analysis of the hyperthermophilic archaeon *S. solfataricus* heat shock response dynamics to temperature rise from 80 to 90°C revealed that the genome encodes at least 26 vapBC of family TA (toxin–antitoxin) loci in its genome. This suggests a possible role in heat shock response (Cooper et al. 2009). However, more research is needed to confirm the role of vapBCs in thermal stress response. In *S. acidocaldarius*, a significant increase in levels of a protein with molecular mass of 64–66 kDa was observed when the temperature raised from 70 to 85°C. Trent et al. (1990) showed that *S. shibatae* B12 exhibited a heat shock response when the cells were subjected to 88°C, accompanied by the increased synthesis of a predominant 55 kDa protein (TF55), the most abundant protein produced during the heat shock response in hyperthermophiles. TF55 belongs to the Cct family of chaperonins. Kagawa et al. (1995) found that TF55 is composed of two subunits forming two homo-oligomeric rings joined in a structure called a rosettasome, and they referred to this complex as the archaeal HSP60/*S. shibatae* HSP60 (SshHSP60). The α and β subunits of the protein with molecular mass of 59.72 and 59.68 kDa, respectively, were found to be approximately 55% identical at the amino acid level.

In addition to thermal stress, oxidative damage also needs to be mentioned here. A Dps-like protein has been identified and characterized from *S. solfataricus* (SsDps) in response to oxidative stress. Dps ($\sim$20 kDa) is a Fe binding and storage protein. It protects the bacterial genome from oxidative stress. A homologous SsDps-like sequence (85% similar) has been identified even in the mesophilic bacterium, *Gloeobacter violaceus* PCC 7421. Even though the amino acid sequences are highly conserved, the nucleotide sequences are not. The G+C content of the *SsDps* gene (40%) is low in hyperthermophiles in contrast to the high G+C content in mesophilic organism (60%) (Wiedenheft et al. 2005). This uneven composition in G+C content reveals uncertainty about the distribution of this gene in prokaryotes. Also virulence has been attributed to the expression of Dps protein in bacteria (Colangeli et al. 2009; Hasley et al. 2004).

Genus Pyrococcus The hyperthermophilic archaeon, *P. furiosus* expresses a small, α-crystallin-like protein in response to extreme temperatures, above 103°C. The *P. furiosus* small heat shock protein (Pfu-sHSP or *Pyrococcus furiosus* sHSP) gives cellular protection from extremely high temperatures (Laksanalamai et al. 2003). Another heat shock response in *P. furiosus* showed the expression of repair and stabilizing proteins for resisting thermal stress. The detailed expression profile is given in Table 1 (Shockley et al. 2003).

A Dps-like protein which functions as a powerful antioxidant has been isolated and characterized in *P. furiosus* (PfDps-like or *Pyrococcus furiosus* Dps-like) in response to oxidative stress (Ramsay et al. 2006). A tRNAm5C methyltransferase has been isolated from *P. abyssi* (Auxilien et al. 2007). An α-subunit-ThsA (thermosome alpha) chaperonin and 2 HSPs, HtpX and sHSP, has been identified in *P. abyssi* (Macario et al. 1999). However, the absence of HSP70/DnaK from *P. abyssi* is noteworthy.

Genus Thermococcus In *T. kodakarensis*, two molecular chaperonins (HSP60), CpkA and CpkB were expressed in response to heat shocks. The expression of CpkA was higher in both logarithmic and stationary phases at 60°C, while CpkB was not expressed in either phase. At 85°C, though CpkA and CpkB were expressed in both phases, the CpkA level decreased in the stationary phase. At 93°C, CpkA was expressed only in the logarithmic phase, and not in the stationary phase. In contrast, CpkB was highly expressed in both the phases. These in vitro studies indicate that CpkA and CpkB are important for cell growth at lower and higher temperatures (Fujiwara et al. 2008).

At lower temperatures, for example, in the frigid polar waters the organisms have to cope with high concentrations of dissolved oxygen leading sometimes to oxidative stress. An Msr gene homolog, encoding an MsrA–MsrB fusion protein ($MsrAB_{Tk}$) is induced in *T. kodakarensis*. The high levels of this protein at lower temperatures (30°C), rather than 85°C may be a novel strategy to deal with low-temperature environments in which the dissolved oxygen concentration increase. The presence of this protein in *T. kodakarensis* is exceptional among the hyperthermophiles and it points out to its significance (Fukushima et al. 2007). Four prefoldin (proteins that promote misfolded protein folding) genes encoding two α subunits (pfdA and pfdC) and two β subunits (pfdB and pfdD) are expressed in *T. kodakarensis*. The PfdA/PfdB complex was expressed in all tested temperatures, but the PfdC/PfdD expression in heat stress was at higher temperatures (93°C). This indicates that the first complex plays a crucial role in lower

Table 1 Thermal stress response in *P. furiosus* (Shockley et al. 2003)

No.	Protein	Function
1	ATP-dependent proteases	
	ATPase component of the 26S proteasome	Protein folding
	ATP-dependent La (Lon)	Degradation of abnormal proteins
	Proteasome	Protein metabolism
2	ATP-independent proteases and peptidases	
	ArgE/peptidase	Protein metabolism
	HtpX	Protein metabolism
	YtoP (similar to eno-1,4-glucanase)	Protein metabolism
	D-Aminopeptidase	Protein metabolism
	Signal sequence peptidase I (SECII)	Protein metabolism
	Methionine aminopeptidase (MAP)	Protein metabolism
	Putative proline dipeptidase	Protein metabolism
	HSP X	Protein metabolism
	Carboxypeptidase I	Protein metabolism
	Pyrolysin	Protein metabolism
	Subtilisin-like proteases	Protein metabolism
3	Chaperone-related proteins	
	sHSP (HSP20 homolog)	Heat shock resistance and protein folding
	Prefoldin (β subunit)	Heat shock resistance and protein folding
	Thermosome (single subunit)	Heat shock resistance and protein folding
	VAT homologs (chaperones)	Heat shock resistance and protein folding
4	DNA repair proteins (RadA and RadB)	DNA repair and recombination
5	DNA damage inducible protein (DinF homolog)	DNA repair and recombination
6	Glycoside hydrolases	
	Chitinase	Carbohydrate metabolism
	Endo-β-1,3-glucanase	Carbohydrate metabolism
	Putative methyltransferase	Carbohydrate metabolism
	Putative α-dextrin-endo-1,6-α glucosidase	Carbohydrate metabolism
	α-Amylase	Carbohydrate metabolism
	β-Glucosidase	Carbohydrate metabolism
	β-Mannosidase	Carbohydrate metabolism
7	Putative sugar binding protein	Sugar metabolism
8	Putative trehalose synthase	Sugar metabolism
9	Spermidine synthase	Spermidine synthesis
10	Trehalose/maltose-binding protein	Sugar metabolism

temperatures while the other complex helps to maintain at higher temperatures (Danno et al. 2008). An OsmC protein which is involved in cellular defense mechanism is also reported in *T. kodakarensis* in response to oxidative stress (Park et al. 2008). In the Antarctic bacteria, anerobiosis outcompete aerobiosis suggesting that the disposition to express increased viability under reducing conditions is a strategy to counteract stress due to supersaturation of oxygen in the cold lacustrine environment (Loka Bharathi et al. 1999).

The studies in *T. thermophilus* confirmed the expression of osmolytes, trehalose and MG. MG was also produced in the halotolerant strains of *T. thermophilus* under salt stress. Two pathways for the synthesis of trehalose, i.e., TPS/TPP and Tres pathway are involved in *T. thermophilus* (Empadinhas and Costa 2006). The involvement of these pathways in sugar metabolism may help to meet the high-energy demands during the stress-tolerant mechanisms.

Eubacteria

Little is known about the thermal response of hyper-thermophilic eubacteria; however, homologs of DnaK and GroEL are present in both the *Thermotoga maritima* and *Aquifex aeolicus* genomes suggesting that the heat shock responses in these organisms are very similar to those of

mesophilic response. The hyperthermophilic archaea, *Thermococcus* sp. ES4 growing optimally at 98–100°C (Pledger and Baross 1991) was found to produce increased levels of a 98 kDa protein in response to a temperature change from 95 to 102°C (Holden and Baross 1993). An *hsp70* gene was later cloned and sequenced from *Halobacterium marismortui* (Gupta and Singh 1992). The presence of HSP60-related proteins has been suggested in *Methanobacterium thermoautotrophicum* (Thole et al. 1988).

Analysis of the thermal stress response

In response to heat shock, the predominant protein induced in Crenarchaea (*Sulfolobus* genera) was HSP60 (TF55/SshHSP60). Euryarchaea expressed HSP70. Western blot analysis showed that SshHSP60 was similar to those found in other members of Crenarchaea, but not evident in Euryarchaea. TF55 was shown to be unrelated to bacterial DnaK, but homologous nature with a eukaryotic protein showed the evolutionary relatedness between archaea and eukaryota. The clear absence of a homolog to bacterial HSP70/DnaK protein in the genomes of some archaeal species such as *M. jannaschii*, *Archaeoglobus fulgidus*, *Pyrobaculum aerophilum*, *Pyrococcus horikoshii*, or *P. furiosus* (Kawarabayasi et al. 1998; Fitz-Gibbons et al. 1997; Klenk et al. 1997; Bult et al. 1996) is still an evolutionary puzzle.

Psychrophily

Psychrophiles inhabit extremely cold, typically subzero aquatic environments, including the deep sea (−1 to 4°C), Arctic and Antarctic marine habitats (seawater and sediments near −1°C and sea ice, where internal fluids remain liquid to −35°C in winter time), and glacial and lake ice (down to −5°C). A psychrophile is one which is capable of low growth at or below 0°C but unable to grow above 20°C (Fuge et al. 1994; Watson 1987). The polar regions of the earth, especially the Arctic regions, are undergoing rapid environmental changes globally (http://www.ipcc.ch), such that the permanent residents of frozen ice conditions are almost near extinction. Since the advent of the new fascinating field of biology–astrobiology demands to locate polar regions as habitat analogues for possible life elsewhere is growing, the attempts to document biodiversity at high latitudes is full-fledged (Deming and Huston 2000; DesMarais and Walter 1999). The presence of polar ice caps in Mars (Baker 2001) and the vast oceanic region below the ice in Jupiter's ice-covered moon, Europa, showed the probability of the occurrence of microbial communities (Chyba and Phillips 2002).

In such a context, it would be relevant to analyze the molecular adaptive mechanisms of such extremophiles. In cold environments, the physical parameters such as low temperature, high hydrostatic pressure, increased salt concentration reinforces the direct relationship between psychrophily–piezophily (Deming and Baross 2000) and psychrophily–halophily (halotolerance) (Deming and Baross 2001; Stanley et al. 2001). Extensive studies have been conducted on the induction of proteins, such as cold shock proteins and EF-2. Cold shock proteins are induced in psychrophiles as the temperature drops below the physiological tolerance level.

Cold shock response

A cold shock response involves the transient induction of a subset of proteins termed CSPs. CSPs are synthesized to enable gene expression and protein synthesis to continue at low temperature (Phadtare et al. 1999; Yamanaka 1999). They help the cold-shocked cells by decreasing the membrane fluidity and acting as RNA chaperones (Yamanaka et al. 1998). They are different from CAPs. CSPs are induced in response to a sudden shock to a low temperature, whereas CAPs are specifically synthesized to acclimatize during continuous growth at cold temperatures. To describe these induced proteins, Graumann and Marahiel (1996) proposed a new term, CIPs.

The cold shock response studies in psychrophiles are still in its infancy. Most of the present knowledge available on physiological and molecular response of bacteria to rapid temperature changes originating from studies on mesophilic microorganisms such as *Escherichia coli* and *Bacillus subtilis* (Thieringer et al. 1998; Graumann and Marahiel 1996; Jones and Inouye 1994; Wolffe 1995). In this review, the cold stress mechanism has been analyzed by categorizing the microbes into the following three groups: obligate psychrophiles, non-obligate psychrophiles and psychrophilic yeasts.

Obligate psychrophiles

CSPs and proteins involved in unsaturated fatty acid synthesis have been identified in the genomes of *Desulfotales psychrophila*, *Colwellia psychrerythraea* 34H, *Pseudoalteromonas haloplanktis* TAC125 and archaea *Methanogenium frigidum* and *Methanococcoides burtonii*. Lipid desaturases and genes involved in the maintenance of cell membrane fluidity have been found in the *P. haloplanktis* genome. Proteins concerned with membrane fluidity: β-keto-acyl-carrier proteins, β-keto-acyl-CoA synthetases and fatty acid *cis–trans* isomerase have been identified in *C. psychrerythraea*. To resist the reactive oxygen species (ROS), the genome of *C. psychrerythraea* and

D. psychrophila encodes catalases and superoxide dismutases. *P. haloplanktis* counteract ROS by the suppression of metabolic pathways producing ROS (Amico et al. 2006). Proteins concerned with energy metabolism, transcription–translation and in protein quality control were recognized at 4°C in *M. burtonii* (Goodchild et al. 2004). A prolyl *cis–trans* isomerase was identified in *Shewanella* sp. strain SIB1 (Suzuki et al. 2004).

Non-obligate psychrophiles

Genus Arthrobacter

A. globiformis In *A. globiformis* SI55, CapA is produced very rapidly within 20 min after a cold shock from 25 to 4°C, demonstrating that *capA* expression is an immediate response to low temperature. It has been observed that growth of *A. globiformis* S155 after cold shock is correlated with CapA synthesis. The *capA* gene was homologous to the *cspA* gene of *E. coli* (Berger et al. 1997). The induction of CSPs also reported in *A. globiformis* by Berger et al. (1996).

Genus Listeria

L. monocytogenes The proteomic analyses in *L. monocytogenes* when the temperature drops from 37 to 5°C, revealed the induction of ten proteins with apparent molecular masses of 74, 66.5, 55.5, 47, 42, 38, 34.7, 31, 26.3, and 18 kDa. Among the low molecular mass proteins, a CSP close to 18 kDa (17.6–19 kDa) was highly induced in the three different strains of *L. monocytogenes* and in *L. innocua* CHUT 861156 (Phan-Thanh and Gormon 1995). In *L. monocytogenes* LO28, the 18 kDa CSP was particularly over-expressed several hours after the temperature dropped to 5°C. Such an observation led to categorize this small protein as CAP rather than CSPs (Hebraud and Potier 1999). The sequence analysis showed that the CSP belongs to the family of Flps (ferritin like proteins) (Hebraud and Guzzo 2000). Flps form a novel family of bacterial proteins with diverse functions, such as DNA binding, iron storage and cell activation.

Genus Vibrio

V. vulnificus Forty proteins were induced when the bacterial cells were subjected to a temperature decrease from 23 to 13°C in *V. vulnificus*. Induced proteins fell into three classes. For the 12 class I proteins, induction increased, decreased, and then increased again as the cells resumed growth at the new temperature. Induction of the 26 class II proteins peaked to a maximum and then declined. The two class III proteins were induced only after 4 h in the cold. It is speculated that the first class represents titratable factors that are synthesized to high levels and then maintained. The second group may represent true survival factors, needed only as cells adjust to growth at new lower rates (McGovern and Oliver 1995).

Fungi

Psychrophilic yeasts and thermal stress response

In the Antarctic psychrophilic yeast, *Candida psychrophila*, cells grown at 15°C and heat shocked at 25°C for 3 h, acquired tolerance to a rise in temperature to 35°C and 100 mM hydrogen peroxide. A novel heat shock-inducible protein of about 110 kDa was induced, in addition to the presence of HSP90, 70 and 60. But the absence of HSP104 was conspicuous. HSP110 is supposed to play a role in stress tolerance in psychrophilic yeasts similar to that of HSP104 in mesophilic species (Deegenaars and Watson 1997). In one of the few reports on heat shock-induced protein synthesis in eukaryotic microorganisms from polar regions, the induction of 12 proteins ranging from 32 to 84 kDa was reported in the Arctic psychrotrophic yeast, *Trichosporon pullulans* (Julseth and Inniss 1990).

Analysis of cold shock response in psychrophiles

The cold shock response generally induced the synthesis of specific CSPs/CAPs and proteins which control the overall metabolism of the cell. In fact, majority of the induced proteins in different bacteria were involved in signal transduction (chemotaxis), metabolism (protein folding, translation) and energy maintenance (energy pathways, sugar uptake). The main function of the induced proteins in obligate psychrophiles was the maintenance of membrane rigidity/fluidity.

Halophily

Halophilic and halotolerant microbes live in highly ionic environments such as Dead Sea, salt lakes, salt brines, salt deposits, etc. Normally, high or extreme solute concentrations do not necessarily impose stress upon a microbial cell; changes in osmotic conditions will cause adaptational responses. Microbes that have been adapted during evolution (genotypic adaptation) to grow optimally at high solute concentrations are not stressed by these conditions. They may be severely stressed by lowering of the solute concentrations.

Osmotic stress response

It can be defined as a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme

production, gene expression, etc.) as a result of a stimulus indicating an increase or decrease in the concentration of solutes outside the organism or cell (http://amigo.geneontology.org). Most microbial cells are stressed by either high and low salt or solute concentrations. Based on this, osmotic stresses are distinguished into two: (1) solute stress/hyperosmotic stress (stress by high external solute concentrations) and (2) dilution stress/hypo-osmotic stress (stress due to decreased external solute concentrations). Abrupt changes in the external osmolarity either by increase or decrease of the solute concentration are called up-shock or down-shock. Halophily and halotolerance are entirely different terms as halophily describes the requirement and halotolerance refers to the tolerance of an organism to high salt concentrations (Imhoff 1999).

Osmo-adaptation mechanisms are better examined at proteome levels than genome levels. An investigation of the hyper- and hypo-osmotic conditions in archaea and bacteria showed that hypersalinity created a more general stress response than hyposaline conditions.

Archaea

The study of stress proteins in archaea is less advanced and very little information is available on the anti-stress mechanism. The detailed in vivo and in vitro experiments conducted in extreme halophiles such as *Haloarcula marismortui*, *Haloferax volcanii* and *Halobacterium* sp. NRC-1 are reviewed here.

Genus Haloarcula

H. marismortui Halophiles may experience hyposaline shocks after rain or flooding. In extreme halophiles, it is interesting to notice that such conditions induce the synthesis of a 45 kDa protein (P45), showing chaperone activities. In vivo experiments in the extreme halophile *H. marismortui* (normal growth at 3–4 M NaCl) showed that P45 is induced when cells are exposed to a low-salt (2.5 M NaCl) environment. The proteome analysis showed the presence of P45 protein in vitro studies. P45 forms complexes with halophilic malate dehydrogenase during its salt-dependent denaturation/renaturation and decreases the rate of deactivation of the enzyme in an ATP-dependent manner. P45 is also identified in *H. salinarum* (Franzetti et al. 2001).

Genus Haloferax

H. volcanii The extremely halophilic archaeon *H. volcanii* is able to grow over a wide range of NaCl concentrations from about 8% to saturation (Mullakhanbhai and Larsen 1975). Osmotic stress proteins (OSPs which are either HSPs/GSPs) with a transient response after a shift to low (10% SW) or high (30% SW) salinities, and other proteins showing a long-term response in the adaptation to certain salinity, have been identified. GSPs are termed as 'General Stress' proteins because they are induced by different stresses such as heat shock, salt stress, oxidative stress and nutrient starvation.

Hyperosmotic shock induced a general stress response. Notable transient responses were those of 21, 46 and 98 kDa OSPs (Mojica et al. 1997). HSP of 98 and 18–22 kDa have also been found in other archaeal groups (methanogens and extreme thermophiles) (Macario and Macario 1994). A 46 kDa OSP was induced both under hyper- and hypo-osmotic shocks and could correspond to DnaJ-like chaperones. Therefore, they are considered as GSP. Notable proteins in the long-term response to medium-high salinities were a 16 kDa, a 48 kDa, and a set of around 70 kDa (72, 70, and 68 kDa) species (Mojica et al. 1997). Low-saline (10% SW) conditions induced certain unique as well as strong general stress responses. The more important long-term responses were shown by the 63, 44, 34, 18, 17, and 6 kDa proteins. Among them, the 63 and 44 kDa species were unique to low-salt condition. Most of these proteins which were over-expressed in the long-term response to low-salt conditions, gradually increased their synthesis after a downshift, but particularly strong transient responses were shown by 63, 34 and 18 kDa low-salt proteins (Mojica et al. 1997).

The *cct1* and *cct2* genes are induced in *H. volcanii* in response to hyposaline and heat shocks (Kuo et al. 1997). It is obvious that the *cct* gene system plays a vital role in both heat and osmotic stresses. The genes/proteins expression profile in *H. volcanii* is given in Table 2.

Genus Halobacterium

Halobacterium sp. NRC-1 The extreme halophile *Halobacterium* sp. NRC-1 has evolved a unique and novel strategy of adaptation to extreme salinity and other stresses. In *Halobacterium* NRC-1, the proteins induced after salt stress perform a set of diverse functions: assisting in the proper folding of damaged proteins, membrane stabilization and protection from oxidative and DNA damages. Transcription of several K and Na ion transporter genes was affected greatly by hypo- and hyper-saline conditions. Table 3 shows the genes greatly affected by high salt concentrations (2.9 M NaCl). The genes which are greatly affected by low-salt levels (5 M NaCl) at 42°C are listed in Table 4. The common induction of genes such as *app A & B*, *car A & B*, *cxp* and *sod 1 & 2* in both high and low-salt concentrations is of great significance in stress tolerance mechanism (Coker et al. 2007).

Table 2 Salt stress response in *H. volcanii* (after Kuo et al. 1997 and Mojica et al. 1997)

No.	Genes	Proteins	Remarks
1	*Cct1 & Cct2*	–	*Cct* genes induced both in halophiles and thermophiles
2	–	21, 46, 98 kDa	46 kDa is induced both under hyper- and hyposaline conditions
3	–	16, 48, 70 (68, 72, 70)	–
4	–	6, 17, 18, 34, 44 (45, 46), 63	The induction of 44 and 63 kDa were unique to hyposalinity

Table 3 Genes affected by high salt conditions in *Halobacterium* sp. NRC-1 (Coker et al. 2007)

No.	Gene	Function
1	*appA*	Oligopeptide binding protein
2	*appB*	Oligopeptide ABC permease
3	*car A & B*	Carbamoyl phosphate synthase: large and small subunits
4	*cxp*	Carboxypeptidase
5	*hik5*	Sensory histidine protein kinase
6	*hsp5*	Heat shock protein
7	*Ifl3*	Long chain fatty acid CoA ligase
8	*mdhA*	L-Malate dehydrogenase
9	*nosY*	Nitrite/nitrate ABC transporter
10	*phnC*	Phosphonate transport ATP-binding
11	*pyrB*	Aspartate carbamoyltransferase catalytic subunit
12	*pyrI*	Aspartate carbamoyltransferase regulatory chain
13	*sfuB*	Iron-transporter-like protein
14	*sirR*	Transcription repressor
15	*sod 1 & 2*	Superoxide dismutases

Eubacteria

The osmo-adaptation studies in eubacteria included *Halobacillus dabanensis* D-8^{T}, *Halomonas elongata* and *Listeria monocytogenes.*

Genus Halobacillus

H. dabanensis D-8^{T} Moderately halophilic bacteria are a versatile group adapted to a wide variation in salinity ranging from 0.1 to 32.5% salt (Ventosa et al. 1998). The proteome analysis of the salt shock treatment (1–25% salinity for 5 min or 50 min) in *H. dabanensis* D-8^{T} showed the over-expression of eight proteins. Among them, three are GSPs, i.e., ATPases with chaperone activity (ATPases can unfold proteins and disaggregate preformed protein aggregates to target them for degradation to restore their functions), ClpC ATPase (a stress protein belonging to the HSP100/Clp family, is a class of highly conserved proteins implicated in stress tolerance mechanisms of many prokaryotic organisms) (Schirmer et al. 1996) and a class I HSP. These GSPs assist proper folding of other proteins and/or ensure synthesis of newly induced proteins as part of the cell's adaptation to salt stress (Feng et al. 2006). A molecular chaperonin GroEL homologue (hp GroEL) have been purified and characterized from the moderately eubacterial halophile *Pseudomonas* sp strain #43 (Tokunagaa et al. 1997).

Genus Listeria

L. monocytogenes *L. monocytogenes* can survive a variety of environmental stresses, such as 10% NaCl solutions (McClure et al. 1989) and a range of temperatures from 0.1 to 45°C (Walker et al. 1990), pH as low as 3.5 after an adaptation phase at pH 5.5 (O'Driscoll et al. 1996). This high degree of adaptability is one reason for the difficulty in controlling the pathogen in a number of food products, since treatments used in food processing and preservation often utilize stressing agents and parameters to which *L. monocytogenes* is resistant. *L. monocytogenes* is frequently isolated from food containing high quantities of salt, such as smoked salmon (Vogel et al. 2001). A better knowledge of the adaptive mechanisms of *L. monocytogenes* to salt stress could lead to better control and prevention of this pathogen in food-processing plants.

The 2-D gel electrophoretic treatment in *L. monocytogenes* induced 12 SSIPs. They belong to two groups: the

Table 4 Genes affected by low-salt conditions in *Halobacterium* sp NRC-1 (Coker et al. 2007)

No.	Gene	Function
1	*app A & B*	Oligopeptide binding protein and ABC permease
2	*aspC2*	Aspartate aminotransferase
3	*atp A, F, C, E, K, I*	H^+-transporting ATP synthase subunits A, F, C, E, K, I
4	*carA & B*	Carbamoyl phosphate synthase-large and small subunits
5	*cbiQ & N*	Cobalt transport proteins
6	*cspD1*	Cold shock protein
7	*cxp*	Carboxypeptidase
8	*dfr*	DinF-related damage inducible protein
9	*dnaK*	Heat shock protein
10	*dppB1 & C1*	Dipeptide ABC transporter permeases
11	*dppD1*	Oligopeptide ABC transporter
12	*glnA*	Glutamine synthetase
13	*gst*	Glutathione-*S*-transferase
14	*hsp1*	Small heat shock protein
15	*Htr 12 & 13*	Htr 12 and 13 inducers
16	*nhaC3*	Na^+/H^+ antiporter
17	*rpl22p*	50S ribosomal protein L22P
18	*rpl23p*	50S ribosomal protein L23P
19	*rpl29p*	50S ribosomal protein L29P
20	*rpl2p*	50S ribosomal protein L2P
21	*sod 1 & 2*	Superoxide dismutases
22	*tbpC*	Transcription initiation factor IID
23	*trkA6*	K^+ (potassium) transporter system protein
24	*trxA1*	Thioredoxin
25	*ush*	UDP-sugar hydrolase
26	*xup*	Xanthine/uracil permease family protein

SSPs, which are rapidly but transiently over-expressed (Kilstrup et al. 1997), and the SAPs, which are more or less rapidly induced but still over-expressed several hours after the downshifts. A brief summary of the protein expression profile is given in Table 5.

Among the six SSPs induced, two GSPs—DnaK and Ctc—were identified. Three other over-expressed SSPs belonged to the general metabolism. First an alanine dehydrogenase has been identified which presently does not seem to be involved in any other stress response (Siranosian et al. 1993) but catalyzes the formation of pyruvic acid. The second protein, CysK is involved in cysteine biosynthesis. The amino acid, cysteine takes part in the formation of pyruvate. The last identified protein is Gap, an enzyme of glycolysis. Gap is an essential enzyme in the glycolytic pathway, where it catalyzes the synthesis of 1,3-diphosphoglyceric acid, a high-energy intermediate in the pyruvate synthetic pathway. It is clearly observed that these three proteins (alanine dehydrogenase, CysK, and Gap) induced after salt stress take part in the metabolism of pyruvic acid, which is necessary for acetyl-coenzyme A synthesis, one of the key components of fatty acid synthesis. Among the 11 SAPs detected, 7 of them have been identified. The first is GbuA, which is an osmoprotectant transporter accumulated in response to salt stress by *L. monocytogenes* and many bacteria, such as *B. subtilis* (Gerhardt et al. 2000). The second SAP, EF-2, is implicated in protein folding and/or protection from stress

Table 5 Salt stress response in *L. monocytogenes* (Kilstrup et al. 1997) in response to hyposalinity

No.	Proteins	Function
1	Alanine dehydrogenase	Catalyzes pyruvic acid synthesis
2	CcpA	Control carbon catabolism
3	Ctc	Role in general stress response
4	CysK	Cysteine synthesis
5	DnaK	Heat shock resistance
6	EF-2	Protein folding
7	Gap	Essential in glycolytic pathway
8	GbuA	Osmoprotectant transporter
9	GuaB	Essential in GMP
10	Homolog of PTS enzyme, IIAB	Sugar metabolism
11	PdhA and PdhB	Pyruvate decarboxylation

in *E. coli* (Caldas et al. 1998). Salt stress also induced higher levels of GuaB, an enzyme, involved in the first step of GMP biosynthesis from IMP. Since purines (adenine and guanosine) are the major components of DNA, the over-expression of GuaB in *L. monocytogenes* showed the necessity of synthesizing purines in stressed cells where DNA is being repaired (Duche et al. 2002). Some induced SAPs were related to glycolysis. The CcpA, which controls the pathways of carbon catabolism, is a regulator of glycolysis in several microorganisms (Mahr et al. 2000; Tobisch et al. 1999; Behari and Youngman 1998).It is induced after cold stress in *Lactococcus lactis* (Wouters et al. 2000). A homolog of mannose-specific PTS enzyme, IIAB and two pyruvate dehydrogenase subunits (PdhA and PdhD) were also identified.

Summarizing the protein expression profile, in *L. monocytogenes*, the salt stress response is connected with general stress response, with two GSPs (DnaK and Ctc), and over-expression of two osmoprotectants, GbuA and GuaB. The synthesis of CcpA, IIAB, Gap, CysK, PdhA and PdhD are related to the metabolic processes of the cell, responsible for the two significant energy producing cycles, glycolysis and glyoxylate cycle. The stress-tolerant mechanism is an energy consuming process. To compensate for this energy utilization, there is increase in sugar uptake. This sugar is catabolized to produce high-energy packets of ATPs with the help of metabolic enzymes such as alanine dehydrogenase, CysK and Gap. All these observations reveal that salt stress response is a complex process which remains to be elucidated by understanding the detailed function of the SSPs and SAPs (Duche et al. 2002).

Genus Halomonas

H. elongata Within the two prokaryotic domains, Bacteria and Archaea, the species of the moderately halophilic genus *Halomonas* is reported to have the widest growth spectra in relation to salt from 0.1% to saturation (Vreeland 1984; Vreeland et al. 1980). In *H. elongata*, low salinity-induced proteins of 6, 15, 42 and 60 kDa while high salinity produced 15.5, 20, 24 and 39 (Mojica et al. 1997). Hypo-osmotic shock also induced typical transient general stress responses, such as expression of 85, 79, 71 and 46 kDa species.

Analysis of stress protein expression profile in halophiles

The hypo-osmotic shock induced a general stress response. In the studies described here, it can be seen that the hypo-osmotic shock mimicked a heat shock response. The 85 and 79 kDa species in *H. elongata* could correspond to the similar-molecular-weight HSPs described for *H. volcanii* (Daniels et al. 1984). HSPs with molecular masses of about 70 and 45 kDa have been described (Gupta and Singh 1992; Daniels et al. 1984) for haloarchaeal species of the genera *Haloarcula* (*H. marismortui*) and *Halobacterium* (*H. trapanicum*) but not for *Haloferax* (*H. volcanii* and *H. mediterranei* R-4) (Rodriguez-Valera et al. 1983) or for other *Haloarcula* (*H. hispanica* Y-27) or *Halobacterium* (*H. salinarium*) species (Daniels et al. 1984). The data in this study suggest the presence of GSPs or chaperones of about 70 and 46 kDa in *H. volcanii.* A gene encoding a protein of about 70 kDa, homologous to the universally present HSP70 protein has been found in the haloarchaeon *Haloarcula* (previously *Halobacterium*) *marismortui* (Gupta and Singh 1992). All proteins with typical transient responses after a down- or up-shift are considered GSPs of 98, 85, 79, 71, 46, and 21 kDa (Mojica et al. 1997). Besides, a DnaJ homolog with an approximate molecular mass of 43 kDa has been found in a methanogenic archaeon (Macario et al. 1993). Chaperones of about 46 kDa, homologous to the *E. coli* DnaJ protein, also present in eukaryotes (Caplan and Douglas 1991; Zhu et al. 1993) show a transient accumulation in response to salt stress. The universal presence of such HSP70-like and DnaJ-like chaperones among haloarchaea, with a possible role under osmotic stress is highlighted here.

Piezophily

In deep-sea realm where the microbes form the predominant biotic community, the distribution and survival of organisms is controlled by the crucial factor, pressure (Whitman et al. 1998; Somero 1990; Yayanos 1986). Deep-sea habitat is characterized by a lower temperature limit of −7.5°C (Bedford 1933), and the upper temperature limit is reported to be 113°C (Blochl et al. 1997). The microbes inhabiting the deep sea can grow at 2–3°C and hundreds of bars of hydrostatic pressure and those living in Challenger Deep, which is the deepest known oceanic site, must be adapted to survive at pressures >100 megapascals (MPa) (1 bar = 0.1 MPa). Presently the term "piezophile" which was previously referred to as barophile, is used to describe those microorganisms with optimal growth at pressures >0.1 MPa.

The field of piezomicrobiology was born more than 100 years ago (Simonato et al. 2006), but is still in an infant stage because of the limited number of scientists and labs involved in the field most probably due to the lack of specialized and expensive collection vehicles. Many basic properties of piezophiles that enable their survival at extreme pressures remain to be elucidated (Kato and Bartlett 1997). The major challenges in piezophysiology

are to discover whether the physiological responses of living cells are relevant to their growth and to identify the critical factors in cell viability and lethality under high pressure (Abe 2007).

There is growing interest in understanding microbes and potential applications of them in extreme environments that have significant impacts on them (Simonato et al. 2006; Bartlett 2002; Abe and Horikoshi 2001; Abe et al. 1999; Yayanos 1995). The biomedical applications of piezophiles are wide ranging. Proteins extracted from obligate piezophiles such as *Photobacterium profundum* SS9, *Shewanella violacea* DSS12, *Colwellia hadaliensis* BNL-1 and *Pyrococcus abyssi* are adapted to work both at high pressures and low temperatures. High-pressure effects on bacterial membranes and enzymes suggest potential applications in cheese ripening (Malone et al. 2002). *P. profundum* SS9, the genome of which has been completely sequenced is an excellent model organism for piezophilic studies. A number of vectors have been developed for the cloning and expression of genes in this bacterium (Lauro et al. 2005). In the food industry, high-pressure treatment is a very useful strategy in the pasteurization of food without a heating process. A number of food products treated under high-pressure conditions have been commercialized (Knorr et al. 2006; Hayashi 2002; Smelt et al. 2002; Knorr 1999). High-pressure treatment (200–800 MPa) of bacteria and other microorganisms is useful in food sterilization (Buzrul and Alpas 2004). Sterilization at high pressures preserves color and flavor of food items (Ludwig et al. 1996).

Piezotolerance

Piezotolerance and stress strategies

Deep-sea environment is characterized by high pressure, low temperature, fluctuations in oxygen supply, salinity and pH. Microorganisms, which are not piezophiles may respond to high-pressure environments by the induction of stress proteins termed, PIPs, which are either heat or cold shock or ribosomal proteins (Marteinsson et al. 1999). The piezotolerant mechanisms are reviewed here with three different groups, i.e., piezophiles, hyperthermophiles and mesophiles. The limited availability of literature on the expression of stress proteins in piezophiles suggests that piezomicrobiology needs further studies in depth to understand the biochemical mechanisms thoroughly.

Piezophilic studies in bacteria, archaea and fungi

The piezophilic studies conducted in deep-sea bacteria, archaea, mesophiles and fungi are reviewed here.

Deep-sea bacteria

Genus Photobacterium P. profundum SS9 The piezophile, *P. profundum SS9* (SS9) is suitable for studies since the genome is sequenced and is capable of growth at temperatures of <2 to >20°C with an optimal temperature at 15°C and at pressures varying from 0.1 to 90 MPa with an optimal at 28 MPa. Two important aspects of piezophilic adaptation of SS9 are its pressure–temperature responsive genes and its high metabolic diversity (Vezzi et al. 2005).

Hydrostatic pressure influences the induction of cell-surface proteins in deep-sea bacteria. SS9 modulated the abundance of several OMPs in response to hydrostatic pressures (Chi and Bartlett 1993; Bartlett et al. 1989). OmpH was found to be highly induced at 28 MPa. A second OMP, designated OmpL (outer membrane protein low pressure), was repressed at elevated pressure while a third OMP, OmpI, was induced at pressures above the pressure optima of SS9 and was most abundant at 40 MPa. Even though the function of these proteins is not clear, it is suggested that the OmpH protein facilitates the uptake of substrates >400 Da (Bartlett and Welch 1995). Mutational studies of *ompH* gene showed that it is required for high-pressure conditions (Chi and Bartlett 1993). In SS9, the OmpH–OmpL regulation is carried out by ToxR–ToxS system. ToxR is a transmembrane protein that binds to genes via its DNA-binding domain and is modulated by ToxS. Mutational studies of *toxR* proved that ToxR is essential for *ompL* activation and *ompH* repression (Welch and Bartlett 1998). To confirm the exact role of ToxR–ToxS regulatory system which activates the virulence genes in *V. cholerae* in SS9, further genomic studies would be needed.

The genome of SS9 encodes genes for piezo-adapted F1F0 ATP-synthases (Vezzi et al. 2005). The up-regulation of glycine reductase, a key enzyme in Stickland reaction and some other genes suggests that SS9 gains ATP by the above reaction under anaerobic conditions (Martin et al. 2002). Further studies are needed to confirm their exact role under high pressure. Under low pressure of 0.1 MPa, SS9 showed up-regulation of HtpG, DnaJ, DnaK and GroEL (Vezzi et al. 2005). The exact roles of these proteins under such conditions remain to be elucidated. The same pressure condition also showed the over-expression of genes in SS9 involved in DNA repair: *mutT*, *recN*, *uvrA* and *uvrD* orthologs (Aertsen et al. 2004). Allen and Bartlett (2002) reported that genomic analysis of SS9 showed that monounsaturated fatty acids in membrane lipids were required for growth at high pressure and low temperature. High proportions of monounsaturated fatty acids, octadecenoic and tetradecenoic acids (Kato et al. 1998) and polyunsaturated fatty acids, eicosapentanoic acid (EPA)

and docohexanoic acid (DHA) were induced in obligate piezophiles, *Shewanella* sp. strain DB21MT-2 and *Moritella* sp. strain DB21MT-5 (optimal growth pressure 80 MPa), respectively (Simonato et al. 2006) (in vivo studies).

Osmolytes play a vital role in piezophilic adaptation in *P. profundum* SS9. SS9 showed the accumulation of primary osmolytes, glutamate and glycine betaine at atmospheric pressures and high concentrations of β-hydroxybutyrate (β-HB) and oligomers of β-HB which were later termed as 'piezolytes' at optimum growth pressures of 28 MPa (Martin et al. 2002). Vezzi et al. (2005) reported the induction of TMAO (trimethylamine-*N*-oxide) reductase, a protein stabilizer at 28 MPa. The same study revealed a slight up-regulation of the putative PTS system, trehalose-specific IIBC component and putative trehalose-6-phosphate hydrolase at low pressure. Trehalose protects the cell from different stresses such as desiccation, dehydration, heat, cold and oxidation (Elbein et al. 2003). The SS9 genome contains 15 ribosomal operons, the maximum number so far reported in a bacterial genome. In addition to this, there is a high intragenomic variation among the rRNA operons which could be the major reason for different functionality at high pressure (Simonato et al. 2006).

Deep-sea archaea

Genera Thermococcus and Pyrococcus *T. barophilus* and *P. abyssi* The high-pressure induced protein studies have been carried out in two deep-sea hyperthermophilic species, *T. barophilus* and *P. abyssi*, grown under experimentally reproduced deep-sea and shallow hydrothermal vent conditions. In *T. barophilus*, an HSP (P60) belonging to the GroEL chaperonin family of HSPs was induced at low pressures of 0.3 MPa hydrostatic pressure and 0.1 MPa atmospheric pressure. It decreased under pressures >10 MPa, but increased at 40 MPa and 98°C. This is the optimal temperature limit for growth of this organism at high pressure (Marteinsson et al. 1999). Under optimum conditions, another protein, P35, was highly induced, but decreased at low pressure, a feature which was just opposite to the induction of P60 protein. Identical protein profiles were also produced in *P. abyssi* (Marteinsson et al. 1999).

Mesophiles

In the deep-sea habitat, the interactions of different parameters like pressure and cold produce complex stress response in mesophiles and it is practically very difficult to simulate the conditions in the laboratory. Model organisms such as *E. coli, B. subtilis, Lactobacillus sanfransicensis*, *S. cerevisiae* and *Schizosaccharomyces pombe* have been useful as powerful genetic tools and the piezophilic studies conducted in them are described here.

Genus Escherichia

E. coli The piezophilic studies in *E. coli* resulted in the induction of 55 PIPs, including 11 HSPs and 4 CSPs. Among these, DnaK and GroEL (molecular chaperones) are induced at 54.6 MPa. Even though, the pressure-induced protein unfolding is not evident in living *E. coli* cells, the presumed unfolded proteins may be substrates for DnaK and GroEL (Welch et al. 1993). The induction of both HSPs and CSPs, which is unique to pressure stress response, can be an attempt by *E. coli* to cope with the damaging effects of elevated pressure on membrane integrity and the stability of macromolecules (Bartlett 2002). Three pressure-inducible promoters of genes, *dnaK, lon* and *clpPX* have been recovered after high-pressure treatment for 15 min. They are marginally induced by subjecting the cells to a pressure of 75 MPa, but they are substantially induced at a higher pressure of 150 MPa. The HSPs encoded by these genes prevent cellular damage and/or aid recovery after high-pressure treatment (Abe 2007). High-pressure treatments in *E. coli* triggered the SOS response which induces DNA repair proteins such as LexA, RecA (Aertsen et al. 2004). A recent discovery shows the induction of an *mrr* gene encoding a cryptic endonuclease under SOS response (Aertsen et al. 2005). The mechanism of activation of *mrr* gene still remains to be known. Another notable effect of SOS response is the stoppage of cell division via the protein SulA. When the DNA damage is repaired the SOS response is switched off and cell division continues. In such cases bacteria may escape the classical detection methods, presenting serious health problems (Aertsen and Michiels 2007).

Genus Lactobacillus

L. sanfransicensis A sub-lethal pressure of 45 MPa for 30 min in *L. sanfransicensis* induced ribosomal proteins such as S2, L6 and L11 involved in the binding between 30S subunit and aa-tRNA (Pavlovic et al. 2005).

Fungi

Genera Saccharomyces and Schizosaccharomyces *S. cerevisiae* and *S. pombe* A 30-min-high hydrostatic pressure of 200 MPa in *S. cerevisiae* up-regulated *ole* 1 gene product (stearoyl-CoA desaturase) which might be to increase the proportion of unsaturated fatty acids (Fernandes et al. 2004). The exposure of yeast cells to sub-lethal pressure of 30 MPa resulted in the up-regulation of genes *ino*1,

*opi*3, *pst*1, *rta*1, *sed*1 and *prm*5 which play crucial roles in the response to membrane-structure stresses (Iwahashi et al. 2005). The induction of high levels of Tat1 and Tat2 proteins provides increased cell growth in *S. cerevisiae* under high pressures (Abe and Horikoshi 2000). In *S. cerevisiae*, the up-regulation of genes involved in carbohydrate metabolism (glycolysis, gluconeogenesis, glycogen metabolism) (Fernandes et al. 2004) during high pressures could be a stress response to meet the high-energy requirements (Yale and Bohnert 2001).

In yeasts, *S. cerevisiae* and *S. pombe*, the growth is arrested at pressures greater than 50 MPa (Abe 2007). It has been observed that conditions of 100 MPa pressure and temperatures higher than the optimum for growth (e.g., 42°C) enhances the synthesis of HSPs. Among the HSP104, HSC70 expressed, HSP104 plays an essential role in acquired tolerance by unfolding denatured intracellular proteins in an ATP-dependent manner (Sanchez and Lindquist 1990). HSC70 also has a role in piezotolerance in *S. cerevisiae* (Iwahashi et al. 2001). HSP12 and 26 were also observed to be induced along with HSP104 under lethal pressure of 180 MPa at 4°C (Iwahashi et al. 2003).

Analysis of *Hsp* genes in high pressure

In a study conducted by Fernandes et al. (2004) to understand the role of HSPs in high-pressure growth, it was observed that two genes encoding HSP12 and 30 were strongly induced under high pressure of 200 MPa at room temperature for 30 min in *S. cerevisiae*. HSP30 was induced in response to a variety of stresses like organic acid stress, high ethanol condition, entry into stationary phase and glucose scarcity. The other genes linked to up-regulation were also involved in energy metabolism (*pau* genes), oxidative stress (*grx1* and *cct1*) and heat shock response (*hsp12, hsp26, hsp104*, *hsp150*, *sse2* (Iwahashi et al. 2003). In another study, a subset of *hsp* genes was analyzed in *S. cerevisiae* to identify the genes responsible for growth under high pressure (Miura et al. 2006). It has been observed that with the loss of *hsp31*, cell growth is significantly retarded at a pressure of 25 MPa, suggesting the role of *hsp31* in high-pressure growth. HSP31 is 25.5 kDa protein and a possible chaperone and cysteine protease with similarity to *E. coli* HSP31 (Malki et al. 2005).

In piezophiles, there is induction of both HSPs and CSPs. Here, the pressure coexists with temperature, and the induction of HSPs even at low temperature, has its own critical role in cell survival. How HSPs and other heat-inducible factors protect cells from high-pressure stress is yet unknown, but they could be responsible for protecting membrane or protein stability.

Figure 1 provides a functional interaction map of stress proteins in a disturbed bacterial cell under piezophilic conditions. In order to shield the cell from a variety of stresses such as membrane disruption to DNA damage, stress proteins directs the cell to adopt a protective stationary phase until the damaged cell organelles are repaired. In the stationary phase, the stress proteins repair the DNA components and synthesize the related factors which help the exit of stationary phase and make the cell ready for further growth. They derive energy for all these functions by activating the ATP producing mechanisms/pathways.

Amino acids and piezophilic studies

Changes in pressure and temperature easily disrupt the chemical bonds in proteins which hold the molecules and polypeptide chains altogether. Not much information is available on the amino acid basis of piezophilic adaptation due to the lack of reports on bacterial sequences from deep-sea habitat. A study on the obligate piezophile, *Shewanella* sp. reported that amino acid composition in SSB protein greatly influenced the pressure adaptation. The increasing pressure parameter for the bacterium was accompanied by a reduction in two amino acids, namely, proline responsible for helix-breaking and glycine for helix-destabilization. It was observed that a decrease in proline and glycine residues reduced the flexibility of SSB in *Shewanella* PT99 (Chilukuri and Bartlett 1997). This means minimal glycine and proline residues favored the piezophilic adaptation. A proline to glycine substitution in staphylococcal nuclease increased the stability of the protein under hyperbaric conditions (Royer et al. 1993).

pH homeostasis in acidophiles and alkaliphiles

The genome of *Picrophilus torridus*, a thermoacidophile, has been extensively studied to explore the biochemical mechanism. Under pH variations, the DNA integrity of the bacterium is maintained by repair endonucleases (types III, IV and V), repair DNA helicases, proteins with MutT-like domains, and the repair proteins RadA-RadB-Rad50-MRE11 (archaeal homologs of recombinases) together with RecJ exonuclease homolog, topoisomerases and ligases. The chaperones, HSP60, 70, VAT protein, Lon2-related ATPase, HSP20 have been identified in the bacterium. The genome contains a large number of transporters involved in sugar–peptide–amino acid uptake systems, drug and trace elements transport. Most importantly, the occurrence of high ratio of secondary to primary solute transport systems indicates the predominant use of proton-driven secondary transport as a relevant strategy of the organism to the super acidic environment (Futterer et al. 2004).

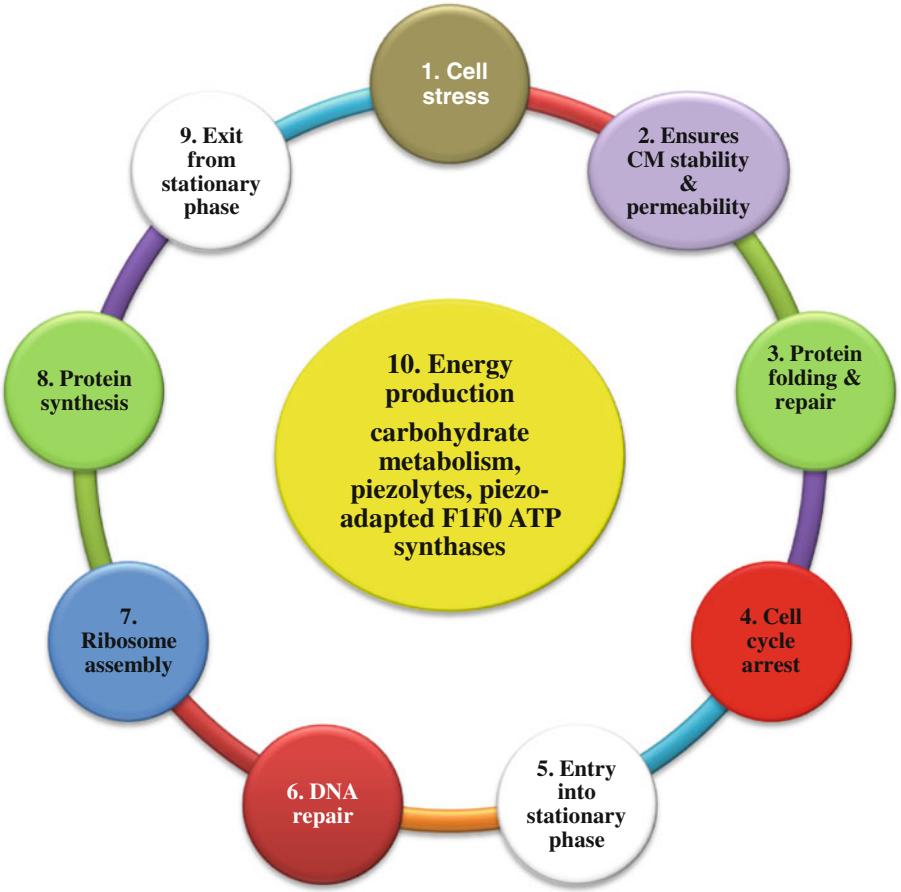

Fig. 1 Functional interaction map of stress proteins in piezophiles

The pH homeostasis in the facultative alkaliphile *Bacillus halodurans* C-125 has been well reviewed by Kitada et al. (2000). The study characterized a *shaA* gene encoding Na^+/H^+ antiporter system found to be essential for the survival. DNA regions homologous to *shaA* have been found in the genome of another alkaliphile *B. firmus* OF4. Generally, alkaliphiles maintain the pH homeostasis by H^+ coupled ATP synthase activity, high induction of monovalent cation/proton antiporters, changes in cell-surface characteristics and metabolic acid production by deaminases. Among these, anti-porters play an essential role in pH regulation (Mesbah and Wiegel 2008).

Radiation resistance in *Deinococcus*

The extremophilic, ubiquitous bacterium *Deinococcus radiodurans* is famous for its extraordinary radiation resistance mechanism. Research is in progress for the use of *D. radiodurans* as an effective agent for nuclear waste disposal. In such a context it would be relevant to examine the biochemical resistance mechanism in this microbe. In response to DNA damage, proteins such as RecA, KatA and EF-2 are synthesized. Even though many DNA repair genes and pathways have been predicted, only very few have been evaluated for their biochemical activities. The UvrA protein, a component of nucleotide excision repair, UV endonuclease-beta, uracil DNA glycosylase, DNA polymerase I and deoxyribophosphodiesterase have been identified in *D. radiodurans* (Makarova et al. 2001). Earl et al. (2002) identified that *IrrE* gene plays a central regulatory role in DNA damage repair mechanism in *D. radiodurans*. The genome of *D. radiodurans* revealed that the bacterium acquired the resistance genes such as topoisomerase IB, RNA binding proteins and *lea* from eukaryotes via lateral gene transfer (Marri et al. 2007). The increased expression levels of the general stress protein, DR1199, in *D. radiodurans* is suggested to be involved in the detoxification of the cell from ROS (Fioravanti et al. 2008).

Summary

The functional role and expression pattern of all the genes and proteins encountered in this study are summarized in Table 6. About ten proteins were commonly induced in different extremophiles (Table 7). Interestingly, among all the extremophilic conditions, halophily demands the

Table 6 Detailed gene/protein expression profile in different extremophiles of present review

No.	Function	Gene	Protein	Family
1. Thermophily				
1	Binding to unfolded proteins		TF55	Cct
2	Degradation of abnormal proteins		Protease La	ATP-dependent protease
3	Degradation of abnormal proteins		Subtilisin-like protease	Subtilisin-like protease
4	Folding of denatured proteins		Cpk A, B	Cct
5	Folding of misfolded proteins		Pfd A, B, C, D	Prefoldin
6	Folding of proteins		GroEL homolog	HSP60
7	Folding of proteins		DnaK homolog	HSP70
8	Folding of proteins, peptide-binding and mitotic spindle formation	*Cct1 & 2*	Cct1 & Cct2	Cct
9	Folding/refolding of denatured proteins		Thermosomes	HSP60
10	Function unknown in thermophiles		VapBC	Vap
11	Heat shock resistance		64–66 kDa	HSP70
12	Heat shock resistance		98 kDa	HSP100
13	Heat shock resistance		HSP X	sHSP
14	Hydrolysis of methionine from peptide		Methionine aminopeptidase	Methionine aminopeptidase
15	Osmotic stress prevention		Malto-oligosyl-synthase (MTSase)	Glycosyl-hydrolase 13
16	Oxidative stress resistance		CO dehydrogenase	Oxidoreductases
17	Oxidative stress resistance		MsrA–MsrB	MsrAB
18	Oxidative stress resistance		Peroxiredoxin	Peroxidases
19	Oxidative stress resistance		OsmC	OsmC
20	Protection from heat-induced apoptosis	*hsp70*	HSP70	HSP70
21	Protein metabolism		Proteasomes	Proteasomes
22	Protein metabolism		Endo-1,4-β-glucanase	Peptidase
23	Protein metabolism		Proline dipeptidase	Metalloprotease
24	Protein metabolism		Pyrolysin	Serine protease
25	Protein metabolism		Signal peptide I	Type I peptidase
26	Protein metabolism		Carboxypeptidase I	Carboxypeptidase
27	Protein metabolism		D-aminopeptidase	Aminopeptidase
28	Repair mechanism of DNA		Dps-like protein	Dps
29	Repair mechanism of DNA		Rad A & B	RecA
30	Repair mechanism of DNA		DinD	DinI-like family
31	Sugar metabolism		Chitinase	Glycosyl-hydrolase 18
32	Sugar metabolism		Trehalose/maltose-binding protein	ABC
33	Sugar metabolism		β-Glucosidase	Glycosyl-hydrolase 1
34	Sugar metabolism		Deacetylase	Sir2
35	Sugar metabolism		Endo-1,3-β-glucanase	Peptidase
36	Sugar metabolism		α-Amylase	α-Amylase
37	Sugar metabolism		β-Mannosidase	Glycosyl-hydrolase 1
38	Sugar metabolism		α-Dextrin-endo-1,6-α-glucanase	Glycosyl-hydrolase 13
39	Sugar metabolism		Trehalose synthase	Glycosyl-hydrolase 13
40	Surface protein expression		HtpX	sHSP
41	Synthesis of arginine		ArgE	Peptidase
42	Synthesis of glutamate		GdhA	GdhA
43	Unfolding and refolding of proteins		VAT homologs	AAA
44	Unfolding of protein and translocation into the 20S proteasome		PAN	AAA

No.	Function	Non-protein
45	Oxidative stress tolerance	Mannosyl glycerate (MG)
46	Oxidative stress tolerance	Trehalose

Table 6 continued

No.	Function	Protein	Family
2. Psychrophily			
1	Cold shock resistance	Cap	DEAD-box
2	Cold shock resistance	CSPs	CspA
3	Maintenance of membrane fluidity	Lipid desaturase	Fatty acid desaturase
4	Maintenance of membrane fluidity	β-Keto-acyl carrier protein	Transferases
5	Maintenance of membrane fluidity	β-Keto-acyl-coA	FAE
6	Maintenance of membrane fluidity	Fatty acid *cis–trans* isomerase	CTI
7	Protein folding	Prolyl *cis–trans* isomerase	PPIase family
8	Resist ROS	Catalases	Catalase
9	Resist ROS	Superoxide dismutase	SOD

No.	Function	Gene	Protein	Family
3. Halophily				
1	Adaptation to high salinity	*nhaC3*	Na^+/H^+ antiporter	NhaC
2	Amino acid metabolism	*aspC2*	Aspartate aminotransferase	GRPs
3	Biosynthesis of carbamoyl phosphate	*carA & B*	Carbamoyl phosphate synthase genes A, B	CarA
4	Cellular metabolism	*mdhA*	L-Malate dehydrogenase	Malate dehydrogenase
5	Cold shock resistance	*cspD1*	Cold shock protein D1	CSD
6	Converts pyruvate to Acetyl CoA		PdhA & B	Oxidoreductase
7	DinF-related damage inducible protein	*dfr*	Dihydrofolate-reductase	Dihydrofolate-reductase
8	Fatty acid metabolism	*Ifl3*	Long chain fatty acid CoA ligase 3	Ligase
9	Folding of protein		ATPase	AAA
10	Folding of protein		ClpC ATPase	Clp
11	Fosfomycin resistance	*gst*	Glutathione-*S*-transferase	GST
12	Global growth regulator	*hik5*	Histidine kinase	Histidine kinase
13	Glycolysis		Gap	Oxidoreductase
14	Heat shock resistance	*hsp5*	Heat shock protein5	HSP20
15	Heat shock resistance	*dnaK*		HSP70
16	Hydrolysis of UDP sugar	*ush*	UDP-sugar hydrolase	5′-nucleotidase
17	Iron starvation resistance	*sirR*	Staphylococcal iron-regulator repressor	DtxR
18	Osmotic shock resistance	*cct 1 & 2*	Cct 1 & 2	Cct
19	Oxidative stress resistance	*sod 1 & 2*	SOD 1 & 2	SOD
20	Oxidative stress resistance	*trxA1*	Thioredoxin	Thioredoxin
21	Peptide ABC transporters	*dppB1, C1, D1*	Dipeptide ABC transporter permease B1, C1, D1	ABC transporter
22	Protein metabolism	*cxp*	Carboxypeptidase	Carboxypeptidase
23	Reduction of pyruvate to alanine		Alanine dehydrogenase	Oxidoreductase
24	Salt stress tolerance	*htr12 & 13*	Htr 12 & 13	Htr
25	Salt stress tolerance		DnaK	HSP70
26	Salt stress tolerance		Ctc	L25
27	Signal transduction	*nosY*	NosY	Crp
28	Sugar uptake		Homolog of PTS enzyme, IIAB	Mannose family PTS transporters
29	Synthesis of cysteine		CysK	Transferases
30	Synthesis of glutamine	*glnA*	Glutamine synthetase	Crp
31	Synthesis of guanine		GuaB	IMPDH
32	Transcription regulator		GbuA	Secondary transporters
33	Transcription regulator		CcpA	LacI/GalR
34	Transcription repressor	*tbpC*	TATA-binding protein	TBP
35	Translation	*l2*	Protein belongs to large subunit of ribosome	L2
36	Translation	*l22*	Protein belongs to large subunit of ribosome	L22
37	Translation	*l23*	Protein belongs to large subunit of ribosome	L23
38	Translation	*l29*	Protein belongs to large subunit of ribosome	L29
39	Translocation of peptidyl tRNA from A-site to P-site of ribosome		EF-2	GTP-binding translation-elongation factor

Table 6 continued

No.	Function	Gene	Protein	Family
40	Transport of cobalt	*cbiN*	Cobinamide dicyanide N	CbiN
41	Transport of cobalt	*cbiQ*	Cobinamide dicyanide Q	CbiQ
42	Transport of H^+ ions	*atp* (*A*, *F*, *C*, *E*, *K*, *I*)	H^+ transporting ATP synthase subunits A, F, C, E, K, I	F1 ATPase
43	Transport of potassium	*trkA6*	K^+ transporter system protein	Trk
44	Transport of xanthine/uracil	*xup*	Xanthine/uracil permease	Slc26
45	Unknown function	*appA & B*	Periplasmic phosphoanhydride phosphohydrolase A, B	ABC transporter

No.	Function	Gene	Protein	Family
4. Piezophily				
1	Binding between 30S subunit and aa-tRNA		S2	S5P
2	Binding between 30S subunit and aa-tRNA		L11	L5P
3	Binding between 30S subunit and aa-tRNA		L6	L6E
4	Cell growth under high pressure		Tat1 & 2	Permeases
5	Destruction of misfolded proteins	*clpPX*	ClpP, ClpX	Clp
6	Energy metabolism	*pau*	Seripauperin	PAU
7	Essential for cell growth under high pressure	*hsp31*	HSP31	ThiJ
8	Essential for OmpL activation and OmpH repression		ToxR	OmpR
9	Folding of proteins	*lon*		ATP-dependent proteases
10	Folding of proteins		DnaK, DnaJ	HSP70
11	Folding of proteins		HSP60	HSP60
12	Heat shock response		HtpG	HSP90
13	Heat shock response		HSP30	HSP30
14	Hypothesized role in membrane—stability and heat shock response	*hsp12 & 26*	HSP12 & 26	sHSP
15	Hypothesized role in membrane stability	*hsp150*	HSP150	Pir
16	Hypothesized role in membrane stability	*sse2*		HSP70
17	Hypothesized role in membrane stability and unfolding of denatured intracellular proteins	*hsp104*	HSP104	HSP104
18	Maintain membrane structure composition		OmpI	OmpH
19	Oxidative stress resistance	*grx1*	Glutaredoxin1	GRX
20	Oxidative stress resistance	*cct1*	Cct1	Cct
21	Promotes the uptake of substrates >400 Da under high-pressure conditions		OmpH	OmpH
22	Repair of DNA		LexA	Winged-helix-turn-helix23
23	Repair of DNA	*mutT*	Mutator	Nudix
24	Repair of DNA		RecA	Recombinases
25	Repair of DNA	*recN*	RecN	SMC
26	Repair of DNA	*uvrA, D*		UvrA and UvrD
27	Resistance from membrane-structure stresses	*ino1*	Ino1	Inositol–choline regulated gene family
28	Resistance from membrane-structure stresses	*opi3*		Inositol–choline regulated gene family
29	Resistance from membrane-structure stresses	*pst1*		ABC transporters
30	Resistance from membrane-structure stresses	*prm5*		GAL
31	Resistance from membrane-structure stresses	*rta1*		LTE
32	Resistance from membrane-structure stresses	*sed1*		SED
33	Role in piezotolerance		HSC70	HSP70
34	SOS response	*mrr*		Mrr family
35	Stoppage of cell division-during SOS response		SulA	SulA
36	Synthesis of ATP		F1-F0 synthase	F1-F0 ATPase
37	Synthesis of ATP by Stickland reaction under anaerobic conditions		Glycine reductase	Oxidoreductase
38	Synthesis of monosaturated fatty acids	*ole1*		Fatty acid desaturase

No.	Function	Non-proteins
1	Carbohydrate metabolism	Putative trehalose-6-phosphate hydrolase
2	Cell growth at high pressures	Primary osmolytes: (1) glutamate, (2) glycine betaine

Table 6 continued

No.	Function	Non-proteins
3	Cell growth at high pressures	Piezolytes: (1) β-hydroxybutyrate (β-HB), (2) oligomers of β-HB
4	Cell growth at high pressure and low temperature	Monosaturated fatty acids: (1) octadecenoic acid, (2) tetradecenoic acid
5	Cell growth at high pressure and low temperature	Polysaturated fatty acids: (1) eicosapentanoic acid, (2) docohexanoic acid
6	PEP-dependent sugar phosphotransferase system, trehalose transport	Trehalose-specific IIBC component
7	Phosphotransferase system	Putative PTS system
8	Protection from desiccation, dehydration heat, oxidation	Trehalose
9	Protein stabilizer	TMAO (trimethylamine-*N*-oxide reductase)

No.	Function	Protein	Family
5. pH resistance			
1	Degradation of abnormal proteins	Lon2-related ATPase	ATP-dependent protease
2	DNA repair	Endonucleases	Endonuclease
3	DNA repair	DNA helicases	ATPase motors
4	DNA repair	Rad A, B, 50	RecA
5	DNA repair	Topoisomerases	Topoisomerase
6	DNA repair	DNA ligases	ATP-dependent ligase family
7	Heat shock resistance	HSP20	Shsp
8	Heat shock resistance	HSP60	HSP60
9	Heat shock resistance	HSP70	HSP70
10	Heat shock resistance and protein folding	VAT	AAA
11	Regulation of cation/proton transportation	Na^+/H^+ antiporter	NhaC
12	Transportation	Transporters	–

No.	Function	Gene	Protein	Family
6. Radioresistance				
1	Detoxification		DR1199	DJ-1
2	Detoxification		KatA	Kat gene family
3	DNA repair		RecA	Recombinases
4	DNA repair		UvrA	UvrA
5	DNA repair		UV endonuclease-β	Endonuclease
6	DNA repair		Uracil DNA glycosylase	Helix–hairpin–helix DNA glycosylase
7	DNA repair		DNA polymerase I	DNA polymerase
8	DNA repair		Deoxyribophosphodiesterase	Phosphodiesterase
9	DNA repair		Topoisomerase IB	Type IB topoisomerase
10	Radiation resistance	*IrrE*		GntR
11	Radiation resistance	*lea*		Lea
12	Translocation of peptidyl -tRNA in ribosomes		EF-2	GTP-binding translation-elongation factor

expression of highest number of transcripts/proteins (Table 8). Strangely, there are seven unifunctional but only three multifunctional proteins (Table 9).

The members of Cct family are found only in archaea and eukarya. The homologous nature of TF55 in Crenarcaheota with a eukaryotic protein showed evolutionary relatedness between archaea and eukarya. The absence of a homolog of bacterial HSP70/DnaK in some archaeal species such as *Methanococcus jannaschii*, *Archaeoglobus fulgidus*, *Pyrobaculum aerophilum*, *Pyrococcus horikoshii*, or *P. furiosus* was conspicuous. In psychrophilic yeasts, the absence of HSP104 is compensated by the induction of HSP110. In halophiles, the hypo-osmotic shock resembled heat shock response. Only in piezophiles, the simultaneous induction of both CSPs and HSPs has been reported.

Contrary to the accepted paradigm that there is one protein induced for one stress factor, we have noticed that it is not always true. The review highlights that some of the proteins are not specific but multifunctional and they switch from one role to another depending on the stress factor. An overlap of expression of proteins is observed in extremophiles. HSP70 and 60 are of universal occurrence with versatile functions such as heat shock resistance, folding of misfolded and/or denatured proteins and are expressed in all extremophiles irrespective of the stress factor. DNA repair proteins like RecA, RadA/B, UvrA/D

Table 7 Expression of overlapping proteins in extremophiles

Protein	Extremophile	Function
Hsp70 (DnaK)	Thermo-Psychro-Halo-Piezo-Acido	Heat-salt shock resistance and protein folding
RecA (RadA, B)	Thermo-Psychro-Piezo-Radiation-Acido	DNA repair mechanism
Hsp60	Thermo-Psychro-Piezo-Acido	Protein folding and heat shock resistance
F1F0 ATPases	Psychro-Halo-Piezo	ATP synthesis coupled proton transport
Cct	Thermo-Halo-Piezo	Protein folding and osmotic shock resistance
Cxp	Thermo-Halo	Protein metabolism
Csp	Psychro-Halo	Cold shock resistance
Sod	Psychro-Halo	Prevent ROS damage
UvrA	Piezo-Radiation	DNA repair mechanism
Na^+/H^+ antiporter	Halo-Alkali	pH homeostasis

Thermo thermophiles, *Psychro* psychrophiles, *Halo* halophiles, *Piezo* piezophiles, *Acido* acidophile, *Alkali* alkaliphile, *Radiation* radiation-resistant bacteria

Table 8 Summary table on the number of genes/proteins induced in different extremophiles

No. of genes/proteins	Extremophiles
44	Thermophily
9	Psychrophily
45	Halophily
38	Piezophily
12	Extremes in pH-resistant bacteria
12	Radio-resistant bacteria

Table 9 Number of unifunctional/multifunctional proteins in extremophiles

No.	No. of unifunctional proteins	No. of multifunctional proteins
1	CSP	HSP60
2	*Cxp*	HSP70
3	F1F0 ATPases	Cct
4	RecA	
5	*Sod*	
6	UvrA	
7	Na^+/H^+ anti-porter	

The metabolic proteins are given in italics

are also induced in all extremophiles. Proteins concerned with metabolism, i.e., Cxp, Gap, Pdh, CysK, RecA, Na^+/H^+ antiporter and F1F0 ATPases are expressed in considerable amounts indicating their crucial roles in extreme environments. Another interesting aspect from the review was the induction of vapBC and Dps in the genus *Sulfolobus*. VapBC is the virulence-associated protein induced in response to thermal stress. It is very similar to the induction/activation of RelA enzyme in bacterial survival strategies like stringent response and VBNC (viable but non-culturable) state while retaining the pathogenic potential in such states. Dps protein is reported to be an important virulence factor protecting the microbial genome from oxidative stress. There could be similar multifunctional proteins awaiting discovery in future.

Conclusion

Extremophiles inhabit the most hostile environments on earth. To acclimatize with the variations in physical and chemical parameters like temperature, salinity, pressure, radiation and pH they should possess highly flexible and dynamic biomolecular machinery. Though some proteins like OMPs in piezophiles are unique a commonality in stress resistance mechanism in extremophiles has been observed. Stress resistance mechanism is a complex but highly regulated macromolecular chain reaction cascading from extracellular to core region of cell, i.e., the stress factor invokes genes/proteins of signal transduction (membrane maintenance), metabolic pathways, DNA repair mechanism, protein and ATP synthesis. Most of the present knowledge on extremophilic microbes is from the in vitro studies. Simulated studies under piezophilic conditions are few and restricted to some laboratories in JAMSTEC, Japan, or University of Marseille, France. Such attempts in research would strengthen or give new perspectives on the adaptation mechanisms in extremophiles.

Extremophilic research: future perspectives

Stress proteins have various applications in industrial, biomedical and environmental realms. The properties such as resistance to elevated temperatures and low pH, allow *shsp* gene to be applied as a food-grade selection marker for *Streptococcus thermophilus* and other lactic acid bacteria (Hassan et al. 2003). Another important application of

chaperones in biomedical field is to increase the stability of vaccines to make them easier to deliver to remote parts of the world. Chaperones prevent the breakdown of proteins in the living cells found in certain vaccines or of the proteins in vaccine additives known as adjuvants that are necessary for making a vaccine effective. Dr. Frank Robb and his colleagues (University of Maryland, Center of Marine Biotechnology) are working towards these aspects of chaperones. Two examples of vaccines that might be improved through such research are those for typhoid and cholera. The application of *Deinococcus* spp. in the bioremediation of nuclear waste disposal is an active study area. Research is progressing in the application of HSPs as powerful markers for environmental monitoring. *Hsp70* has been used successfully recently as a first marker for the assessment of environmental chemicals (Mukhopadhyay et al. 2003).

Extremophiles have been a fascinating area of research not only because they represent the ancient forms of life on earth, but also because of their stable biochemical machinery over time and generations. In the present context of the controversial Panspermia theory of origin of life, the scientists are actively involved in research on the probability of bacterial migration to earth from other planets and perhaps from earth to space. Understanding life under extreme conditions would help this pursuit and the best models for such studies would be microorganisms like bacteria and archaea. Microbes flourish in diverse habitats such as those that thrive near the boiling point of water, in deep-sea pressures above 1000 bars and in poles below the freezing point of water. The advanced branch of biology, Astrobiology concentrates on the discovery of life or signs of life on planets beyond earth. Elucidating the participation of crucial cellular components which shield the bacterial genome from challengeable conditions would enable furthering the research in that direction.

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General glossary

AAA	ATPases associated with a variety of cellular activities
ABC	ATP-binding cassette
ArgE	Acetylornithine deacetylase
AspC2	Aspartate aminotransferase
CAP	Cold acclimation protein
CarA	Carbamoyl phosphate synthase A
CcpA	Catabolite control protein A
Cct	Chaperones containing t (tailless)-complex polypeptide
CIP	Cold induced proteins
ClpPX	Caseinolytic proteases: ClpP and ClpX
CO	Carbon monoxide
Cpk A and B	Alpha, beta subunits of chaperonin from *P. kodakaraensis*
Crp	Cyclic AMP receptor protein
CSP	Cold shock protein
Ctc	General stress protein
CTI	*Cis–trans* isomerase
Cxp	Carboxypeptidase
CysK	Cysteine synthase
DEAD box	D(asp)-E(glu)-A(ala)-D(asp) box family of helicases
DinD	DNA damage-inducible protein
DinI-like	DNA damage-inducible protein-like
DnaK	Prokaryotic analogue of eukaryotic HSP70
DnaJ	Prokaryotic analogue of eukaryotic HSP40
Dps	DNA-binding protein from starved cells
DtxR	Diphtheria toxin repressor
EF-2	Elongation factor 2
F1F0	ATPases-H^+ transporting two-sector ATPase
FAE	Fatty acid elongation
FKBP	FK 506 binding protein
Gap	Glyceraldehyde-3-phosphate dehydrogenase
GbuA	A subunit of the glycine betaine transport system GbuABC
GdhA	Glutamate dehydrogenase
GlnA	Glutamine synthetase
GMP	Guanosine monophosphate pathway
GntR	Repressor of gluconate operon in *Bacillus subtilis*
GroEL	Prokaryotic homolog of HSP60
GRP	Glucose regulated protein
Grx1	Glutaredoxin 1
GSP	General stress protein
GST	Glutathione-*S*-transferase
GuaB	Inosine-5′-monophosphate dehydrogenase
HSC	Heat shock cognate
HSP	Heat shock protein
HtpG	High-temperature protein G
HtpX	‘Protein for high temperature production X
Htr	Halobacterial transducer
Htr 12 and 13	Halobacterial transducers 12, 13
IMP	Inosine monophosphate
IMPDH	Inosine monophosphate dehydrogenase
Ino1	Inositol-1-phosphate synthase encoding gene
IrrE	Regulator of radiation resistance

KatA	Catalase A
L 6 & 11	Protein belongs to the large (L) subunit of ribosome
LacI/GalR	Lactose galactose repressors
Lea	Late embryogenesis abundant protein
LexA	DNA-binding transcriptional repressor of SOS regulon
Lon	ATP-dependent Lon serine protease
LTE	Lipid-translocating exporter
MG	Mannosyl glycerate
Mrr	Methylated adenine and cytosine restriction protein
MsrAB	Methionine sulfoxide-reductase
MRE11	DNA repair protein
MutT	Mutator
NhaC3	Na^+/H^+ antiporter
NosY	Nitrous oxide reductase Y
Nudix	Nucleotide diphosphate linked to an X moiety
Ole1	Delta (9) fatty acid desaturase gene which synthesizes oleic acid
OMP	Outer membrane protein
OmpH	Outer membrane protein high pressure
OmpH/Skp	Outer membrane protein H/Seventeen kilo Dalton protein
Ompl	Outer membrane protein lipoprotein
OmpL	Outer membrane protein low pressure
OmpR	Outer membrane protein regulator
Opi3	Overproducer of inositol protein 3
OsmC	Osmotically inducible protein C
OSP	Osmotic stress protein
PAN	Proteasome-activating nucleotidase
Pau	Seripauperin gene
Pdh A & B	Pyruvate dehydrogenase alpha, beta subunits
Pfd A to D	Prefoldin
PIP	Pressure-induced proteins
Pir	Proteins with internal repeats
Prm5	Pheromone-regulated membrane protein
Pst1	Phosphate-specific transporter 1
PTS	Phosphotransferase system
RadA, B, 50	Archaeal recombinase protein homologs
RecA, N	Recombinase A, N
RecJ	Helicase exonuclease protein
Rta1	Resistance to 7-aminocholesterol gene
S2	Protein belongs to the small (S) subunit of ribosome
SAP	Stress acclimation protein
Sed1	Suppressor of Erd2 deletion gene
ShaA	Sodium–hydrogen antiporter gene A
sHSP	Small HSP
Sir2	Silent information regulator2
Slc26	Solute carrier 26
SMC	Structural maintenance of chromosomes
SOD	Superoxide dismutase
SSB	Single-stranded DNA binding protein
Sse2	*Saccharomyces cerevisiae* HSP70 protein
SSIP	Salt stress-induced protein
SSP	Salt shock protein
SulA	SOS cell division inhibitor
Tap A and B	Temperature-adapted proteins A, B
Tat 1and 2	Tryptophan permeases
TBP	TATA-binding protein
TF55	Thermophilic factor55
ThiJ	Thiamine biosynthesis protein
ToxR	Regulation of cholera toxin protein
ToxS	19 kDa Transmembrane regulatory protein
TPS	Trehalose phosphate synthase
TPP	Trehalose phosphate phosphatase
Tres	Trehalose synthase
Trk	K^+ transporter
UvrA, D	UV light repair A, D
VapBC	Virulence-associated protein B, C
VAT	Valosine-containing protein-like ATPase from *Thermoplasma acidophilum*

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