

Biology and Applications of Halophilic Bacteria and Archaea: A Review

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Abstract

Extremophiles include a diverse group with ability to survive in highly saline conditions, the halophiles. Halophiles are characterized in major life domains including Archaea as well as Bacteria. Archaea represents halophiles with heterotrophic, chemotrophic aerobes and anaerobes. A large number of members from these domains are found growing in diverse habitats including both thalassohaline and athalassohaline environment. Well-developed strategies of adaptation allow halophiles to sustain their survival in saline extremes. Certain important biotechnological and ecological features are grabbing the attention of scientists to study halophilic biology more deeply. This review presents overview of the halophilic biology with special focus on taxonomy, diversity, habitat, osmoadaptation and biotechnological applications of Bacteria and *Archaea*.

Keywords: Halophiles; Extremophile; Osmoadaptation; Thalassohaline; Archaea.

1. Introduction

The first chemical stress encountered during the evolution of life on earth may have been salt stress. Thus, from the beginning, organisms must have evolved strategies and effective mechanisms for the stabilization of protoplasmic structures and ion regulation [1]. These mechanisms are of diverse nature including membrane composition modification, variations in membrane fluidity, Na⁺/H⁺ antiporter activity, general salinity tolerance proteins etc [2]. Genes conferring salt tolerance to certain bacterial strains have been identified and isolated. These isolated genes have been successfully transferred for the formation of transgenic plants showing resistance to high saline conditions [3,4].

Microorganisms growing optimally at salt concentrations higher than 100 g/L fall under the category of halophiles. Halophilic bacteria thrive in widely varying concentrations of NaCl ranging from (0.2 – 5.1M or 2 – 30%). On the basis of this varying NaCl concentration, halophiles are categorized as slight, moderate and extreme halophiles [5]. The largest family of halophilic *Archaea* is represented by

Halobacteriaceae comprising of 36 genera with 129 species. Moreover Halobacteriaceae holds the most salt requiring strains [6]. Halophiles live in diverse range of habitat including solar salterns, deep sea brines, salt mines, hypersaline and alkaline lakes, marshy lagoons and hydrothermal [7].

1.1 Taxonomy

Methods of chemotaxonomy, multilocus sequence analysis, numerical taxonomy, comparative genomics and proteomics have allowed taxonomists to classify halophiles. These versatile microorganisms occupy all three major domains of life i.e., Archaea, Bacteria and Eukarya. Slightly and moderately halophilic microorganisms are represented by eukarya and bacteria whereas the extreme halophiles are represented by Archaea [8,6] (Figure 1).

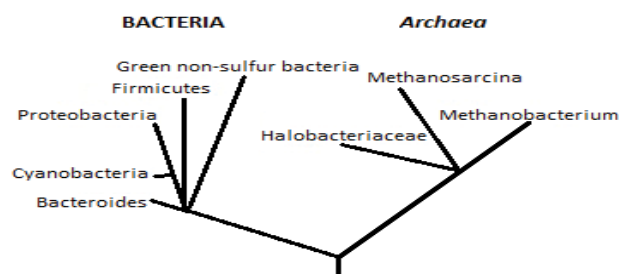


Figure 1. The universal phylogenetic tree of life as based on small subunit rRNA gene sequences, and distribution of halophilic Bacteria and Archaea [1].

The domain Archaea has been further divided into two subdomains, Halobacteria and Methanogenic Archaea. Halobacteria is represented by one of the largest halophile family, Halobacteriaceae with 36 genera and 129 species requiring high NaCl concentrations which discriminate them from other halophiles. They cannot even survive in fresh water and lyse [9]. The role of Methanogenic Archaea is vital in the environment but characterization of only a few halophilic species has been possible yet. Among all five orders of methanogenic Archaea, only Methanocarcinales is the one containing halophilic species. These strictly anaerobic species form methane to obtain energy and are placed in the family Methanosarcinaceae [10].

1.2 Diversity

The uniqueness of hypersaline environment is that it allows a great variety of microbes to inhabit it besides high salt concentrations. A wide variety of halophiles including heterotrophic, chemoautotrophic, chemolithotrophic aerobes and anaerobes could be observed transforming diverse range of substrates in hypersaline habitats [12,13]. The largest family of Archaea, the Halobacteriaceae holds its name as the extremely halophilic group containing the highest salt requiring microorganisms. High sodium chloride concentration allows members of Halobacteriaceae to degrade organic compounds aerobically [7]. Halophilic Archaea including the members of genus Haloarcula and Haloferax possess the ability to grow anaerobically in the presence of nitrate as electron acceptor resulting in the formation of nitrous oxide and gaseous nitrogen [14].

1.3 Habitat

Hypersaline habitats favour microbial species having complexity in their composition and nature [15]. Even with recent advancements in technology in molecular science, the fraction of discovered microorganisms is minor and exploration is still needed [16]. Halophiles have been discovered from diverse habitats including the most noxious environments on the planet. This fluctuation in the environment from the normal condition has allowed these extremophiles to develop rare skills for survival in nature [17-19]. Diversification of halophiles is largely because of their acclimatization ability in all hypersaline biotopes. These hypersaline environments are increasing as a result of manmade global as well as natural changes [13].

Habitats like soda lakes, thalassohaline, athalassohaline environments, Dead Sea, carbonate springs, salt lakes, alkaline soils and many others favors the existence of halophiles. Variations in

composition and nature of these habitats do exist. Formation of thalassohaline environments occurs by the evaporation of seawater leading to the increase in sodium and chloride ions with slightly alkaline pH.

The athalassohaline environment faces difference in ionic composition as compared to sea water, containing salts of non-marine proportion. In sea water high concentration of K^+ and Mg^+ salts is left as a result of NaCl precipitation [20]. Soda lakes prove themselves to be the most productive ecosystems exhibiting pH nearly 11, greatly favoring salt loving microbes to flourish. The exploration of soda lakes is less because of their inaccessibility [21] but recently this biota has received attention of microbiologists [21]. Dead Sea exhibits its uniqueness as the concentration of monovalent cations is less than divalent cations in it. Being the largest hypersaline environment, dense microbial blooms are supported by it [19,20] (Tables 1 and 2).

In solar salterns, variation in salinity content has been observed. These ponds depending upon climatic conditions, water retention time and status of the deposited nutrients allows variety of halophilic biota to thrive. Often extensive growth of pigmented microbes imparts red color to these crystallizer ponds [22]. In Asia (Pakistan), Khewra Salt mines also provide an extensive and rich habitat for salt loving micro biota. Saline soils mostly assist the growth of halotolerant microbes rather than halophiles. Other halophiles have been successfully isolated from meat, salted fish, desert plants and animals [23].

1.4 Physiology

a) Internal ion concentration

For survival in extreme saline conditions, halophiles maintain their internal ion concentration in such a way that their cytoplasm is not affected by the external

Table 1. Main characteristics of major halophilic gram-negative members.

Feature	<i>Halomonas elongata</i>	<i>Halomonas halodurans</i>	<i>Halomonas halophile</i>	<i>Halomonas salina</i>	<i>Halomonas israeliensis</i>
Morphology	Rods	Rods	Rods	Short rods	Rods
Size	ND	0.4-0.6×1.5-2.0	0.5-0.7×1.5-2.0	0.7-0.8×2.0-2.5	ND
Pigmentation	None	None	Cream	Yellowish or cream	Cream
Motility	+	+	+	-	ND
NaCl Range (%)	3.5-20	3.5-20	2-30	2.5-20	3.5-20
NaCl Optimum (%)	3.5-8	8	7.5	5	8
Temp Range (°C)	15-45	4-37	15-45	15-40	15-45
pH Range	5-9	5.5-8.5	5-10	6-10	5-9
Habitat	Salterns	Estuarine water	Saline Soils	Saline soils	Dead sea
Phylogenetic Branch	Gamma <i>Proteobacteria</i>	Gamma <i>Proteobacteria</i>	Gamma <i>Proteobacteria</i>	Gamma <i>Proteobacteria</i>	Gamma <i>Proteobacteria</i>

*ND, Not determined; -, negative; +, positive

osmotic conditions. This osmotic balance is achieved by accumulating salts internally [24] (Table 3).

b) Tolerance to metals

Metal tolerance has been distinctively observed in many halophilic species from both domains, Archaea and Bacteria. Heterogenous response to arsenic, cadmium, chromium and copper was shown by multiple halophilic cultured and fresh strains. *Flavobacterium* strains proved to be the most metal tolerant whereas *Acinetobacter* strains were most sensitive to metals. Variations in salinity directly affected tolerance in halophiles. In cultured strains, reduction in yeast extracts content lead to decrease in metal tolerance [25].

c) Osmoadaptation

Inhabiting environments with high salinity and being ubiquitous in nature, halophiles encounter varying salt concentrations with effective osmotic strategies [26]. Different genera of halophiles have evolved multiple mechanisms favoring their survival in the noxious environments. Mostly halophiles rely upon the usage of compatible solutes and Na^+/H^+ antiporter system based sodium pumps for the maintenance of internal osmotic environment [27]. Recently a number of such compatible solutes have been discovered with high biotechnological importance and application [28,29].

Nature of compatible solutes used by halophiles varies with the provided environment and type of microbe. Mostly two major groups of compatible solutes including polyols and sugars and amino acid derivatives are used for osmotic balancing. The complex nature of these compatible solutes has still to be studied thoroughly as presently lack of highly advanced molecular tools is being faced [30]. Archaea and bacteria use different compatible solutes. Mostly anionic solutes derived from carbohydrates of bacteria are utilized by Archaea [31]. Polyol phosphodiester are also preferentially used by Archaea. Considering bacteria, α -glutamate and proline, α -amino acids derivatives are the widespread compatible solutes which allow them to maintain osmotic balance [32,33]. For certain energetic reasons, rather than biosynthesis, uptake of compatible solutes is preferred including glycine betaine, ectoine, glycerol and others (Figure 2). The most widely used compatible solute glycine betaine has at least one transporter in all Archaea and bacteria [33]. For example, *Methanosarcina mazei* possesses *Ota* and *Otb* glycine betaine transporters comprising of three subunits [34,35]. Through transcription analysis, it was revealed that when *Ota* is expressed, glycine betaine transportation is carried out but its deletion results in halting of the solute transport [33] (Figure 2).

Table 2. Main characteristics of major halophilic gram-positive members.

Feature	<i>Halobacillus halophilus</i>	<i>Halobacillus litoralis</i>	<i>Bacillus halophilus</i>	<i>Marinococcus halophilus</i>	<i>Saliococcus hispanicus</i>
Morphology	Cocoid	Rods	Rods	Cocci	Cocci
Size	1.0-2.0×2.0-3.0	0.7-1.1×2.0-4.5	0.5-1.0×2.5-9.0	1.0-1.2	1.0-2.0
Pigmentation	Orange	Orange	None	Yellow-Orange	Reddish-Orange
Motility	+	+	+	+	-
NaCl Range (%)	2-20	0.5-25	3-30	2-25	0.5-25
NaCl Optimum (%)	10	10	15	10	10
Temp Range (°C)	15-40	10-43	15-20	15-37	15-37
pH Range	7-9	6-9.5	6-8	ND	5-9
Habitat	Saline soils, salterns	Great Salt Lake	Rotting wood from sea water	Salterns, saline soils	Salterns, saline soils
Phylogenetic Branch	Low-G1C gram positive	Low-G1C gram positive	ND	Low-G1C gram positive	ND

*ND, Not determined; -, negative; +, positive

Table 3. Intracellular ionic concentrations of moderately halophilic bacteria.

Bacterial Strains	Medium Concentration _v			Intracellular Concentration		
	Na	K	Cl	Na	K	Cl
<i>Halomonas elongata</i>	0.06	0.02	-	0.04	0.02	-
<i>Halomonas canadensis</i>	0.6	0.04	-	0.05	0.34	-
<i>Halomonas israelensis</i>	2.0	-	-	1.14	-	-
<i>Pseudomonas halosaccharolytica</i>	1.0	0.006	1.0	0.90	0.71	0.71
<i>Salinivibrio costicola</i>	1.0	0.004	-	0.68	0.22	-
<i>Micrococcus varians</i>	1.0	-	-	1.17	0.03	-
<i>Bacillus haloalkaliphilus</i>	3.4	-	-	3.52	0.31	2.7

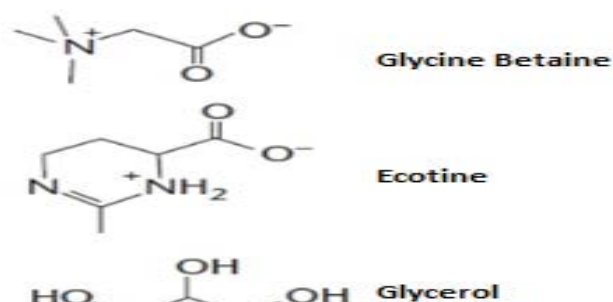


Figure 2. Structure of three common compatible solutes in halophiles [20].

Some alkaliphilic halophiles possess cytoplasmic polyamines and acidic polymers in their cell wall to protect cell against fluctuating pH. Excessive accumulation of Na^+ leads to high cytotoxicity and may cause cellular death. The evolution of ATP driven efflux pumps as well as Na^+/H^+ antiporters have favored halophiles to avoid this intense cytotoxicity. It has been observed that the expression of Na^+/H^+ antiporter genes contributes in Na^+ homeostasis, thus regulating salt concentration [36,37]. The combine action of antiporter genes as well as efflux pumps support halophiles to cope up with the challenging and life threatening salinity extremes. Their mode of action helps in the maintenance of a stable internal osmotic balance without losing cellular integrity placing halophiles in a state where their survival is ensured [38].

1.5 Halophilic enzymes and biotechnological applications

Applications of halophilic microorganisms are varied and strains from both domains possess great biotechnological potential [2]. As halophiles possess the ability to flourish in saline environment which limits the growth of other microbial life, are used in producing transgenic crops with salt tolerant character. Halobacteriaceae contributes the production of certain carotenoid pigments helping absorption of light and increasing evaporation in saltern crystallizer ponds. Halobacterium salinarum produces an integral membrane protein, bacteriorhodopsin, which is being used as holographic storage material in computer memory and processing units. Certain strains from Archaea, belonging to generas Halobacterium, Haloferax and Haloarcula contribute in the degradation of certain hydrocarbons and insecticides [9] (Table 4).

Table 4. Applications of halophilic Bacteria and Archaea.

Archaea	
<i>Halobacterium salinarum</i>	Bacteriorhodopsin (Holographic storage material, computer memory and processing units)
<i>Haloferax mediterranei</i>	Poly-3-hydroxybutyrate (PHB)

<i>Haloferax volcanii</i>	Extracellular polysaccharides
<i>Natronomonas pharaonic</i>	Chymotrypsinogen B-like protease
Bacteria	
<i>Salicola marasensis</i>	Lipase production
<i>Pseudoalteromonas ruthenica</i>	Haloprotease production
<i>Halomonas elongata</i>	Ecotine and hydroxtecotine production
<i>Halorhodospira halophila</i>	Production of glycine, betaine, ectoine and trehalose

A vast variety of halophiles are being used and targeted for their unique commercial applications for example as food coloring pigments in food industry, stress protectant metabolites, additives in cosmetic industry etc. [39]. Salt tolerant exoenzymes including amylases, proteases and lipases have been successfully produced from halophilic Archaea and bacteria at commercial level [40]. Multiple halophilic strains from both domains are being used as drug targets in seeking cure for certain lethal diseases [2].

Halophilic bacteria favor biodegradation of certain hazardous organic and inorganic compounds in both aerobic and anaerobic conditions. Wastewater containing high concentrations of such compounds is being treated with these bacteria in hypersaline environments [41]. Bioremediation of heavy metals like cadmium, mercury, arsenic etc from wastewater is being carried out by halophilic bacteria. Compounds like phenol, formaldehyde, organophosphorous and others are successfully degraded by halophilic bacteria including members of family Halomonadaceae, *Marinobacter hydrocarbonoclasticus* degrades oil hydrocarbons in saline conditions and proves itself as hydrocarbon degrader. Halophiles are also being considered to be used in sustainable bioenergy technology to overcome the expected shortage of petroleum and concerns regarding global warming [42-44].

2. Conclusion

Halophiles bacteria and Archaea are a unique and diverse group extremophilic microbes that are well adapted to harsh, hypersaline conditions. They possess the ability to compete the denaturing effects of salts. The diversity of these microbes in hypersaline environments is of growing interest. A few hypersaline environments have been extensively studied using molecular methodologies. Molecular mechanisms of adaptation to hypersaline conditions have only recently been studied and modern findings of bacterial and Archaea metabolic activities suggests that these unique microbes possess genes conferring salt tolerance by synthesizing compatible solutes or osmolytes that can withstand the denaturing effects of salts. These genes and their regulation have become

the focus of recent investigations for the production of transgenic crops and novel products.

Halophiles possess the potential to provide significant opportunities for biotechnology. Natural and man-made global changes are resulting in increasing hypersaline environments. Moreover, by the concentration of sea water in arid environments, hypersaline environments may easily be created. These facts, along with the presence of novel and stable biomolecules in halophilic bacteria and Archaea, suggest that these microorganisms will prove even more treasured in coming.

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