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Mini Review

MICROBES THRIVING IN EXTREME ENVIRONMENTS: HOW DO THEY DO IT?

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Abstract

Our knowledge about habitat of microorganisms appears diminutive when we witness amazing flexibility in choice of survival under various conditions. Extremophiles refers to the organisms living and carrying out vital life processes at extreme conditions of temperature, pressure, pH, salt concentration among others and this is why they have attracted attention of researchers worldwide. There is a continuous quest to unreveal the probable mechanism or structural and functional adaptations that make extremophiles survive under other holistic conditions. There occur modifications primarily in cell membrane, DNA, RNA, protein and enzymes in order to render fit microbial cell to its external environment. Thus, extremophiles are robust source of high temperature and alkali stable enzymes. Various enzymes as lipase and protease have found several applications in food and cosmetic industry while Taq polymerase from bacteria *Thermus aquaticus* has revolutionized entire scene of molecular biology. Present review focuses on extremophiles, their structural and molecular adaptations to overcome unfavorable conditions of environment.

Key words: Archaebacteria; halophile; Thermophile; Barophile

Introduction

Microbes are present diverse habitats, ranging from suitable environment of soil, water or can be isolated from hostile habitats such as desert, caves, hydrothermal vents, saline lakes, glaciers etc. Term Extremophile is coined for the organisms growing optimally under extreme environmental conditions of temperature, pressure, salinity and pH but cannot grow under normal conditions. Although extremophiles include different taxa of bacteria and eukarya but archaea dominate such ecological niches and even some of them survive where conditions are prohibitive for other life forms. Extremophiles are well adapted to unfavorable environmental factors and have huge biotechnological potential. In order to adapt in harsh conditions of temperature, pH, alkali etc. microbes modify their cellular and molecular components and sustain well (Bertemont and Gerday, 2011). Enzymes synthesized by them have enhanced activity (stability), as a rule, and can function under high temperatures, salt concentrations (haloenzymes), conditions of high alkalinity (alkaloenzymes) and under other extreme conditions (high pressure, acidity and so on (Bertemont and Gerday, 2011). Extremophile enzymes express increased stability to several environmental factors that make possible their wide use during industrial biotechnology elaboration. The majority of the industrial enzymes known to date have been derived from both bacteria and fungi (Naidu and Saranraj, 2013). The global annual enzyme market is around five billion

Euros (Kunamneni *et al.*, 2005). Biologically active substances, synthesized by extremophiles have already found a way to medicine, cosmetology and food industry (Vieille and Zeikus, 2001). In addition, proteins and enzymes from extremophiles are usually suitable for biochemical and structural analyses due to their physico-chemical stability and a lot of proteins from these organisms have paved the way for elucidating the structure-function relationships in their mesophilic homologs. Microbes in such extreme environments as a rule deploy certain strategies according to the external environmental. These adaptations can be at any level viz., cellular, molecular or cytoplasmic level.

Thermophiles

For temperature as a factor, microorganisms are divided into psychrophiles (optimal growth temperature $\leq 15^{\circ}\text{C}$), mesophiles ($T_{opt} \approx 37^{\circ}\text{C}$), thermophiles ($T_{opt} > 50^{\circ}\text{C}$) and hyperthermophiles ($T_{opt} > 80^{\circ}\text{C}$ (Madigan and Martino, 2006). Certain members of all three domains of life bacteria, eukarya and archaea are equipped to grow and survive well at higher temperature. Thermophiles are among the best studied of the extremophiles. Thermophilic microorganisms are more widespread than hyperthermophiles and are represented by various bacterial species, including photosynthetic bacteria (cyanobacteria, purple and, green bacteria), enterobacteria (*Bacillus*, *Clostridium*, and some others), Thio bacteria bacteria (*Thiobacillus*), and archaeabacteria (*Pyrococcus*, *Thermococcus*, *Sulfolobus* and

methanogens) (Vieille and Zeikus, 2001). The maximum known temperature limit of growth (113°C) was found in nitrate reducing chemolithoautotrophic archae *Pyrolobus fumarii* (Kashefi and Lovley, 2003). Some of these features are modifications in the metabolic pathways of synthesis of cofactors like heme, acetyl CoA, acyl CoA, and folic acid which are either greatly reduced or are eliminated in thermophiles because of high temperature constraints (Merkley *et al.*, 2010). Presently, more than 70 species, 29 genera, and 10 orders of thermophiles are known, interestingly, most of them are archae (Madigan and Martino, 2006). Then, hyperthermophilic bacteria were found that grow even at 121°C (Daniel and Cowan 2000) and cannot live under a temperature lower than 80–90°C temperature.

1. Cell Membrane Modifications

Molecular mechanisms used by two distinct groups of hyperthermophiles archae and bacteria to survive under high temperatures, differ markedly. Membrane lipids of archae differ from that of bacterial cells by the presence of an L-isomer of glycerin, instead of D-glycerin, the presence of saturated polyisoprene phytanol (3,7,11,15-tetramethylhexadecyl) (C20) with four side -CH₃ groups instead of unbranched saturated fatty acids with length of 16–18 carbon atoms in the structure of membranes studied earlier, and also by the presence of simple ether bonds between L-glycerin and phytanol (with one oxygen atom) (van de Vossenberg *et al.*, 2001). Branching of side chains can cause the integration of phytanol molecules inside one layer and with the other layer with biphytanol formation (C40) in the composition of special “monolayer” membranes, which are 2.5–3.0 nm thick and are peculiar to archaebacteria, unlike the typical phosphodiester (C18) “bilayer” membranes of bacteria (De Rosa *et al.*, 1986). The unique lipids—macrocyclic archaeol and transmembrane caldarchaeol nonitolcardarchaeol—found only in archaebacteria, cause 10–17 fold decrease of membrane permeability, and the presence of cyclopentane circles and phosphatidylinositol leads to tighter lipid packing that decrease lateral mobility of molecules in membranes of thermophilic archaebacteria (Vossenberg *et al.*, 2001; Kitano *et al.*, 2003). Such modifications of membrane lipids, as acylation, saturation, branching and (or) formation of cyclic structures of hydrocarbon chains have been found in bacteria, growing under high temperatures (Tolner *et al.*, 1998).

2. DNA and RNA Modifications

Denaturation resistance of DNA under extreme conditions of archaebacteria habitation is attributed by positively charged DNA binding proteins—the analogs of eucaryotic histones, polyamines with

relatively high content of nucleotide guanine—cytosine (G–C) pairs and (or) high intracellular salt concentrations (Hamana *et al.*, 2003). Several structural variations of cellular polyamines, stabilizing DNA, as well as secondary structures of RNA in extremophilic archaebacteria exist viz., Putrescine (H₂N–(CH₂)₄–NH₂), linear triamines spermidine (H₂N–(CH₂)₃–NH–(CH₂)₄–NH₂), norspermidine (H₂N–(CH₂)₃–NH–(CH₂)₃–NH₂) and homospermidine, tetraamines spermine (H₂N–(CH₂)₃–NH–(CH₂)₄–NH–(CH₂)₃–NH₂) and norspermine, penta and hexaamines, guanidoamine agmatin, quaternary branched pentaamine N4 bis (aminopropyl) spermidine and acetylated penta-amine are arranged nonuniformly inside the cell of archaebacterial genera; however, as a whole, their structural diversity and relative content is higher than bacteria and eucaryotes (Hamana *et al.* 2003). Comparative analysis of intracellular polyamines of bacteria and archaebacteria, isolated from mesophilic and hyperthermophilic sources, showed that several polyamines (norspermine and norspermidine) are exclusive to hyperthermophilic archaebacteria only (Kneifel *et al.*, 1986). The higher content of G–C pairs can be also the reason for additional stabilization of DNA structure in hyperthermophiles, as it is more stable due to an additional hydrogen bond in comparison with the adenine-thymine (A–T) pair. As a result of the studies of G–C pair content in DNA, 23S, 16S, and 5S rRNA, and also tRNA, the direct correlation between optimal growth temperature of microorganism (*T*_{opt}) and the content of G–C pairs in rRNA and tRNA was revealed, whereas such correlation for DNA was not found (Wang *et al.*, 2006). The alternative mechanisms, that can stabilize secondary structure of a DNA molecule, are its association with cationic proteins (HMf and Sac families, DNABPII), which provides additional DNA spiralization and nucleosome formation, and also the presence of ATP-dependent topoisomerase type I (reverse gyrase) in all hyperthermophilic bacteria and archaebacteria, due to which the DNA molecule has a positive direction of spiralization. A number of authors advocate that the presence of reverse gyrase in hyperthermophilic archaebacteria is the very authentic adaptive mechanism providing for the survival of microorganisms under high temperature (Brochier-Armanet and Forterre, 2007).

The increase of stability of secondary and tertiary structures of tRNA is also attained by posttranslational nucleoside modification; a number of such modifications are hallmark of archaebacteria (Edmonds *et al.*, 1991). The most frequently observed modification is 2'O-methylation of ribose, the degree of methylation increases with the temperature growth

of microorganism habitation. e.g three ribonucleoside modifications were found in *Pyrococcus furiosus* cells, growing at 70, 85, and 100°C, as the temperature grows their relative portion grows to provide conformational stability (Robb *et al.*, 2001).

3. Enzymes and Protein Modifications:

Extremophile enzymes are characterized with increased thermostability and higher reaction rate, with activity optimum at more than 90°C, as a rule (Hough and Danson, 1999). While, enzymes, isolated from mesophiles, exhibited their maximum activity at 25–60°C and that of psychrophiles at 5–25°C (Vieille and Zeikus, 2001). The comparative analysis of available crystallized structures of proteins from hyperthermophilic bacteria and their analogs, isolated from thermophilic and mesophilic organisms, revealed marked differences responsible for providing stability of protein molecule under extremely high temperatures (Adams and Kelly, 1994). To date no single factor is responsible for increased stability of thermophilic enzymes. Several factors seem to confer stability as: the lower content of thermolabile amino acid residues, such as asparagine and cysteine; higher number of hydrophobic interactions; interaction of aromatic groups and higher number of ionic pairs, hydrogen bonds and saline bridges; the alteration of α -helix to β -layer ratio; compactness of molecule, decrease of the number and size of peptide loops at the molecule surface; oligomerization and inter subunit interactions, reduced ratio of surface to volume; and increased thermophobia and surface charge, less exposed thermolabile amino acid and truncated amino and carboxy terminus (Hough and Danson, 1994). Mobility of hyperthermophilic proteins under room temperature is lower than that of thermophilic and mesophilic organisms as the conformational mobility of atom increases with increase in temperature (Závodscky *et al.*, 1998).

Heat shock proteins (HSP) are found in many extremophilic microorganisms, and are considered as one of the main “responses” to the influence of stress factors, of extreme temperature values, dehydration, chemical stress and starvation (Laksanalamai and Robb 2004). HSP are classified by a value of molecular weight to high molecular (HSP100, HSP90, HSP70, HSP60) and low molecular (sHSP with molecular weight from 15 to 42 kDa). Most of those proteins establish functions of molecular chaperons, preventing protein aggregation, providing refolding of denatured proteins, and also participating in the folding of newly synthesized ones (Laksanalamai and Robb, 2004). Homologs of heat shock proteins were found in genomes of all the classes of extremophilic organisms.

Psychrophiles

More than 80% of the earth's biosphere is permanently below 5°C, and a surprising variety of microorganisms have been discovered from these seemingly inhospitable regions across the globe (van de Vossenberg *et al.*, 1998), polar regions, mountains, and deepwater seas, freshwater and marine water lakes (Deming, 2002). A psychrophilic and slightly halophilic methanogen, *Methanococcoides burtonii* was isolated from perennially cold, anoxic hypolimnion of Ace Lake, Antarctica suggesting that members in the domain archaea are also capable of psychrophilic life style (Franzmann *et al.*, 1992). Low temperature level of life subsistence is determined by the point of water freezing inside a cell. Crystallization of intracellular water is lethal for every organism, except nematode *Panagrolaimus davidi*, which is capable of outliving the freezing of all body water (Wharton and Ferns, 1995). Members of *Shewanella*, *vibrio*, *Micrococcus*, *Methangenium*, *Bacillus* are Psychrophilic (Lloyd, 1961). The genus *Moritella* are known to be psychrophiles only (Richard *et al.*, 2004). Archaea, cold loving organisms that inhabit areas below 15°C and often thrive at temperatures near the freezing point of water. Permanent exposure to cold has necessitated adaptations to overcome two main challenges: low temperature and the viscosity of aqueous environments (D'Amico *et al.*, 2006). These two challenges are addressed by a variety of adaptations as: reduced enzyme activity, membrane fluidity, genetic expression, protein function, altered transport of nutrients and waste products, and intracellular ice formation (D'Amico *et al.*, 2006). The aim is to preserve the structural integrity, supply the necessities for metabolism, and regulate metabolism in response to the changes in the environment. Sometimes they synthesize glycoproteins and peptides as a means of antifreeze properties. These substances significantly decrease the freezing point of the fluid in cytoplasm and organelles due to anticoagulation processes and called as thermal hysteresis proteins (Feller and Gerday, 1997). Psychrophilic bacteria have exceptional ability to survive and grow at low temperatures. Several mechanisms are responsible for their adaptation to low temperature such as the ability to modulate membrane fluidity, ability to carry out biochemical reactions at low temperatures, ability to regulate gene expression at low temperatures, and capability to sense temperature (Shivaji and Ray, 1995; Ray *et al.*, 1998).

A. Membrane fluidity and polyunsaturated fatty acids

Membranes and enzymes become rigid at low temperatures. Reduced membrane fluidity results in reduced membrane permeability and adversely affects the transport of nutrients and waste products across the membrane (Goodchild *et al.* 2004). The ability of psychrophiles to function at

low (but not moderate) temperatures is due to adaptations in cellular proteins and lipids that form a barrier between the cytoplasm and the extreme environment and aid maintenance of optimal membrane fluidity.

Unlike mesophilic bacteria, psychrophiles have higher levels of unsaturated fatty acids that can further increase with reduction in temperature, hence modulating membrane fluidity. It was found that thermophilic, mesophilic, psychophilic species of Clostridia contained an average of 10, 37, and 52% of unsaturated fatty acid, respectively. Phenotypic adaptation is also characterized by increase in fatty acid unsaturation and chain shortening in response to lowered temperatures (Nichols *et al.*, 1992, 2004). Increased contents of unsaturated, polyunsaturated, and methyl-branched fatty acids with high proportion of cis-unsaturated double bonds and antiesio-branched fatty acids allow the membrane to remain in a liquid crystalline state (through reduced gel-liquid-crystalline phase transition temperature, T_m) at low temperatures (Chintalapati *et al.*, 2004; Russell, 1990). These changes in composition increase membrane fluidity by introducing steric constraints that hinder molecular interactions within the membrane. An appropriate level of cytoplasmic membrane permeability is essential for the provision of energy, metabolites, and intracellular environment needed for metabolism and, consequently, is a determining factor of maximum temperature for growth (Russell, 1990). Recent studies have linked membrane permeability (and thus fatty acid unsaturation) to proton motive force (PMF; directed into cell) and sodium motive force (SMF; directed out of cell). These translocated, energy-coupling ion pumps result in an electrochemical gradient that catalyzes energy transduction. Proton permeability of liposomal membranes derived from various Archaea that live in different temperatures was observed and found to be maintained within a narrow range at their respective growth temperatures (van de Vossenberg *et al.*, 1999). Thus, the role of the cell membrane in survival is to adjust lipid composition in order to maintain constant homeo-proton permeability (Nichols *et al.*, 2004). Carotenoids are also shown to regulate membrane fluidity due to their temperature-dependent synthesis (Jagannadham *et al.*, 2000).

B. Ice crystallization and antifreeze proteins

The debilitating effects of ice crystallization pose a major threat to all psychophilic organisms. Archaeal homologues of antifreeze proteins (AFPs) are distributed in bacteria, plants and fish

are believed to function similarly in psychophilic archaea to allow growth and survival in subzero environments. AFPs possess large surface area well-suited for effectively binding to ice crystals, inhibiting extensive nucleation and thus reducing the freezing temperature (Lillford and Holt, 2002). AFP's are large, have relatively flat surface and believed to bind irreversibly to ice through van der Waals and hydrogen bonding (Jia and Davies, 2002). Low concentrations ($5\text{-}160 \mu\text{g ml}^{-1}$) of AFPs are also extremely effective at inhibiting ice recrystallization during the thawing process by adsorbing to the ice and blocking the addition of water molecules to the more energetically favorable crystals (Carpenter and Hansen 1992). High concentrations ($>1.54 \text{ mg ml}^{-1}$) of AFPs, however, damage the cells by inducing preferential growth of ice around the cells during the thawing process. Since the capacity of a species to produce AFPs is reflective of the species' living circumstances, increasing efforts are being made to explore the potential of psychophilic Archaea by manipulating the freezing temperatures and ice recrystallization.

The presence of cold active enzymes and the ability to support transcription and translation in psychrophiles is also studied. These studies have also revealed the presence of certain genes which were active at low temperature (Janiyani and Ray, 2002). Differential phosphorylation of membrane proteins and probably lipoproteins (LPS) has also been attributed to temperature sensing. Recent studies of low-temperature induced changes in the composition of LPS highlight the importance of LPS in cold adaptation. Sea-ice microbes produce polymeric substances, which could serve as cryoprotectants both for the organisms and their enzymes (Anchordoquy and Carpenter, 1996). In response to sudden changes in environmental temperature (cold shock), psychrotrophs and psychrophiles, such as *Trichosporon pullulans*, *Bacillus psychrophilus*, *Aquaspirillum arcticum* and *Arthrobacter globiformis*, synthesize cold shock and cold-acclimation proteins, which function as transcriptional enhancers and RNA-binding proteins (Berger *et al.*, 1996).

Pressure

Under high pressure conditions, progress of chemical reactions with volume increase is impossible, besides, densification of spatial organization of lipids occurs under the influence of pressure factor, which leads to the damage of the cellular wall and cytoplasmic membrane. High pressure can be a reason for decrease of cell growth rate and biomass new production because of the suppression of

interaction of proteins with various substrates and enzyme inactivation (Zobell and Johnson, 1949).

ZoBell and Morita (1957) were among the first to attempt to isolate microorganisms specifically adapted to grow under high pressures, and they called these bacteria barophiles. The first barophilic bacteria to be isolated were reported in 1979 (Yayanos *et al.*, 1979). Barophilic bacteria are defined as those displaying optimal growth at pressures >40 MPa, whereas barotolerant bacteria display optimal growth at a pressure <40 MPa and can grow well at atmospheric pressure. Piezophiles (barophiles) can be found up to the depth of more than 10 km (the Mariana Trench—10898 m) under the pressure of 130 MPa. Optimal pressure ranging from 70–80 MPa for obligate piezophiles to 100–200 MPa for certain members. Whereas pressure value lower than 50 MPa serves as critical for their growth. These unique ecological niches as bottom of sea trenches are offered not only with high pressure, but also with extreme temperature value (from 1–2°C to 400°C near hydrothermal sources), that's why the piezophile group can be further divided to psychrophilic, mesophilic, and thermophilic microorganisms in accordance with growth temperature (Kato *et al.*, 1995). Pressure increase, results in enzyme dissociation protein–protein interactions are very sensitive to pressure. Gross *et al* (1993) demonstrated that *E. coli* ribosomes dissociate under the pressure of more than 60 MPa. Pressure exceeding 300 MPa causes denaturation of monomeric proteins, as the volume of denatured protein is less than that of native one (Heremans and Smeller, 1998).

When genomes of barophilic microorganisms with bacteria living in atmospheric pressure (e.g. *E. coli*) were compared it showed the presence of a conservative regulatory system, responsible for organism adaptation to high pressure (Horikoshi, 1998). During a study on gene expression in psychrophilic piezophiles *Photobacterium profundum* SS9 under conditions of high pressure it was found that the OmpH protein, which is responsible for transport of nutrients into the cell, was found and its maximum expression level was observed under optimal pressure for microorganism growth (28 MPa). Under conditions of normal pressure value (0.1 MPa), this protein was not synthesized and its function was provided by the other protein (OmpL) (Welch and Bartlett 1996). Besides that, it was shown that the rpoE like locus of SS9 strain participates in the regulation of expression of OmpH protein and is necessary both under conditions of high pressure and under low growth temperature (Chi and Bartlett 1995). This conservative operon was then identified in many other deepwater bacteria (Li *et al.*, 1998). Further it was proposed that transmembrane protein ToxR and ToxS take part in the regulation system of gene expression. The complex of these proteins ToxR–ToxS serves as sensor of fluctuation of temperature, pH, salt concentration, and nutrients in *Vibrio*

cholerae and participates in transcription. In *Shewanella violacea* DSS12 under conditions of high pressure gene expression is controlled by the sigma 54 promoter (Kawano *et al.*, 2004). Transcription activation through RNA polymerase sigma 54 is controlled by specific proteins—NtrB and NtrC—that were earlier identified as signal proteins, participating in nitrogen exchange (Nakasone *et al.*, 2002).

pH

Most biological processes occur at neutral pH but certain organisms have successfully adapted themselves to cope with external acidic or alkaline pH. Depending on the environmental pH, various microorganisms are referred to as acidophiles (growth optimum at pH<2), neutrophils (growth optimum at pH 6–8) and alkaliphiles (that are active at pH>9) (Morozkinaa *et al.*, 2010).

Archaeabacteria can grow under extremely low environmental pH. Aerobic heterotrophic archaeabacteria *Picrophilus oshimae* and *Picrophilus torridus* isolated from Japanese soils soaked with sulphuric acid (1.2 M) were able to grow at pH 0.7 and 60°C. Grown as a result of hydrogen sulphide oxidation by sulphur-oxidizing bacteria. These microorganisms maintain intracellular pH value at 4.6, whereas that of the other extreme acidophiles amounts to 6.0 (Schleper *et al.*, 1995). Acid mine outlets are a source of thermophilic acidophiles, capable to oxidize (*Leptospirillum thermoferrooxidans*) and reduce (*Acidiphilum* spp.) iron. Archaeabacteria *Sulfolobus acidocaldarius* grow under pH 3.0 and 80°C. Thermophilic haloalkalo-tolerant bacteria *Anaerobranca* spp. were isolated from ecological niches with high salt concentrations and extreme pH and temperature values (Morozkinaa *et al.*, 2010).

Acidophiles maintain their internal pH close to neutral and therefore, maintain a large chemical proton gradient across the membrane. Acidophilic and alkaliphilic microorganisms have effective mechanisms to maintain intracellular ionic homeostasis and keep pH values close to neutral inside cells. Acidophiles have high protonic chemical membrane gradient (ΔpH) that, in *Picrophilus oshimae*, for instance, amounts to more than 4 pH units and serves as the basic proton_driving force. Thus, acidophilic microorganisms have to compensate ΔpH with the change of the charge of transmembrane potential ($\Delta\Psi$) from negative to positive inside a cell, forming it by active cell accumulation of positively charged ions, K^+ . Proton movement into the cell is minimized by an intracellular net positive charge; the cells have a positive inside membrane potential caused by amino acid side chains of proteins and phosphorylated groups of nucleic acids and metabolic intermediates that act as titrable groups. As a result, the low intracellular pH leads to protonation of titrable groups and produces net intracellular positive charge. In *Dunaliella acidophila*, the surface charge and inside membrane potential are positive, which is expected to reduce influx of

protons into the cells. It also over expresses a potent cytoplasmic membrane H⁺-ATPase to facilitate efflux from the cell. The acid stability of rusticyanin (acid-stable electron carrier) of *T. ferrooxidans* has been attributed to a high degree of inherent secondary structure and hydrophobic environment in which it is located in the cell. A relatively low level of positive charge has been linked to acid stability of secreted proteins, such as thermopsin (a protease of *Sulfolobus acidocaldarius*) and an *a*-amylase of *Alicyclobacillus acidocaldarius*, by minimizing electrostatic repulsion and protein folding. Alternatively bacteria use H⁺ containing vesicles to avoid cytoplasmic acidity (Sorokin, 2003).

Alkalophiles

Alkalophiles are the microorganisms growing under extremely high environmental pH values (10.0–11.0). They may be divided into two physiological groups: essential alkalophiles and haloalkalophiles. Alkalophiles grow under pH 9.0 and higher, whereas haloalkalophiles need not only alkaline conditions (pH ≥ 9.0), but also high NaCl concentrations (≥33%). Haloalkalophiles are usually found in extreme habitats, such as soda lakes in Eastern Africa, the United States and the Russian Federation. Microorganism communities, dwelling under pH 10.0 and 10% mineralization degree, contain mainly alkalophilic cyanobacteria. There occur high diversity within genera of alkalophilic microorganisms and are represented with various aerobic (*Bacillus*, *Micrococcus*, *Pseudomonas*, *Streptomyces*), anaerobic (*Clostridium thermohydrosulfurican*), thermophilic (*Anaerobranca horikoshii*, *Clostridium paradoxum*) bacterium species and hyperthermophilic archaeabacteria (*Thermococcus alcaliphilus*) (Zavarzin *et al.*, 1999). Haloalkalophilic microorganisms are also represented with different genera of bacteria, archaeabacteria, cyanobacteria (*Synechocystis*, *Nostoc*), methanogens (*Methanosa*l_s *zhilinaeae*) and sulphur oxidizing microorganisms (Sorokin, 2003).

When growing in the environment with alkaline pH values and, therefore, low proton and high Na⁺ ion outer support, alkalophilic microorganisms need not only osmoadaptation, but also keep pH homeostasis inside a cell. Protons, being transported from the cell, cannot make effective work during respiration after their return into cytoplasm, as they would move against the concentration gradient. Replacement of protons with Na⁺ ions provided the solution of this problem and the secondary Na⁺/H⁺ antiport became the basic mechanism for Na⁺ ion elimination. Chemiosmotic reverse ΔpH is formed due to the electrochemical Na⁺ gradient. Antiporter uses Na⁺ ions instead of protons in transmembrane electrogenic processes, which results in Na⁺ ion release due to electrochemical protonic potential. Thus, alkalophiles need well-balanced work of Na⁺-ATPase and Na⁺/H⁺ (K⁺/H⁺) antiporters to maintain proton driving force (Grant *et al.*, 1990).

It is also proposed that cell wall forms defense barrier from extreme environmental conditions in an alkalophilic cell. The cell wall contains the components with high number of carboxylic groups negatively charged that push off hydroxyl ions and adsorb protons and sodium ions (Jones *et al.*, 1998).

Salt Concentrations

Microorganisms are distributed within a wide range of soluble salt concentrations as they can be isolated from freshwater reservoirs, as well as from sea biotopes and hyper salty lakes, where NaCl concentration exceeds saturation value (Oren, 1999). Among halophilic microorganisms are a variety of heterotrophic and methanogenic archaea; photosynthetic, lithotrophic, and heterotrophic bacteria; and photosynthetic and heterotrophic eukaryotes. Examples of well-adapted and widely distributed extremely halophilic microorganisms include archaeal *Halobacterium* species, cyanobacteria such as *Aphanothece halophytica*, and the green alga *Dunaliella salina*. Among multicellular eukaryotes, species of brine shrimp and brine flies are commonly found in hypersaline environments. Halophiles can be loosely classified as slightly, moderately or extremely halophilic, depending on their requirement for NaCl. The extremely halophilic archaea, in particular, are well adapted to saturating NaCl concentrations and have a number of novel molecular characteristics, such as enzymes that function in saturated salts, purple membrane that allows phototrophic growth, sensory rhodopsins that mediate the phototactic response, and gas vesicles that promote cell flotation. Halophiles are found distributed all over the world in hypersaline environments, many in natural hypersaline brines in arid, coastal, and even deep sea locations, as well as in artificial salterns used to mine salts from the sea. They may be classified according to their salt requirement: slight halophiles grow optimally at 0.2– 0.85 molL⁻¹ (2–5%) NaCl; moderate halophiles grow optimally at 0.85–3.4 molL⁻¹ (5–20%) NaCl; extreme halophiles grow optimally above 3.4–5.1 molL⁻¹ (20–30%) NaCl. In contrast, nonhalophiles grow optimally at less than 0.2 molL⁻¹ NaCl (Oren, 1999).

These microbes employ two types of mechanisms in order to prevent water losses during the osmotic process as they have to keep high intracellular osmotic pressure to survive under salt stress conditions (Oren, 1999). Firstly, creation of salt in the cytoplasm, this is accumulation of non-organic ion accumulation inside a cell to concentration, comparable to the outer one, secondly, salt elimination from cytoplasm, while turgor pressure is formed due to the accumulation of high concentrations of organic molecules—osmolytes (“compatible regulators”) (Pfluger and Muller, 2004).

When an isoosmotic balance with the medium is achieved, cell volume is maintained. The compatible solutes or osmolytes that accumulate in halophiles are usually amino

acids and polyols, e.g. glycine betaine, ectoine, sucrose, trehalose and glycerol, which do not disrupt metabolic processes and have no net charge at physiological pH. A major exception is for the halobacteria and some other extreme halophiles, which accumulate KCl equal to the external concentration of NaCl (Zhilina *et al.*, 1996). Halotolerant yeasts and green algae accumulate polyols, while many halophilic and halotolerant bacteria accumulate glycine betaine and ectoine. Compatible solute accumulation may occur by biosynthesis, de novo or from storage material, or by uptake from the medium.

Conclusion

Extremophiles appear to be nature's magnificent example to show how microbes can push their limits in order to survive under extremities of environmental habitats. These adaptations range from genomic to cellular and molecular levels, and due to this versatility extremophiles have already found several applications in biotechnology and industry. Researches solely dedicated to find novel extremozymes hold immense potentials to revolutionize biotechnological applications.

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