

Review Paper:

Osmoadaptation in halophilic bacteria and archaea

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Abstract

Microbial diversity characterized from saline environments shows a great adaptation ability to grow under a wide range of salt concentrations. Halophiles are salt loving organisms that grow in salt affected environments and can tolerate a wide range of salt concentrations. According to their salt requirements, they can be categorized into slight, moderate and extreme halophiles. These organisms have a unique ability to survive and grow at high salt concentration and thus could serve as tremendous model systems to understand the molecular basis of high salt adaptation.

The physiology of the halophiles is affected by change in salt concentration, pH, growth temperature and nature of available nutrients. Halophilic bacteria and archaea have evolved two strategies to deal with high osmotic stress. Most of the bacteria use 'compatible solute strategy' in which they accumulate different organic compounds like proline, ectoine, glycine betaine and glutamine. Halophilic archaea and anaerobic bacteria cope with high salt stress by 'salt in strategy' in which they maintain their internal environment by accumulation of high concentration KCl ions.

Keywords: Halophilic bacteria, Haloarchaea, Osmoregulation, Compatible solutes, Inorganic ions.

Introduction

Halophiles represent an interesting class of extremophiles. They are salt-loving organisms that grow in saline environments. Halophilic organisms can grow in hypersaline environments which require at least 0.2M NaCl for their growth. They can be classified as slightly, moderately or extremely halophilic depending on their requirement for sodium chloride. Slight halophiles are microorganisms which can grow at 0.2-0.85M NaCl (1-5%) concentrations, moderate halophiles that grow optimally at 0.85-3.4M NaCl (5-20%) concentrations and extreme halophiles which can grow optimally at 3.4-5.1M NaCl (20-30%) concentrations. Halotolerant organisms have ability to grow in the saline environments but they can also grow in the absence of a high concentration of salt.^{1,2}

Hypersaline environment is usually described as that contains significant salt concentrations with salinity level more than ocean water (3.5% of total dissolved salts). Oceans are the largest body of saline water in the world

which constitutes approximately 99% of the biosphere. Based on their origin, most of the hypersaline bodies are classified into thalassic environments with salt concentration similar to sea water. These environments were usually derived from evaporation of sea water. They have Na⁺ and Cl⁻ dominating ions and the pH is near neutral to slightly alkaline. Hypersaline thalassic bodies such as brines, salt lakes often display bright red colour due to red pigments of haloarchaea.³

Two large and best-studied hypersaline lakes are the Great Salt Lake in the USA and the Dead Sea in the Middle East. A great diversity of microbial life is observed in such environments with salinity upto 3-3.5M salt concentrations. Hypersaline lakes and ponds of Mediterranean salterns in France contain saline water (3.1-3.6M salt concentrations) from where sodium chloride precipitates in the form of halite crystals.⁴ In Pakistan, Khewra Salt Mine also provides a rich and extensive habitat for halophytes and halophile microorganisms. Rhizosphere microbiome of halophytes contains a wide diversity of halotolerant and halophilic microorganisms.⁵ These habitats have harsh environment conditions such as high salinity, low oxygen availability, high temperature, high light intensity and extremely alkalinity.

Halophiles have evolved two basic strategies to survive in salt stress conditions. In 'compatible solute' strategy, most of the halophilic bacteria cope with their external environments by accumulating small, highly water-soluble organic compounds like glycine, betaine, proline, glutamine, potassium, glutamic acid and ectoine. In 'salt in' strategy, bacterial cells maintain internal environment by accumulating high concentrations of potassium chloride.^{1,3} Halophilic bacteria can also be used in food (fermentation of soy and fish sauces) and nutraceutical industries (β -carotene production).^{4,6} Mostly halophilic bacteria have ability to degrade certain toxic compounds and have role in bioremediation of heavy metals.⁷ Halophiles can also be used in biofuel production to overcome the expected shortage of petroleum and concerns regarding global warming.⁸ Halophiles have enzymes (extremozymes) that enable these organisms to function in such extreme environments.^{9,10} In this review, we mainly focused on the osmoregulation of halophilic microorganisms specially bacteria and archaea. Different strategies used in osmoadaptation of halophiles were explained thoroughly.

Halophilic bacteria

Mostly halophilic bacteria are cocci, rod, triangular and even square-shaped. Some strains are pleiomorphic especially

when the ionic conditions of the media are altered and most lie below the NaCl level of 1M/L. The physiology of the moderate and extreme halophilic bacteria is affected by change in salt concentration, growth temperature and nature of available nutrients.¹¹⁻¹³ Extremely halophilic bacteria generally grow slowly. Halophilic bacteria and archaea can be identified commonly by phenotypic characterization as well as 16S rRNA gene sequences.¹⁴ Moderately halophilic bacteria are dominant in mostly hypersaline environments and they constitute a major proportion of total microbial population in the hypersaline environments and play a major ecological role.

Currently, a large number of bacterial species related to different bacterial phyla especially gram-positive showed moderately halophilic response. Moderately halophilic bacteria include members of *Proteobacteria* (*Halomonas*, *Chromohalobacter*, *Pseudomonas*, *Marinobacter*, *Rhodospirillum*, *Aeromonas*, *Alteromonas*, *Rhodovibrio*, *Halovibrio* and *Alcaligenes*), *Firmicutes* (*Halobacillus*, *Virgibacillus*, *Oceanobacillus*, *Staphylococcus*, *Gracilibacillus*, *Clostridium*, *Pontibacillus*, *Sporosarcina* and *Planococcus*), *Actinobacteria* (*Kocuria*, *Streptomyces* and *Rubrobacter*), *Actinomycetes* (*Nocardia*, *Nocardiopsis*, *Streptomonospora*, *Actinopolyspora* and *Nesterenkonia*) and *Bacteroidetes* (*Flavobacterium*, *Salinibacter* and *Polaribacter*).

Mostly extreme halophilic bacteria contain a variety of carotenoids as carotenoids help membrane stabilization in *Thermus thermophiles*, *Rubrobacter radiotolerans* and help to tolerate the high osmotic stress in *Halobacterium* spp.¹³⁻¹⁵ Many gram-positive bacteria isolated from different saline environments (salt lakes, salt mines and salt marshes) also have carotenoids which indicate the crucial role of carotenoids in osmotolerance of these bacteria.¹⁶

Cyanobacteria are characterized by the presence of chlorophyll and phycobilin pigments. They are photosynthetic bacteria (planktonic biomass) and form microbial mats in many hypersaline lakes.¹⁷ *Aphanothece halophytica* is an extreme halophile form of brown layer of microbial mat on the water surface. Mostly cyanobacteria use glycine betaine as the major compatible solute which they take from the medium or synthesize from choline.¹⁸ Many genera related to halophilic cyanobacteria have been described from the Dead Sea, the Great Salt Lake, Solar Lake and other salt lakes and ponds but the cyanobacterial diversity has not been studied extensively from the hypersaline environments.¹⁹

Moderately halophilic purple sulfur bacteria like *Chromatium* spp. have ability to store sulfur granules inside cells and they grow phototrophically by using glycerol or glycolate. *Rhodospirillum salaxigens* (purple non-sulfur bacterium) can use glycine, betaine or ectoine as osmolytes. Sulfur oxidizing bacteria are halophilic gram-positive, filamentous CO₂ fixing bacteria. They oxidize sulfur and

hydrogen sulfide to form sulfates. For example, *Achromatium volutans*, a filamentous bacterium was isolated from solar lakes and *Thiobacillus halophilus* a halophilic chemoautotrophic bacterium was isolated from the hypersaline lake, Australia.²⁰

Haloarchaea

The extremely halophilic archaea are members of the class Halobacteria.²¹ They are cocci, rods or a multitude of involution forms from disks to triangle. They grow in the presence of amino acids and salt with at least 1.5M NaCl (9%) concentration and lack muramic acid containing peptidoglycan in the cell envelope. They are mostly pigmented bacteria (orange to red) due to presence of carotenoids.²² They have bacterioruberins, a light-sensitive protein which provides chemical energy for the cell by using sunlight to pump out of the cell.

The intracellular enzymes of halobacteria have a requirement for high levels of KCl with 3M-5M concentration. They have many antibiotic resistant genes to survive in hypersaline environments such as salt mines, salt lakes, soda lakes, salterns and marine environments. Haloarchaeal genera *Halorubrum*, *Halobacterium*, *Halococcus*, *Haloflex*, *Natronococcus* and *Haloarcula* belonged to the Halobacteriaceae. Intracellular gas vesicles are synthesized by several halophilic bacteria and archaea. These vesicles are usually filled by different gases and provide buoyancy and enable cells to regulate their position in the aquatic environments.^{1,6}

Halobacterium salinarum contains fibrillary structures (fibrocrystalline bodies) which represent the presence of cytoskeleton-like organelle in haloarchaeal cells.²³ Some anaerobic archaeal genera especially methanogens have been reported from the hypersaline environments. They use methyl related substrates for their growth and have been isolated from the Great Salt Lake and solar salterns. *Methanohalophilus* and *Methanohalobium*, extreme halophilic and thermophilic methanogen, generally grow with optimum 4.4M NaCl concentrations and 50°C. These bacteria have intra-cellular high salt concentration.²⁴ Methanogenesis is a process of organic matter degradation by bacteria and archaea especially in marine sediments. During this process, bacteria produce methane by sulfate reduction pathway as well as acetate pathway.²⁵

Osmoregulation by halophilic bacteria and archaea

Water is one of the most important prime elements responsible for life. Living organisms have ability to adapt to changes in external environment (osmotic pressure) to survive under harsh conditions.²⁶ In exposure to hypersaline environments, water usually moves out of the microbial cells along the osmotic gradient and it causes dehydration of the cytoplasm. Halophilic bacteria and archaea have adapted genetic and physiological modifications to survive in extreme (hypersaline) environments. They can be classified

according to their salt dependency or salt requirement. Therefore halophiles have adapted different mechanisms of osmoadaptation to survive in hypersaline environments.^{27,28} Halophilic bacteria and archaea generally use two mechanisms of adaptation to hypersaline environments: (1) the accumulation of compatible solutes (osmolytes) to the imposed osmotic pressure, (2) the maintenance of high intracellular ionic (K^+) concentrations and adaptation of entire intracellular enzymatic machinery to function in hypersaline conditions (Fig. 1 and 2).

Saum and Müller²⁹ explained different mechanisms used for osmoregulation of *Halobacillus* spp. as shown in fig. 1. Mostly halophilic bacteria and archaea have two component regulatory systems. They have osmosensor and osmoregulator proteins to recognize osmotic imbalance. Regulator proteins bind DNA and regulate gene expression and sensor proteins (histidine kinases) detect the signals. This system controls the changes in osmotic pressure on both sides of the cell membrane.³⁰

Compatible solute strategy: Mostly halophilic bacteria balance their cytoplasm with the high salt concentration of the medium by the accumulation of osmolytes (compatible solutes). These compatible solutes are excreted in the cytoplasm either by the bacterial cell itself or can be taken from the medium. Osmolytes can be classified into two main groups; organic solutes (trehalose, ectoine, betaine, proline), inorganic solutes (K^+ , Mg^{2+} , Na^+).^{31,32} Mostly bacteria lack intracellular systems for active transport of water to cope with external osmotic stress. Therefore, they balance internal environment by transport or synthesis of a group of organic solutes (compatible solutes) without affecting metabolic function of the cell.^{33,34} Compatible solutes can be classified according to their chemical nature: (1) anionic solutes, (2) zwitterionic solutes and (3) non-charged solutes (Fig. 2).

Organic anions: Mostly halophilic bacteria have high intracellular K^+ and have negative potential inside the cell. Organic anions (negatively charged solutes) are used to balance internal environment to survive at high salt concentrations (Fig. 2). Some halophilic bacteria such as *Halomonas* usually synthesize ectoine and *Halobacterium* can synthesize L-glutamate as an osmolytes.³⁵ These organic anionic solutes have a carboxylate group which supplies the negative charge or may contain sulfate or phosphate groups.³ Methanogens and halobacteria increase their glutamate levels with increasing external salt concentrations. The negatively charged glutamates are accumulated at concentrations comparable to the intracellular K^+ concentration.^{36,37}

Polyols: The polyol compounds like sorbitol, arabinol, mannitol and glycerol are used as compatible solutes by halotolerant bacteria, algae, plants and some fungi.²⁸ Halotolerant bacteria included *Bacillus*, *Pseudomonas*, *Aeromonas*, *Stenotrophomonas* and *Zymomonas* using sorbitol, mannitol and glucose (osmolytes) for

osmoadaptation (Fig. 2). *Cyanobacteria* and *Proteobacteria* have ability to synthesize glucosyl glycerol or use it from the medium in salt stress environments.³⁸⁻⁴⁰

Zwitter solutes: Halophilic bacteria have some neutral amino acids which are used as compatible solutes in salt stress conditions. But most of the neutral amino acids are utilized at high concentrations due to lack of availability of these amino acids (Fig. 2). As free polar amino acids are used as intermediates in protein synthesis process, bacterial cells accumulate or synthesize some zwitterionic molecules derived from amino acids as osmolytes.^{41,42}

Betaine: Betaine is a natural compound having a negatively charged ions carboxylate group and a positively charged phosphonium ions or ammonium ions. It is used as osmolyte for the protection of cells to cope high osmotic stress, high salinity and high temperature.⁴³ Different intracellular enzymes are involved for the accumulation of betaine. They maintain internal balance by regulation of water inside the cells and thus protect the cells from dehydration (Fig. 2).

Different halophilic bacteria (*Halomonas*, *Virgibacillus*, *Oceanobacillus*, *Kocuria* and *Polaribacter*) can synthesize betaine from glycine with the primary amine methylated to form a quaternary amine.^{44,45} Betaine concentration inside the cells increases with increase in salt concentrations. Some methanogens (*Methanohalophilus* and *Methanohalobium*) can accumulate and also synthesize betaine as an osmolyte either by methylation of glycine or oxidation of choline.^{24,42}

Mostly halotolerant and halophilic bacteria use two metabolic pathways to synthesize betaine. Gram-positive bacteria (*Virgibacillus*, *Oceanobacillus* and *Staphylococcus*) synthesize betaine by oxidation of choline with a single enzyme (choline oxidase).⁴⁶ In gram-negative bacteria (*E. coli*), it is coded by *bet* operon with four genes and can occur as membrane associated system. The *bet* operon includes *betA* gene (choline dehydrogenase), *betB* (betaine aldehyde dehydrogenase), *betI* (putative regulator) and *betT* (choline transporter).⁴⁷ These enzymes belong to family of oxidoreductases. Choline is changed into an intermediate compound (glycine betaine aldehyde) by choline dehydrogenase and finally betaine (osmolyte) is produced by a catalytic enzyme (Fig. 1).

Sometimes microorganisms prefer to accumulate the betaine as synthesizing it. This process is because of the limited availability of precursors (substrates) rather than the result of a low expression level of the genes. Some extreme halophiles like methanogens (*Methanohalophilus* and *Methanohalobium*) and haloalkalophilic sulfur bacteria like *Ectothiorhodospira* usually synthesize betaine by dimethylglycine and sarcosine. This process involves two enzymes, dimethylglycine methyltransferase and sarcosine methyltransferase. Concentration levels of K^+ ion and sarcosine usually regulate the biosynthesis of betaine inside the cell.⁴⁸

Ectoine: Ectoine, a cyclic tetrahydropyrimidine is used as an osmolyte in halotolerant and halophilic bacteria (Fig. 1 and 2). It is either accumulated from the external medium or synthesized by bacterium itself. It was detected in a halophilic, phototrophic bacterium, *Halorhodospira halochloris* isolated from the hypersaline Mono Lake.⁴⁹ Recently, ectoine has been detected in a number of halotolerant and halophilic bacteria related to different phylogenetic groups (*Halomonas*, *Oceanobacillus*, *Marinococcus* and *Nesterenkonia*).^{34,36} Some methylophilic bacteria *Methylophaga* and *Methylarcula* can also synthesize ectoine to balance their intracellular environment under the osmotic stress.²⁴

The biosynthesis and regulation of ectoine has been studied in a large number of halophilic bacteria especially with detail in *Halomonas* and *Oceanobacillus*.^{35,50,51} Aspartate aldehyde is used as a precursor molecule in the biosynthesis of ectoine. This molecule is converted into 2, 4-diaminobutyric acid and finally as a result of acetylation, ectoine is formed.²⁸ The biosynthesis of ectoine is involved by the enzymatic action of three genes: *ectA*, *ectB* and *ectC*. Diaminobutyric acid acetyltransferase is encoded by the gene *ectA*, diaminobutyric acid aminotransferase is encoded by *ectB* and ectoine synthase is encoded by *ectC*.^{52,53} The enzymes involved in biosynthesis of ectoine were functionally characterized in *Halomonas elongata*. Activity of diaminobutyric acid acetyltransferase and aminotransferase is regulated by intracellular K⁺ ion concentration.

Non-charged Solutes: Some polar and non-charged organic molecules have been detected as compatible solutes in halotolerant and halophilic bacteria (Fig. 2). Some halophilic bacteria from soil and marine environments accumulate glycerol as an osmolyte to protect cells in salt stress conditions.^{20,33} Glycerol has also been detected in some eukaryotes like halotolerant yeast *Debaryomyces hansenii* and *Hortea werneckii* under osmotic stress environments.⁵⁴ Negatively charged derivatives of both myo-inositol and glycerol are accumulated by some haloarchaeal strains.²²

Carbohydrates: Some sugar molecules are also used as osmolytes for balancing of intracellular environment in salt stress conditions. They are usually reducing in nature and react with surface proteins of the cell. Some non-charged organic molecules like glycerol bind with reactive end of the sugar to overcome the reducing nature of the sugar molecules (Fig. 2).

Different sugars with neutral molecules like glycerol or glyceramide have been detected from halotolerant and halophilic bacteria.⁵⁵ Trehalose is a disaccharide sugar molecule used as a compatible solute identified in a variety of organisms like bacteria, fungi and plants.⁵⁶ It helps cells to cope with desiccation, heat, cold and hypersaline environments. It is also used as a signaling molecule and energy source in different metabolic pathways.^{57,58}

Some *Proteobacteria* and marine *Cyanobacteria* are known to accumulate sucrose as an osmolyte in osmotic stress conditions.^{28,59} *Cyanobacteria* catalyzed sucrose by two pathways, one with sucrose-6-phosphate synthase (SPS) and the other involved sucrose-6-phosphate phosphatase (SPP).

Proteins (Amino acids and peptides): Proline, acetylated glutamine dipeptide and carboxamine are the main osmolytes from the class proteins (Fig. 2). They are mostly detected from marine phototrophic bacteria (*Ectothiorhodospira mobilis*) and halophilic purple sulfur bacteria. Protein solute molecules included Carbamoyl-L-glutamine 1-amide detected from the halophilic marine phototrophic bacteria and a dipeptide N-acetyl glutaminyl glutamine amide is usually synthesized by halophilic purple sulfur bacteria.^{60,61}

Salt-in strategy: This strategy is used by two phylogenetically unrelated groups: anaerobic halophilic bacteria (Haloanaerobiales) and aerobic halophilic archaea (Halobacteriales). These groups use inorganic ions (K⁺, Mg²⁺, Na⁺) to cope with external osmotic stress (high salt concentrations). All intracellular machinery is adapted to the high salt concentration in the external environment of the cell (Fig. 1 and 2).^{1,43} The composition and concentration of these inorganic ions usually vary among different species and even within the same species by means of synthesis or transport. KCl is generally used to balance intracellular salt concentrations in the family Halobacteriaceae.

In halophilic archaea, all the intracellular machinery (enzymes and other cell proteins) are adapted to the presence of high salt concentrations in the external medium.^{62,63} Different enzymes with unique characteristics are involved in balancing of internal environment of the cell. These enzymes have been structurally modified with excess of acidic amino acids and low content of hydrophobic amino acids.

Halophilic enzymes and other proteins from the haloarchaea maintain their proper conformation and can function only in the presence of high salt. Therefore halophilic archaea and bacteria can flourish only in the presence of high salt.⁶⁴⁻⁶⁶ Halophilic enzymes from the haloarchaea are defined either according to the salt tolerance of the organism from which they are detected or with respect to their own salt requirements for activity, stability or solubility.

Conclusion

Microorganisms living under saline environments have special genetic and physiological characteristics to survive under harsh conditions. Halophiles can grow in wide range of salt concentrations and can be classified into slight, moderate and extreme halophiles according to their salt requirements. Halotolerant microorganisms have ability to grow either under hypersaline conditions or even in the absence of high salt concentrations. These organisms usually use two basic mechanisms for adaptation to high salt

concentrations. Most of the bacteria use ‘compatible solute strategy’ in which they accumulate different organic

compounds like proline, ectoine, glycine betaine and glutamine.

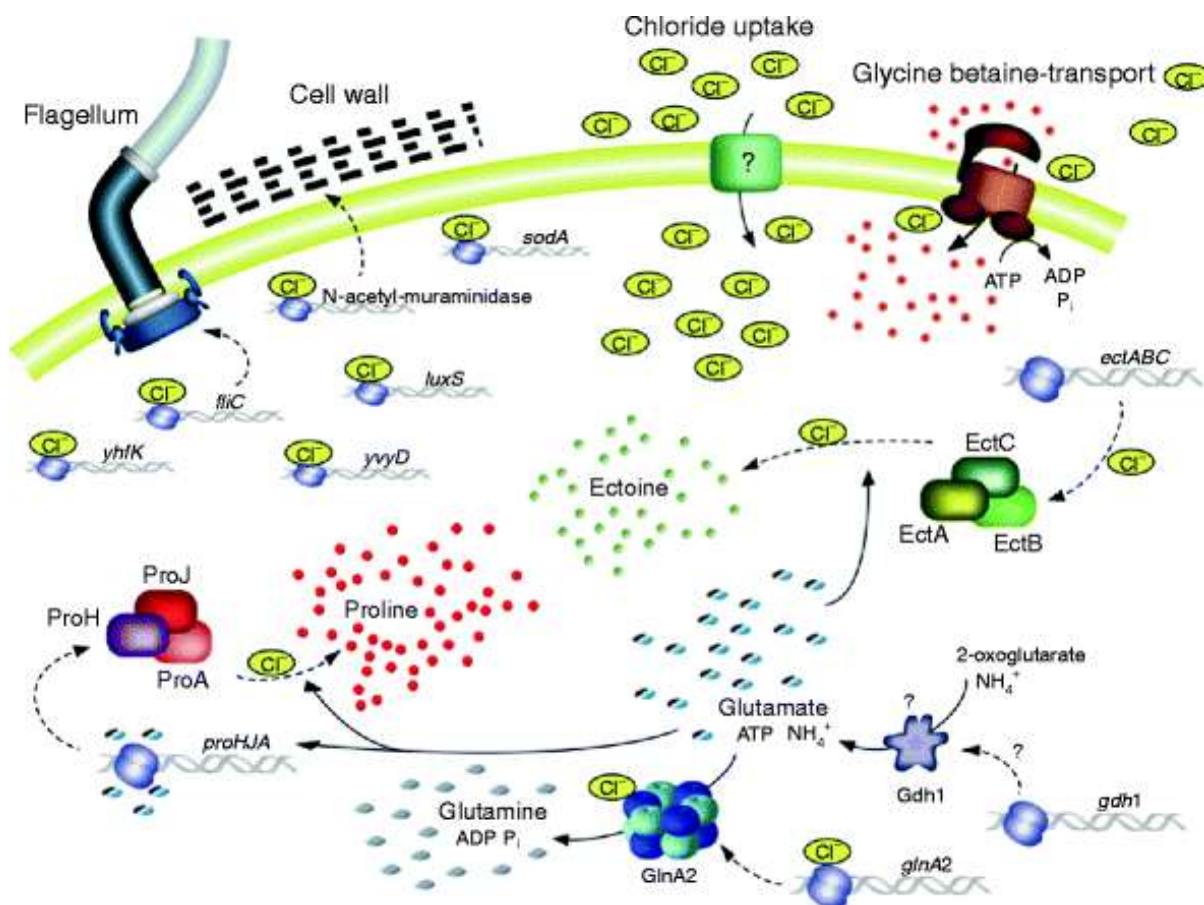


Figure 1: Overview of osmoregulation in halophilic bacteria and archaea

Osmoadaptation in Halophilic Bacteria and Archaea

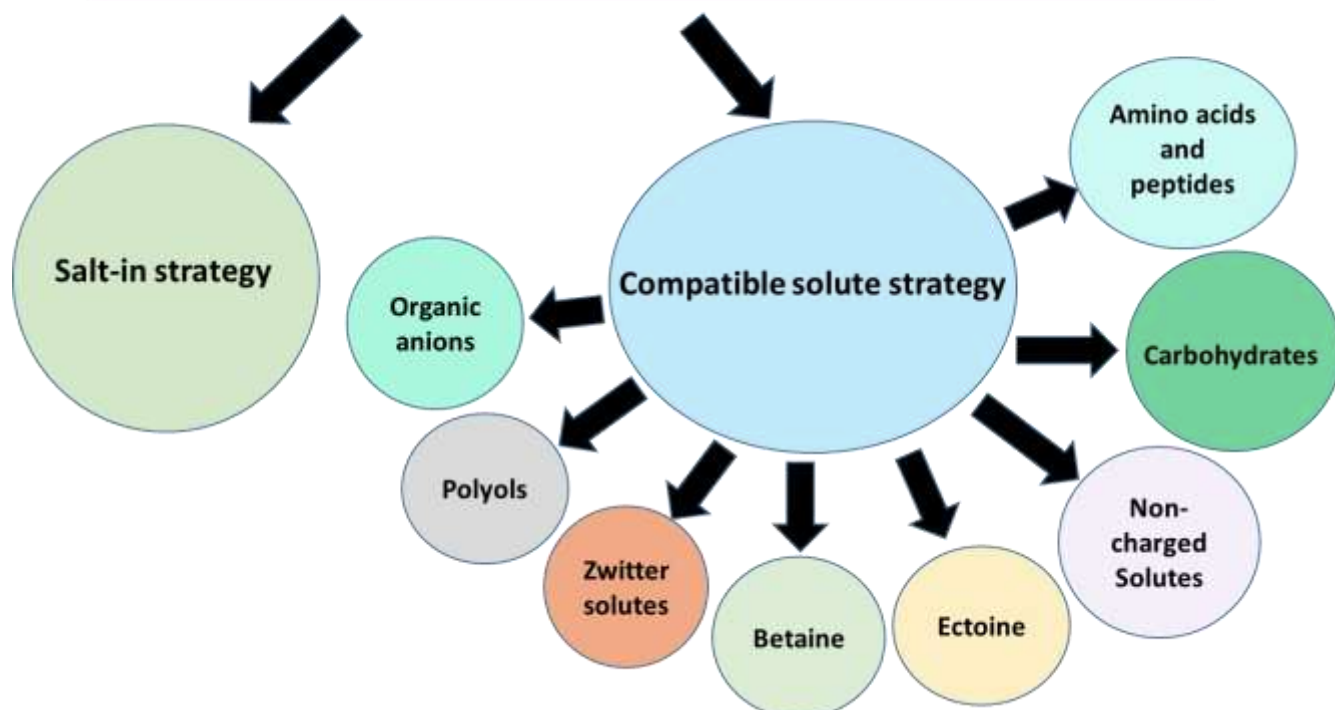


Figure 2: Different strategies used for osmoregulation in halophilic bacteria and archaea

Halophilic archaea and anaerobic bacteria cope with high salt stress by 'salt in strategy' in which they maintain their internal environment by accumulation of high concentration KCl ions. The molecular mechanisms used for osmoregulation to salinity stress conditions are now studied using modern technologies such as omics approaches, gene knock-outs and DNA micro-arrays.

References

1. Das Sarma S. and Das Sarma P., Halophiles and their enzymes: negativity put to good use, *Curr Opin Microbiol*, **25C**, 120-126 (2015)
2. Purdy K.J., Cresswell-Maynard T.D., Nedwell D.B., McGenity T.J., Grant W.D., Timmis K.N. and Embley T.M., Isolation of haloarchaea that grow at low salinities, *Environ Microbiol*, **6**, 591-595 (2004)
3. Pinar G., Kraková L., Pangallo D., Piombino-Mascali D., Maixner F., Zink A. and Sterflinger K., Halophilic bacteria are colonizing the exhibition areas of the Capuchin Catacombs in Palermo, Italy, *Extremophiles*, **18**, 677-691 (2014)
4. Oren A., Taxonomy of the family Halobacteriaceae: a paradigm for changing concepts in prokaryote systematic, *Int J Syst Evol Microbiol*, **62**, 263-271 (2012)
5. Mukhtar S., Mirza M.S., Awan H.A., Maqbool A., Mehnaz S. and Malik K.A., Microbial diversity and metagenomic analysis of the rhizosphere of Para Grass (*Urochloa mutica*) growing under saline conditions, *Pak J Bot*, **48**, 779-791 (2016)
6. Sawers R.G., Little red floaters: gas vesicles in an *Enterobacterium*, *Environ Microbiol*, **18(4)**, 1091-1093 (2016)
7. Bonfa M.R.L., Grossman M.J., Piubeli F., Mellado E. and Durrant L.R., Phenol degradation by halophilic bacteria isolated from hypersaline environments, *Biodegradation*, **24**, 699-709 (2015)
8. Sarwar M.K., Azam I. and Iqbal T., Biology and applications of halophilic bacteria and archaea: a review, *E J Bio*, **11**, 98-103 (2015)
9. Sánchez-Porro C., Martín S., Mellado E. and Ventosa A., Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes, *J Appl Microbiol*, **94**, 295-300 (2003)
10. Biswas J. and Paul A.K., Production of extracellular enzymes by halophilic bacteria isolated from solar salterns, *Int J App Biol Pharma Technol*, **4**, 30-36 (2013)
11. Fourcans A., Solé A., Diestra E., Ranchou-Peyruse A., Esteve I., Caumette P. and Duran R., Vertical migration of phototrophic bacterial populations in a hypersaline microbial mat from Salins-de-Giraud (Camargue, France), *FEMS Microbiol Ecol*, **57**, 367-377 (2006)
12. Ventosa A., Gutierrez M.C., Kamekura M., Zvyagintseva I.S. and Oren A., Taxonomic study of *Halorubrum distributum* and proposal of *Halorubrum terrestris* sp. nov., *Int J Syst Evol Microbiol*, **54**, 389-392 (2004)
13. Amoozegar M.A. et al, *Oceanobacillus halophilus* sp. nov., a novel moderately halophilic bacterium from a hypersaline lake, *Int J Syst Evol Microbiol*, **66**, 1317-1322 (2016)
14. Fendrihan S. et al, Spherical particles of halophilic archaea correlate with exposure to low water activity-implications for microbial survival in fluid inclusions of ancient halite, *Geobiology*, **10**, 424-433 (2012)
15. Sasaki T., Razak N.W., Kato N. and Mukai Y., Characteristics of halorhodopsin-bacterioruberin complex from *Natronomonas pharaonis* membrane in the solubilized system, *Biochemistry*, **51**, 2785-2794 (2012)
16. Li Y., Liu S., Man Y., Li N. and Zhou Y.U., Effects of vitamins E and C combined with β -carotene on cognitive function in the elderly, *Exp Ther Med*, **9**, 1489-1493 (2015)
17. Baxter B.K., Gunde-Cimerman N. and Oren A., Salty sisters: The women of halophiles, *Front Microbiol*, **5**, 192 (2014)
18. Boonburapong B., Laloknam S. and Incharoensakdi A., Accumulation of gamma-aminobutyric acid in the halotolerant cyanobacterium *Aphanothece halophytica* under salt and acid stress, *J Appl Phycol*, **28**, 141-148 (2016)
19. Tripathi K., Sharma N.K., Kageyama H., Takabe T. and Rai A.K., Physiological, biochemical and molecular responses of the halophilic cyanobacterium *Aphanothece halophytica* to Pi-deficiency, *Eur J Phycol*, **48**, 461-473 (2013)
20. Sorokin D.Y., Abbas B., Erik V.Z. and Muyzer G., Isolation and characterization of an obligately chemolithoautotrophic *Halothiobacillus* strain capable of growth on thiocyanate as an energy source, *FEMS Microbiol Lett*, **354**, 69-74 (2014)
21. Grant W.D., Kamekura M., McGenity T.J. and Ventosa A., Class III, Halobacteria class, nov. In Boone D.R. and Castenholz R.W., eds., The Archaea and the Deeply Branching and Phototrophic Bacteria, In Garrity G.M., eds., *Bergey's Manual of Systematic Bacteriology*, 2nd ed., Springer-Verlag, New York, 294-334 (2001)
22. Reitter J.N., Cousin C.E., Nicastrì M.C., Jaramillo M.V. and Mills K.V., Salt-Dependent Conditional Protein Splicing of an Intein from *Halobacterium salinarum*, *Biochemistry*, **55**, 1279-1282 (2016)
23. Andersson M., Structural dynamics of light-driven proton pumps, *Structure*, **17**, 1265-1271 (2009)
24. Attar N., A new phylum for methanogens, *Nat Rev Microbiol*, **13**, 739-745 (2015)
25. Maltby J., Sommer S., Dale A.W. and Treude T., Microbial methanogenesis in the sulfate-reducing zone of surface sediments traversing the Peruvian margin, *Biogeosciences*, **13**, 283-299 (2016)
26. Irshad A., Ahmad I. and Kim S.B., Cultureable diversity of halophilic bacteria in foreshore soils, *Braz J Microbiol*, **45**, 563-571 (2014)
27. Mukhtar S., Mirza M.S., Mehnaz S., Mirza B.S. and Malik K.A., Diversity of *Bacillus*-like bacterial community in the

- rhizospheric and nonrhizospheric soil of halophytes (*Salsola stocksii* and *Atriplex amnicola*) and characterization of osmoregulatory genes in halophilic *Bacilli*, *Can J Microbiol*, **64**, 567-579 (2018)
28. Youssef N.H., Savage-Ashlock K.N., McCully A.L., Luedtke B., Shaw E.I., Hoff W.D. and Elshahed M.S., Trehalose/2-sulfotrehalose biosynthesis and glycine-betaine uptake are widely spread mechanisms for osmoadaptation in the Halobacteriales, *ISME J*, **8**, 636-649 (2014)
29. Saum S.H. and Müller V., Regulation of osmoadaptation in the moderate halophile *Halobacillus halophilus*: chloride, glutamate and switching osmolyte strategies, *Saline Sys*, **4**, 4 (2008b)
30. Foo Y.H., Gao Y., Zhang H. and Kenney L.J., Cytoplasmic sensing by the inner membrane histidine kinase EnvZ, *Prog Biophys Mol Biol*, **118**, 119-129 (2015)
31. Nath A., Insights into the sequence parameters for halophilic adaptation, *Amino Acids*, **48**, 751-762 (2016)
32. Anbu P. and Hur B.K., Isolation of an organic solvent-tolerant bacterium *Bacillus licheniformis* PAL05 that is able to secrete solvent-stable lipase, *Biotechnol Appl Biochem*, **61**, 528-534 (2014)
33. Petrovic U., Cimerman N. and Plemenitas A., Cellular responses to environmental salinity in the halophilic black yeast *Hortaea werneckii*, *Mol Microbiol*, **45**, 665-672 (2004)
34. Moghaddam J.A., Boehringer N., Burdziak A., Kunte H.J., Galinski E.A. and Schäberle T.F., Different strategies of osmoadaptation in the closely related marine myxobacteria *Enhygromyxa salina* SWB007 and *Plesiocystis pacifica* SIR-1, *Microbiology*, **162**, 651-661 (2016)
35. Tanimura K., Matsumoto T., Nakayama H., Tanaka T. and Kondo A., Improvement of ectoine productivity by using sugar transporter-overexpressing *Halomonas elongate*, *Enzyme Microb Technol*, **89**, 63-68 (2016)
36. Rajan A.L., Joseph T.C., Thampuran N., James R., Ashok K.K., Viswanathan C. and Bansal K.C., Cloning and heterologous expression of ectoine biosynthesis genes from *Bacillus halodurans* in *Escherichia coli*, *Biotechnol Lett*, **30**, 1403-1407 (2008)
37. Borjian F., Han J., Hou J., Xiang H. and Berg I.A., The methylaspartate cycle in haloarchaea and its possible role in carbon metabolism, *ISME J*, **10**, 546-557 (2016)
38. Roder A., Hoffmann E., Hagemann M. and Berg G., Synthesis of the compatible solutes glucosylglycerol and trehalose by salt-stressed cells of *Stenotrophomonas* strains, *FEMS Microbiol Lett*, **243**, 219-226 (2005)
39. Klahn S. and Hagemann M., Compatible solute biosynthesis in cyanobacteria, *Environ Microbiol*, **13**, 551-562 (2011)
40. Mukhtar S., Mirza M.S., Mehnaz S., Mirza B.S. and Malik K.A., Isolation and characterization of halophilic bacteria from the rhizosphere of halophytes and non-rhizospheric soil samples, *Braz J Microbiol*, **50**, 85-97 (2019)
41. Knief C. et al, Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice, *ISME J*, **6**, 1378-1390 (2012)
42. Rajan L., Toms C.J., Nirmala T., Roswin J., Viswanathan C. and Kailash C.B., Functional characterization and sequence analysis of choline dehydrogenase from *Escherichia coli*, *Genetic Eng Biotechnol J*, **2010**, GEBJ12 (2010)
43. Karan R., Capes M.D. and Das Sarma S., Function and biotechnology of extremophilic enzymes in low water activity, *Aquat Biosyst*, **8**, 4-10 (2012)
44. Ates O., Toksoy E. and Arga K.Y., Genome-scale reconstruction of metabolic network for a halophilic extremophile *Chromohalobacter salexigens* DSM 3043, *BMC Syst Biol*, **5**, 12 (2011)
45. Ying X., Liu Y., Xu B., Wang D. and Jiang W., Characterization and application of *Halomonas shantousis* SWA25, a halotolerant bacterium with multiple biogenic amine degradation activity, *Food Add Cont*, **33**, 674-682 (2016)
46. Zou H., Chen N., Shi M., Xian M., Song Y. and Liu J., The metabolism and biotechnological application of betaine in microorganism, *Appl Microbiol Biotechnol*, **100**, 3865-3876 (2016)
47. Zhao S. et al, Discovery of new enzymes and metabolic pathways by using structure and genome context, *Nature*, **502**, 698-702 (2013)
48. Wu W.L. et al, Phosphoproteomic analysis of *Methanohalophilus portucalensis* FDFIT identified the role of protein phosphorylation in methanogenesis and osmoregulation, *Scient Rep*, **6**, 29013 (2016)
49. Culla R.A., Diaz M.R., Taylor B.F. and Roberts M.F., Organic osmolytes in aerobic bacteria from Mono Lake, an alkaline, moderately hypersaline environment, *Appl Environ Microbiol*, **63**, 220-226 (1999)
50. Roberts M.F., Organic compatible solutes of halotolerant and halophilic microorganisms, *Saline Sys*, doi:10.1186/1746-1448-1-5 (2005)
51. Schubert T., Maskow T., Benndorf D., Harms H. and Breuer U., Continuous synthesis and excretion of the compatible solute ectoine by a transgenic, nonhalophilic bacterium, *Appl Environ Microbiol*, **73**, 3343-3347 (2007)
52. Reshetnikov A.S., Khmelenina V.N., Mustakhimov I.I. and Trotsenko Y.A., Genes and enzymes of ectoine biosynthesis in halotolerant methanotrophs, *Method Enzymol*, **495**, 15-30 (2011)
53. Gao S., Zhang L., Li D., Liu S. and Li X., Comparison of ectoine synthesis regulation in secreting and non-secreting strains of *Halomonas*, *Annal Microbiol*, **64**, 1357-1361 (2014)
54. Rhaisa A., Studies of *Debaryomyces hansenii* killer toxin and its effect on pathogenic bloodstream Candida isolates, Master thesis, University of Nebraska, Lincoln, Nebraska (2016)
55. Sila Z., Bores N., Martins L.O., Wait R., Da Costa M.S. and Santos H., Effect of the growth temperature and salinity of the

- medium on the accumulation of compatible solutes by *Rhodothermus obamensis*, *Extremophiles*, **9**, 163-172 (2009)
56. Empadinas N. and Costa M.S., Osmoadaptation mechanisms in prokaryotes: distribution of compatible solutes, *Int Microbiol*, **11**, 151-161 (2008)
57. Nobre A., Alarico A., Fernandes C., Empadinhas N. and Costa M.S., A unique combination of genetic systems for the synthesis of trehalose in *Rubrobacter xylanophilus*: properties of a rare actinobacterial TreT, *J Bacteriol*, **8**, 7939-7946 (2008)
58. Liu W., Wang Q., Hou J., Tu C., Luo Y. and Christie P., Whole genome analysis of halotolerant and alkalotolerant plant growth-promoting rhizobacterium *Klebsiella* sp. D5A, *Scient Rep*, <https://doi.org/10.1038/srep26710> (2016)
59. Lunn J.E., New complexities in the synthesis of sucrose, *Curr Opin Plant Biol*, **6**, 208-214 (2009)
60. Besse A., Peduzzi J., Rebuffat S. and Carre-Mlouka A., Antimicrobial peptides and proteins in the face of extremes: lessons from archaeocins, *Biochimie*, **118**, 344-355 (2015)
61. Raizel R., Leite J.S., Hypólito T.M., Coqueiro A.Y., Newsholme P., Cruzat V.F. and Tirapegui J., Determination of the anti-inflammatory and cytoprotective effects of l-glutamine and l-alanine, or dipeptide, supplementation in rats submitted to resistance exercise, *Br J Nutr*, **116**, 470-479 (2016)
62. Srivastava P. and Kowshik M., Mechanisms of Metal Resistance and Homeostasis in Haloarchaea, *Archaea*, <https://doi.org/10.1155/2013/732864> (2013)
63. Fernandez A.B., Ghai R., Martín-Cuadrado A.B., Sánchez-Porro C., Rodríguez-Valera F. and Ventosa A., Prokaryotic taxonomic and metabolic diversity of an intermediate salinity hypersaline habitat assessed by metagenomics, *FEMS Microbiol Ecol*, **88**, 623-635 (2014)
64. Oren A., Halophilic microbial communities and their environments, *Curr Opin Microbiol*, **33**, 119-124 (2015)
65. Kumar V., Saxena J. and Tiwari S.K., Description of a halocin-producing *Haloferax larsenii* ha1 isolated from pachpadra salt lake in rajasthan, *Arch Microbiol*, **198**(2), 181-192 (2016)
66. Kavitha P., Lipton A.P., Sarika A.R. and Aishwarya M.S., Growth characteristics and halocin production by new isolate, *Haloferax volcanii* KPS1 from Kovalam solar saltern (India), *Res J Biol Sci*, **6**, 257-262 (2011).

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