

- Introduction
- Halophilicity and Osmotic Protection
- Hypersaline Environments
- Eukaryotic Halophiles
- Prokaryotic Halophiles
- Biotechnology
- Conclusions and Future Prospects

Halophiles

Shiladitya DasSarma, *University of Massachusetts, Amherst, Massachusetts, USA*

Priya Arora, *University of Massachusetts, Amherst, Massachusetts, USA*

Halophiles are salt-loving organisms that inhabit hypersaline environments. They include mainly prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts.

Introduction

Halophiles are salt-loving organisms that inhabit hypersaline environments. They include mainly prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts. 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. Their novel characteristics and capacity for large-scale culturing make halophiles potentially valuable for biotechnology.

Halophilicity and Osmotic Protection

Although salts are required for all life forms, halophiles are distinguished by their requirement of hypersaline conditions for growth. They may be classified according to their salt requirement: slight halophiles grow optimally at 0.2–0.85 mol L⁻¹ (2–5%) NaCl; moderate halophiles grow optimally at 0.85–3.4 mol L⁻¹ (5–20%) NaCl; and extreme halophiles grow optimally above 3.4–5.1 mol L⁻¹ (20–30%) NaCl. In contrast, nonhalophiles grow optimally at less than 0.2 mol L⁻¹ NaCl. Halotolerant

organisms can grow both in high salinity and in the absence of a high concentration of salts. Many halophiles and halotolerant microorganisms can grow over a wide range of salt concentrations with requirement or tolerance for salts sometimes depending on environmental and nutritional factors.

High osmolarity in hypersaline conditions can be deleterious to cells since water is lost to the external medium until osmotic equilibrium is achieved. To prevent loss of cellular water under these circumstances, halophiles generally accumulate high solute concentrations within the cytoplasm (Galinski, 1993). 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. 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.

Hypersaline Environments

Though the oceans are, by far, the largest saline body of water, hypersaline environments are generally defined as those containing salt concentrations in excess of sea water (3.5% total dissolved salts). Many hypersaline bodies derive from the evaporation of sea water and are called thalassic. A great diversity of microbial life is observed in thalassic brine from marine salinity up to about 3–3.5 mol L⁻¹ NaCl, at which point only a few extreme halophiles can grow, e.g. *Halobacterium*, *Dunaliella*, and a few bacterial species. Athalassic waters are those in which the salts are of nonmarine proportion, found for example after the concentration of sea water leads to precipitation

of NaCl, leaving a high concentration of potassium and magnesium salts. This point marks the upper limit of resistance of all biological forms.

The two largest and best-studied hypersaline lakes are the Great Salt Lake, in the western United States, and the Dead Sea, in the Middle East. The Great Salt Lake is larger (3900 km²) and shallower (10 m), and contains salts that are close in relative proportion to sea water. The Dead Sea is smaller (800 km²) and deeper (340 m), and contains a very high concentration of magnesium salts. Both of these lakes are close to neutral pH, although the Great Salt Lake is slightly alkaline while the Dead Sea is slightly acidic. Compared to smaller hypersaline ponds, the compositions of these lakes are fairly constant as a result of their size, although recent human activities have had significant effects on the chemistry and biology of both. For example, a railroad causeway built in 1959 divided the Great Salt Lake into northern and southern sections, leading to dilution of the southern section, which receives the greatest inflow of fresh water from streams, and the concentration of the northern section to nearly saturating salinity. Diversion of incoming freshwater streams for irrigation in the Dead Sea basin in recent years has also had significant impact on its size and salinity (Watzman, 1997).

Many small evaporation ponds or sabkhas are found near coastal areas, where sea water penetrates through seepage or via narrow inlets from the sea. Notable among these are Solar Lake, Gavish Sabkha and Ras Muhammad Pool near the Red Sea coast, Guerrero Negro on the Baja

California coast, Lake Sivash near the Black Sea, and Sharks Bay in western Australia. Hypersaline evaporation ponds have also been found in Antarctica (e.g. Deep Lake, Organic Lake and Lake Suribati), several of which are stratified with respect to salinity.

A number of alkaline hypersaline soda brines also exist, including the Wadi Natrun lakes of Egypt, Lake Magadi in Kenya, and the Great Basin lakes of the western United States (Mono Lake, Owens lake, Searles Lake and Big Soda Lake), several of which are intermittently dry. Soda brines are lacking in magnesium and calcium divalent cations because of their low solubility at alkaline pH. Many smaller hypersaline pools represent especially dynamic environments, experiencing significant seasonal variations in size, salinity and temperature.

In addition to natural hypersaline lakes, numerous artificial solar salterns have been constructed for the production of sea salts (Figure 1). These usually consist of a series of shallow evaporation ponds connected by pipes and canals. As evaporation occurs, brine is directed into ponds with progressively greater salinities until sequential precipitation of calcium carbonate, calcium sulfate (gypsum) and NaCl (halite) occurs. After NaCl precipitation, the concentrated potassium and magnesium chloride and sulfate brines ('bitterns') that remain are usually returned to the sea.

Hypersaline environments also occur in subterranean evaporite deposits and deep-sea basins created by the evaporation and flooding of ancient seas. Deep-sea brines



Figure 1 Halobacterial bloom in a solar saltern. Dense growth of halophilic microorganisms in hypersaline environments, like this saltern near San Francisco, leads to reddening of the brine. Cell densities of 10^7 ml⁻¹ species *Halobacterium* are not uncommon. Courtesy of Drs Nicole Tandeau de Marsac and Germaine Stanier, Pasteur Institute, Paris.

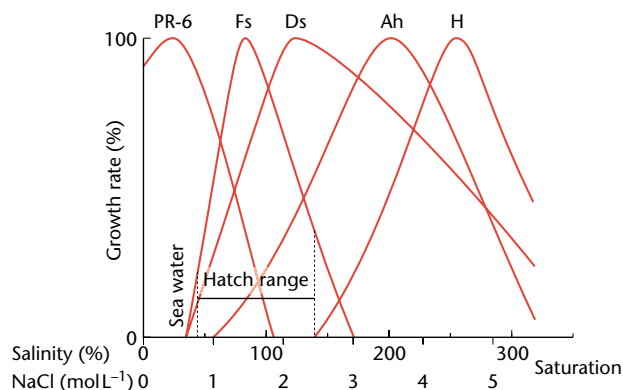


Figure 2 Salt-tolerance of halophilic organisms. Relative growth rate is plotted against both percentage salinity and NaCl concentration. The five microorganisms are *Agmenellum quadraplicatum* (PR-6), a slightly halotolerant cyanobacterium, *Fabrea salina* (Fs), a moderately halophilic protozoan, *Dunaliella salina* (Ds), a halophilic green alga, *Aphanothece halophytica* (Ah), an extremely halophilic cyanobacterium, and *Halobacterium* sp. (H), an extremely halophilic archaeon. The salinity of sea water and the hatch range for brine shrimp are noted.

are relatively stable as a result of their higher density and have been found in the Red Sea and Gulf of Mexico (MacDonald *et al.*, 1990). Preliminary studies have suggested that microbial activity occurs in some deep-sea hypersaline basins and viable microorganisms may be recoverable from brine inclusions in ancient salt deposits over 100 million years old.

A common phenomenon in hypersaline environments is the production of gradients of salinity owing to the evaporation of sea water. This process leads to sequential blooms of diverse microbial species adapted to different ranges of salinity (Figure 2). As brine is concentrated from 1 mol L^{-1} NaCl to about 3.5 mol L^{-1} , dense algal populations are supported, on which brine shrimp and larvae of brine flies feed. Protozoa are also found, as are yeasts and other fungi. Microbial mats, containing predominantly photosynthetic unicellular and filamentous cyanobacteria, and purple and green sulfur and non-sulfur bacteria, cover the bottom of many hypersaline ponds. In the anoxic zones of the mats and in the sediment below, a variety of sulfur-oxidizing, sulfate-reducing, homoacetogenic, methanogenic and heterotrophic bacteria and archaea occur. From about 4 mol L^{-1} NaCl to saturation ($> 5.2 \text{ mol L}^{-1}$ NaCl), red-orange halobacteria (halophilic archaea) dominate the brine pools and most other microbial activity ceases.

Eukaryotic Halophiles

Multicellular eukaryotes

Few such organisms can tolerate hypersaline conditions and the highest salinity at which any vertebrates have been observed (e.g. *Tilapia* species) is about 1 mol L^{-1} NaCl. A variety of obligate and facultative halophytic plants, e.g. *Atriplex halimus* and *Mesembryanthemum crystallinum*, can survive in moderately high saline soils. There are also a surprising number of invertebrates that can survive in hypersaline environments. Some examples are rotifers such as *Brachionus angularis* and *Keratella quadrata*, tubellarian worms such as *Macrostomum* species, copepods such as *Nitocra lacustris* and *Robertsonia salsa*, ostracods such as *Cypridis torosa*, *Paracyprideinae* spp., *Diacypris compacta*, and *Reticypriis herbsti*. Some insects from hypersaline environments include brine flies *Ephydra hians* and *E. gracillis* and brine shrimp *Artemia franciscana* and related species. Some hypersaline environments help to support many birds, one of the most spectacular of which is the pink flamingo.

Algae

At moderately high salinities ($1\text{--}3.5 \text{ mol L}^{-1}$ NaCl) dense populations of green algae ($> 10^5 \text{ ml}^{-1}$), are supported. These are obligately aerobic, photosynthetic, unicellular eukaryotic microorganisms, some species of which produce large quantities of orange-coloured β -carotene at high salinities. Green algae of the genus *Dunaliella*, e.g. *Dunaliella salina*, *D. parva*, and *D. viridis*, are ubiquitous and are the main source of food for brine shrimps and the larvae of brine flies. Most species of green algae are moderate halophiles, with only a few extremely halophilic species, e.g. *Dunaliella salina* and *Asteromonas gracilis*, which can grow even in saturated NaCl.

Algae predominantly use polyols as compatible solutes. In *Dunaliella salina*, glycerol is synthesized in response to osmotic stress. The cytoplasmic concentration of glycerol can reach 7 mol L^{-1} when grown in medium containing 5 mol L^{-1} NaCl and can constitute over 50% of the dry weight of the cells. The intracellular sodium concentration has been reported to be less than 100 mmol L^{-1} over a wide range of external salt concentrations. During moderate dilution stress, glycerol does not leak out of cells but is metabolized and transformed into osmotically inactive reserve material.

Diatoms are algae surrounded by silica cell walls and are commonly found but rarely abundant in hypersaline environments. A variety of species have been found at about 2 mol L^{-1} NaCl, although the upper limit for diatom growth is about 3 mol L^{-1} NaCl. Examples of common diatoms include *Amphora coffeaeformis* and *Nitzschia* and *Navicula* species. Although osmoregulation has not been studied extensively in diatoms, accumulation

of proline and oligosaccharides has been reported in some species.

Protozoa

A large variety of protozoa, which are cell wall-less chemoheterotrophic protists that ingest algae and bacteria, have been described from hypersaline environments. Identified species include the moderate halophile *Fabrea salina* from a west Australian lake (Post *et al.*, 1983), and the extreme halophile *Porodon utahensis* from the Great Salt Lake. Although protozoa are known to regulate osmotic pressure in freshwater with contractile vacuoles that expel water, their mechanism of osmoregulation in hypersaline brine has not been investigated.

Fungi

Yeasts and other fungi are chemoheterotrophic cell-walled eukaryotes, some of which are well adapted to tolerate hypersaline environments. They grow best under aerobic conditions on carbohydrates at moderate temperatures and acidic to neutral pH. *Debaromyces hansenii* is a halotolerant yeast, isolated from sea water, that can grow aerobically up to salinities of 4.5 mol L^{-1} NaCl. It produces glycerol as a compatible solute during the logarithmic phase and arabitol in the stationary phase. A saprophytic hyphomycete, *Cladosporium glycolicum*, was found growing on submerged wood panels at a salinity exceeding 4.5 mol L^{-1} NaCl in the Great Salt Lake. Halophilic fungi, e.g. *Polypaecilum pisce* and *Basipetospora halophila*, have also been isolated from salted fish.

Prokaryotic Halophiles

Cyanobacteria

Cyanobacteria (or 'blue-green algae') are bacterial prokaryotes that are characterized by the presence of chlorophyll *a* and phycobilin pigments and carry out oxygenic photosynthesis. They dominate the planktonic biomass and form microbial mats in many hypersaline lakes. The top brown layer of microbial mats contains a common unicellular cyanobacterial species, *Aphanothece halophytica*. It can grow over a wide range of salt concentrations, from $2\text{--}5 \text{ mol L}^{-1}$ NaCl, is an extreme halophile with a salt optimum of 3.5 mol L^{-1} , and lyses in distilled water. It uses glycine betaine as the major compatible solute, which it can take up from the medium or synthesize from choline. *A. halophytica* and similar unicellular cyanobacteria have been described from the Great Salt Lake, Dead Sea, Solar Lake and artificial solar ponds. A planktonic cyanobacterium reported from the Great Salt Lake is *Dactylococcopsis salina*.

A variety of filamentous cyanobacteria, e.g. in the order Oscillatoriales, such as *Oscillatoria neglecta*, *O. limnetica*, *O. salina* and *Phormidium ambiguum*, have also been described that develop in the green second layer of mats in hypersaline lakes. These are more moderate halophiles, usually growing optimally at $1\text{--}2.5 \text{ mol L}^{-1}$ NaCl, and form heterocysts that fix nitrogen. Another common species in the same family is *Microcoleus chthonoplastes*. The diversity of cyanobacteria occurring in hypersaline environments has not been studied extensively.

Other phototrophic bacteria

Phototrophic bacteria occur beneath the cyanobacterial layers in anaerobic but lighted zones in hypersaline microbial mats. They usually grow anaerobically by anoxygenic photosynthesis, although many also have the capacity to grow aerobically as heterotrophs. They can use reduced sulfur (hydrogen sulfide, elemental sulfur), organic compounds or hydrogen as electron donors. They include green and purple sulfur and non-sulfur bacteria that are characterized by bacteriochlorophyll pigments. The green sulfur bacteria, such as the slight to moderately halophilic *Chlorobium limicola* and *C. phaeobacteriales*, deposit elemental sulfur granules outside their cells and are capable of nitrogen fixation. *Chlorobium limicola* can take up glycine betaine from the environment and synthesize trehalose for use as an osmolyte. The moderately halophilic, filamentous green non-sulfur bacteria such as *Chloroflexus aurantiacus* are also slightly thermophilic.

Halophilic purple sulfur bacteria such the Chromatiaceae, which deposit sulfur granules inside cells, include mainly moderate halophiles, e.g. *Chromatium glycolicum*, which grows photoorganotrophically using glycolate and glycerol, *C. violescens* and *C. salexigens*. They synthesize *N*-acetylglutaminylglutamine amide as a minor component of their compatible solute and use sucrose and glycine betaine from their environment. The moderate halophiles *Thiocapsa roseoparsarcina* and *T. halophila* from Guerrero Negro both synthesize sucrose and take up glycine betaine from the environment. *T. halophila* also synthesizes glycine betaine and *N*-acetylglutaminylglutamine amide for osmoprotection. The moderately halophilic purple non-sulfur bacterium *Rhodospirillum salexigens* from evaporated seawater pools and *R. salinarum* from a saltern both use glycine betaine, and *R. salexigens* also uses ectoine as an osmolyte.

The purple sulfur bacteria, *Ectothiorhodospira* species, dominate alkaline soda lakes in Egypt and Central Africa. The moderate halophile *Ectothiorhodospira marismortui* is a strict anaerobe and uses carboxamines as compatible solutes and uses the osmolyte *N*- α -carbamoyl-L-glutamine-1-amide. The extreme halophile *Ectothiorhodospira halochloris* isolated from Wadi Natrun, was the first bacterium shown to synthesize and accumulate ectoine, a

cyclic amino acid, which it uses along with glycine betaine and trehalose as compatible solutes.

Sulfur-oxidizing bacteria

Below the cyanobacteria and the phototrophic bacteria in microbial mats, halophilic, filamentous, carbon dioxide-fixing bacteria oxidize hydrogen sulfide (and elemental sulfur) to sulfate. Examples include the filamentous *Achromatium volutans* from Solar Lake, *Beggiatoa alba* from Guerrero Negro, and *B. leptiformis* from Solar Lake. A unicellular halophilic, chemoautotrophic sulfur-oxidizing bacterium, *Thiobacillus halophilus*, from a hypersaline western Australian lake, has also been described.

Anaerobic bacteria and archaea

A large variety of facultative and strictly anaerobic bacteria and archaea inhabit the bottom layers of microbial mat communities and sediment in hypersaline lakes. These include fermentative bacteria, homoacetogenic bacteria, sulfate-reducing bacteria and methanogenic archaea. Fermentative anaerobic bacteria that grow at saturated NaCl concentrations have been described. One example is *Haloanaerobacter chitinovorans*, isolated from a saltern, which is capable of fermenting chitin contained in brine shrimp and brine flies. Other more moderate halophilic isolates are *Haloanaerobacter saccharolytica*, which ferments carbohydrates, *Halobacterioides acetothylicus*, from an oil well, and *Halocella cellulolytica*, which ferments carbohydrates including cellulose. *Sporohalobacter lorretii* and *S. marismortui* are sporogenous and ferment carbohydrates.

Several homoacetogens, strict anaerobes that produce acetate from oxidation of sugars or amines, have been described. For example, *Haloicola saccharolytica* ferments carbohydrates and *N*-acetylglucosamine and can grow at a wide range of NaCl concentrations. *Acetohalobium arabaticum*, which grows from 1–4.5 mol L⁻¹ NaCl, grows on glycine betaine and trimethylamine. *A. arabaticum*, isolated from Lake Sivash, also has the ability to reduce carbon dioxide to acetate and is a likely competitor of sulfate-reducing bacteria for hydrogen.

Sulfate-reducing bacteria use sulfate as the terminal electron acceptor, although many can also utilize other sulfur compounds, nitrate, and fumarate. They differ in their ability to oxidize different compounds, though most use low-molecular weight organic species such as lactate, pyruvate, ethanol and volatile fatty acids or hydrogen as electron donors. A few can use carbon dioxide as the sole carbon source. Although many slightly halophilic sulfate reducers have been isolated, mostly from marine environments, relatively few that can survive at an extremely high salinity have been cultured.

Desulfohalobium retbaense, isolated from Lake Retba, Senegal, and *Desulfovibrio halophilus*, from Solar Lake, are two moderately halophilic sulfate-reducing species that have been described. These can grow at up to 4 mol L⁻¹ NaCl, but only relatively slowly. Another isolate, from the deep-sea hypersaline pools in the Red Sea, is similar to *D. halophilus*. The osmoregulation of sulfate-reducing bacteria has not been studied extensively; preliminary indications are that they do not synthesize compatible solutes but accumulate salts internally.

Methanogens from hypersaline environments generally use methylotropic substrates rather than carbon dioxide, acetate and hydrogen, and are strict anaerobic archaea. Several, mostly moderate halophilic, methanogens have been identified, including *Methanohalophilus halophilus* from a microbial mat, *M. muhii* from the Great Salt Lake, and *M. portucalensis* from a saltern. The slight halophile *Methanosalsus zhilinae* is also an alkaliphile and a slight thermophile. The extremely halophilic methanogen, *Methanohalobium evestigatum*, with an NaCl optimum of 4.5 mol L⁻¹, is also a thermophile with a temperature optimum of 50°C. Methanogenesis has also been reported from deep-sea brine pools in the Gulf of Mexico that contain moderately high salinity. Methanogens use β -amino acids (β -glutamine, *N*- ϵ -acetyl- β -lysine) as compatible solutes and also play an important role in the anaerobic degradation of glycine betaine in their environments. Their intracellular salt concentration is somewhat higher than that of most bacteria, about 0.6 mol L⁻¹ KCl, but is significantly lower than for the halophilic archaea (halobacteria) (see below).

Aerobic and facultative anaerobic Gram-negative bacteria

Many moderately halophilic, heterotrophic Gram-negative bacteria belonging to the *Halomonas* and *Chromohalobacter* genera have been described. Other genera with halophilic representatives include *Salinivibrio*, *Arhodomonas*, *Dichotomicrobium*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Alteromonas*, *Acinetobacter*, and *Spirochaeta*. Most of these are heterotrophs, and include *Arhodomonas aqueoli*, isolated from a subterranean brine associated with an oil field and capable of nitrate reduction; *Chromohalobacter marismortui* from the Dead Sea, also capable of nitrate reduction; *Pseudomonas beijerinckii* from salted beans preserved in brine; *Pseudomonas halophila* from the Great Salt Lake; and *Salinivibrio costicola*, originally isolated from Australian bacon. Several *Halomonas* species are capable of nitrate reduction, including *H. elongata*, isolated from a solar saltern, and *H. halodentrificans*, isolated from meat-curing brines. Others include *H. eurihalina*, isolated from saline soil, which produces an extracellular polysaccharide; *H. halodurans*, from estuarine waters, which is capable of degrading aromatic

compounds; *H. halophila*, from saline soil; *H. panteler-iense*, from alkaline saline soil, which grows at a pH optimum of 9; *H. salina*, from saline soil; and *H. subglaciescola*, from beneath the ice of Organic Lake in Antarctica. These organisms use primarily glycine betaine and ectoine as the compatible solutes. Genes for uptake of glycine betaine from the medium (*betT*) and for its synthesis from choline (*betI*, A, and B) and for synthesis of ectoine (*ectA*, B, and C) have been cloned from *Halomonas* species and some other halophiles.

Among spirochaetes, the moderate halophile *Spirochaeta halophila*, found in Solar Lake, is a chemolithotroph capable of iron and manganese oxidization. The flavobacteria *Flavobacterium gondwanense* and *F. salegens*, are psychrotolerant halophiles isolated from Antarctic Lakes.

Gram-positive bacteria

This group includes moderately halophilic species of the genera *Halobacillus*, *Bacillus*, *Marinococcus*, *Salinococcus*, *Nesterenkonia*, and *Tetragenococcus*. They include cocci such as *Nesterenkonia halobia*, isolated from salterns, which produce yellow-red carotenoid pigments; *Tetragenococcus halophilus*, from fermented soy sauces and squid liver sauce, and from brine for curing anchovies, which are capable of lactic acid fermentation; and several *Salinococcus* species from salterns. Other examples include *B. diposauri*, from the nasal cavity of a desert iguana; *B. haloalkaliphilus*, from Wadi Natrun; and *B. halodenitrificans*, from a solar saltern in southern France. *Halobacillus litoralis* and *H. trueperi* are found in the Great Salt Lake. *Sporosarcina halophila* is an endospore-forming bacterium, from which the compatible solute *N*- ϵ -acetyl-lysine was originally isolated. Many of these organisms use proline, ectoine or *N*-acetylated diamino acids, which they are capable of synthesizing, as a compatible solute.

Actinomycetes from saline soils include *Actinopolyspora halophila*, which grows best at moderate NaCl concentrations and is one of the few heterotrophic bacteria that can synthesize the compatible solute glycine betaine, and *Norcardopsis halophila*, which uses a hydroxy derivative of ectoine and β -glutamate as compatible solutes.

Halobacteria

These extreme halophiles grow best at the highest salinities (3.4–5 mol L⁻¹ NaCl) (Figure 2), forming dense blooms (up to 10⁸ cells ml⁻¹), and resulting in the red colour of many brines (Figure 1). Common species of halobacteria are rod-, cocci- or disc-shaped, although triangular and even square-shaped species exist. Many are pleiomorphic, especially when the ionic conditions of the media are altered, and most lyse below 1–1.5 mol L⁻¹ NaCl. Halobacteria are classified as archaea (and are also called halophilic archaea or haloarchaea) and belong to the

family Halobacteriaceae. Ten genera have been reported, *Halobacterium*, *Haloarcula*, *Halococcus*, *Haloferax*, *Halorubrum*, *Halobaculum*, *Natronobacterium*, *Natronococcus*, *Natrialba* and *Natromonas*, and an eleventh genus, *Haloterrigena*, has recently been proposed.

The first microbiological analysis was conducted on several closely related *Halobacterium* strains (originally designated *H. halobium*, *H. salinarium*, and *H. cutirubrum*) isolated in the mid-twentieth century from salted fish and meat from northern Europe and North America. These are generally amino acid-utilizing facultative aerobes that require a number of growth factors and slightly elevated temperatures (38–45°C) for optimal growth. Most have distinctive features such as gas vesicles, purple membrane and red-orange carotenoids. Many have the ability to grow in the absence of oxygen via dissimilatory nitrate reduction and denitrification, fermentation of different sugars, breakdown of arginine, and use of light energy mediated by retinal pigments.

Several more recently isolated species will oxidize carbohydrates, e.g. *Haloarcula marismortui*, *Haloarcula vallismortis*, and *Haloferax volcanii* from the Dead Sea; *Haloferax mediterranei* and *Halorubrum saccharovororum* from salterns; and *Halorubrum lacusprofundi*, a psychrotolerant species from Deep Lake, Antarctica. Glucose is oxidized by a modified Entner–Doudoroff pathway and the pyruvate resulting is further oxidized by pyruvate oxidoreductase and the tricarboxylic acid cycle. Several strains are capable of growth on single carbon sources such as sugars, glycerol and acetate.

Some halobacterial species growing in alkaline lakes are alkaliphilic and others are rather acid-tolerant. *Natronobacterium pharaonis* from Wadi Natrun, and *Natronococcus occultus* from Lake Magadi, have pH optima in the range 9.5–10 and do not grow below pH 8.5. Slight acidophiles, such as *Haloferax volcanii* and *Haloferax mediterranei*, grow at pH values as low as 4.5.

The intracellular salt concentration of halobacteria has been measured to be extremely high and it is generally assumed that organic compatible solutes are not accumulated in these extreme halophiles. Potassium ions are accumulated internally up to 5 mol L⁻¹ concentration. In addition, the content of sodium ions appears to be in the molar range, although the ratio of cytoplasmic potassium to sodium is high. The potassium gradient is maintained by the combination of an electrogenic sodium ion/proton antiporter and a potassium ion uniporter (Figure 3). Amino acid uptake is carried out by a sodium ion/amino acid symporter.

Proteins of halobacteria are either resistant to high salt concentrations or require salts for activity. As a group, they contain an excess ratio of acidic to basic amino acids, a feature likely to be required for activity at high salinity. This characteristic is shared with proteins from some halophilic bacteria. Surface negative charges are thought to be important for solvation of halophilic proteins, and to

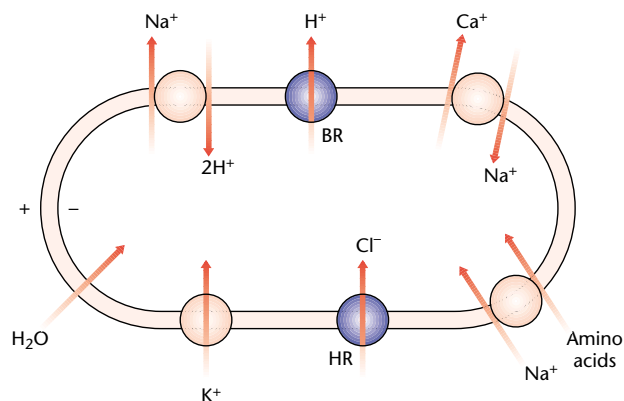


Figure 3 Flux of ions and molecules across the membrane of halobacteria. The light-driven proton and chloride pumps, bacteriorhodopsin (BR) and halorhodopsin (HR) (respectively), are shown in blue, and the sodium/proton antiporter, potassium uniporter, sodium/amino acid symporter and calcium/sodium antiporter are shown in pink. The directionality of ions, amino acids and water is indicated by arrows, and the electrical potential across the membrane is indicated by the + and - charges at the left (- inside).

prevent the denaturation, aggregation and precipitation that usually results when nonhalophilic proteins are exposed to high salt concentrations. The structure of one enzyme of halobacteria, malate dehydrogenase, has been determined by X-ray crystallography (Dym *et al.*, 1995) (Figure 4).

A unique feature of halobacteria is the purple membrane, specialized regions of the cell membrane that contain a two-dimensional crystalline lattice of a chromoprotein, bacteriorhodopsin. Bacteriorhodopsin contains a protein moiety (bacterioopsin) and a covalently bound chromophore (retinal) and acts as a light-dependent transmembrane proton pump (Krebs and Khorana, 1993) (Figure 3). The membrane potential generated can be used to drive ATP synthesis and support a period of phototrophic growth. Bacteriorhodopsin is induced by low oxygen tension and high light intensity and can cover more than 50% of the surface of the cells.

Halobacteria produce large quantities of red-orange carotenoids. Carotenoids have been shown to be necessary for stimulating an active photorepair system for repair of thymine dimers resulting from ultraviolet radiation. The most abundant carotenoids are C-50 bacterioruberins, although smaller amounts of biosynthetic intermediates such as β -carotene and lycopene are also present. Retinal is produced by oxidative cleavage of β -carotene, a step that requires molecular oxygen. Several retinal proteins, in addition to bacteriorhodopsin, are also produced by halobacteria, including halorhodopsin, which is an inwardly directed light-driven chloride pump, and two sensory rhodopsins, which mediate the phototactic response (swimming towards green light and away from blue and ultraviolet light) (Spudich, 1993).

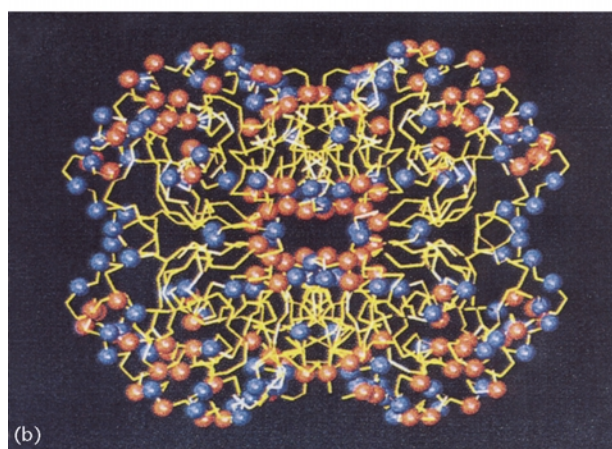
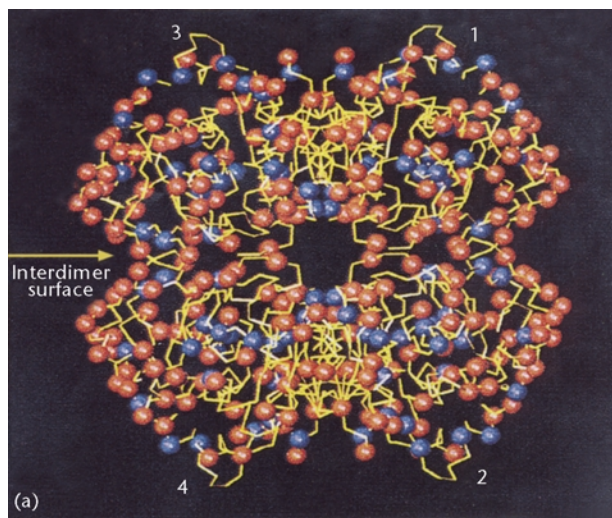


Figure 4 X-ray structure of a halophilic enzyme. (a) The structure of a tetramer of halobacterial malate dehydrogenase. (b) The structure of a homologous nonhalophilic lactate dehydrogenase. The red spheres indicate acidic residues and the blue spheres indicate basic residues.

Halobacteria produce buoyant gas vesicles, like many aquatic bacteria (DasSarma and Arora, 1997). Gas vesicles are hollow proteinaceous structures surrounding a gas-filled space. The function of gas vesicles for halobacteria, organisms whose primary metabolism is aerobic and that live in concentrated brines in which the solubility of molecular oxygen is low (especially at high temperatures), is to enable the cells to float to the more oxygenated surface layers. This also increases the availability of light for purple membrane-mediated photophosphorylation.

Halobacterial mutants that have lost purple membrane, gas vesicles or carotenoids occur spontaneously at high frequency. Genetic analysis has shown that recombinational events promoted by transposable insertion sequences are responsible for the observed genetic instabilities. Halobacteria also contain large dynamic plasmids, which are reservoirs of transposable insertion

sequences. The finding of plasmids and insertion sequences has aided the development of genetic tools for analysis and manipulation of these organisms.

Halobacteria and methanogens are phylogenetically distinct from both bacteria and eukaryotes, and are classified as archaea. As such, they exhibit features characteristic of the archaea, including eukaryotic-like transcription and translation machinery, ether-linked lipids and, like some bacteria, a cell wall S-layer composed of a glycoprotein.

Biotechnology

Although current commercial uses of halophiles are quite significant (fermentation of soy and fish sauces, β -carotene production, aquaculture), the many novel and unique properties of many of these organisms suggest that they have even greater potential for biotechnology (Rodríguez-Valera, 1992). Halophiles can survive and flourish in environments that limit the growth of most other organisms. Hypersaline environments are ubiquitous and they are spreading as a result of irrigation and other uses of fresh water. Many natural geological formations, such as petroleum reserves, are associated with hypersaline brines. Many industrial processes also use salts and frequently release brine effluent into the environment. Halophiles are likely to be useful for bioremediation of contaminated hypersaline brine.

Halophiles produce a large variety of stable and unique biomolecules that may be useful for practical applications. Halophilic microorganisms produce stable enzymes (including many hydrolytic enzymes such as DNAases, lipases, amylases, gelatinases and proteases) capable of functioning under conditions that lead to precipitation or denaturation of most proteins. Halophilic proteins compete effectively with salts for hydration, a property that may result in resistance to other low-water-activity environments, such as in the presence of organic solvents. Novel halophilic biomolecules may also be used for specialized applications, e.g. bacteriorhodopsin for bio-computing, gas vesicles for bioengineering floating particles, pigments for food colouring, and compatible solutes as stress protectants.

Conclusions and Future Prospects

Halophiles are an interesting class of extremophilic organisms that have adapted to harsh, hypersaline conditions. They are able to compete successfully for water and resist the denaturing effects of salts. They include mostly microorganisms, e.g. photosynthetic green algae, cyanobacteria and green and purple bacteria, sulfur-oxidizing bacteria, anaerobic fermentative, homoaceto-

genic, sulfate-reducing bacteria, Gram-negative and Gram-positive heterotrophic bacteria, and methanogenic and halophilic archaea.

Adaptation to hypersaline conditions is interesting from an evolutionary standpoint. The earliest prokaryotic fossils in ancient stromatolites more than 3.5 billion years old are very similar in appearance to the microbial mats found in modern hypersaline ponds. The halobacteria and methanogens are both members of the archaeal branch of the phylogenetic tree that likely appeared very early in evolution. These findings, and the likelihood of concentration of brines during prebiotic evolution, suggest that adaptation to salts may have been among the earliest evolutionary inventions (Dundas, 1998). Future studies will provide greater insights into these fascinating questions.

The diversity of microorganisms in hypersaline environments is also of growing interest. Few hypersaline environments have been carefully surveyed using molecular methods. The recent finding of bacterial and archaeal metabolic activities suggests that these environments may harbour diverse consortia of microbes that are not easily cultured. The extremes of hypersaline environments, e.g. in salinity (high magnesium and potassium concentration in bitterns), in anaerobic zones, and in high and low pH and temperature habitats, may yield especially interesting species.

Molecular mechanisms of adaptation to hypersaline conditions have only recently been studied. Halophilic organisms appear either to exclude salts by synthesis of an equally high concentration of uncharged compatible solutes or osmolytes, or to contain stable macromolecules that can withstand the denaturing effects of salts. The genes involved in synthesis and accumulation of compatible solutes and their regulation have become the focus of recent investigations. For halobacteria, early studies showed the intracellular concentration of salts to be extremely high, but, with few exceptions, their highly acidic salt-resistant proteins have not been studied in detail. Interestingly, some halobacterial DNA sequences have been shown to adopt a left-handed Z-DNA conformation, but any biological role of this unusual structure is unknown. Recent development of tools for genetic and biochemical analysis of halophiles will aid future work with these organisms.

Finally, halophiles are likely to provide significant opportunities for biotechnology. As a result of natural and man-made global changes, hypersaline environments are on the increase. Moreover, hypersaline environments may easily be created by the concentration of sea water in arid environments, two commodities in plentiful supply. These facts, together with the occurrence of novel and stable biomolecules in halophiles, suggest that these organisms will prove even more valuable in the future.

References

- DasSarma S and Arora P (1997) Genetic analysis of the gas vesicle gene cluster in haloarchaea. *FEMS Microbiology Letters* **153**: 1–10.
- Dundas I (1998) Was the environment for primordial life hypersaline? *Extremophiles* **2**: 375–377.
- Dym O, Mevarech M and Sussman JL (1995) Structural features that stabilize halophilic malate dehydrogenase from an archaeobacterium. *Science* **267**: 1344–1346.
- Galinski EA (1993) Compatible solutes of halophilic eubacteria: molecular principles, water-solute interactions, stress protection. *Experientia* **49**: 487–496.
- Krebs MP and Khorana HG (1993) Mechanism of light-dependent proton translocation by bacteriorhodopsin. *Journal of Bacteriology* **175**: 1555–1560.
- MacDonald IR, Reilly II JF, Guinasso NL Jr *et al.* (1990) Chemosynthetic mussels at a brine-filled pockmark in the northern Gulf of Mexico. *Science* **248**: 1096–1099.
- Post FJ, Borowitzka MA, Mackay B and Moulton T (1983) The protozoa of a western Australian hypersaline lagoon. *Hydrobiologia* **105**: 95–113.
- Rodriguez-Valera F (1992) Biotechnological potential of halobacteria. In: Danson MJ, Hough DW and Lunt GG (eds) *The Archaeobacteria: Biochemistry and Biotechnology*, pp. 135–147. London: Portland Press.
- Spudich JL (1993) Color sensing in the archaea: a eukaryotic-like receptor coupled to a prokaryotic transducer. *Journal of Bacteriology* **175**: 7755–7761.
- Watzman H (1997) Left for dead. *New Scientist* Feb. 8, 37–41.

Further Reading

- Cohen Y and Rosenberg E (eds) (1989) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*. Washington, DC: American Society for Microbiology.
- DasSarma S, Fleischmann EM, Robb FT, Place AR, Sowers KR and Schreier HJ (eds) (1995) *Archaea, a Laboratory Manual – Halophiles*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Javor B (1989) *Hypersaline Environments, Microbiology and Biogeochemistry*. Berlin: Springer-Verlag.
- Olliver B, Caumette P, Garcia J-L and Mah RA (1994) Anaerobic bacteria from hypersaline environments. *Microbiology Reviews* **58**: 27–38.
- Rodriguez-Valera F (ed.) (1991) *General and Applied Aspects of Halophilic Microorganisms*. New York: Plenum Press.
- Tindall BJ (1992) The family Halobacteriaceae. In: Balows A, Trüper HG, Dworkin M *et al.* (eds) *The Prokaryotes, A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd edn, pp. 768–808. New York: Springer-Verlag.
- Ventosa A, Nieto J and Oren A (1998) Biology of moderately halophilic aerobic bacteria. *Microbiology and Molecular Biology Reviews* **62**: 504–544.
- Vreeland RH and Hochstein LI (eds) (1993) *The Biology of Halophilic Bacteria*. Boca Raton, FL: CRC Press.