

APPLICATION OF HALOPHILIC BACTERIA IN MEDICINE

Abstract

Halophiles are potential competitors because of the outstanding capacity for thrive in difficult environmental factors. Thanks to novel advancements in genetic engineering, halophiles are now functioning better for industrial applications. We are most fascinated by organisms that can live in hard environments due to their hostile physiological traits, hostile growth surroundings, and synthesis of beneficial bioactive constituents. Due to their specialised cellular catalytic mechanism, halophilic microbes can endure very salinized habitats. The capability of these microbes to grow in hypersaline environments has been connected to the elevated acidic amino acid content in their proteins, which increases the negative protein surface potential. Given that they successfully use hydrocarbons as their only source of carbon and energy, these bacteria may show to be effective bioremediation agents for the treatment of salty effluents and hypersaline waters contaminated with dangerous compounds that are hard to breakdown. This is true of microorganisms (bacteria, archaea, and fungus), which can produce biomolecules with therapeutic value and live best in very saline habitats. As the microflora is explored by extensive sequencing, new visions are exposing the environmental factors under which the chemicals will generated in the microbial population without incurring any greater pressure beyond providing the similar substrate amongst other competitors, the salinity.

Keywords: Halophilic bacteria, anticancer compounds, antimicrobial compounds, archaea and fungi, biomedicine, biomolecules;.

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I. INTRODUCTION

A diverse group of microorganisms known as halophilic microorganisms can withstand extremely high salinity levels in a variety of environments. They are regarded as a particular form of extremophile, which are microbes that need extremely harsh environments to grow. Mac Elroy coined the phrase "extremophile" in 1974. Extreme conditions vary depending on the organism, thus what might be extreme for one creature may not be essential for another to survive. Numerous different microorganisms live in hypersaline settings, and they frequently do so in extremely high colony densities. Archaea, Bacteria, and Eucarya are the three basic categories of life. Halophiles that are heterotrophic, phototrophic, and chemoautotrophic in nature and capable of converting a variety of substrates. Extremophile organisms are those that can survive in conditions with extreme salt concentration, pH, temperature, as well as other factors.

Extremophiles are organisms that can only thrive in an environment that would kill most of many other species. These extremophiles divided into five groups depending on where they live: acidophiles, alkalophiles, halophiles, thermophiles, and psychrophiles. Halophiles are extremophiles that can endure conditions with high salt concentrations. These microorganisms live in the saltiest places on earth, like flats, evaporation ponds, hypersaline lakes, and tidal marine settings. Halophilic bacteria can counteract the osmotic pressure of their surroundings and withstand salts' denaturing effects. These organisms grouped in to mild (2–5 percent NaCl), moderate (5–15 percent NaCl), or extreme halophiles depending on their halotolerance or even the salt concentration at which they survive (15-30 percent NaCl). Halophilic microorganisms employ a variety of methods to survive in high-salinity- surroundings. For example, they produce suitable solutes in their cells or have transporters that enable them to endure such extreme conditions. These bacteria were found to include osmoregulatory solutes like proline, potassium ion, ectoine, betaine and glutamate. Some halophiles manufacture acidic proteins that can work in environments with a lot of salt. They have been isolated from various salinity settings, ranging from saturating salinities to natural brines and hypersaline lakes. Eukarya, Archaea, & bacteria are included in the diverse halophilic community. The Bacteria and Archaea, including *Salinibacter*, *Halobacterium* *Halomonas*, are the most frequently seen halophiles. One of the recognized excellent source of carotenoids in nature is the halophilic Eukarya, like *Dunaliella salina*. Halophiles may survive in conditions that range in salt concentration from hypersaline (0.6 M) to saturated (> 5 M NaCl). To react to a salt-stress condition, they have evolved a variety of molecular and cellular mechanisms. A common 'salt-in strategy' halophilic Archaea and Bacteria. The most well-known mechanism is the so-called referred to as the 'salt-out strategy' or 'organic-osmolyte mechanism,' this mechanism allows for osmotic adaptation by excluding salts and/or synthesising de novo compatible solutes.. Archaea and Bacteria both use this strategy. Furthermore, the molecular basis of protein halotolerance and adaptation of halophilic enzymes to high salinity is increased protein charges and hydrophobicity. Halophiles have been regarded for biotechnological applications over the last few decades. Diverse halophile response mechanisms under high-salinity conditions result in the production of a variety of valuable biomolecules. Halophiles are also known to be major sources of stable enzymes that function in extremely high salinity, a condition that causes denaturation and aggregation in most conventional proteins. For example, the -amylase isolated from *Haloarcula* sp. works

best at 4.3 M salt at 50°C and is stable in benzene, toluene, and chloroform. Furthermore, halophiles are thought to be potential sources for the discovery of bioactive compounds, compatible solutes, novel enzymes, and other potential biotechnological applications. At the moment, only two industrial processes that use halophilic microorganisms are in operation: carotenoid production by the halophilic alga *Dunaliella* and ectoine production by the halophilic bacterium *Halomonas elongate*. There are numerous bioactive compounds derived from halophiles that exhibit various biological activities ranging from antioxidant, sunscreen, and antibiotic actions. Several studies show that some halophiles can synthesise large amounts of compatible solutes like glycine betaine and ectoine. These small organic molecules can act as biomolecule stabilisers or stress protectants. A wide range of microorganisms live in hypersaline environments, and they are often found in extremely high community densities. The most common are coloured by the pigment carotenoid and others; halophilic microorganisms can be found without the use of a microscope in saltern crystallizer brines and other salt lakes with saturating or near-saturating salt concentrations. Halophilic microorganisms live in a diverse environment. Archaea, Bacteria, and Eucarya have all been found to be salt tolerant up to saturation. Some anaerobic halophilic thermoalkaliphilic bacteria are "polyextremophilic" halophiles, adapted to grow in a combination of extreme measures such like alkaline and salt conditions (a popular combination), low pH and salt (relatively uncommon), or high temperatures and salt.

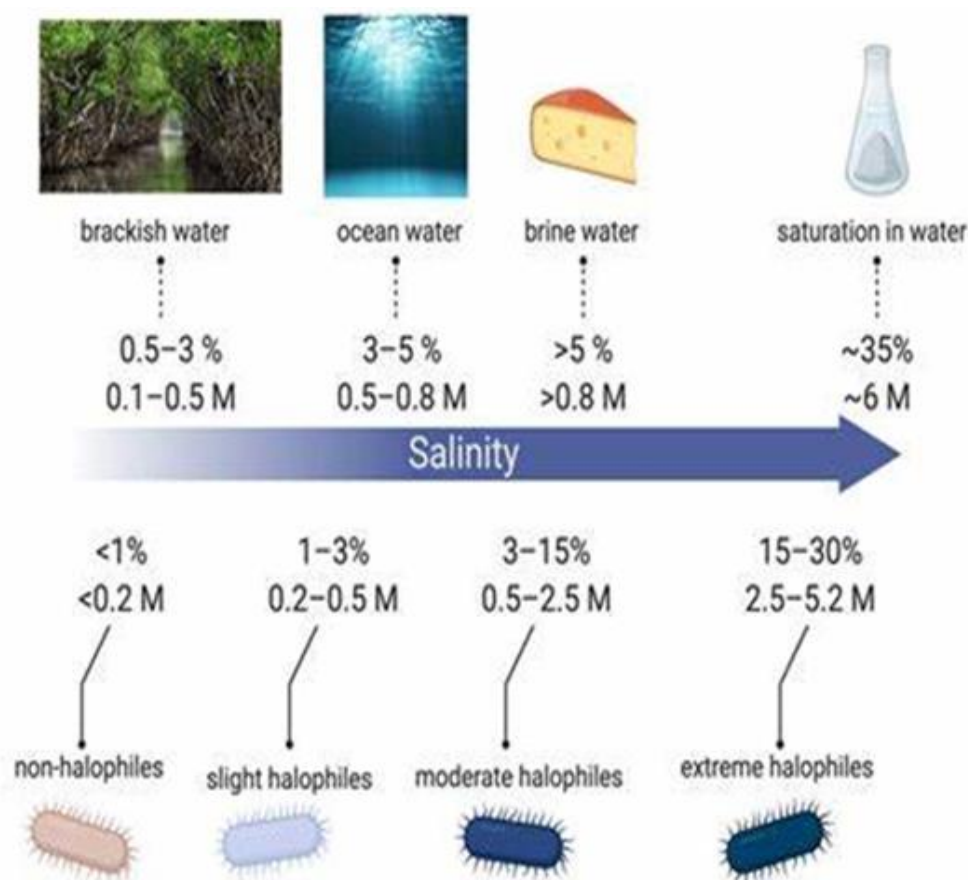


Figure 1: Halophiles Classification Based on Salt Concentration

II. MECHANISMS FOR SALINE TOLERANCE

The salt-out and salt-in strategies are two halotolerance mechanisms used by halophilic bacteria. Osmoprotectant synthesis or uptake is part of the salt-out strategy. They are small molecules that present in the cytoplasm of cells to defend them from cell disruption caused by osmotic pressure changes. They include ectoines, amino acids, sugars, and betaines. *Salinibacter ruber*'s salt-in strategy involves the accumulation of counter ions in the cytoplasm as a result of potassium ion influx. For high ion concentrations, the latter mechanism necessitates structural differences in biomolecules. Moreover, because osmoprotectants can also stabilise biomolecules in high ion concentrations, the former is usually the most prevalent. As a result, no major structural changes are required. The structural differences observed in potassium influx osmoregulation mechanisms include an increase in acidic content and charged amino acids on the protein surface, as well as an increase in glutamic acid due to its superior ability to bind water molecules.

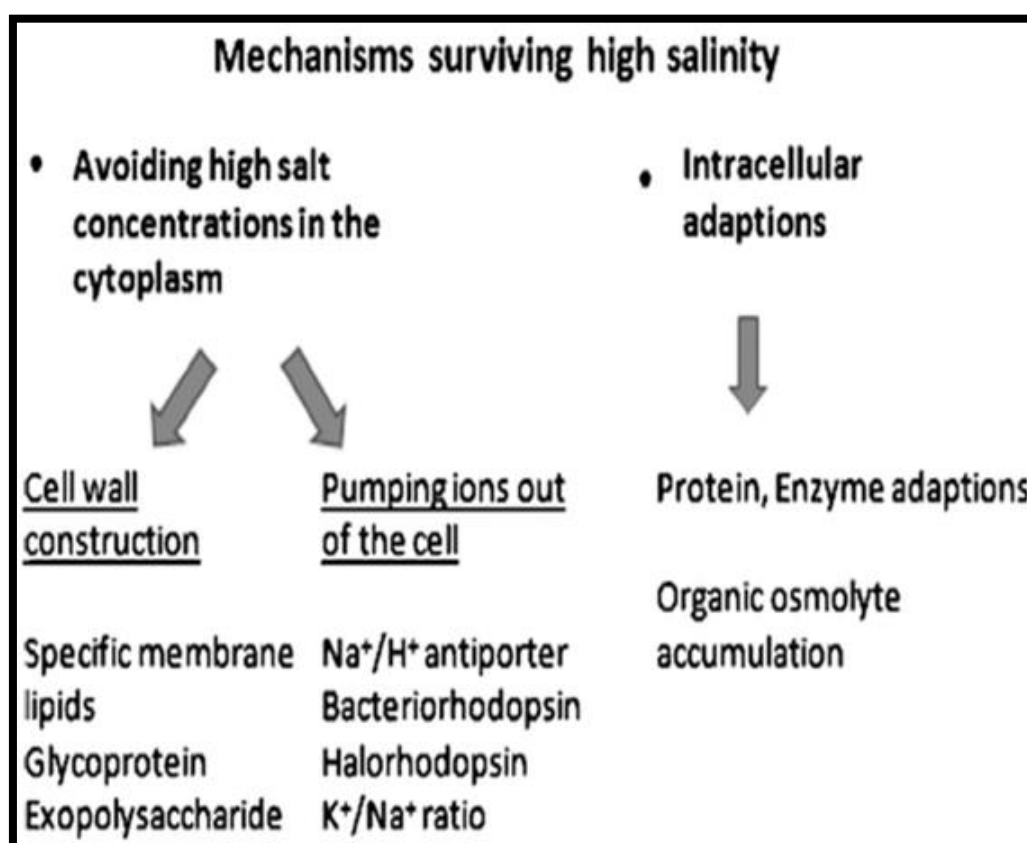


Figure 2: High Salinity Survival Mechanism

Halophiles are organisms that include archaea, bacteria, and eukarya and are defined by their salinity demand, halophilic "loving salt." Natural microbial communities of hypersaline ecosystems all over the world are made up of halophilic microorganisms. For growth and metabolism, they need sodium ions. Temperature, pH, and growth medium all influence tolerance parameters and salt requirements. Halophiles adapt to and are limited by specific environmental factors in this way. Polyextremophiles are microorganisms that can thrive and survive in a variety of environments range of extreme environmental conditions.

The halophilic bacterium can also be an alkaliphile, known as a haloalkaliphile, and it can thrive or grow exceptionally well at pH levels higher than 9.0, but not at the pH level of 6.5, which is close to neutral. Halophilic microorganisms often have low nutritional needs, resilience to high salt concentrations, and the capacity to maintain a stable environment's osmotic pressure. They have a wide range of physiological characteristics, including aerobic, anaerobic, phototrophic, heterotrophic and chemoautotrophic organisms. Halophilic microorganisms live in a variety of ecosystems with salinities greater than salt water, i.e., 3.5 percent sodium chloride, ranging from salt lakes, hypersaline soils to springs and other commonly occurring saltwater areas along the coast. Human intervention has resulted in other known habitats such as oil fields, brines, salted foods, saltern ponds and tanneries.

III. HALOPHILIC ARCHAEA AND BACTERIA: EVOLUTION, GENOMICS AND TAXONOMY

Taxonomists have been able to classify halophiles using methods such as numerical taxonomy, multilocus sequence analysis, chemotaxonomy, comparative genomics, and proteomics. These adaptable microorganisms exist as Archaea, Bacteria, and Eukarya. Eukarya & bacteria represent mildly and moderately halophilic microorganisms, whereas Archaea represents extreme halophiles. Archaea is further subdivided into Halobacteria & Methanogenic Archaea. Halobacteria is represented by Halobacteriaceae, one of the largest halophile families, with 36 genera and 129 species that require high sodium chloride concentrations, distinguishing them from other halophiles. They can't live in fresh water. Only Methanocarcinales contains halophilic species among the five orders of methanogenic Archaea. These strictly anaerobic species, which are classified as Methanosarcinaceae, produce methane to obtain energy.

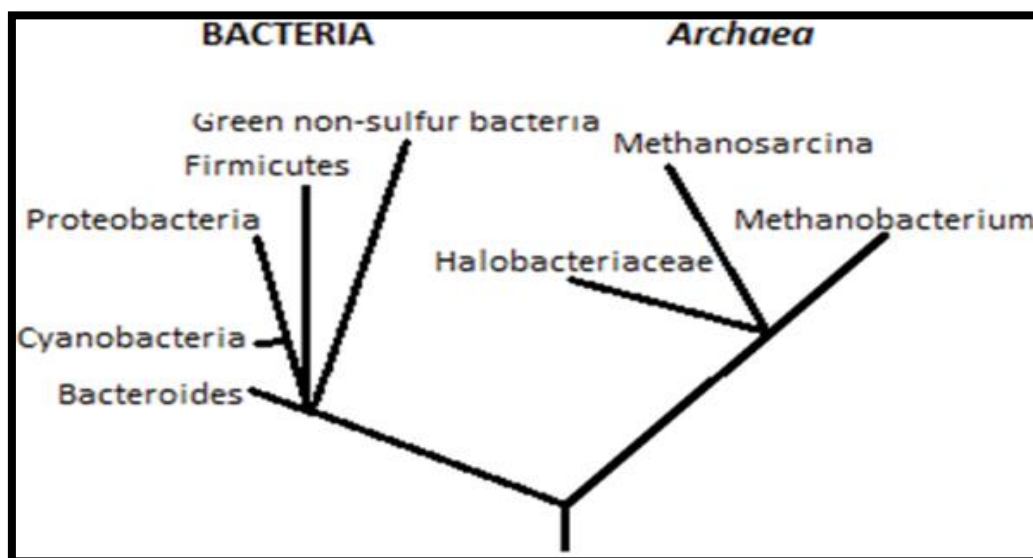


Figure 3: The Distribution of Halophilic Bacteria and Archaea on Focus on Small Subunit Rrna Gene Sequences, the Universal Phylogenetic Tree of Life

Haloarcula marismortui, Natronomonas pharaonis, aloquadratum, walsbyi, Halorubrum, lacusprofundi, Halomicrobium, mukohataei, Halorhabdus, utahensis,

Halogeometricum, *borinquense*, *Haloterrigena*, *turkmenica*, and *Haloferax*, *volcanii* were included among the recently completed genomes. Among those on the list are a haloalkaliphilic species, a cold-adapted species, a species adapted to life in low-Na⁺-high-Mg²⁺ environments, and isolates with exciting cell morphologies. These genomes range in size from 2.6 to 5.4 Mb. The presence of large megaplasmids and minichromosomes, as well as core acidic proteomes, are among the conserved properties of haloarchaeal genomes. Sequencing of new isolates' genomes are becoming easier & less expensive, and it will most likely become routine in the near future. This discovery will undoubtedly have far-reaching implications for halophile taxonomy until comparative taxonomy can be used.

Multilocus sequence analysis (MLSA) is becoming popular tool to strain correlation in evolutionary and taxonomic studies. The data analysis shows that homologous recombination occurs frequently, to the point where alleles are associated at random, as it is characteristic of sexually reproducing species. The two mechanisms, natural competence and conjugation (similar to *Haloferax*'s mating mechanism) are used for lateral gene transfer. There are currently only a few sequenced genomes of halophilic and halotolerant Bacteria. It excludes *Halomonas elongata*, an organism that has become a popular model organism and has found biotechnological applications since it was discovered 30 years ago. The genome sequences of *Halo rhodospirahalophila*, an anoxygenic halophilic phototroph, an extremely salt-tolerant alkaliphilic sulfur-oxidizing bacterium of the genus *Thioalkalivibrio*, the thermophilic anaerobic halophile *Halothermothrix orenii*, and the aerobic heterotrophic *Chromohalobacter salexigens* and *Salinibacter ruber* are all available. Comparative analyses show that the genomes of *Salinibacter ruber* have a mosaic structure with conserved and hypervariable regions. In total, ten percentage of the genes encoded in the *Salinibacter* M8 genome are missing from the type strain *Salinibacter* M31. These two isolates' metabolic profiles also differed.

IV. VIRUSES: HALOPHILIC

There are a wide range of halophilic viruses are present in the environment. Viruses' role in hypersaline ecosystems was previously unknown. There are new viruses that attack Archaeal halophiles. The discovery of pleomorphic viruses with single or double stranded DNA genome and an envelope of lipid shows that viral diversity in marine habitats is far greater than previously thought. Siphoviridae virus BJ1 and Myoviridae virus BJ2 infect *Halorubrum kocurii* with novel lytic head/tailed viruses. Sequencing of a few virus archeal genome is also possible.

Predators and protozoa are absent in saltern crystallizer ponds so there are large groups of prokaryotes and virus-like particles are present. The salterns have stable & predictable species of Archaea and viruses, which are distinct from the "Kill-the-winner" behaviour, as expected in such an environment, with rapid cycling of viral predators and microbial taxa. The distribution of microbial taxa and viral taxa in the salterns near San Diego, according to a metagenomic analysis of the viruses, the occurrence of viral genotypes and microbial strains has remained stable over time, but with strong dynamic fluctuations. Populations of specific strains and viral genotypes also fluctuate at the fine level in a "kill-the-winner" fashion. Virus action may also have an impact on the spread

of highly halophilic bacteria like *Salinibacter* (Bacteroidetes). A viral metagenome from a saltern pond, where *Salinibacter* represents about fifteen percentage of the group of prokaryotic organisms. Bioinformatic analysis (G+C content and dinucleotide frequency analysis) suggests that approximately 24% of the recovered viral sequences are *Salinibacter* phages. Phages that infect *Salinibacter* seem to be more active in the habitat than phages that infect *Haloquadratum*, which may explain why *Haloquadratum* outnumber *Salinibacter* in every environment to support their development.

V. FUNGI: HALOPHILIC

Only in the last decade has the significance of halophilic fungi, long overlooked as high saline environments' inhabitants, been recognised. These include the black yeast *Hortaea werneckii*, which grows up to 5 M NaCl, the true halophile *Wallemia ichthyophaga*, which grows up to saturation with at least 1.5 M NaCl, and *Aureobasidium pullulans*, which grows up to 3 M NaCl. They all are noticed in high salinity lakes, as well as wide range of other habitats, such as domestic kitchen appliances, ice caps, and possibly even spider webs in desert caves. The fungus halophilic and halotolerant retain low salt concentrations in its cytoplasm by using polyols like glycerol, arabitol, mannitol and erythritol as osmotic solutes. In *Hortaea werneckii* and *Wallemia ichthyophaga* osmotic adaptation was studied at the molecular level. *HwHal2*, a possible determinant of halotolerance in *H. werneckii* and a prospective transgenic to enhance halotolerance in crops, was identified and its structural characteristics were studied. The HOG (high osmolarity glycerol) pathway has been thoroughly studied, the knowledge is used for future research for the creation of enhanced salt-resistant crops. Both *Wallemia* and *Hortaea* produce glycerol, with the help of dehydrogenase. Halotolerance can be recovered in *Saccharomyces cerevisiae* through the heterologous production of the enzyme's gene.

VI. DIVERSITY

Aside from high salt concentrations, the hypersaline environment is unique in that it can support a wide range of microbes. A wide variety of halophiles, including chemolithotrophs, heterotrophs, chemolithotrophic aerobes, and anaerobes, have been documented in hypersaline settings converting a wide variety of substrates. The Halobacteriaceae, the largest Archaea family, is known for being the most salt requiring microbes in the halophilic group. Because of high sodium chloride concentrations, Halobacteriaceae members can aerobically degrade organic compounds. Halophilic Archaea, which include members of the genera *Haloarcula* and *Haloferax*, can use nitrate as an electron acceptor & grow anaerobically, producing nitrous oxide and gaseous nitrogen. Based on small subunit rRNA, microorganisms that can withstand at salinity levels up to sodium chloride saturation (>300 g/l salt) can be observed

In salt saturated ponds around the world, the unicellular alga *Dunaliella salina* (Chlorophyceae, Eucarya), the square archaeon *Haloquadratum walsbyi* (Halobacteriales, Euryarchaeota), and *Salinibacter ruber* can be found (Bacteroidetes, Bacteria). Halophiles are found in a variety of phyla within the domain Bacteria, most notably the Cyanobacteria, Gammaproteobacteria and Bacteroides. Large number of different types of cyanobacteria grow at significant salt content but not on a very high levels available in

saturated brines. The Halomonadaceae family is probably the best known group of halophilic or highly halotolerant Bacteria (Gammaproteobacteria). Halophilic organisms are frequently related to organisms in the aerobic groups that have little salt tolerance and requirement. Halanaerobiaceae and Halobacteriodaceae included, order Halanaerobiales. The red aerobic *S. ruber* (Bacteroidetes) is a new addition to the group of real halophiles and is now acknowledged as a significant contributor to the Archaea, the biota of salt lakes and salterns that are close to salt saturation is dominated by, the halobacteriaceae family. This organism is phylogenetically related to the Firmicutes and the Mollicutes. It survives through denitrification or fermentation. It is the sole recognised member of the family Haloplasmataceae and order Haloplasmatales. As evidenced by the brine shrimp *Artemia*, which has been discovered at concentrations of salt higher than 150–200 g/l and occasionally greater, eukarotic life is also feasible when the salinity content is excessive.

The green algae in the genus *Dunaliella*, some of which appear orange rather than green due to significant b-carotene buildup, are without a doubt the most well-studied halophilic eukaryotes. It is vital to the life of heterotrophic communities in salt lakes because it is the only or principal producer when the salt content is high. Microorganisms that are heterotrophic and halophilic, as well as their presence and significance have only lately come to light. In the salt lake, various types of ciliate protozoa and flagellate are present up to sodium chloride saturation, and other studies reveals that many more are still unknown. Over 40 phylotypes of protists were discovered using 18S rRNA studies. Fungi, in hypersaline ecosystems all over the world, black yeasts and other melanized kinds such as *Hortaea*, *Trimmatostroma*, *Candida*, *Pichia*, *Yarrowia*, and others play a crucial role. The prevalence of this variety and whether it belongs to a different, unidentified bigger group of organisms are unknown.

Diversity in Phylogeny of the Halophile World According to small subunit rRNA, microorganisms that can thrive at salt concentrations up to sodium chloride saturation (>300 g/l salt) can be found all throughout the tree of life. As a result, red-pigmented organisms such as *Salinibacter ruber* (Bacteroidetes, Bacteria), *Haloquadratum walsbyi* (Halobacteriales, Euryarchaeota), and *Dunaliella salina* (Chlorophyceae, Eucarya) are typically seen in ponds used to crystallise salt all over the world. Extreme halophiles have focused the most on the aerobic Archaea of the family Halobacteriaceae. In the order Halobacteriales, only this family is described. The square archaeon *Haloquadratum walsbyi*, which was previously mentioned and has long defied attempts at culture but is now available for study, is a member of this family. The Euryarchaeota phylum of the Archaea contains all known cultivated forms because there are no known halophiles in the Crenarchaeota phylum of the Archaea. There are also truly halophilic methanogens in the Methanosarcinaceae family, with *Methanohalobium evestigatum* being the most salt tolerant and salt requiring. Halophiles are found in a variety of phyla within the domain Bacteria, most notably the Cyanobacteria, Gammaproteobacteria, Firmicutes, and Bacteroidetes. There are variety of unicellular and filamentous cyanobacteria that require high salt concentrations for growth, though not at the highest concentrations found in saturated brines. There are known phages that attack halophilic Archaea and Bacteria, and such viruses abound in the (few) hypersaline systems. Virus particles may outnumber prokaryotes by one to two orders of magnitude in most freshwater and seawater environments. We can found a variety of halophiles, including ds DNA viruses and ss DNA viruses with membrane envelopes, spherical, spindle-shaped, and head-and-

tail viruses of halophilic Archaea.

- 1. Halophiles metabiologic diversity:** Given the significant evolutionary diversity of creatures found at high salt concentrations, the halophile community is as varied as one could anticipate with the help of techniques employed to extract energy and carbon for growth. However, certain processes that have been studied in low-salt environments seem to break down at sodium chloride level greater than 100–150 g/l. Aerobic methane oxidation, autotrophic nitrification, methanogenesis from hydrogen and carbon dioxide or acetate, are some examples.
- 2. Oxygenic and anoxygenic phototrophs:** The variety among phototrophs thriving, when the salt content is high, somewhat limited, yet oxygenic photosynthesis continues until salt saturation. The sea water phytoplankton can't live in concentrated brines. The only two major families of phototrophs that function at the highest salt concentrations are cyanobacteria and green unicellular algae of the genus *Dunaliella*, despite the fact that some species of diatoms can be found at salt concentrations of up to 100–150 g/l. Plankton and shallow benthic biofilm sheets are both popular places to find cyanobacteria in salt lakes and saltern evaporation ponds. These include filamentous and unicellular organisms (*Aphanothece halophytica* and kindred forms) (e.g., *Microcoleus chthonoplastes*, *Halospirulina tapeticola*, *Phormidium* spp., and others). Nitrogen-fixing microbes were never found in salt concentrations greater than 70 g/l. At high salt concentration that is above 200–250 g/l nitrogen fixing cyanobacteria are never found and only oxygenic phototrophs are found. By using sulphide as an electron donor at high salt concentration the process of anoxygenic photosynthesis is taking place. In hypersaline microbial mats where cyanobacteria predominate in the upper aerobic layers, purple layers of anoxygenic phototrophs are frequently present. Phototrophic sulphur microbes are those that have intracellular sulphur as elemental form as well as those that excrete sulphur. Some species can tolerate high pH values and high salinity. Some photoheterotrophic *Halophiles* and purple bacteria like *Rhodovibrio salinarum*, *R. sodomense*, and *Rhodothalassium salexigens*, can withstand a salt concentration of 200–240 g/l. An entirely new sort of anoxygenic phototrophic life that thrives at the highest salinities uses the light energy absorbed by retinal-based pigments like bacteriorhodopsin and related pigments like xanthorhodopsin. The main retinal pigment of *Halobacterium* and certain related halophilic Archaea, bacteriorhodopsin, can promote anaerobic growth in the light in *Halobacterium salinarum*. Photoautotrophy does not occur in this group because organic substrates are used as carbon sources. Light can be used as an additional energy source in the phylogenetically unrelated but physiologically very similar *Salinibacter*. Its proton pumping retinal protein, xanthorhodopsin, harvests light using the membrane carotenoid salinixanthin.
- 3. Aerobic heterotrophs:** Aerobic heterotrophs are prevalent in all three domains of life, and they make up the majority of the halophilic and halotolerant microorganisms known. In environments with salt concentrations of up to 200–250 g/l, bacteria are primarily responsible for heterotrophic activity, and some species are particularly adaptable in terms of the range of salt concentrations tolerated and the variety of resources used for growing. The list of organic materials that support development in environments with lots of salt is shorter than that known for freshwater environments,

but it includes several hydrocarbons and aromatic compounds. At high salinities, groups such as the Halomonadaceae cease to exist, and aerobic degradation of organic compounds is assumed by two groups of prokaryotes: the Halobacteriaceae (Archaea) and *Salinibacter* (Bacteria). The halophilic Archaea can grow to saturating salt concentrations, by oxidizing simple sugars and amino acids as limited substrates. It is ineffective to employ hydrocarbons and aromatic chemicals. We previously believed that the only aerobic heterotrophs capable of functioning at 200–250 g/l of salt were members of the Halobacteriaceae; however, *Salinibacter* has now been found to be a significant part of the biota in saltern crystallizer ponds. *Salinibacter* is not very versatile in terms of growth substrates.

- 4. Anaerobic respiration:** There are numerous types of respiration that use electron acceptors than molecular oxygen when the concentration of salt is high. Arsenate and selenate, two less common chemicals, are also utilised as electron acceptors and their reduction is crucial in some hypersaline settings. Nitrate and sulphate are also used in this capacity. For respiration, as an electron acceptor using nitrate a variety of halophilic and halotolerant prokaryotes are present. Many moderately halophilic Halomonadaceae members reduce nitrate to nitrite, and some halophilic Bacteria also denitrify nitrate to gaseous nitrogen. Some halophilic Archaea, such as *Haloarcula marismortui*, *Haloarcula vallismortis*, and *Haloferax mediterranei*, can grow anaerobically and release gaseous nitrogen and/or nitrous oxide when nitrate is present as an electron acceptor. Dimethylsulfoxide, trimethyl-N-oxide, and fumarate reduction are further anaerobic growth methods used by some organisms. It is not surprising that the ultimate stage in the degradation of organic molecules in anaerobic marine and hypersaline seawater-derived settings is dissimilatory sulphate reduction given the high sulphate content of seawater. However, when compared to freshwater and marine systems, certain types of sulfate-reducing bacteria appear to be absent in high-salt environments.

All of the halophilic and highly halotolerant dissimilatory sulphate reducers described thus far are "incomplete oxidizers," i.e., instead of oxidising the substrates all the way to carbon dioxide, they oxidise substrates like ethanol, pyruvate and lactate and excrete acetate. Sulfate reduction can also take place in highly alkaline and hypersaline environments. With an optimal pH of 9.5 to 10, *Desulfonatronospira thiodismutans* and *Desulfonatronospira delicata* oxidise lactate and ethanol between 1 and 4 M total salt. Additionally, they undergo sulfite or thiosulfate dismutation to grow chemoautotrophically. Sulphate reduction may be achievable at high salt concentrations, according to some evidence. For instance, dissimilatory sulphate reduction may occur in the Dead Sea bottom sediments (>300 g/l total salts), according to stable isotope data, but no details on the mechanism or the species involved are currently known. "Complete oxidizers," which convert substrates like acetate to carbon dioxide, don't seem to exist in hypersaline settings. *Desulfobacter halotolerans* is the microorganism that can withstand the most salt. It can grow slowly up to 130 g/l and does best at salt concentrations of 10–20 g/l. Halophilic/haloalkaliphilic bacteria can develop anaerobically with the aid of arsenate and selenate, which are less frequent electron acceptors for microbial growth. The optimal growth conditions for *Haloarsenatibacter silvermanii* are 350 g/l salt and pH 9.4, which can be achieved either through chemoautotrophic sulphide oxidation using

arsenate as an electron acceptor or through heterotrophic use of a variety of organic electron donors. Other electron acceptors used for respiration include Fe(III) or elemental sulphur. A halophilic selenate reducer that transforms Se(V) into a combination of Se(III) and elemental selenium is *Selenihalanaerobacter shriftii*.

5. Methanogens and homoacetogens: Acetate splitting to create methane and carbon dioxide and hydrogen reduction of carbon dioxide account for the majority of the methane produced in low-salinity conditions. Both of these responses were not seen at high salt concentrations. *Methanocaldococcus halotolerans*, the most halotolerant methanogen known to grow on these substances, reaches its growth peak at a NaCl concentration of 50 g/l and does not continue to grow over 120 g/l. Despite the fact that acetate-splitting methanogens are anticipated to be even less salt resistant, anaerobic sediments in hypersaline lakes are frequently a rich source of methane, even at close to saturation salt concentrations. Although the two widely stated processes are not used by methanogens, they do occasionally use dimethylsulfide and methylated amines like trimethylamine as substrates. Even at salinities of 240–250 g/l, *M. evestigatum* and *Methanohalophilus portucalensis* can flourish on such substrates. It is somewhat remarkable that homoacetogenic bacteria may convert the identical substrates to acetate since no methanogenesis is known to occur from hydrogen and carbon dioxide at high salt concentrations.

6. Fermentation: By converting organic substances, it is possible, with in the lack of an external electron acceptor until the maximum salt concentrations, growth of anaerobes can be induced. Fermentative halophiles are found in a variety of phylogenetic sections. Aerobes that employ oxygen as an electron acceptor are the Halobacteriaceae family's Archaea. However, certain species have the ability to develop anaerobically by denitrifying nitrogen or by utilising light energy received by bacteriorhodopsin. Species of the genus *Halobacterium* may also grow anaerobically in the dark while collecting energy through the fermentation of arginine, which results in the formation of ornithine, ammonia, and carbon dioxide. The nonpigmented microbe *Halorhabdus tiamatea* coming under Halobacteriaceae that grows by fermentation. It only grows on complex substrates, and the mode of fermentation is unknown. Only obligate anaerobic halophiles make up the order Halanaerobiales, which has two families, the Halanaerobiaceae and the Halobacteroidaceae, and almost all of them get their energy through fermentation.

Most species can thrive at salt concentrations of 100 to 200 g/l and ferment simple carbohydrates to create ethanol, acetate, hydrogen, and carbon dioxide. Several fermenting prokaryotes in the domain Bacteria that aren't Halanaerobiales-classified. The rare *Haloplasma contractile*, which can grow up to 180 g/l salt, produces lactate as a fermentation byproduct, and is unconnected to any other Bacteria group, and *Clostridium halophilum* (Firmicutes), are two examples.

7. Methanotrophs and chemoautotrophs: Although some aerobic chemoautotrophic systems seem to be restricted to low salinities, others seem to be able to function at extremely high salt concentrations. At high salt concentrations, there is no information on the autotrophic oxidation of nitrite to nitrate. Reduced sulphur compounds undergo chemoautotrophic oxidation at even higher salt concentrations.

Thiosulfate, elemental sulphur, and tetrathionate can all be oxidised by *Halothiobacillus halophilus* at concentrations up to 240 g/l salt, with 50 to 60 g/l being ideal. The highly halophilic *Thiohalorhabdus denitrificans* develops between 11 and 290 g/l NaCl, with an optimum concentration of 175 g/l. Using nitrate as an electron acceptor, it can also grow anaerobically as a chemoautotroph. The organisms *Thiohalospira halophila*, *T. alkaliphila*, and *Thioalkalivibrio halophilus* may thrive at pH values of 8 to 9 and above as well as in solutions containing up to 5 M of Na⁺. Arsenic is biogeochemically cycled in the alkaline hypersaline habitat by chemoautotrophic oxidation of As(III) to As(V) and anaerobic reduction of As(V) (arsenate) to As(III) (V). Gammaproteobacterium *Alkalilimnicola ehrlichii* can grow heterotrophically or as a chemoautotroph on arsenite with oxygen or nitrate as the electron acceptor at salt concentrations up to 190 g/l (optimum 30 g/l). Anaerobic sediments can create methane up to very high salt concentrations. Methane-oxidizing bacteria have been identified from salt lakes, including "*Methylobacterium modestohalophilum*," however their ideal salt concentration for growth is only approximately 20 g/l.

VII. HABITAT

Hypersaline environments favour microbial species with a diverse order and environment. The fraction of found microbes is small, even with recent advances in molecular science techniques, and further studies are required.

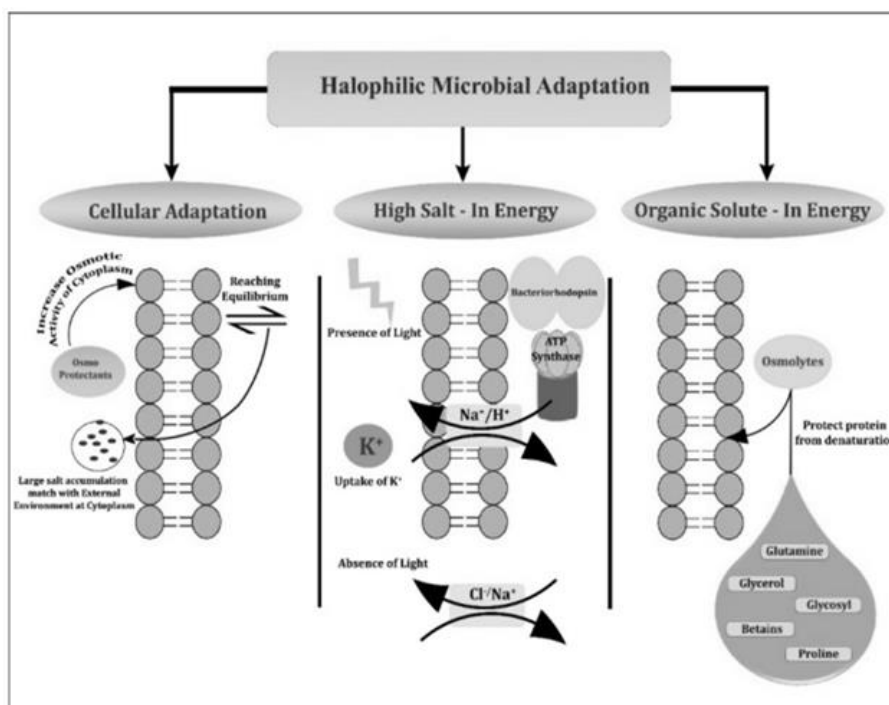


Figure 4: Common halophilic microbial adaptation strategies include cellular adaptation, high salt-in energy, and organic solute-in energy.

Halophiles are found in a variety of habitats, including the world's most toxic environments. These extremeophiles have acquired unique abilities for surviving in the natural world thanks to this deviation from the usual environmental circumstances. The diversity of halophiles is largely a result of their capacity to survive in all hypersaline biotopes. Due to both man-made and natural global change, these hypersaline settings are becoming more prevalent. Halophiles can be found in a variety of habitats, including soda lakes, thalassohaline and athalassohaline ecosystems, the Dead Sea, carbonate springs, salt lakes, alkaline soils, and many others. These environments vary in terms of their nature and makeup. Thalassohaline habitats are created when seawater evaporates, increasing the amount of sodium and chloride ions and their pH level to a slightly alkaline state.

The ionic composition of the athalassohaline environment differs from that of sea water because it contains salts in non-marine proportions. As a result of NaCl precipitation, high concentrations of K⁺ and Mg⁺ salts are left in sea water. The most productive habitats are soda lakes, where pH levels close to 11 encourage the growth of salt-loving bacteria. Due to their difficulty in exploration, soda lakes have only recently drawn the attention of microbiologists. The concentration of monovalent cations in the Dead Sea is lower than that of divalent cations. It supports dense microbial blooms because it is the largest hypersaline environment.

VIII. VARIOUS SALT STRESS COPING MECHANISMS FOR A MICROBE TO BE ABLE TO SURVIVE AT HIGH SALT CONCENTRATIONS

Osmotically comparable cytoplasmic contents to the surrounding media are essential. If the internal osmotic pressure of a cell is lower than that of the salt solution around it, the cell will rapidly lose water via its water-permeable cell membrane. The osmotic pressure inside the cell must be greater than that of its medium in order to sustain turgor (in all cells with the possible exception of the very halophilic Archaea of the family Halobacteriaceae). Numerous strategies for osmotic stabilisation of halophilic bacteria have been developed by nature. One thing that all halophiles have in common is that sodium is constantly avoided the cytoplasm as much as possible by strong, energy-intensive sodium pumps (in prokaryotes, these pumps are typically based on Na⁺ /H⁺ antiporter systems). However, depending on the type of microbe, different substances can be used to maintain osmotic balance in place of sodium chloride. One method is to build up chloride and potassium ions to levels that are identical to the medium's ionic concentrations. To stop water loss from the cell by osmosis, the second and much more typical technique is to greatly restrict ions and collect tiny electrically neutral organic solutes (also known as "compatible solutes"). Due to the requirement that all cytoplasmic enzymes and processes operate when there was molar amounts of KCl, the first technique (salt-in to balance "salt-out") calls for considerable modifications to the intracellular apparatus. High salt levels also decrease the solubility of proteins and enzymes. As a result, the proteome as a whole has to be altered. A distinctive characteristic of halophilic proteins is their extremely an acidic character, with a preponderance of acidic amino acids (glutamate, aspartate) over basic amino acids. Halophilic proteins are produced by microorganisms that collect KCl for osmotic equilibrium (lysine, arginine). These proteins are very negatively charged compared to their non-halophilic counterparts. Additionally, the proportion of hydrophobic amino

acids in halophilic proteins is minimal. These protein modifications necessitate the presence of molar quantities of salts at all times in order to maintain the proteins in their native and active states; dilution of the salt results in unfolding and denaturation. Because of this, "salt-in" strategy-using microorganisms are typically obligate halophiles with limited flexibility in the range of salt concentrations that allow growth, and they are thus suited to living at as steady and extremely high salinity as possible. The "salt-in" approach to osmotic adaptation has the advantage of requiring less energy than manufacture of molar quantities of organic osmotic solutes, even though the latter method also expels sodium ions.

The halophilic Archaea of the family Halobacteriaceae, the exceptionally halophilic red aerobic Bacteroidetes *S. ruber*, and members of the anaerobic, primarily fermentative order Halanaerobiales (Firmicutes) are the only prokaryotes known to employ the "salt-in" technique. The intracellular KCl content in the Halobacteriaceae was found to be above 4.5 M in cells growing at saturating NaCl conditions. Although active chloride transport has been shown, employing the light-driven main chloride pump halorhodopsin and/or cotransport with Na⁺ ions, potassium ions can enter the cell through specific channels. *S. ruber*'s genome, which is phylogenetically unrelated to the Halobacteriaceae, showed notable parallels to Halobacterium and cousins in terms of its features, including a proteome that is extremely acidic. Since *Salinibacter* and the halophilic Archaea share a common environment and have experienced similar environmental pressures throughout their evolutionary histories, there may have been extensive gene exchange between the two groups. Select members of the anaerobic fermentative Halanaerobiales (*Halanaerobium praevalens*, *Halanaerobium acetethylicum*, and *Halobacteroides halobius*) had intracellular ionic concentrations that showed the presence of molar KCl concentrations, indicating that their enzymes need a lot of salt to function properly.

The genome of the similarly fermentative anaerobic thermophile *Halothermothrix orenii* was not discovered to have an acidic proteome. The process of osmotic adaptation of this fascinating polyextremophilic bacterium is yet unknown because no genes for any of the known organic osmotic solutes have been found. In the microbial world, the buildup of organic osmotic solutes is a considerably more prevalent osmotic adaptation method. The strategy's drawback is the high energy cost of making the solutes, but its advantage is that it is highly flexible and can be adjusted to a wide range of salt concentrations. In response to ambient salinity, the cell can simply alter the intracellular solute concentration because solutes typically have little impact on protein structure and enzyme activity. The quantity of energy required for de novo biosynthesis can be significantly reduced when suitable solutes are found in the medium and can frequently be taken up from the outside. Examples include polyols like glycerol and others, simple sugars like sucrose and trehalose, a number of amino acid derivatives such glycine betaine and ectoine, among others. The bulks are uncharged or zwitterionic at physiological pH. Glycerol is utilised by various yeasts and fungi as well as the unicellular algae *Dunaliella*. The chemical must have a very low cytoplasmic membrane permeability in order to function as an osmotic solute. Since glycerol is very permeable to most membranes, it has never been discovered in prokaryotes as an osmotic solution. As prokaryotic oxygenic phototrophs, cyanobacteria utilise glucosylglycerol, glycine betaine, or sugars (trehalose, sucrose), with the latter substance being favoured by the

most halotolerant species. Numerous bacteria can accumulate glycine betaine from the medium and use it to stabilise the osmotic pressure, but only a few prokaryotes can synthesise the substance. The amino acid metabolic intermediary ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) and its hydroxy derivative, produced from aspartate- β -semialdehyde, are more frequently encountered suitable solutes. A single organism frequently contains mixtures of osmotic solutes; for instance, the cells of the Gammaproteobacterium *Halorhodospira* may contain glycine betaine, ectoine, and trehalose. As was already mentioned, KCl is used by the Archaea of the Halobacteriaceae to stabilise osmotic pressure. But 2-sulfotrehalose, an organic osmotic solute, was found in some haloalkaliphilic individuals, where it collaborates with KCl to keep the osmotic equilibrium. The osmotic solutes that methanogenic Archaea appear to utilise include glycine betaine, β -glutamine, β -glutamate, and *N*-acetyl- β -lysine.

IX. HALOPHILES IN UNUSUAL ENVIRONMENTS AND HALOPHILES STRESSED IN MULTIPLE WAYS

The majority of halophile habitats are thalassohaline settings, which reflect the ionic makeup of saltwater and have a pH range of nearly neutral to slightly alkaline. They are the result of seawater evaporation. Some extreme habitat which gives fascinating settings in which to hunt for novel bacteria. They are anaerobic, have elevated salinity in addition to abrupt, distinctive brine-seawater interfaces. Some brines also have elevated temperatures and metal concentrations. Brine is anaerobic, has a different ionic composition from seawater in general, and occasionally has a higher temperature than seawater. *Salinisphaera shabanensis*, a facultative anaerobe that can grow in different salt ranges, ranging , 1 to 28 percent, *Halorhabdus tiamatea*, a non-pigmented member of the Halobacteriales that prefers an anaerobic lifestyle, and *Flexistipes sinusarabici*, an anaerobe tolerant, are just a few of the interesting microorganisms in the group. Many athalassohaline habitats combine the requirement for life to flourish at alkaline pH with high temperatures, and creatures that grow there do so at the physicochemical edge of life. *Natranaerobius jonesii* and *Natranaerobius grantii* are two species. *Natranaerobius jonesii* has a very high chloride ion demand and is unable to develop at concentrations lower than 1.4 M Cl. Even in saturated soda brines, sulphide generation was active, although under these conditions, elemental sulphur and thiosulfate produced much more sulphide than sulphate. A prevalent pattern in soda lake isolates was thiosulfate and sulfite dismutation. A chloride brine with low Na⁺, high Mg²⁺, high Ca²⁺, and a somewhat acidic pH is unusual, although it exists in the Dead Sea. Solar salterns have salinity contents that vary. Depending on the climate, the length of water retention, and the condition of the deposition of nutrients, these ponds enable a diversity of halophilic biota to flourish. These crystallizer ponds usually have a reddish tint from the massive development of bacteria that produce pigment. Salt-loving bacteria can also find a huge and varied habitat in salt mines. In contrast to halophiles, halotolerant bacteria thrive in saline soils.

X. PHYSIOLOGY

- 1. The amount of internal ions:** In order to thrive in extremely saline environments, halophiles regulate their internal ion concentration in a way that they prevents their cytoplasm from being impacted by external osmotic conditions. The goal of osmotic

balance is achieved by internal salt buildup.

- 2. Metals tolerance:** Numerous halophilic organisms from the Archaea and Bacteria domains have been found to be metal-tolerant. Diverse halophilic fresh and cultured strains showed disparate reactions to arsenic, cadmium, chromium, and copper. It was discovered that *Acinetobacter* strains were the most metal sensitive, whereas *Flavobacterium* strains were the most metal tolerant. Variations in salinity had an immediate effect on halophile tolerance. When the amount of yeast extracts is decreased, metal tolerance in cultivated strains is also decreased.
- 3. Osmoadaptation:** Halophiles, which are ubiquitous in nature and live in high salinity environments, utilize efficient osmotic techniques to handle fluctuating salt concentrations. Many different strategies have evolved in different halophile taxa to help them survive in toxic surroundings. Halophiles typically rely on suitable solutes and sodium pumps based on the Na⁺/H⁺ antiporter system to sustain their internal osmotic environment. Recently, a number of these suitable solutes with significant biotechnological applications have been found. Depending on the environment and the type of microorganism, halophiles utilise different types of suitable solutes. Polyols, sugars, and derivatives of amino acids are examples of two main families of compatible solutes that are frequently employed to balance osmotic pressure. Due to the lack of very sophisticated molecular tools at this time, the complicated nature of these suitable solutes still has to be thoroughly researched. Bacteria and archaea both utilise several compatible solutes. Anionic solutes produced from bacterial carbohydrate sources are predominantly used by Archaea. Polyol phosphodiester are additionally used favourably by Archaea. The most frequent compatible solutes that enable bacteria to maintain osmotic equilibrium are α-glutamate, proline, and derivatives of α-amino acids. Due to certain energy considerations, absorption of suitable solutes like glycine betaine, ecotine, glycerol, and others is favoured over production. Every Archaea and bacterial species possesses at least one transporter for glycine betaine, the most popular suitable solute. For instance, the glycine betaine transporters Ota and Otb in *Methanosarcina mazei* include three subunits each. Through transcriptional research, it was found that glycine betaine transportation starts when Ota is expressed but stops when it is removed.

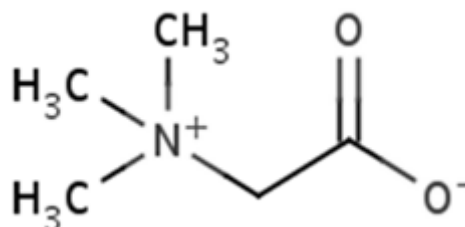


Figure 5: Glycine Betaine

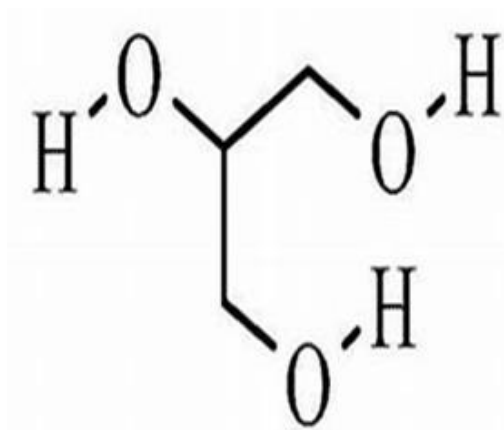


Figure 6: Glycerol

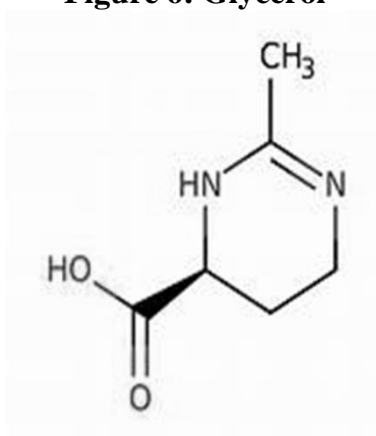


Figure 7: Ecotine

To protect against pH fluctuations, some alkaliphilic halophiles have cytoplasmic polyamines and acidic polymers in their cell wall. Excessive Na⁺ accumulation causes cytotoxicity and may result in cellular death. In order to prevent the severe cytotoxicity, ATP-driven efflux pumps and Na⁺/H⁺ antiporters have been advantageous for halophiles. It has been discovered that Na⁺/H⁺ antiporter gene expression helps maintain Na⁺ homeostasis, which controls salt concentration. Halophiles are able to endure the difficult and sometimes fatal salinity extremes thanks to the joint action of efflux pumps and antiporter genes. Halophile survival is ensured by their mode of action, which contributes to the preservation of a stable internal osmotic equilibrium without compromising cellular integrity.

XI. HALOPHILES : SIGNIFICANT CHARACTERISTICS

- 1. Enzymes:** Catalysts like enzymes are useful in a variety of industries, including metal recovery, leather processing, food, detergent formulation, and others. Extracellular enzymes in particular, which are extremophile enzymes because they can survive and catalyse reactions under unusual habitats, have been found to have significant applications in industries.
- 2. Nucleases:** *M. varians* subsp. *halophilus* has been found to have a nuclease (nuclease H) that is both DNase and RNase active. Another halophilic nuclease was also

generated by *Bacillus halophilus* (an exonuclease that releases 5'-mononucleotides from both DNA and RNA).

- 3. Amylases:** *Acinetobacter*, *Halobacterium salinarum*, *M. varians* subsp. *Halophilus*, *N. halobia*, *Natronococcus amylolyticus*, *Halomonas meridiana*, and *Haloferax mediterranei* were used to purify and characterise a few α -amylases. The enzyme from *Haloarcula* sp. S-1 was highly resistant to organic solvents. At varying $\text{Na}^+/\text{Mg}^{2+}$ concentration ratios, the impact of ionic strength on amylase activity has also been documented.
- 4. Proteases:** Proteases produced by halophilic microorganisms have novel applications because they are highly stable at saturated salt concentrations or because they can withstand organic solvents. An extracellular serine protease that *Halobacterium salinarum* makes has the potential to be employed in peptide synthesis. The other serine proteases were isolated from *Natrialba magadii*, *Natronococcus soccultus*, and *Natronomonas pharaonis*. Other proteases from *Natrialba baasiatica*, *Haloferax mediterranei*, and other halophilic isolates were purified and characterised. Several studies have been conducted in recent years to characterise extracellular proteases from the *Salinivibrio* genus, *Pseudoalteromonas* sp. strain CP76, *Natrialba magadii*, *Halobacterium mediterranei*, and *Bacillus clausii*. The haloalkaliphilic archaeon *Natronomonas pharaonis* also had a chymotrypsinogen B-like protease.
- 5. Lipases:** One of the most crucial hydrolytic enzymes, lipase has uses in both the pharmaceutical and agricultural sectors. It has been reported that moderately or very halophilic microbes such as *Salinivibrio* sp., *Natronococcus* sp., haloarchaeal strains, and others produce lipase that is also stable at high temperatures.
- 6. Cellulose-degrading enzymes:** *Halocella cellulolytica*, an obligate anaerobic organism, can use cellulose as its sole carbon source. In a different investigation, a large number of Archaea that are very halophilic and consume cellulose were found. Extracellular hydrolytic enzymes of subterranean rock salt halophilic microorganisms have the presence of cellulose.
- 7. Pigments:** Microorganisms that are halophilic are a rich source of natural compounds. One of the natural substances responsible for the red, orange, yellow and purple colors observed in a variety of plants, animals, and microbes is carotenoid pigment. Carotenoid pigments are particularly abundant in hypersaline environments. The reddish and orange colour of hypersaline habitats is a result of the presence of pigmented microorganisms such as the β -carotene-rich *Dunaliella*, the bacterioruberin-producing Haloarchaea, and the halophilic *Salinibacter ruber*. These organisms also create the carotenoid salinixanthin. *Halorubrum* sp. TBZ126, an extremely halophilic archaeon isolated from Urmia Lake, has been identified as a potential carotenoids producer. Melanin is a nearly ubiquitous pigment with enormous application potential in cosmetics, agriculture and pharmaceutical industries (photoprotection and mosquitocidal activity isolated from *Streptomyces*). On potato dextrose agar, the halophilic black yeast *Hortaea werneckii* developed a dark pigment that was diffusible. Additionally, it prevented the growth of potential pathogens, with action seen in *Vibrio parahaemolyticus* and *Salmonella typhi*.

- 8. Antibiotic resistance:** Antibiotic resistance develops through an evolutionary process based on the selection of organisms with improved tolerance to previously fatal antibiotic dosages. Different antibiotics such as penicillin, erythromycin, and gentamycin, which were previously successful treatments are no longer as effective due to bacteria's increased resistance. The halophilic bacteria Halobacteriaceae, Halomonas elongate, and Bacillus cereus SIU1, they are of various regions, were discovered to be resistant to antibiotics.
- 9. Antimicrobial activity:** At different bacterial biodiversity most actinobacterial isolates can produce antibacterial and antifungal metabolites. Antibacterial and antifungal activity of halophiles have vast actions against pathogens. Marine environments may be a source of new antimicrobial and antifungal agents, according to the results of antibacterial and antifungal experiments on halophiles (protein crude extract). In the presence of pathogenic bacteria and yeasts, Microbacterium oxydans and Streptomyces fradiae showed antibacterial activity.
- 10. Anticancer compounds:** Bioactive compounds, commonly referred to as natural products, are anticancer medications made by living things. Microorganisms are a great alternative to plant cells for producing anticancer natural products because of their diversity, ease of manipulation, and capacity for physiological screening to find new antitumor natural products, despite the fact that plant cells have previously and reliably yielded anticancer natural compounds. Although bacterial cells have other experimental communication ways with tumour cells than metabolites, bacterial metabolites have been considered the most traditional tactic against cancer cell viability. Extremophiles are gaining more focus as a potential fresh resource of innovative biomolecules. In hypersaline conditions, halophilic and halotolerant microorganisms are recognised as viable sources of anticancer compounds with few side effects. The use of halophilic microorganism metabolites in the treatment of cancer has been the subject of several investigations in recent years.

XII. HALOPHILES PRODUCING STRAINS OF NOVEL ANTIMICROBIALS

The WT6 and R4A19, they are strains which produce antimicrobials and are active against *E. coli* and *B. cereus*. The novel bacterium *Paenibacillus sambharensis* inhibit the growth of *S. aureus* by the production of the compound bacitracin A, with a molecular mass of 1421.749 Da. Some novel halophiles shows antibacterial activity against *K. pneumoniae*. *Pseudomonas syringae* and *Agrobacterium tumefaciens* also have antibacterial activity. The Andaman and Nicobar Islands' unexplored deep-sea habitats provided a source of novel halophilic species with antibacterial activity against Gram-positive and Gram-negative strains, including *P. mirabilis*, *V. cholerae*, *E. coli* and *S. pneumoniae* also have antibacterial activity against other strains. Halophilic strains *Halobacillus karajiensis* and *Alkalibacillus mallahensis* partially purified biosurfactants inhibited the growth of *K. pneumoniae* (94%) and *A. flavus* (80.4%). From the halophilic *Nocardiopsis gilva* p-terphenyl 1 indicates its activity against *F. avenaceum*, *F. graminearum*, and *F. culmorum* with MICs of 8, 6, and 128 g/mL. Compound 1 has antifungal activity against *Candida albicans* with a MIC of 32 g/mL, *B. subtilis* with a MIC of 64 g/mL, and Novobiocin 4 with MICs of 16 g/mL and *S. aureus* with a MIC of 64 g/mL. Even though the salty environment generates antimicrobials, some salty

habitats are still unknown and need urgent exploration in order to find new antimicrobials and other biological properties of applied relevance.

XIII. HALOARCHAEA AND HALOPHILIC BACTERIA'S BIOTECHNOLOGICAL IMPORTANCE / INTEREST

The creation of novel compounds with potential applications in biomedicine requires a special emphasis on extremeophile exploitation. The majority of current activities are directed at addressing critical health needs, particularly those that pose significant hazards to the world, such as antibiotic resistance and cancer. Halophilic microorganisms are promising drug discovery candidates due to their high metabolic versatility, low nutritional needs and genetic mechanisms for coping with challenging environmental factors such as dehydration, nutrient deprivation, high ionic strength and high levels of solar radiation. A multitude of encoding genes responsible for the production of a variety of active compounds in response to extreme conditions are being revealed by ongoing advancements in "omics" and bioinformatic tools. Deciphering the physiological, molecular, and metabolic mechanisms for the synthesis of new bioactive substances is made possible by the concurrent application of cutting-edge technology. The production of hydrolytic enzymes, retinal proteins, carotenoid pigments, and suitable solutes by halophilic microbes has been linked to their potential application as biopolymers, biofertilizers, and stabilisers for large molecules. Halophilic bacteria and very halophilic aerobic archaea, also known as haloarchaea, play an important role in the industry with a variety of purposes, including cosmetics, photoelectric devices, fermented food products, preservatives, making bioplastics, biosensors, artificial retinas, holograms, and so forth. Antimicrobial Substances Antibiotics resistance propagation is currently posing a worldwide danger to humens. Antibiotics are used for several diseases. But their continuous usage leads to the formation of multi-drug resistant bacteria (MDR), which reduces and eliminates their efficacy. Complications from MDR infections are the leading cause of death among inpatients. One of the most difficult challenges for science is to prevent a go back to the days before antibiotics. Utilization of all renewable and natural resources, including severe conditions, as a prospective source for novel therapeutic development is being driven by the urgent need for more effective antimicrobial medicines. The term "haloclin" is used to refer to compounds secreted by certain Halobacterium species that have the power to lyse and kill nearby microorganisms. Haloarchaea produces halocins and they are called halocins or AMPs. Although various halocins play important ecological and environmental roles, less is known about how they interact with human diseases. While antimicrobial activity against ESKAP (high risk group of human pathogen): *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, the clinical symptoms are highly specific.

The inhibitory activity of Actinobacteria are the cause of decreased activity of humen pathogens. Actinobacteria are heterotrophic bacteria that are more commonly found in soils in salty and hypersaline settings. *Nocardiosis* and *Streptomyces* species are the primary metabolite producers and they are the most frequently reported organisms. Some bacterial strains produces novobiocin. Some pecess anti-bacterial activity against different Gram bacteria. Some have anti-fungal and cytotoxic activity. Halophiles have a number of distinctive and advantageous qualities like production of substrates with low cost for industrial biotechnology, and because of these distinctive

properties, they constitute a crucial instrument for competitive and affordable production that industrial biotechnology cannot avoid using them. Alkaliphilic, or able to develop and thrive in hostile environments, many halophiles are particularly tolerant of high salt salinities. Halophiles take advantage of the high salt concentration for growth and survival because a conventional cell will experience plasmolysis when exposed to high salt concentrations. They have the ability to endure salt and water stress and adapt to hostile settings. Halophiles, especially extreme halophiles, thrive in brines due to their abundance and capacity to exclude nonhalophilic pollutants, creating the perfect environment for numerous biotechnological transformations. Halophiles can get their energy from a number of different places. They can employ less costlier and more renewable substrates, such kitchen and agricultural waste, in substitute of costly raw materials. Due to their halophilic (salt-loving) nature, they can also consume recycled seawater during bioprocessing, which lowers the need for freshwater. Equipment made of inexpensive materials, such as ceramics, plastics, or carbon steels, instead of the pricey stainless steel, can be utilised during fermentation since the medium has a high salt concentration, which lowers the high cost of equipment maintenance (stainless steel). Unsterile and continuous bioprocessing of halophiles can also save energy for intensive sterilisation and increase process efficiency. Halophilic is produced with little harm to the environment because it is bio-based, and bio-based goods may be broken down by nature. There for it contributes less environmental pollution.

XIV. HALOPHILE GENETIC MANIPULATION

Genetic manipulation is critical for improving halophile performance for industrial applications. It takes a lot of effort to change halophiles so that they express foreign genes by giving them a variety of genetic tools. These currently accessible genetic tools must be improved, nevertheless, as they have some limitations. By utilising synthetic biology and genetic modification technologies like recombineering, gene knockout, gene overexpression, and others, many of them have increased accelerated cell growth, high cell density, downstream separation simplification, enlarged space for more inclusion body accumulation (increased cell size for PHA granules), production yield, and reduced bioproduction costs. They increased PHB and PHBV production by deleting the *prpC* gene, which encodes 2-methylcitrate synthase, and the *phaZ* gene, which encodes PHA depolymerase, as well as overexpressing the *udhA* gene, which encodes soluble pyridine nucleotide transhydrogenase. The deletion of the *prpC* gene in *Halomonas* TD01 increased the conversion efficiency of propionic acid to 3-hydroxyvalerate (3HV) monomer fraction from 10% to nearly 100% in random PHBV copolymers of 3-hydroxybutyrate (3HB) and 3HV, resulting in cell growth to accumulate 70% PHBV in dry weight (CDW) consisting of 12 mol percent 3HV from 0.5 g/L propionic acid in glucose mineral medium. Furthermore, manipulation of PHA granule-associated proteins leads to an increase in PHA granule size, allowing for more natural separation. By creating an overexpression plasmid (pBBR1MCS1-oriC-Pporin-phaCABLS) of PHA synthesis genes encoding genes for PHA synthase *phaC*, some researchers were able to increase the PHA synthesis capacity of *Halomonas campaniensis* LS21.

Recently, overexpression of recombinant, leaderless thioesterase I (TesA) from *Escherichia coli* and cyanobacteria increased free fatty acid production. *coli*. For biofuel production, a TesA homolog from the moderately halophilic bacterium *Chromohalobacter salexigens* was identified, cloned, and recombinantly expressed in *E.*

coli strains BL21 and M15. Additionally, the *alsS* gene from *Bacillus subtilis*, as well as the *ilvC* and *ilvD* genes from *Corynebacterium glutamicum*, were overexpressed to produce KIV (2- Ketoisovalerate) for isobutanol production. By creating a *Halomonas elongata* transformant (*H. elongata*/pHS15N-lysC) overexpressing *lysC* and contrasting it to a control strain carrying an empty vector (*H. elongata*/pHS15N), the researchers were able to boost ectoine synthesis from glucose. After 4 hours of cultivation, *H. elongata*/pHS15N produced 174 mmol/kg FW of ectoine and 0.31 mol of ectoine/mol of glucose, while *H. elongata*/pHS15N-lysC produced 207 mmol/kg FW of ectoine and 0.39 mol of ectoine/mol of glucose. Ectoine production from glucose was 1.2-fold greater in *H. elongata*/pHS15N-lysC than in the control, and the yield was marginally higher. This suggests that *lysC* overexpression enhances ectoine production from glucose.

XV. HALOPHILE PHARMACEUTICAL APPLICATIONS AND FUTURE PROSPECTS

The pharmaceutical and healthcare sectors are anticipated to use halophilic microbial products extensively. Pharmaceutical substances are made with the aid of proteolytic enzymes. Bioactive chemicals are important and sources of bioactivities, that have not been well examined, like anti-bacterial, anti-viral and anti-tumor compounds and are produced by a variety of halophilic bacteria. As a powerful group in the development of drugs like curacin and cryptophycin, both of which are currently undergoing clinical research, marine cyanobacteria have attracted a lot of interest. Studies on the antioxidant and anticancer effects of peptide compounds produced from marine diatoms have also been conducted. Halophile biosurfactants are becoming more and more common in vaccines, immunomodulators, dermal fibroblast stimulators, antiviral, antibacterial, antifungal, anticancer, and antiviral treatments. Halophilic bacteria need to produce novel medicines, anti-oxidants, sunscreens, suitable solutes, and hydrolytic enzymes from uncharted territories in order to eventually achieve a major milestone in future. Due to recent advancements in the incorporation of halogenated compounds into peptoids (oligomers of N-substituted glycines), brominated analogues exhibit 32-fold increased activity against MRSA and 16-64-fold increased activity against *P. aeruginosa* and *E. coli*, respectively, which improve antimicrobial efficacy against multi-drug-resistant pathogens. Future halogenated medications might be more effective against microorganisms that are resistant to them. OMIC technologies offer tremendous promise for the future identification of novel biomolecules originating from halophiles due to the recent findings of halophilic bacteria, even in terrestrial habitats.

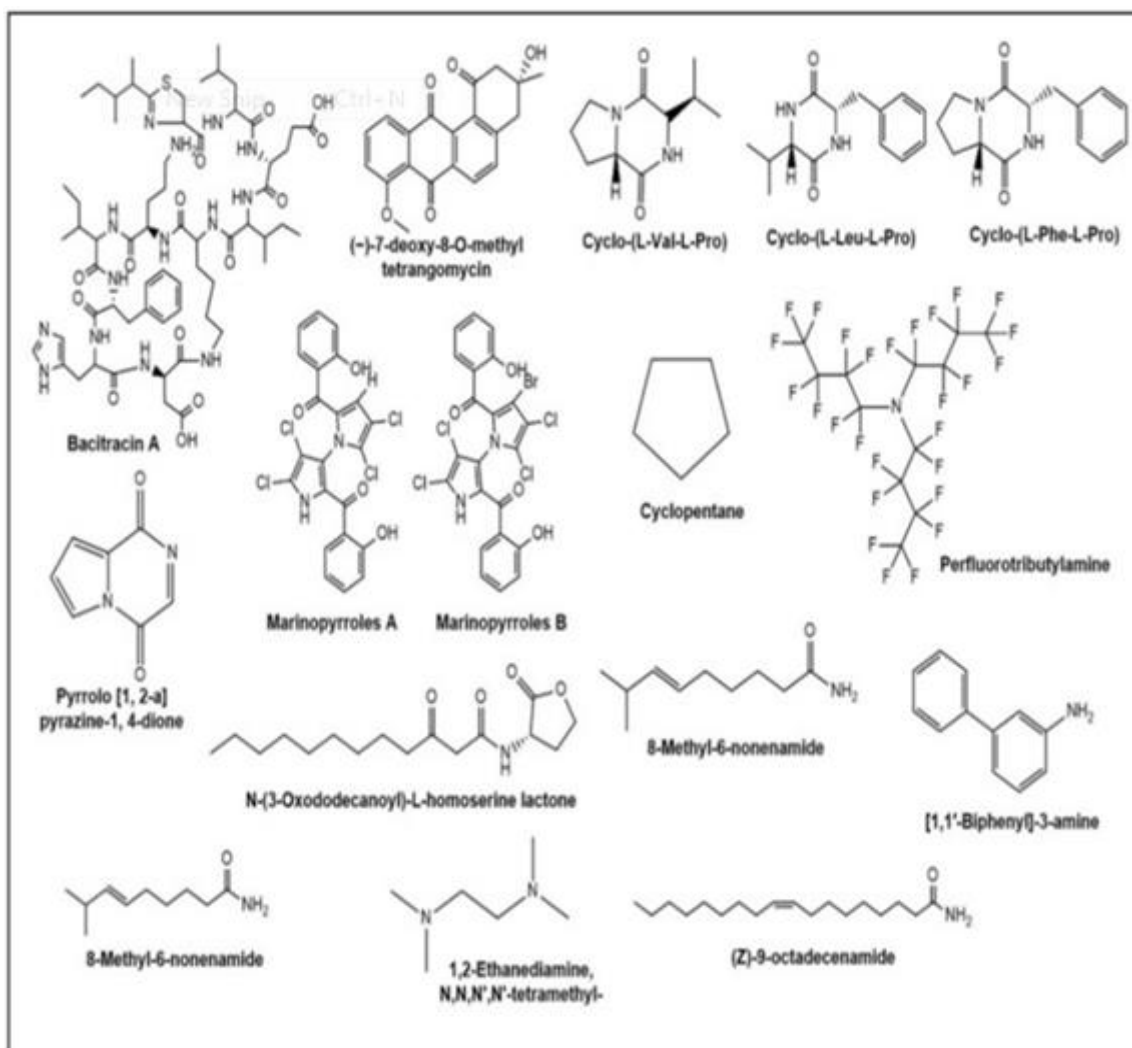


Figure 8: Antimicrobial substances produced by halophilic microorganisms are their chemical structure.

The evolution, diversification, and global distribution of halophilic bacteria during the first stages of life on Earth need to be studied further. Their ability to synthesise suitable solutes, biopolymers, and other compounds through biotechnology has significant industrial implications. To fully realise their clinical potential, more study is needed. By focusing on their physical makeup and modes of action, doctors will be able to anticipate which molecule will have the desired therapeutic outcome.

XVI. CONCLUSION

Halophiles are extremophiles that can live in high salt concentration. This habitat is a great source of various bioactive compounds with high economic value in chemical, pharmaceutical, agricultural, industrial, and biotechnological applications. Future industrial and biotechnological activities could benefit greatly from the diversity of microorganisms. Novel biomolecules in halophilic ecosystems are primarily motivated by the human pathogens that are resistant to medication. Antibacterial activity in

Haloarchaeon has not been discovered. Isolation of new and more halophilic microbes are required to combat with drug resistant human pathogens. It has already been proven that the predominant actinomycete macromolecules and the halophilic representatives of the Bacillus are efficient against human infections with treatment resistance. Halophilic microorganism enzymes have not yet been shown to be efficient in combating human illnesses. Clinical investigations should concentrate more on halophile-produced antimicrobials, nevertheless, as it is unknown how halo-antimicrobials work against organisms that are drug-resistant. Thus, it is feasible that many more members of this incredibly diverse collection of bacteria will eventually be included in the biotechnological applications of halophiles or genes produced from them.

REFERENCE

- [1] Larcher W. (2001). Physiological plant ecology: Ecophysiology and stress physiology of functional groups. *Biochem. Cell Biol.* **64**: 675-680
- [2] DasSarma P, Coker JA, Huse, V, DasSarma S. (2010). Halophiles, Biotechnology. In: Flickinger MC (ed.) *Encyclopedia of Industrial Biotechnology, Bioprocess, Bioseparation, and Cell Technology*. John Wiley & Sons Ltd, 2769-2777.
- [3] Zuleta LMC, Cavalheiro AJ, Silva DHS, et al. (2003). Seco Iridoids from *Calycophyllum spruceanum* (Rubiaceae). *Phytochemistry*. **64**: 549-553.
- [4] Klahn S, Hagemann M (2011) Compatible solute biosynthesis in cyanobacteria. *Environmental Microbiology*. **13**: 551-562.
- [5] DasSarma S. (2006). Extreme halophiles are models for astro-biology. *Microbe*. **1**: 120-127.
- [6] Oren A. (2012). Taxonomy of the family Halobacteriaceae: a paradigm for changing concepts in prokaryote systematic. *International Journal of Systematic and Evolutionary Microbiology*.
- [7] Oren A. (2006). The order Halobacteriales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, (eds) *The prokaryotes. A handbook on the biology of bacteria*. Vol. III. (Ed. 3) Springer, New York. 113-164.
- [8] De la Haba RR, Arahal DR, Marquez MC, Ventosa A. (2010). Phylogenetic relationships within the family Halomonadaceae based on 23S and 16S rRNA comparative sequence analysis. *Int. J. Syst. Evol. Microbiol.* **60**: 737-748.
- [9] Oren A. (2010). Industrial and environmental applications of halophilic microorganisms. *Environmental Technology*. **31**: 825-834.
- [10] Das Sarma S, Arora P. (2001). Halophiles. *Encycl. of Life. Sci.* 1-9.
- [11] Boone DR, Whitman WB, Koga Y. (2001). Order III. Methanosarcinales ord. nov. In: Boone DR, Castenholz RW, Garrity GM, (eds) *Bergey's manual of systematic bacteriology. The Archaea and the deeply branching and phototrophic Bacteria*. Springer, New York. 287-289.
- [12] De la Haba RR, Sanchez-Porro C, Marquez MC, Ventosa A. (2010). Taxonomic study of the genus *Salinicola*: transfer of *Halomonas salaria* and *Chromohalobacter salarius* to the genus *Salinicola* as *Salinicola salaries* comb. nov. and *Salinicola halophilus* nom. nov., respectively. *Int. J. Syst. Evol. Microbiol.* **60**: 963-971.
- [13] Oren A. (2002). *Halophilic microorganisms and their environments*, Kluwer Academic Publishers, London.
- [14] Loiko NG, Soina VS, Sorokin DY, Mitiushina LL, El'-Registan GI. (2003). Resting forms of gram negative chemolithoautotrophic bacteria *Thioalkalivibrio versutus* and *Thioalkalimicrobium aerophilum*. *Microbiologia*. **72**: 328-337.
- [15] Oren A (2015) Halophilic microbial communities and their environments. *Curr Opin Biotechnol* **33**: 119-124.
- [16] Oren A (2014) Taxonomy of halophilic Archaea: current status and future challenges. *Extremophiles* **18**: 825-834.

- [17] Gunde-Cimerman N, Ramos J, Plemenitas A (2009) Halotolerant and halophilic fungi. *Mycol Res* 113: 1231–1241
- [18] Hosseini TA, Shariati M (2009) *Dunaliella* Biotechnology: methods and applications. *J Appl Microbiol* 107: 14–35.
- [19] Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* 62: 504–544
- [20] Waditee R, Bhuiyan MN, Rai V, et al. (2005) Genes for direct methylation of glycine provide high levels of glycinebetaine and abiotic-stress tolerance in *Synechococcus* and *Arabidopsis*. *Proc Natl Acad Sci USA* 102: 1318–1323.
- [21] DasSarma S, DasSarma P (2015) Halophiles and their enzymes: negativity put to good use. *Curr Opin Microbiol* 25: 120–126.
- [22] Arakawa T, Tokunaga M (2005) Electrostatic and hydrophobic interactions play a major role in the stability and refolding of halophilic proteins. *Protein Pept Lett* 11: 125–132.
- [23] *Macromol.* 117, 493–522. Nagata, S., et al., 2008. Efficient cyclic system to yield ectoine using *Brevibacterium* sp. JCM 6894 subjected to osmotic downshock. *Biotechnol. Bioeng.* 99 (4), 941–948.
- [24] Hutcheon GW, Vasisht N, Bolhuis A (2005) Characterization of a highly stable alpha-amylase from the halophilic archaeon *Haloarcula hispanica*. *Extremophiles* 9: 487–495.
- [25] Fukushima T, Mizuki T, Echigo A, et al. (2005) Organic solvent tolerance of halophilic alpha-amylase from a Haloarchaeon, *Haloarcula* sp. strain S-1. *Extremophiles* 9: 85–89.
- [26] Waditee-Sirisattha R, Kageyama H, Sopun W, et al. (2014) Identification and upregulation of biosynthetic genes required for accumulation of Mycosporine-2-glycine under salt stress conditions in the halotolerant cyanobacterium *Aphanothece halophytica*. *Appl Environ Microbiol* 80: 1763–1769.
- [27] Chen D, Feng J, Huang L, et al. (2014) Identification and characterization of a new erythromycin biosynthetic gene cluster in *Actinopolyspora erythraea* YIM90600, a novel erythronolideproducing halophilic actinomycete isolated from salt field. *PLoS One* 9:e108129.
- [28] Falb M, Müller K, Königsmaier L, et al. (2008) Metabolism of halophilic archaea. *Extremophiles* 12: 177–196
- [29] Chen, H.; Simoska, O.; Lim, K.; Grattieri, M.; Yuan, M.; Dong, F.; Lee, Y.S.; Beaver, K.; Weliwatte, S.; Gaffney, E.M.; et al. *Fundamentals, Applications, and Future Directions of Bioelectrocatalysis*. *Chem. Rev.* 2020, 120, 12903–12993.
- [30] Harnisch, F.; Rabaey, K. *Bioelectrochemical Systems*. In *Materials for Low-Temperature Fuel Cells*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2014; pp. 167–184.
- [31] Grattieri, M.; Hasan, K.; Minter, S.D. *Bioelectrochemical Systems as a Multipurpose Biosensing Tool: Present Perspective and Future Outlook*. *ChemElectroChem* 2017, 4, 834–842.
- [32] Zheng, T.; Li, J.; Ji, Y.; Zhang, W.; Fang, Y.; Xin, F.; Dong, W.; Wei, P.; Ma, J.; Jiang, M. *Progress and Prospects of Bioelectrochemical Systems: Electron Transfer and Its Applications in the Microbial Metabolism*. *Front. Bioeng. Biotechnol.* 2020, 8, 1–10.
- [33] Wang, H.; Ren, Z.J. A comprehensive review of microbial electrochemical systems as a platform technology. *Biotechnol. Adv.* 2013, 31, 1796–1807.
- [34] Menon, G., et al., 2010. Isolation, purification, and characterization of haloalkaline xylanase from a marine *Bacillus pumilus* strain, GESF-1. *Biotechnol. Bioprocess Eng.* 15 (6), 998–1005.
- [35] Schröder, U.; Harnisch, F.; Angenent, L.T. *Microbial electrochemistry and technology: Terminology and classification*. *Energy Environ. Sci.* 2015, 8, 513–519.
- [36] Santoro, C.; Arbizzani, C.; Erable, B.; Ieropoulos, I. *Microbial fuel cells: From fundamentals to applications. A review*. *J. Power Sources* 2017, 356, 225–244.
- [37] Mamo, G., et al., 2009. An alkaline active xylanase: insights into mechanisms of high pH catalytic adaptation. *Biochimie* 91 (9), 1187–1196.

- [38] Kretzschmar, J.; Böhme, P.; Liebetrau, J.; Mertig, M.; Harnisch, F. Microbial Electrochemical Sensors for Anaerobic Digestion Process Control—Performance of Electroactive Biofilms under Real Conditions. *Chem. Eng. Technol.* 2018, 41, 687–695.
- [39] Grattieri, M.; Minteer, S.D. Self-Powered Biosensors. *ACS Sens.* 2018, 3, 44–53.
- [40] Abrevaya, X.C.; Sacco, N.J.; Bonetto, M.C.; Hilding-Ohlsson, A.; Cortón, E. Analytical applications of microbial fuel cells. Part I: Biochemical oxygen demand. *Biosens. Bioelectron.* 2015, 63, 580–590.
- [41] Koch, C.; Korth, B.; Harnisch, F. Microbial ecology-based engineering of Microbial Electrochemical Technologies. *Microb. Biotechnol.* 2018, 11, 22–38.
- [42] Goldman, Y., et al., 1981. Conversion of halophilic algae into extractable oil. 2. Pyrolysis of proteins. *Fuel* 60 (2), 90–92
- [43] Zhu, Y.-H., Jiang, J.-G., 2008. Continuous cultivation of *Dunaliella salina* in photobioreactor for the production of β -carotene. *Eur. Food Res. Technol.* 227 (3), 953–959.
- [44] Quillaguamán, J., et al., 2008. Poly (3-hydroxybutyrate) production by *Halomonas boliviensis* in fed-batch culture. *Appl. Microbiol. Biotechnol.* 78 (2), 227–232.