Application of Halophilic Bacteria in Medicine

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ABSTRACT

Halophiles are potential candidates due to their unique ability to grow under harsh environmental conditions. Recent advances in genetic manipulations have been established to better the performance of halophiles for industrial applications. The organisms thriving under extreme conditions better than any other organism living on Earth, fascinate by their hostile growing parameters, physiological features, and their production of valuable bioactive metabolites. The unique cellular enzymatic machinery of halophilic microbes allows them to thrive in extreme saline environments. That these microorganisms can prosper in hypersaline environments has been correlated with the elevated acidic amino acid content in their proteins, which increase the negative protein surface potential. Because these microorganisms effectively use hydrocarbons as their sole carbon and energy sources, they may prove to be valuable bioremediation agents for the treatment of saline effluents and hypersaline waters contaminated with toxic compounds that are resistant to degradation. This is the case of microorganisms (bacteria, archaea, and fungi) that grow optimally at high salinities and are able to produce biomolecules of pharmaceutical interest for therapeutic applications. As along as the microbiota is being approached by massive sequencing, novel insights are revealing the environmental conditions on which the compounds are produced in the microbial community without more stress than sharing the same substratum with their peers, the salt.

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

I. INTRODUCTION

Halophilic microorganisms comprise a heterogeneous group of microorganisms which can survive extreame salinity conditions in a wide range of environment. They are being considered as the type of extremophiles, Extremophiles are those microorganisms which require extreme environment for growth. The term extremophile was first used by MacElroy in 1974. Extreme environment is a relative term, since environments that are extreme for one organism may be essential for the survival of another organism. Hypersaline environments are inhabited by a great variety of microorganisms, and these are often present in extremely high community densities. Thanks to the fact that some of the most prevalent types are colored by carotenoid and other pigments, no microscope is needed to see halophilic microorganisms in environments such as saltern crystallizer brines and other salt lakes with saturating or near-saturating salt concentrations. There are three domains of life, Archaea, Bacteria, and Eucarya. Aerobic as well as anaerobic halophiles, heterotrophic, phototrophic, and chemoautotrophic types, able to transform a wide variety of substrates. Extremophiles are those microorganisms which live in extreme environment like high or low temperature, salt concentration, pH etc.

Extremophiles live under conditions that would kill most other creatures and many cannot survive in the normal global conditions. On the bases of their habitation these extremophiles can be categorized as: acidophiles, alkalophiles, halophiles, thermophiles and psychrophiles. Among extremophiles halophiles are those which are able to survive in high salt concentrations. These microorganisms inhabit the world's most saline environments, like hypersaline lake, solar saltern, evaporation pond, salt flats and tidal marine environments. Halophilic bacteria have the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts. On the basis of their halotolerance or optimum salt concentration wherein these organisms grow they are categorized as slight (2-5% NaCl), moderate (5-15% NaCl) or extreme halophiles (15-30% NaCl). To survive in higher saline environment halophilic microorganisms use different types of strategies, they synthesize compatible solutes in cells or possess the transporters that help them to survive in such type of harsh condition. Osmoregulatory solutes such as potassium ion, glutamate, proline, ectoine and betaine have been reported in these bacteria. Some halophiles produce acidic proteins that have the capacity to function in high salt concentration. They have been isolated from diverse salinity environments, varying from natural brines, hypersaline lakes to saturation salinities.

Diverse halophilic population includes Archaea, Bacteria, and Eukarya .Most commonly observed halophiles are Archaea and Bacteria such as Halobacterium, Halomonas and Salinibacter.The halophilic Eukarya such as Dunaliella salina is one of nature's richest sources of carotenoids known to date .Halophiles are able to thrive in hypersaline (~ 0.6 M) up to saturation salinity (>5 M NaCl) environments .They have evolved several different molecular and cellular mechanisms to respond to the salt-stress condition. A primarily used 'salt-in strategy' can be achieved by raising the salt concentration in the cytoplasm, and their enzymes tolerate or require high-salt concentration which is well-recognized in halophilic Archaea and Bacteria .The well-known mechanism is the so-called ‘salt-out strategy’ or ‘organic-osmolyte mechanism’ allowing an osmotic adaptation by excluding salts and/or synthesizing de novo compatible solutes .This strategy is also used in Archaea and Bacteria. In addition, molecular basis of protein halotolerance and adaptation of halophilic enzymes to high salinity is by increasing a substantial number of protein charges and increased hydrophobicity .Over the past few decades, halophiles have been considered for biotechnological applications. Diverse response mechanisms of halophiles under high-salinity conditions cause the production of various valuable biomolecules. It has been recognized that halophiles are also major sources of stable enzymes that function in very high salinity, an extreme condition that results in denaturation and aggregation of most conventional proteins.For instance, the α-amylase isolated from Haloarcula sp. functions optimally at 4.3 M salt at 50°C, and is stable in solvents benzene, toluene and chloroform.

Furthermore, halophiles are also considered to be potential sources for discovery of bioactive compounds, compatible solutes, unique enzymes including other potential biotechnological uses. Currently only two industrial processes that employ halophilic microorganisms, namely carotenoid production by halophilic alga Dunaliella and ectoine production by halophilic bacterium Halomonas elongate are realized .There are a number of bioactive compounds that show assorted biological activities ranging from antioxidant, sunscreen, and antibiotics actions derived from halophiles .Various studies show that some halophiles are capable of synthesizing massive amounts of compatible solutes such as glycine betaine and ectoine. These small organic molecules are useful as stabilizers of biomolecules or stress protective agents. Hypersaline environments are inhabited by a great variety of microorganisms, and these are often present in extremely high community densities.Some of the most prevalent types are colored by carotenoid and other pigments, no microscope is needed to see halophilic microorganisms in environments such as saltern crystallizer brines and other salt lakes with saturating or near-saturating salt concentrations.The world of the halophilic microorganisms is highly diverse. We find representatives of the three domains of life, Archaea, Bacteria, and Eucarya that are adapted to salt concentrations up to saturation.Aerobic as well as anaerobic halophiles, heterotrophic, phototrophic, and chemoautotrophic types, able to transform a wide variety of substrates .Among the halophiles‘‘polyextremophilic’’ types, adapted to grow at a combination of extremes, for example, of salt and alkaline conditions (a commonly found combination), salt and low pH (relatively rare), or salt and high temperatures; some anaerobic halophilic thermoalkaliphilic bacteria.

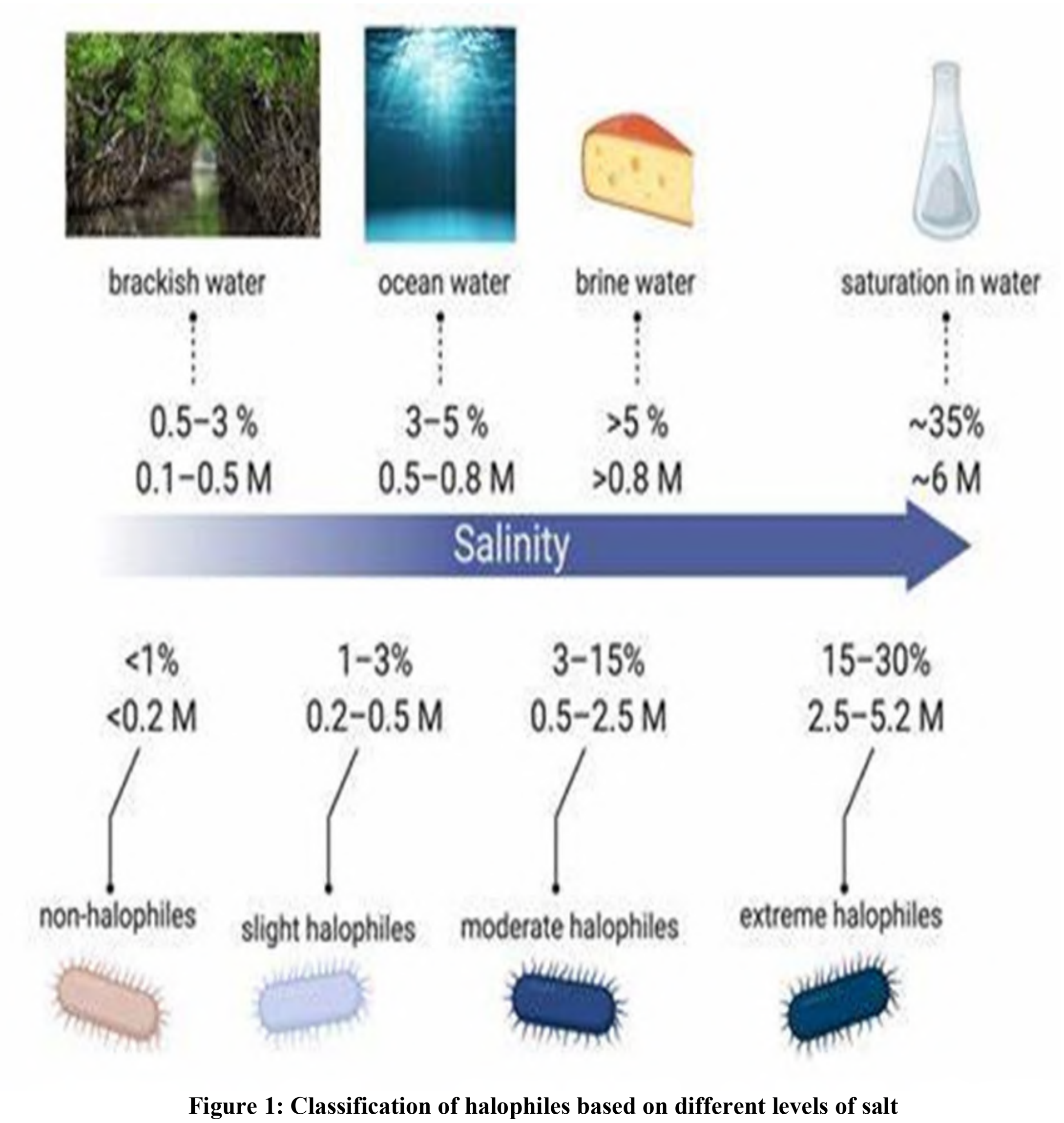

Figure 1: Classification of halophiles based on different levels of salt

II. Mechanisms for Saline Tolerance

Halophilic bacteria utilize two mechanisms for halotolerance known as the salt-out and the salt-in strategies. The salt-out strategy involves the uptake or synthesis of osmoprotectants. Osmoprotectants include small molecules, such as ectoines, amino acids, sugars, and betaines, and accumulate in the cytoplasm to protect the cells from lysis caused by an imbalance of osmotic pressure .The salt-in strategy used by species such as Salinibacter ruber, involves the accumulation of counter ions in the cytoplasm through an influx of potassium ions .The latter mechanism requires structural differences in biomolecules for function in high ion concentrations. However, the former is usually most common since osmoprotectants are also capable of stabilizing biomolecules in high ion concentrations. Therefore, it does not require significant structural changes. Structural differences found in the potassium influx osmoregulation mechanisms include a general increase in acidic content and charged amino acids on the protein surface, and in particular, an increase of glutamic acid due to its ability to bind water molecules better than any other amino acid .These modifications allow for the increased integration of water in the protein solvent shell and result in flexible protein structures that would otherwise be inaccessible due to the high ionic strength.

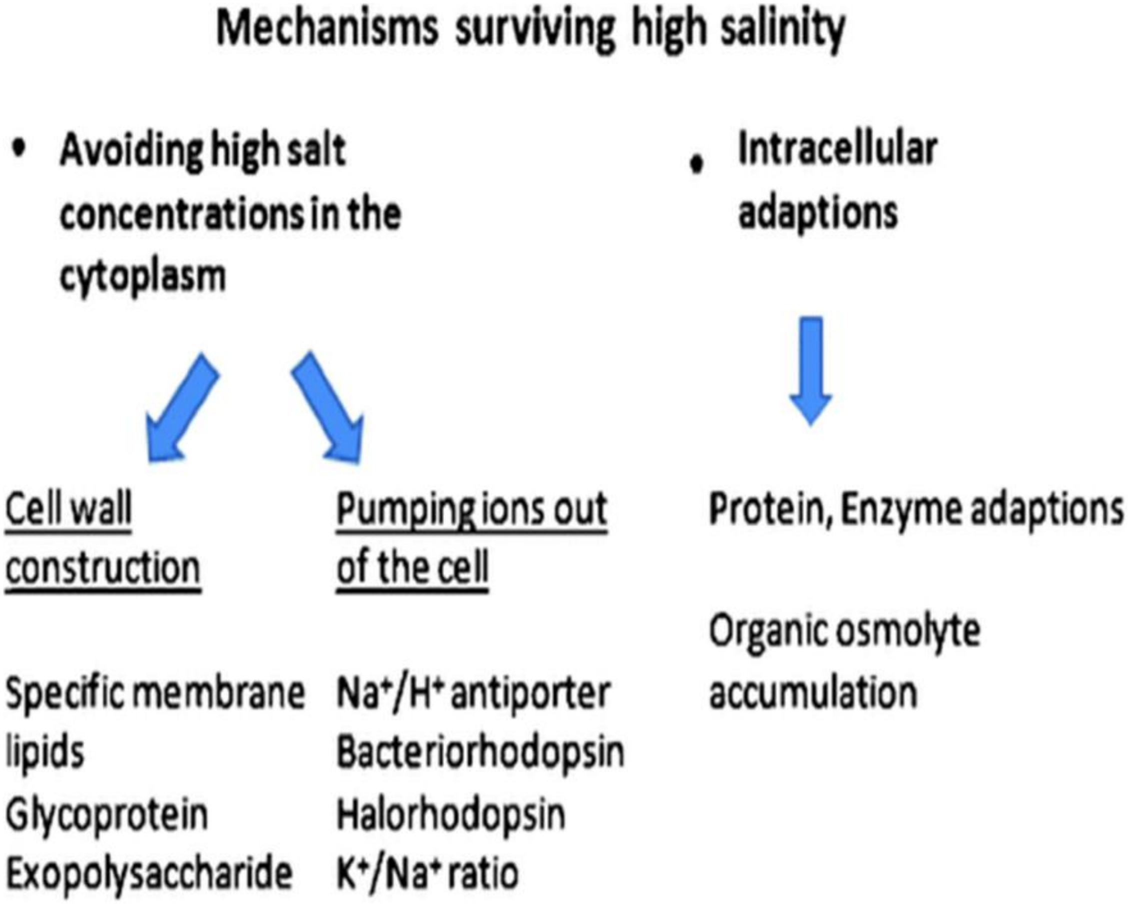

Figure 2: Mechanism of surviving high salinity

Halophiles are organisms represented by archaea, bacteria, and eukarya for which the main characteristic is their salinity requirement, halophilic“salt-loving”.Halophilic microorganisms constitute the natural microbial communities of hyper saline ecosystems, which are widely distributed around the world .They require sodium ions for their growth and metabolism.The tolerance parameters and salt requirements are dependent on temperature, pH,and growth medium. In this way, the halophiles are adapted and limited by specific environmental factors. Those microorganisms able to survive and optimally thrive under a wide spectrum of extreme environmental factors are designed polyextremophiles .In fact, a halophilic microorganism can also be alkaliphile, designated as haloalkaliphile, growing optimally or very well at pH values above 9.0, but cannot grow at the near neutral pH value of 6.5 .The general features of halophilic microorganisms are the low nutritional requirements and resistance to high concentrations of salt with the capacity to balance the osmotic pressure of the environment .They are physiologically diverse; mostly aerobic and as well anaerobic, heterotrophic, phototrophic,and chemoautotrophic.Ecologically, the halophilic microorganisms inhabit different ecosystems characterized by a salinity higher than seawater, i.e., 3.5% NaCl, these niches go from hypersaline soils,springs, salt lakes and other naturally-occurring coastal saline habitats .Other known habitats are the result of human intervention like salted foods,brines, oil fields, saltern ponds and tanneries .The high salinity reduces the number of organisms where just halophilic or halotolerant ones can survive in such hypersaline ecosystem, with archaea typically dominating the higher salinity environments. The better known hypersaline environments are the Great Salt Lake and the Dead Sea, with pH values around 7, and soda lakes with highly alkaline values of pH 9–11.

GENOMICS, EVOLUTION, AND TAXONOMY OF HALOPHILIC ARCHAEA AND BACTERIA

Methods of chemotaxonomy, multilocus sequence analysis, numerical taxonomy, comparative genomics and proteomics have allowed taxonomists to classify halophiles. These versatile microorganisms occupy all three major domains of life i.e., Archaea, Bacteria and Eukarya. Slightly and moderately halophilic microorganisms are represented by eukarya and bacteria whereas the extreme halophiles are represented by Archaea. The domain Archaea has been further divided into two subdomains, Halobacteria and Methanogenic Archaea. Halobacteria is represented by one of the largest halophile family, Halobacteriaceae with 36 genera and 129 species requiring high NaCl concentrations which discriminate them from other halophiles. They cannot even survive in fresh water and lyse .The role of Methanogenic Archaea is vital in the environment but characterization of only a few halophilic species has been possible yet. Among all five orders of methanogenic Archaea, only Methanocarcinales is the one containing halophilic species. These strictly anaerobic species form methane to obtain energy and are placed in the family Methanosarcinaceae.

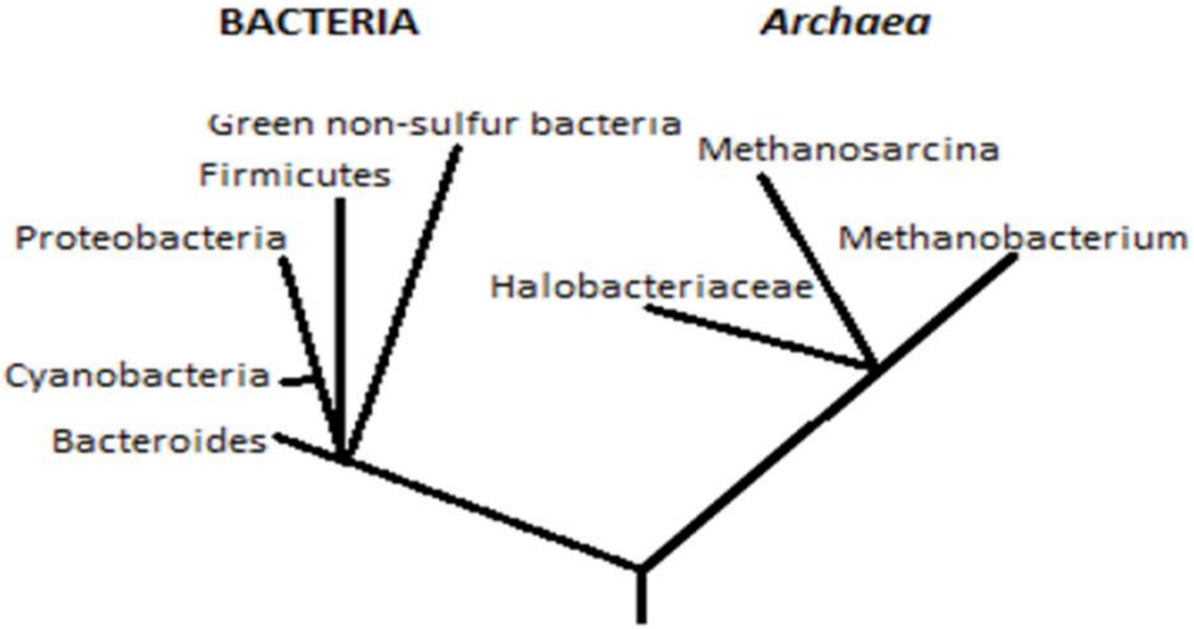

Figure 3: The universal phylogenetic tree of life as based on small subunit rRNA gene sequences, and distribution of halophilic Bacteria and Archaea

Recently completed genomes highlighted included Haloarcula marismortui , Natronomonas pharaonis ,aloquadratum,walsbyi , Halorubrum,lacusprofundi , Halomicrobium,mukohataei ,Halorhabdus,utahensis ,Halogeometricum,borinquense ,Haloterrigena,turkmenica and Haloferax,volcanii.The list includes significant ecological diversity, e.g. a haloalkaliphilic species, a cold-adapted species, species adapted to life in low-Na+-high-Mg2+ environments, and isolates showing interesting cell morphologies. The sizes of these genomes range between 2.6 and 5.4 Mb. Some of the conserved properties of haloarchaeal genomes including the presence of large megaplasmids and minichromosomes and the occurrence of core acidic proteomes. The data analysis also yielded the prediction of an expanding haloarchaeal pan-genome with increasing numbers of novel genes which may have applications in biotechnology.

Environmental genomics studies increasingly show that the genome of individual strains may be only a small fraction of the pan-genome of the species in nature. *Haloquadratum walsbyi* has become an excellent example to illustrate this. Comparisons have been made of the genome diversity within *Haloquadratum* populations in a single saltern crystallizer pond as well as comparisons between populations in similar environments at different geographic locations. The pan-genome of *Haloquadratum walsbyi* is at least 40 times the size of the genome of the type strain, and genomic microdiversity within an extremely simple and relatively constant environment is very high. *Desulfovibrio vulgaris* use a model organism and monitoring genetic changes after exposure of this nonhalophilic bacterium to 0.25 M NaCl for 1,000 generations. Salt-specific mutations and deletions were detected in the salt-resistant phenotype, which used different amino acids as osmoprotectants.

Genome sequencing of new isolates is getting simpler and cheaper and will probably soon become routine. Undoubtedly, this development will have profound implications on the taxonomy of the halophiles. Until taxonomy can be based on comparison of complete genome sequences, multilocus sequence analysis (MLSA) is gaining popularity for the comparison of strains for taxonomic and evolutionary studies. Analysis of the data indicates very frequent occurrence of homologous recombination, to the extent that alleles were randomly associated, as typical of sexually reproducing species. Natural competence and conjugation (like the mating mechanism in *Haloferax*) may be the possible mechanisms for lateral gene transfer. RNA polymerase subunit B′(*rpoB′*) gene can help in reconstructing the phylogeny of the *Halobacteriaceae* . Also for the *Halomonadaceae*, MLSA is becoming a valuable tool for taxonomic studies.For both groups, sets of genes and primers have been defined that give good results consistent with other genotypic and phenotypic traits.

The list of sequenced genomes of halophilic and halotolerant *Bacteria* is as yet short. It does not yet even include *Halomonas elongata*, the organism that, since it was described 30 years ago , has become one of the most popular model organisms and has also found biotechnological applications.Genome sequence information is available for the anoxygenic halophilic phototroph *Halo rhodospirahalophila*, for an extremely salt-tolerant alkaliphilic sulfur-oxidizing bacterium of the genus *Thioalkalivibrio*,for the thermophilic anaerobic halophile *Halothermothrix orenii*, and for the aerobic heterotrophic *Chromohalobacter salexigens* and *Salinibacter ruber*. Extensive environmental genomics data have been collected for *Salinibacter*. Comparative analyses indicate that *Salinibacter ruber* genomes present a mosaic structure with conserved and hypervariable regions. Overall, 10% of the genes encoded in the genome of the *Salinibacter* M8 genome are absent from the type strain *Salinibacter* M31. Metabolomic profiles also differed in these two isolates.

III. HALOPHILIC VIRUSES

The Halophiles 2001 and 2004 symposia in Seville and Ljubljana will be remembered as the events where the importance of fungi in hypersaline ecosystems became clear. Halophiles 2010 can then be described as the congress presenting the importance of viruses. Phages attacking extremely halophilic *Archaea* were first described already in 1974 ,but the role of viruses in hypersaline ecosystems remained largely unexplored.There are novel types of viruses attacking halophilic *Archaea*. The isolation and characterization of pleomorphic viruses possessing a lipid envelope, containing either a single-stranded or double-stranded DNA genome, show that viral diversity in hypersaline environments is much larger than previously assumed. Two novel lytic head/tailed viruses are virus BJ1 of the *Siphoviridae* and virus BJ2 of the *Myoviridae* which infect *Halorubrum kocurii*.Few archaeal virus genomes have been sequenced, and the complete sequence of virus BJ1 (EMBL accession number AM419438) is also done.

Saltern crystallizer ponds are also ideal environments to study virus diversity and dynamics, as protozoa and other predators are absent, and numbers of prokaryotes and virus-like particles are extremely high, typically in the order of $>10^7$/ml and $>10^8$ to 10^9/ml, respectively. At first sight, the salterns present predictable and stable communities of both *Archaea* and viruses, apparently different from the “kill-the-winner” behavior, with rapid cycling of microbial taxa and their viral predators that may be expected in such an environment .Metagenomic analysis of the viruses in the salterns near San Diego showed that the distribution of microbial taxa and viral taxa remained stable over time but with strong dynamic fluctuations of the prevalence of microbial strains and viral genotypes. Thus, at the fine level, the populations of both individual strains and viral genotypes fluctuate in a kill-the-winner fashion. Activity of viruses also may have profound implications on the distribution of the extremely halophilic bacterium *Salinibacter* (*Bacteroidetes*).he metagenome of viral assemblages of saltern pond in which *Salinibacter* accounts for around 15% of the prokaryotic community. Based on bioinformatic analysis (G+C content and dinucleotide frequency analysis), about 24% of the retrieved viral sequences could correspond to *Salinibacter* phages. It seems that phages infecting *Salinibacter* are more active in the environment than phages infecting *Haloquadratum*, and this may possibly explain why *Haloquadratum* outnumbers *Salinibacter* in every environment that supports growth of these organisms.

IV. HALOPHILIC FUNGI

The importance of halophilic fungi, long neglected as members of hypersaline ecosystems, became recognized only in the past decade.These include the black yeasts *Hortaea werneckii* which grows up to 5 M NaCl, the true halophile *Wallemia ichthyophaga* that requires at least 1.5 M NaCl and grows up to saturation, and *Aureobasidium pullulans* that grows up to 3 M NaCl. All of these are commonly found in hypersaline lakes and in a great variety of other, often unexpected, environments: domestic dishwashers, polar ice, and possibly even on spider webs in desert caves. The halophilic and halotolerant fungi use polyols such as glycerol, erythritol, arabitol, and mannitol as osmotic solutes and retain low salt concentrations in their cytoplasm. Molecular studies on osmotic adaptation of *Hortaea werneckii* and *Wallemia ichthyophaga* were done.Identification and structural features of Na^+-sensitive 3′-phosphoadenosine-5′-phosphatase HwHal2, one of the putative determinants of halotolerance in *H. werneckii* and a promising transgene to improve halotolerance in crops, was presented. An in-depth understanding has been obtained of the HOG (high osmolarity glycerol) pathway, and this understanding may be applied in the future to the development of improved salt-resistant crops. Glycerol-3-phosphate dehydrogenase is involved in glycerol synthesis by both *Wallemia* and *Hortaea*, and heterologous expression of the gene encoding the enzyme can restore halotolerance in *Saccharomyces cerevisiae* deficient in glycerol production.

V. DIVERSITY

The uniqueness of hypersaline environment is that it allows a great variety of microbes to inhabit it besides high salt concentrations. A wide variety of halophiles including heterotrophic,chemoautotrophic,chemolithotrophic aerobes and anaerobes could be observed transforming diverse range of substrates in hypersaline habitats .The largest family of Archaea, the Halobacteriaceae holds its name as the extremely halophilic group containing the highest salt requiring microorganisms. High sodium chloride concentration allows members of Halobacteriaceae to degrade organic compounds aerobically. Halophilic Archaea including the members of genus Haloarcula and Haloferax possess the ability to grow anaerobically in the presence of nitrate as electron acceptor resulting in the formation of nitrous oxide and gaseous nitrogen. Microorganisms able to grow at salt concentrations up to NaCl saturation (>300 g/l salt) are found all over the small subunit rRNA-based tree of life. Thus, three red-pigmented organisms are commonly found in salt-saturated saltern crystallizer ponds worldwide: the unicellular alga Dunaliella salina (Chlorophyceae, Eucarya), the square archaeon Haloquadratum walsbyi (Halobacteriales, Euryarchaeota), and Salinibacter ruber (Bacteroidetes, Bacteria). Within the domain Bacteria we find halophiles in a number of phyla, notably in the Cyanobacteria, the Gammaproteobacteria, the Firmicutes, and the Bacteroidetes. Among the cyanobacteria, a variety of both unicellular and filamentous types that grow at high salt, albeit not at the highest concentrations found in saturated brines .The best known group of halophilic or highly halotolerant types within the Bacteria is probably the family Halomonadaceae (Gammaproteobacteria).

In the aerobic Firmicutes and a number of other phyla, halophilic types are often closely related to organisms with little salt tolerance and requirement. Within the Firmicutes we also find the order Halanaerobiales with two families, the Halanaerobiaceae and the Halobacteroidaceae, consisting entirely of anaerobic halophilic, generally fermentative bacteria .A relatively recent addition to the list of true halophiles is the red aerobic S. ruber (Bacteroidetes) ,now known as a major contributor to the biota of salt lakes and salterns close to salt saturation, dominated by Archaea of the Halobacteriaceae. Sometimes the exploration of new environments can lead to the recognition of an entirely new group of halophiles, as shown by the discovery of the anaerobic wall-less, pleomorphic, contractile bacterium Haloplasma contractile in the brine-filled Saban Deep in the Red Sea. Phylogenetically this organism falls between the Firmicutes and the Mollicutes. It grows between 15 and 180 g/l salt, and lives by fermentation or denitrification. It is thus far the only known representative of the order Haloplasmatales, family Haloplasmataceae . Eukarotic life at high salt concentrations is possible as well, even of some macroorganisms, as shown by the brine shrimp Artemia, found at salt concentrations up to 150–200 g/l and sometimes even higher.

The best studied halophilic eukaryotes are undoubtedly the representatives of the green algal genus Dunaliella, some of which are orange rather than green as they accumulate massive amounts of b-carotene. Dunaliella is of great ecological importance in salt lakes as the sole or main primary producer at high salt concentrations, and thus it supports the life of the heterotrophic communities. Only relatively recently was the existence and the importance of heterotrophic halophilic eukaryotic microorganisms recognized. Many types of flagellate and ciliate protozoa were discovered in salt lakes up to NaCl saturation and culture-independent studies show that many more types are waiting to be discovered. Based on 18S rRNA studies, over 40 phylotypes of protists were found in a sample from the lower halocline in the hypersaline anoxic L'Atalante basin in the Eastern Mediterranean (depth 3,501 m, salinity 365‰) .Fungi, especially black yeasts and other melanized types, including species of Hortaea, Trimmatostroma, Candida, Pichia, Yarrowia, Debaryomyces, and others, are an integral part of hypersaline ecosystems worldwide, including salterns and salt lakes such as the Dead Sea.How widespread this type is and whether it may belong to a larger group of yet-to be characterized organisms is still unknown.

Phylogenetic Diversity in the Halophile World Microorganisms able to grow at salt concentrations up to NaCl saturation (>300 g/l salt) are found all over the small subunit rRNA-based tree of life. Thus, three red-pigmented organisms are commonly found in salt-saturated saltern crystallizer ponds worldwide: the unicellular alga Dunaliella salina (Chlorophyceae, Eucarya), the square archaeon Haloquadratum walsbyi (Halobacteriales, Euryarchaeota), and Salinibacter ruber (Bacteroidetes, Bacteria) .Among the extreme halophiles, the aerobic Archaea of the family Halobacteriaceae have been most studied. This family, the only family described in the order Halobacteriales, is a phylogenetically coherent group of salt-requiring prokaryotes .Most of the over a hundred species described grow optimally above 150 g/l salt, and no growth is obtained in its absence. Most species even lyse at salt concentrations below 100 g/l. The recent isolation of species that can survive or even grow at salt concentrations below 100 g/l does not change our general concepts on the ecology and the physiology of the group. The family includes the above-mentioned square archaeon Haloquadratum walsbyi, which for long resisted all attempts toward its cultivation, but is now available for study .No halophiles are yet known within the Crenarchaeota phylum of the Archaea, and all cultured types are classified within the Euryarchaeota. These also include truly halophilic methanogens within the family Methanosarcinaceae, Methanohalobium evestigatum being the most salt tolerant and salt requiring . Within the domain Bacteria we find halophiles in a number of phyla, notably in the Cyanobacteria, the Gammaproteobacteria, the Firmicutes, and the Bacteroidetes. Among the cyanobacteria, we find a variety of both unicellular and filamentous types that grow at high salt, albeit not at the highest concentrations found in saturated brines. Phages attacking halophilic Archaea and Bacteria are known, and such viruses abound in those (few) hypersaline systems where their distribution was studied. As in most freshwater and seawater environments, the number of virus particles may exceed the numbers of prokaryotes by one to two orders of magnitude. Different types of viruses were observed, Diversity of Halophiles including head-and-tail, spherical, and spindle-shaped viruses of halophilic Archaea, lytic types as well as types propagated in a carrier state, and double-stranded DNA viruses as well as single-stranded DNA viruses with a membrane envelope.

A. Metabolic Diversity of Halophiles

With respect to the processes performed to obtain energy and carbon for growth, the halophile world is as diverse as expected in view of the large phylogenetic diversity of the organisms present at high salt concentrations. Still, some processes known from low-salt environments apparently do not function at salt concentrations above 100–150 g/l. Examples are autotrophic nitrification, methanogenesis from hydrogen and carbon dioxide or from acetate, and aerobic oxidation of methane.

B. Oxygenic and Anoxygenic Phototrophs

Oxygenic photosynthesis occurs up to salt saturation, but the diversity of phototrophs growing at high salt concentrations is rather small. Most phytoplankton groups common in seawater have not adjusted to life in concentrated brines. Diatoms are relatively well adapted, and some species are found up to 100–150 g/l salt, but only two major groups of phototrophs function at the highest salt concentrations: cyanobacteria and green unicellular algae of the genus Dunaliella. Cyanobacteria are commonly found both in the plankton and in shallow benthic microbial mats in salt lakes and saltern evaporation ponds. These include both unicellular (Aphanothece halophytica and related forms) and filamentous forms (e.g., Microcoleus chthonoplastes, Halospirulina tapeticola, Phormidium spp. and others) .Heterocystous nitrogen-fixing species were never reported above 70 g/l salt. When the salt concentration increases above 200–250 g/l, also the cyanobacteria disappear, and the only types of oxygenic phototrophs remaining are species of the unicellular eukaryotic genus Dunaliella. Anoxygenic photosynthesis with sulfide as electron donor likewise can function up to high salt concentrations. In hypersaline microbial mats dominated by cyanobacteria in the upper aerobic layers, purple layers of anoxygenic phototrophs are commonly found. The phototrophic sulfur bacteria include types that store elemental sulfur intracellularly (Halochromatium, Thiohalocapsa) and forms that excrete sulfur (Ectothiorhodospira, Halorhodospira). Some Halorhodospira species not only tolerate salt concentrations approaching saturation, but very high pH values as well. Halophilic photoheterotrophic purple bacteria were also described such as Rhodovibrio salinarum, R. sodomense, and Rhodothalassium salexigens, which thrive up to 200–240 g/l salt. A completely different type of anoxygenic phototrophic life that functions at the highest salinities is based on the use of light energy absorbed by retinal-based pigments: bacteriorhodopsin and related pigments such as xanthorhodopsin. Bacteriorhodopsin, the major retinal pigment of Halobacterium and some related halophilic Archaea, can drive anaerobic growth of Halobacterium salinarum in the light .Organic substrates are used as carbon source, and photoautotrophy does not occur in this group. Use of light as supplementary energy source is also possible in the phylogenetically unrelated but physiologically very similar Salinibacter. Its proton pumping retinal protein, xanthorhodopsin, uses the membrane carotenoid salinixanthin as light-harvesting pigment .To what extent this interesting organism is also capable of photoheterotrophic growth remains to be ascertained.

C. Aerobic Heterotrophs

Most halophilic and halotolerant microorganisms described are aerobic heterotrophs, and we find these in the three domains of life. In environments with salt concentrations up to 200–250 g/l, representatives of the Bacteria are responsible for most of the heterotrophic activity, and some groups are very versatile both with respect to the range of salt concentrations tolerated and to the number of substrates used for growth .The list of organic substrates supporting growth at high salt concentrations is smaller than that known for freshwater environments, but it includes a number of hydrocarbons and aromatic compounds. At the highest salinities, groups like the Halomonadaceae no longer function and aerobic degradation of organic compounds is taken over by two groups of prokaryotes: the Halobacteriaceae (Archaea) and Salinibacter (Bacteria). The halophilic Archaea grow up to saturating salt concentrations, but the range of substrates oxidized is rather limited: mainly amino acids and simple sugars. Hydrocarbons and aromatic compounds are poorly used. Until recently is was believed that the Halobacteriaceae are the only aerobic heterotrophs functioning above 200–250 g/l salt, but the discovery of Salinibacter as a major component of the biota of saltern crystallizer ponds has changed our views. Growth experiments and analysis of the Salinibacter genome showed that this organism is not very versatile with respect to its growth substrates.

D. Anaerobic Respiration

Many modes of respiration with electron acceptors other than molecular oxygen are operative at high salt concentrations. Electron acceptors used include not only nitrate and sulfate, but also more unusual compounds such as arsenate and selenate, and their reduction is of ecological importance in some hypersaline environments. Nitrate is used as electron acceptor for respiration in a variety of halophilic and halotolerant prokaryotes. Many moderately halophilic members of the Halomonadaceae reduce nitrate to nitrite, and denitrification of nitrate to gaseous nitrogen is found in some halophilic Bacteria as well .Some halophilic Archaea (e.g., Haloarcula marismortui, Haloarcula vallismortis, Haloferax mediterranei) can grow anaerobically when nitrate is present as the electron acceptor, forming gaseous nitrogen and/or nitrous oxide . Some species also grow anaerobically by reduction of dimethylsulfoxide, trimethyl-N-oxide or fumarate. Seawater is rich in sulfate, and therefore it is not surprising that in anaerobic marine and hypersaline, seawater-derived environments, dissimilatory sulfate reduction is the terminal process in the degradation of organic compounds .However, compared to Diversity of Halophiles freshwater and marine systems, certain types of sulfate reducing bacteria appear to be missing in high-salt environments.

All halophilic and highly halotolerant dissimilatory sulfate reducers described thus far belong to the type of ‘‘incomplete oxidizers,’’ which oxidize substrates such as lactate, pyruvate, and ethanol and excrete acetate rather than oxidize the substrates all the way to carbon dioxide. Desulfohalobium retbaense, isolated from Lake Retba in Senegal, with its growth optimum at 100 g/l salt while tolerating up to 240 g/l, is probably the most halophilic strain known thus far .Sulfate reduction can proceed also under highly alkaline and hypersaline conditions. The haloalkaliphilic species Desulfonatronospira thiodismutans and D. delicata, isolated from the Kulunda Steppe, Altai, Russia, oxidize lactate and ethanol between 1 and 4 M total salt with an optimum at pH 9.5–10. They also grow chemoautotrophically by dismutation of sulfite or thiosulfate .There are indications that sulfate reduction is possible at higher salt concentrations as well. For example, stable isotope data suggested that dissimilatory sulfate reduction may occur in the bottom sediments of the Dead Sea (>300 g/l total salts) but no information is yet available about the process and the organisms performing it. ‘‘Complete oxidizers,’’ which oxidize substrates such as acetate to carbon dioxide, appear to be absent in hypersaline environments. The most salt-tolerant isolate known is Desulfobacter halotolerans, isolated from sediment of Great Salt Lake, Utah. It grows optimally at 10–20 g/l salt only, and slow growth is possible up to 130 g/l .Arsenate and selenate are less common electron acceptors for microbial growth, but these can also support anaerobic growth of halophilic/haloalkaliphilic bacteria. An isolate described as Haloarsenatibacter silvermanii grows optimally at 350 g/l salt and pH 9.4, chemoautotrophically by oxidizing sulfide with arsenate as electron acceptor, or heterotrophically using a range of organic electron donors. It also uses Fe(III) or elemental sulfur as electron acceptors for respiration .An example of a halophilic selenate reducer, which converts Se(V) to a mixture of Se(III) and elemental selenium is Selenihalanaerobacter shriftii, isolated from the Dead Sea – an environment not known for a high content of selenium compounds .

E. Methanogens and Homoacetogens

In low-salinity environments most of the methane generated is derived from two reactions: reduction of carbon dioxide by hydrogen and splitting of acetate to yield methane and carbon dioxide. Neither of these reactions was shown to occur at high salt concentrations. Methanogenesis from hydrogen and carbon dioxide was demonstrated in the alkaline Mono Lake, California at 88 g/l salts and the most halotolerant methanogen shown to grow on these substrates, Methanocalculus halotolerans isolated from an oil well, has its optimum at 50 g/l NaCl and does not grow above 120 g/l .Acetate-splitting methanogens are probably even less salt tolerant, but data are limited. In spite of the above, anaerobic sediments of hypersaline lakes are often a rich source of methane, even at near-saturating salt concentrations. Methanogens growing at such salinities Diversity of Halophiles have been isolated and characterized. They do not use the two common reactions mentioned above, but their substrates are methylated amines such as trimethylamine, and sometimes dimethylsulfide as well .Species such as M. evestigatum and Methanohalophilus portucalensis grow on such substrates even at 240–250 g/l salt. As no methanogenesis is known to occur from hydrogen and carbon dioxide at high salt concentrations, it is somewhat surprising that the same substrates can be converted to acetate by homoacetogenic bacteria. Acetohalobium arabaticum, a representative of the Halanaerobiales (Firmicutes), isolated from a hypersaline lagoon of the Arabay spit, Crimea, grow up to 250 g/l salt.

F. Fermentation

It is the anaerobic growth by conversion of organic compounds in the absence of an external electron acceptor, is possible up to the highest salt concentrations .Phylogenetically, we find fermentative halophiles in disparate groups. The Archaea of the family Halobacteriaceae are basically aerobes using oxygen as electron acceptor. However, some species can grow anaerobically by denitrification or using light energy absorbed by bacteriorhodopsin and species of the genus Halobacterium can grow anaerobically in the dark while obtaining energy by fermentation of arginine with the production of ornithine, ammonia, and carbon dioxide .Another representative of the Halobacteriaceae that probably grows by fermentation is Halorhabdus tiamatea, a non-pigmented isolate from a deep hypersaline anoxic basin near the bottom of the Red Sea. It only grows on complex substrates, and the mode of fermentation has not been ascertained .Specialized fermentative halophiles are found in a few groups in the domain Bacteria. The order Halanaerobiales with two families, the Halanaerobiaceae and the Halobacteroidaceae entirely consists of obligatory anaerobic halophiles, and nearly all obtain energy by fermentation.

Most species ferment simple sugars to products such as ethanol, acetate, hydrogen, and carbon dioxide, and most grow at salt concentrations between 100 and 200 g/l .In the domain Bacteria we also find a few fermentative prokaryotes phylogenetically not classified within the order Halanaerobiales. Examples are Clostridium halophilum (Firmicutes) and the unusual Haloplasma contractile, which grows up to 180 g/l salt, forms lactate as fermentation product, and is unrelated to any other group within the Bacteria.

G. Chemoautotrophs and Methanotrophs

Some aerobic chemoautotrophic processes function up to very high salt concentrations, while others appear to be restricted to low salinities. Autotrophic ammonia oxidation is still found in the alkaline Mono Lake at total salt concentrations between 80 and 90 g/l and 16S rRNA gene sequences related to Nitrosomonas europaea were recovered from the lake .However, no records exist of autotrophic ammonia oxidation above 150 g/l salt, and the most salt-tolerant isolate, described as ‘‘Nitrosococcus halophilus,’’ has its salt optimum at 40 g/l and does not grow above 94 g/l .Information on autotrophic oxidation of nitrite to nitrate at high salt concentrations is lacking altogether. Chemoautotrophic oxidation of reduced sulfur compounds proceeds at much higher salt concentrations. Halothiobacillus halophilus, isolated from a salt lake in Western Australia, oxidizes thiosulfate, elemental sulfur, and tetrathionate up to 240 g/l salt, with an optimum at 50–60 g/l .Thiohalorhabdus denitrificans, found in Siberian salt lakes and in Mediterranean salterns and representing a deep lineage within the Gammaproteobacteria, is even more halophilic: it grows between 11 and 290 g/l NaCl with an optimum at 175 g/l. It also grows anaerobically as a chemoautotroph using nitrate as electron acceptor .A variety of chemoautotrophic sulfur bacteria have been isolated also from soda lakes, such as Thiohalospira halophila, T. alkaliphila, and Thioalkalivibrio halophilus, organisms able to grow at pH 8–9 and above and at $Na+$ concentrations up to 5 M. In the alkaline hypersaline Mono Lake (about 90 g/l salts) and Sears Lake, California (>300 g/l salts), where high concentrations of arsenic occur, a biogeochemical cycle of arsenic functions with anaerobic reduction of As(V) (arsenate) to As(III) (arsenite) and chemoautotrophic oxidation of As(III) to As(V) .An isolate from Mono Lake, Alkalilimnicola ehrlichii(Gammaproteobacteria) grows as a chemoautotroph on arsenite using oxygen or nitrate as electron acceptor at salt concentrations up to 190 g/l (optimum 30 g/l), but can grow heterotrophically as well .Although methane is produced in anaerobic sediments up to very high salt concentrations attempts to show aerobic methane oxidation in hypersaline ecosystems either yielded negative results or very low rates of activity compared to those at lower salinities, as measured in hypersaline basins in the Crimea .A few methane-oxidizing bacteria were isolated from salt lakes such as ''Methylomicrobium modestohalophilum'' showing a moderate salt tolerance, but their optimum salt concentration for growth is only about 20 g/l .

VII. HABITAT

Hypersaline habitats favour microbial species having complexity in their composition and nature .Even with recent advancements in technology in molecular science,the fraction of discovered microorganisms is minor and exploration is still needed. Halophiles have been discovered from diverse habitats including the most noxious environments on the planet. This fluctuation in the environment from the normal condition has allowed these extremophiles to develop rare skills for survival in nature .Diversification of halophiles is largely because of their acclimatization ability in all hypersaline biotopes. These hypersaline environments are increasing as a result of manmade global as well as natural changes.Habitats like soda lakes, thalassohaline, athalassohaline environments,Dead Sea,carbonate springs,salt lakes,alkaline soils and many others favours the existence of halophiles. Variations in composition and nature of these habitats do exist. Formation of thalassohaline environments occurs by the evaporation of seawater leading to the increase in sodium and chloride ions with slightly alkaline pH.

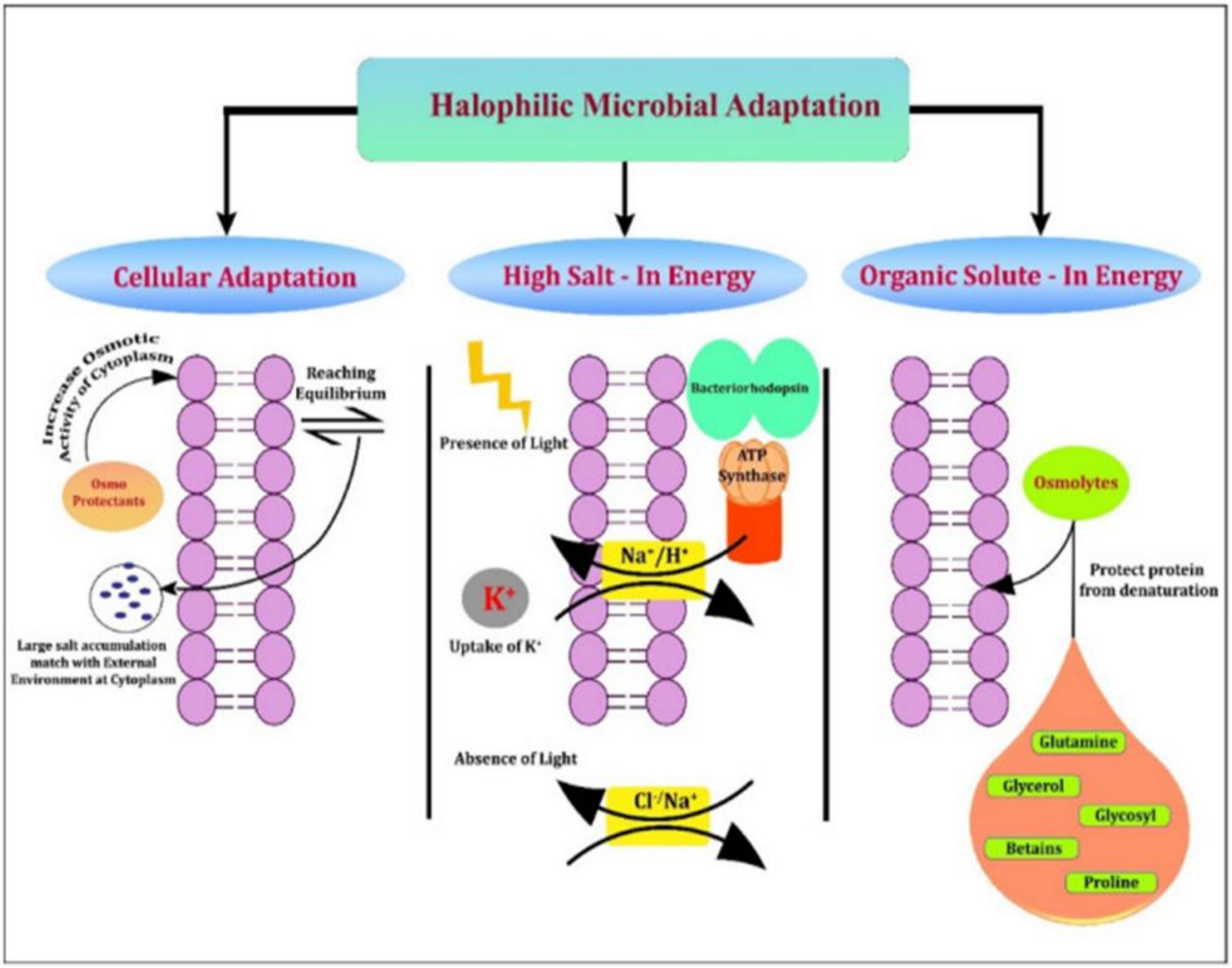

Figure: 4 Common strategies involved in halophilic microbial adaptations consisting of cellular adaptation, high salt-in energy, and organic solute-in energy.

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

VIII. The Diverse Strategies used to Cope with Salt Stress For a microorganism to be able to live at high salt concentrations

It is essential that the cytoplasmic contents are osmotically at least equivalent with the outside medium. A cell with an internal osmotic pressure lower than that of the surrounding salt solution will rapidly lose water through the water-permeable cell membrane. When a turgor is to be maintained (in all cells except possibly the extremely halophilic Archaea of the family Halobacteriaceae), the osmotic pressure inside the cell should even exceed that of its medium. Nature has devised diverse ways to achieve osmotic stabilization of halophilic microorganisms. All halophiles have one feature in common: sodium is always excluded from the cytoplasm as much as possible by means of powerful energy-requiring sodium pumps (in prokaryotes generally based on Na+ /H+ antiporter systems. However, what replaces sodium chloride to provide osmotic balance varies between the different types of microorganisms. One strategy is to accumulate potassium and chloride ions to concentrations similar to the ionic concentrations in the medium. The second strategy, which is much more widespread, is to exclude ions to a large extent and to accumulate small electrically neutral organic solutes (‘‘compatible solutes’’) to prevent loss of water from the cell by osmosis. The first strategy (‘‘salt-in’’ to balance ‘‘salt-out’’) requires far-reaching adaptations of the entire intracellular machinery, as all enzymes and functions in the cytoplasm have to be functional in the presence of molar concentrations of KCl. Moreover, solubility of enzymes and other protein is often lowered at high salt. Therefore, modification of the entire proteome is necessary. A characteristic feature of halophilic proteins from microorganisms that accumulate KCl for osmotic balance is their highly acidic nature, with a great excess of acidic amino acids (glutamate, aspartate) over basic amino acids (lysine, arginine). Such proteins are highly negatively charged compared to their non-halophilic equivalents. In addition, halophilic proteins generally have a low content of hydrophobic amino acids .The result of these protein modifications is that the constant presence of molar concentrations of salts is necessary to maintain the proteins in their native and active form; dilution of the salt leads to unfolding and denaturation. Therefore, those microorganisms that use the ‘‘salt-in’’ strategy are generally obligate halophiles with little flexibility with respect to the salt concentration range that allows growth, and they are thus adapted to life at an as much as possible constant and extremely high salinity. The advantage of using the ‘‘salt-in’’ strategy for osmotic adaptation is that it is energetically cheaper than biosynthesis of molar concentrations of organic osmotic solutes noting that those microorganisms that use the latter strategy also pump out sodium ions .

The ‘‘salt-in’’ strategy is currently known to be used in three groups of prokaryotes only: the halophilic Archaea of the family Halobacteriaceae, the extremely halophilic red aerobic representative of the Bacteroidetes S. ruber, and members of the order Halanaerobiales (Firmicutes), which are anaerobic, mostly fermentative bacteria. In the Halobacteriaceae, intracellular KCl above 4.5 M was measured in cells growing in saturating NaCl concentrations. Potassium ions can enter the cell through special channels, but active transport of chloride has been documented as well, based on the light-driven primary chloride pump halorhodopsin and/ or by cotransport with Na+ ions. Analysis of the genome of S. ruber, phylogenetically unrelated with the Halobacteriaceae, showed a great similarity in properties with those of Halobacterium and relatives, including a highly acidic proteome. Extensive gene exchange may have occurred between Salinibacter and the halophilic Archaea, which share the same environment and have experienced the same environmental stress factors throughout their evolutionary history .Analysis of intracellular ionic concentrations in selected representatives of the anaerobic fermentative Halanaerobiales (Halanaerobium praevalens, Halanaerobium acetethylicum, Halobacteroides halobius) also showed presence of molar KCl concentrations, and their enzymes require high salt for optimal activity .However, analysis of the genome of the anaerobic thermophilic fermentative Halothermothrix orenii, which also belongs to this group, failed to show an acidic proteome. As also no genes were identified for the biosynthesis of any of the known organic osmotic solutes ,the mode of osmotic adaptation of this interesting polyextremophilic bacterium remains to be clarified .The second strategy of osmotic adaptation, that is, accumulation of organic osmotic solutes, is much more widespread in the microbial world.The disadvantage of the strategy is the high energetic cost of the production of the solutes; the advantage is the large degree of flexibility and adaptability to a wide range of salt concentrations. As the solutes generally do not greatly affect protein structure and enzymatic activity, the cell can simply adjust the intracellular solute concentration according to the salinity of the environment. When suitable compatible solutes are found in the medium, these can often be taken up from the outside, thus saving much of the energy needed for de novo biosynthesis. These include glycerol and other polyols, simple sugars such as sucrose and trehalose, different amino acid derivatives such as glycine betaine and ectoine, and others .Most are uncharged or zwitterionic at the physiological pH. Glycerol is used by the unicellular algae Dunaliella and by some yeasts and fungi. A prerequisite for the use of glycerol as osmotic solute is a very low permeability of the cytoplasmic membrane for the compound. Most membranes are highly permeable to glycerol, and it was never found as an osmotic solute in the prokaryote world. The prokaryotic oxygenic phototrophs – the cyanobacteria – use sugars (sucrose, trehalose), glucosylglycerol, or glycine betaine instead, the latter compound being the solute preferred by the most halotolerant types .Glycine betaine can be accumulated from the medium by a wide range of bacteria and used for osmotic stabilization, but only a few prokaryotes synthesize the compound .More commonly encountered compatible solutes are ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) and its hydroxy derivative, synthesized from aspartate-b-semialdehyde –

an intermediate of amino acid metabolism .Often cocktails of osmotic solutes are found in a single organism; thus, Halorhodospira (Gammaproteobacteria) cells may contain glycine betaine, ectoine, as well as trehalose. As explained above, the Archaea of the family Halobacteriaceae use KCl for osmotic stabilization. However, an organic osmotic solute, 2-sulfotrehalose, was found in a number of haloalkaliphilic members, in which it functions together with KCl to provide osmotic balance .The methanogenic Archaea appear to rely on osmotic solutes: glycine betaine, b-glutamine, b-glutamate, and Ne-acetyl-b-lysine.

IX. HALOPHILES IN UNUSUAL ENVIRONMENTS AND HALOPHILES EXPOSED TO MULTIPLE FORMS OF STRESS

Most habitats explored for the presence of halophiles are thalassohaline environments that originated by evaporation from seawater, reflect the ionic composition of seawater, and have a nearly neutral to slightly alkaline pH. Deep-sea brines, found on the bottom of the Red Sea, the Mediterranean Sea, and the Gulf of Mexico, are interesting environments to search for novel microbes. Apart from their increased high salinity, they are anaerobic and form characteristically sharp brine-seawater interfaces, with some of the brines displaying significant increases in temperature and metal concentration. The ionic composition of the brines generally differs from that of seawater; they are anaerobic, and in some cases the temperature can be elevated as well. The microbiology of Shaban Deep and other deep-sea brines in the Red Sea was discussed by André Antunes .These sites, considered sterile in the past, have yielded a number of interesting microorganisms, including *Salinisphaera shabanensis* (a facultative anaerobe growing in a very large range of salt concentrations, from 1 to 28%), *Halorhabdus tiamatea* (a nonpigmented representative of the *Halobacteriales* that prefers an anaerobic life style), *Flexistipes sinusarabici* (an anaerobe tolerating between 3 to 18% NaCl) and *Haloplasma contractile* (a contractile bacterium, phylogenetically equidistant to the *Firmicutes* and the *Mollicutes*)). The sites will be revisited in the near future for further microbiological exploration. In many athalassohaline environments, life at the extremes of high salt is combined with the need to thrive at alkaline pH and elevated temperatures, and organisms growing there do so at the physico-chemical boundary for life.Two new species, designated “*Natranaerobius jonesii*” and “*Natranaerobius grantii*” are currently being characterized. *Natranaerobius jonesii* has an extremely high requirement for chloride ions as it does not grow at less than 1.4 M Cl^-. Other alkaline saline environments subjected to intensive studies in recent years are the soda lakes of the Kulunda Steppe.Dimitry Sorokin summarized the wealth of information obtained from these studies at the level of the characterization of cultures of novel organisms, especially those participating in the reductive part of the sulfur cycle, and from culture-independent studies using molecular markers, as well as measurements of the rates of microbial sulfidogenesis. In general, sulfide production was active even in saturated soda brines, but far more sulfide was produced in these environments from elemental sulfur and from thiosulfate than from sulfate. Dismutation of thiosulfate and sulfite was a major trend in soda lake isolates .
The Dead Sea is a rare example of a low-Na^+, high-Mg^{2+}, and high-Ca^{2+} chloride brine with a slightly acidic pH. Metagenomic studies are now providing information on the microbial diversity in the lake, both at the time of a bloom of microorganisms following dilution of the upper water layers by rain floods in 1992 and during the current drying out of the lake, causing a continuously decreasing ratio of monovalent/divalent cations, making conditions too extreme for even the best salt-adapted microorganisms

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

X. Physiology

a) Internal ion concentration

For survival in extreme saline conditions,halophiles maintain their internal ion concentration in such a way that their cytoplasm is not affected by the external osmotic conditions. This osmotic balance is achieved by accumulating salts internally

b) Tolerance to metals

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

c) Osmoadaptation

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

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

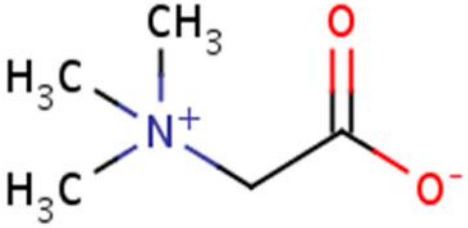

Figure 5: Glycine betaine

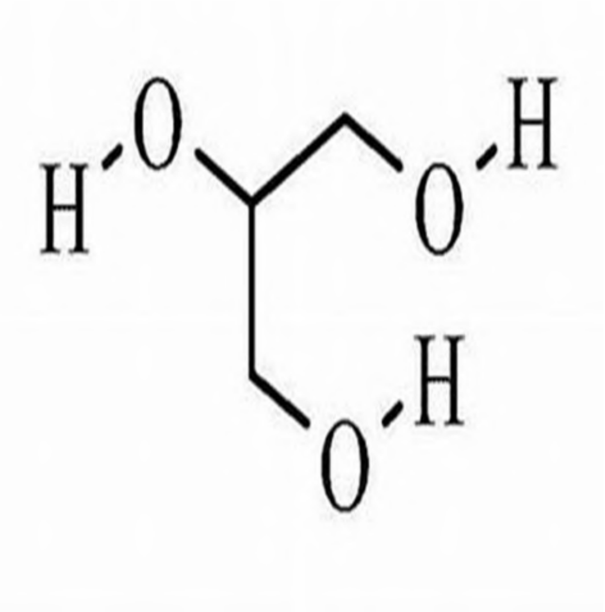

Figure 6:Glycerol

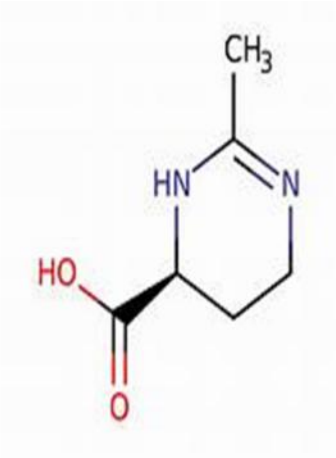

Figure 7:Ecotine

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

XI. Important Characteristics Of Halophiles

A. Enzymes

Enzymes are catalysts which have potential applications in food, detergent formulations, metal recovery, leather processing and in several industries. Extremophile enzymes, especially extracellular, have been found to have potential industrial applications as they can survive and catalyze reactions in unusual environment.

B. Nucleases

A nuclease (nuclease H) is reported from M. varians subsp. halophilus having both DNase and RNase activities. Another halophilic nuclease (an exonuclease, releasing 5 -mononucleotides from both DNA and RNA) was produced by Bacillus halophilus .

C. Amylases

A few -amylases were purified and characterized from halophilic microorganisms, Acinetobacter , Halobacterium salinarum M. varians subsp. Halophilus, , N. halobia Natronococcus amylolyticus, Halomonas meridiana, Haloferax mediterranei . The enzyme from Haloarcula sp. S-1 showed high tolerance to various organic solvents . The effect of ionic strength on the amylase activity, has also been reported at various ratios of Na+ and Mg 2+ concentrations .

D. Proteases

Halophilic microorganisms produce proteases which can have novel applications because of its high stability at saturated salt concentrations or organic solvent tolerance. Halobacterium salinarum produce an extra cellular serine protease that has potential to be used for peptide synthesis. The other serine proteases were isolated from Natronococcu soccultus, Natronomonas pharaonis , Natrial bamagadii .Some other proteases were purified and characterized from Natrial baasiatica and Haloferax mediterranei and other halophilic isolates. In recent years, a number of studies have been conducted to characterize extracellular proteases from Salinivibrio genus, Pseudoalteromonas sp. strain CP76, Natrialba magadii ,Halobacterium mediterranei, Bacillus clausii. There is also a report that a chymotrypsinogen B-like protease was isolated from the haloalkaliphilic archaeon Natronomonas pharaonis .

E. Lipases

Lipase is one of the most important hydrolytic enzymes with potential in various fields of pharmaceutical industry and agriculture. Various moderately or extremely halophilic microorganisms have been reported for the production of lipase which are also stable at high temperature - Salinivibrio sp. ,Natronococcus sp., haloarchaeal strains etc.

F. Cellulose-Degrading Enzymes

Cellulose-utilizing, extremely halophilic bacterium. The obligate anaerobic organism named Halocella cellulolytica is able to utilize cellulose as a sole carbon source. Another work has shown that many cellulose-utilizing extremely halophilic Archaea are present in subsurface salt formation. Extracellular hydrolytic enzymes of halophilic microorganisms from subterranean rock salt revealed the presence of cellulose.

G. Pigments

Halophilic microorganisms are a great source of diverse natural products. Carotenoid pigments are one of these natural products responsible for the yellow, orange, red, and purple colors in a wide variety of plants, animals, and microorganisms .Carotenoid pigments are particularly prominent in hypersaline environment. Red and orangish color of hypersaline habitats is due to the presence of pigmented microorganisms, including Dunaliella, rich in -carotene, Haloarchaea whose main production is bacterioruberin, and halophilic bacteria, such as Salinibacter ruber producing a carotenoid called salinixanthin. An extremely halophilic archaeon isolated from Urmia Lake, Halorubrum sp. TBZ126 is reported as potential producer of carotenoids. Melanin is nearly a ubiquitous pigment having immense application potentials in the field of agriculture, cosmetics and pharmaceutical industries (photoprotection and mosquitocidal activity isolated from Streptomycete). Rani et al reported a halophilic black yeast, Hortaea werneckii which produced a diffusible dark pigment on potato dextrose agar. It also showed inhibitory activity against potential pathogens and activity was observed in Salmonella typhi and Vibrio parahaemolyticus.

H. Resistance to Antibiotics

The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal .Pearson and Carol reported different antibiotics likes penicillin, erythromycin and gentamycin which used to be one of the important cures are now less effective because bacteria have become more resistant. Halophilic bacteria isolated from different region were found resistant against different antibiotics :Halobactericeae, Halomonas elongate, Bacillus cereus SIU1.

I. Antimicrobial Activity

Foreshore soil of Daecheon Beach and Saemangeum Sea of Korea represents an untapped source of bacterial biodiversity and also that most actinobacterial isolates are capable of antibacterial and antifungal metabolite production. The halophiles isolated from Ratnagiri coastal area (marine environments) having antibacterial and antifungal activity. The antibacterial and antifungal assays of halophiles (protein crude extract) have shown that, the marine environments represent a potential source of new antimicrobial and antifungal agents. Microbacterium oxydans and Streptomyces fradiae showed anibactrialactivity against pathogenic bacteria and yeasts.

J. Anticancer Compounds

Natural products are relevant anticancer drugs, which are also called bioactive molecules, produced by organisms. Although, earlier and the well-established anticancer natural products have been obtained from plant cells originally, microorganisms are an excellent alternative, due to the diversity of the microbial world, their easy manipulation, and they can be screened physiologically to discover new natural products with antitumor activity. Although bacterial cells have different communication methods with tumor cells other than metabolites experimentally, bacterial metabolites have been considered the most conventional way against cancer cells viability. Today, more attention is focused on extremophiles as a new source of novel biomolecules. Among extremophiles, halophilic and halotolerant microorganisms, which inhabit hypersaline environments, are considered as reliable sources of antitumor metabolites with fewer side effects. In recent years, several studies have been focused on the importance of metabolites from halophilic microorganisms on cancer treatment.

XII. Recent Antimicrobial Responses of Halophilic Microbes in Clinical Pathogens

Halophilic Bacillus sp**:***Bacillus* and *Virgibacillus* were frequently isolated from saline systems with antimicrobial potential. *Bacillus pumilus* NKCM 8905 *Bacillus pumilus* AB211228 isolates of coastal soil, Arabian Sea, Mumbai, produced antibiotics against *E. coli*, *S. aureus*, *B. subtilis* and *A. niger* . Phospholipid compounds produced from halophilic *B. subtilis* had a better antimicrobial activity than alkaliphilic *B. subtilis* on *S. aureus* with a maximum of 26 mm diameter inhibition zone, whereas alkalic *Bacillus* sp. showed 21 mm. *B. subtilis* derived from Haj Aligholi Salt Deserts and Dagh Biarjmand, Iran, revealed antimicrobial activity against pathogenic fungi and bacteria with MIC ranges from 12.5 to 25 µg/mL, fungus includes *A. flavus*, *F. oxysporum*, *C. albicans*, and the bacterium includes *B. cinerea*, and *N. crassa* with inhibition zones with diameters of 14, 11, 8, 39, and 13 mm. *B. subtilis* isolated from Kovalam Beach waters, Chennai in India, shows activity against clinical pathogens *P. aeruginosa*, *Proteus mirabilis*, *K. pneumonia*, *Salmonella typhi* and *S. typhi* B. The chloroform crude extract of this bacterium containing compound Pyrrolo (1, 2-a) pyrazine-1, 4-dione might be responsible for the reduction in OD (optical density) compared to the control for the aforementioned bacterial species. *Bacillus persicus* 24-DSMisolated from Dead Sea mud provided activity against *B. subtilis* and *E. coli* . Another discovery revealed that the *Bacillus* species DSM2 from the same location has activity against pathogenic fungi, including *C. albicans* ATCC 10231 and *A. brasiliensis* ATCC 16404..

Halophilic Actinomycetes**:** Due to the wide range of biopharmaceutical applications of Actinobacteria, there is a great diversity of halophilic strains being studied. *Nocardiopsis dassonvillei* halophilic actinomycetes showed antibacterial efficacy against human pathogens, such as *S. aureus*, *E. coli*, *B. cereus*, and *P. aeruginosa* . The ethyl acetate extracts of *Kocuria* sp. strain rsk4 inhibit *S. aureus* at the lowest MIC of 30 g/mL by secreting an antibacterial unknown compound with a molecular mass of 473 g/mol .The phenolic extracts of the halophilic actinomycetes isolate GD3007 provided activity at 50 µL/g against different pathogens such as *E. coli*, *S. aureus*, *Vibrio* sp., *P. aeruginosa*, and *K. pneumonia* with inhibition zone diameters of 30, 27, 24, 25, and 26 mm. Corum salterns actinomycetes were found to be active against *B. subtilis*, *E. coli*, and *A. niger*. The most significant activity was obtained from strains belonging to *Streptomyces* providing gene clusters including PKS-I, PKS-II, and NRPS, which were also tested for antibacterial efficacy using similar primers. *Streptomyces* sp. MA05, which was isolated from a salt lake in Chennai, showed antibacterial activity against *S. aureus* with an inhibition zone larger than 15 mm. *Streptomyces* spp. AJ8 was isolated from the Kovalam solar saltern in India, with a single gene fragment of NRPS length and was found to have antagonistic properties against bacterial and fungal pathogens, such as *V. harveyi* (9.2 mm inhibition zone), *A. niger* (9.8 mm), and *C. albicans* (5 mm) .

Other Halophilic Bacterial Species : Other Halomonas taxa isolated from the salty habitat of Northeastern Algeria showed broad antifungal activity against *Fusarium oxyporum*, *Botrytis cinerea*, *Phytophthora capsici*, and *F. verticillioides* .Gamma Proteobacteria from coastal solar salterns, such as *Halomonas smyrnensis* and *Halomonas variabilis*, were found to have antibacterial properties against *S. pasteuri* and *E. coli*. *Salinicoccus roseus* and *Virgibacillus salaries* exhibited activity against *M. luteus*, *A. johnsonii*, *X. oryzae*, *C. lipolytica*, *S. cerevisiae*, and *M. luteus*, *X. oryzae*, *C. lipolytica*, *S. cerevisiae* . The cell supernatants of *Nocardioides* sp. of halo-Antarctic soils containing glycolipids and/or lipopeptides provided antimicrobial activity against *S. aureus* and *X. oryzae*, whereas its salt medium supplemented with various carbon sources provided enzymatic activity

Halophilic Microalgae**:** *Dunaliella salina* alone produced several compounds with antimicrobial potencies against several pathogens. Hexane extract of the microalga *Dunaliella salina* at 97.0 mg mL^{-1} concentration showed an inhibition zone with a diameter of 20 mm against *B. subtilis*, and ethanolic extract at 214.0 mg mL^{-1} showed 21 mm against *B. subtilis* .The methanol and chloroform extract of *Dunaliella salina* possesses antibacterial activity on *Vibrio cholerae* at a maximum 10.4 mm inhibition zone due to the unique compounds such asn-Hexadecane (M.W. 226.2) and 3, 3, 5-Trimethylheptane (M.W. 142.2) .A mixed culture technique using marine and freshwater microalgae, such as *Coelastrum* sp., *Scenedesmus quadricauda*, and *Selenastrum* sp., exhibited growth inhibition on *S. epidermidis*, *S. marcescens*, and *P. fluorescens* via their methanol and hexane extracts proved the antibacterial efficacy of *D. salina* by suppressing the growth of *S. mutants* at 6250 g mL^{-1} using methanol, chloroform, and acetone extracts.

XIII. Novel Antimicrobials and Their Producing Strains from Halophiles

Interestingly, the novel bacterium *Paenibacillus sambharensis* isolated from a salt lake suppressed the growth of *S. aureus* by producing the compound bacitracin A, with a molecular mass of 1421.749 Da .WT6 and R4A19 antimicrobials generating strains were recently retrieved from an Iranian Salt Lake, producing activities against *E. coli* and *B. cereus* .The novel halophilic isolates AH35 and AH10 of the Algerian Sahara showed antibacterial activity (13–45 mm) against *K. pneumoniae*, *Pseudomonas syringae*, and *Agrobacterium tumefaciens*, and AH35 was active against *Salmonella enterica* (13 mm). The phylogenetic clades of these potential strains represent the species *Saccharomonospora paurometabolica*, *Saccharomonospora halophila*, and *Actinopolysporairaqiensis* . The unexplored deep-sea habitats of the Andaman and Nicobar Islands provided a source of novel halophilic species, including Bacilli, Alpha-, and Gamma-Proteobacteria, with antibacterial activity against Gram-positive and Gram-negative strains, including *P. mirabilis* MTCC1429, *V. cholerae* MTCC3904, *K. pneumonia* MTCC109, *E. coli* MTCC443, and *S. pneumoniae* MTCC1935 .The partially purified biosurfactants produced from halophilic strains *Halobacilluskarajiensis* and *Alkalibacillusalmallahensis* suppressed the growth of *K. pneumoniae* (94%) and *A. flavus* (80.4%) .A novel p-terphenyl 1 and a novel p-terphenyl derivative 3 providing a benzothiazole moiety were discovered from halophilic *Nocardiopsisgilva* YIM 90087, thus p-terphenyl 1 signifies its activity against *F. avenaceum*, *F. graminearum*, and *F. culmorum* with 8, 6, and 128 µg/mL MICs. Compound 1 exhibits antifungal activity with MIC 32 µg/mL against *C. albicans*, *B. subtilis* with 64 µg/mL, Novobiocin 4 showed antibacterial efficacy against *B. subtilis* with 16 µg/mL MICs and *S. aureus* with 64 µg/mL MICs .Despite the fact that the saline environment produces antimicrobials, some saline niches still remain unexplored and warrant urgent study for the discovery of novel antimicrobials and other bioactivities of applied interest

XIV. Halo-Microbial Derived Products as Antimicrobials

A. Pigments

A type of carotenoids, bacterioruberin, was retrieved from the halophilic bacterial species *Salinicoccussesuvii* MB597, *Aquisalibacillus elongatus* MB592, and *Halomonasaquamarina* MB598,provided antimicrobial activity against some pathogenic bacteria. Here, *Enterococcus faecium* was suppressed by a maximum inhibition zone diameter of 23 mm, besides wide antifungal activity attained from a pigment derived from *Halomonasaquamarina* MB598 with 98% growth inhibition on *Aspergillus fumigatus* and pigments derived from *Aquisalibacillus elongatus* MB592 showing 96% growth inhibition against the same fungus. Pigment derived from *Salinicoccussesuvii* MB597 gave 96.7% growth inhibition against *Mucor* spp. Red pigment produced by the bacterium *Candidatus chryseobacterium massiliae* isolated from Arabian seawater samples showed higher antibacterial activity among the isolated strains against *B. cerus* (8 mm), *S. aureus* (6 mm), *B. megaterium* (7 mm), *B. subtilis* (6 mm), and *V. cholerae* (8 mm) .A crude extract of bright yellow pigment produced from marine *Brevibacterium* showed antibacterial activity against *S. aureus* (29 mm), *E. coli* (17 mm), *P. aeruginosa* (27 mm), and *B. subtilis* (28 mm) .*Salinococus* sp. isolated from the Nellore sea coast produced a pinkish orange pigment, and its crude extract revealed antimicrobial activity against *K. pneumoniae, P. aeruginosa*, and *S. aureus* with the respective inhibition zone diameters: 16 mm, 14 mm, and 24 mm .In addition, an interesting study says the prodigiosin pigment extracted from marine *Serratia rubidaea* RAM Alex strain with textile fabric coating showed antibacterial activity against *S. aureus* and *E. coli*, which significantly decreased the hospital-acquired infections (HAI) .Marine *P. aeruginosa* producing pyocyanin was shown to act as an anti-chlamydial agent at a concentration of 0.02 µM .Nanomelanin derived from *P. aeruginosa* obtained from the marine sponge *T. citrine* had antibacterial activity against *B. subtilis*, *S. aureus*, and *E.coli* .Marine-derived *V. ruber* DSM 14379 producing prodigiosin showed strong killing efficiency on *B. subtilis* .Marine *Streptomyces* sp. 182SMLY producing quinones exhibited strong antibacterial activity against MRSA .Medermycin-type naphthoquinone-streptoxepinmycin A to D derived from the marine *Streptomyces* sp. XMA39 displayed antibacterial and antifungal activities against *S. aureus*, *E. coli*, and *C. albicans* .As a result of these findings, it appears that marine bacteria create relatively more significant pigments with antimicrobial properties .*Dunaliella* spp. is well-known for creating bioactive pigments from their methanol and chloroform extracts against pathogens, such as *B. subtilis* and *E. coli*, with inhibition zones measuring 20, 19, 18, and 22 mm, respectively. Through GC-MS and HPLC-DAD analyses, the chloroform extract of *Dunaliella* sp. 2 containing active pigments, such as luetin, carotene, and Zeaxanthin, was proven to have the aforementioned activity .*Dunaliella* sp., which produces orange-red pigments, showed antibacterial and antiviral properties

B. Biosurfactants

The partially purified biosurfactants containing compound 1, 2-Ethanediamine N, N, N′, N′ -tetra, 8-Methyl-6-nonenamide, (Z)-9-octadecenamide, and fatty acid derivatives retrieved from *Halomonas* sp. BS4 showed activity against human pathogens, including *S. aureus* (15 mm), *K. pneumoniae* (15 mm), and *S. typhi* (17 mm), and growth inhibition on the fungus *A. niger* .The same team discovered halophilic *Bacilllus* sp. BS3 in Kaniyakumari, India, which produced a lipopeptide biosurfactant comprising compounds such as 13-Docosenamide., (z); Mannosamine,9-; and N,N,N′,N′-Tetramethyl and showed antiviral activity against the White spot syndrome virus (WSSV) by suppressing viral replication at their higher concentrations of 50%, 75% and 100%, respectively. The aforementioned purified biosurfactants were found to have antibacterial activity against *E. coli* and *S. aureus* at 20 µL concentrations, with inhibition zone diameters of 16.0 and 14.06 mm, respectively. Alvionita and Hertadi conducted an intriguing investigation employing *Halomonaselongata* BK-AG18 to bioconvert glycerol into a biosurfactant in a nutritional medium with glycerol as the sole carbon source at an optimal pH 6. The growth inhibition efficacy of a purified biosurfactant was observed against *S. aureus* at 1000 mg/L by reducing its optical density (OD_{600}). The biosurfactants produced from halophilic bacteria, such as *Halomonaselongata*, *Halobacilluskarajiensis*, and *Alkalibacillusalmallahensis*, proved its antimicrobial activity at a 100 µg/mL concentration by reducing the OD value on *S. aureus* (97.75%), *Enterococcus faecalis* (97.6%), and *B. subtilis* (97%) .Antimicrobial glycolipid biosurfactants were recovered from the halophilic bacterium *Buttiauxella* sp., isolated from soils of the Qeshm Island mangrove forest, southern Iran. The antimicrobial activity of the produced biosurfactants was confirmed against the pathogens *B. cereus* (250 µg/mL), *E. coli* (200 µg/mL), *S. enterica* (250 µg/mL), *B. subtilis* (300 µg/mL), *A. niger* (100 µg/mL), and *C. albicans* (150 µg/mL) .*Pseudomonas* sp., isolated from a polluted saltpan, Puthalam district, Kanyakumari, developed biosurfactants with high antibacterial activity to Gram-negative strains *E. coli* (15 mm), *K. pneumoniae* (13 mm), and *V. cholerae* (10 mm) .An interesting report says the anti-biofilm activity of a biosurfactant produced from *Halomonas* sp. isolated from the sediments of the Bay of Bengal showed 99.8% growth inhibition on *S. typhi* and 99.5% on *V. cholerae* at 125 g/mL Con .A new biosurfactant named leu/ile-7 C15 surfactin [M + Na]+ derived from the moderate halophilic bacterium *B. tequilensis* ZSB10 isolated from Crystal salt pond, Las Ventas, showed antifungal action by growth inhibition of *Helminthosporium* sp. at 79.3% and also an IC50 at 1.37 mg/disc .The biosurfactant produced from *Halobacterium salinarum* showed antimicrobial activity against *Bacillus* spp., *Streptococcus* spp., *E. coli*, *Pseudomonas* spp., *S. aureus*, *C. albicans*, and *A. niger* .

C. Exopolysaccharides

Marine bacteria produce exopolysaccharides (EPS) with various sugar and non-sugar compounds such as arabinose, xylose, glucose, acetic acid, and succinic acid from *Bacillus*, *Alteromonas*, *Pseudoalteromonas*, and *Vibrio* species that possess several pharmacological properties, including antimicrobial responses .Several marine bacterial supernatants were shown to exhibit anti-biofilm activity by generating active chemicals ranging from furanones to multifunctional polysaccharides that were shown to be QS (Quorum sensing) inhibitors .The marine *Bacillus altitudinis* MSH2014 isolated from mangrove sediments in Ras Mohamed, Red Sea Coast, Egypt, was able to produce mannuronic acid, glucose, and sulphate-containing heteropolysaccharide that gave an antimicrobial response against *B. subtilis* (17.8 mm), *S. aureus* (18.8 mm), *E. coli* (24.9 mm), *P. aeruginosa* (15.6 mm), and yeast, as well as fungi, including *S. cerevisiae* (17.6 mm), *C. albicans* (17.3 mm), *A. niger* (20 mm), and *F. oxysporum* (10.5 mm) at 200 µg/disc .*Halomonassaccharevitans* AB32 were able to produce EPS at the optimum temperature of 25 °C and pH 9 using lactose and malt extract as their carbon and nitrogen sources with maximum EPS yields at 138 gL^{-1}. The antimicrobial activity of the produced EPS was examined against the pathogenic bacteria *V. fluvialis* and the fungus *A. niger* by growth inhibition at the maximum absolute units of 14.1 and 25.1 .Raffinose carbohydrate was significantly present in the HPLC analysis for the aforementioned EPS with a significant peak at a retention time of 3.910.

Halophilic species such as *Bacillus*, *Halomonas*, *Psychrobacter*, and *Alcaligenes* produced eight EPS compounds with antimicrobial efficacies, and E15 strains were reported to be more active against *B. cereus*, *S. aureus*, *S. saprophyticus*, *Enterobacter cloacae*, *Proteus mirabilis*, MRSA, *Enterococcus faecalis*, *Streptococcus pneumonia*, *Acinetobacter* sp, and *Campylobacter jejuni* with MICs ranging between 250 and 500 µg/mL .E37 also exhibited a wide antimicrobial activity with 250, 62.5, 125, and 500 µg/mL MICs, respectively, against the same pathogens mentioned above. Generally, EPS produced from halophilic isolates displayed more antibacterial action from the genera *Halomonas*, *Chromohalobacter*, *Salinivibrio*, *Nesiotobacter*, *Brevibacterium*, *Virgibacillus*, and *Salinicoccus* against *E. coli, S. pasteuri, B. cereus, P. aeruginosa, M. luteus*, and *S. cerevisiae* .According to the literature, a large number of EPS were produced in saline areas, but only moderate antibacterial activity against microbial pathogens was identified.

XV. Biotechnological Importance/Interest of Haloarchaea and Halophilic Bacteria

The exploitation of extremophiles is having special importance in the development of new molecules with potential applications in biomedicine. Current efforts are focused primarily to cover the urgent health needs, especially those that represent the main global threats, cancer and antibiotic resistance. The great metabolic versatility of halophilic microorganisms, their low nutritional requirements and their genetic machineries of adaptation to harsh conditions, like nutrient starvation, desiccation, high sun radiation, and high ionic strength, make them promising candidates and a hope for drug discovery .Continuous advances in "omics" and bioinformatic tools are revealing uncountable encoding genes for the production of several active compound in response to the extreme conditions .The concomitant application of cutting-edge technologies is helping to deciphering the molecular, physiological, and metabolic mechanisms for the production of new bioactive compounds .Halophilic microorganisms are recognized producers of carotenoid pigments, retinal proteins, hydrolytic enzymes, and compatible solutes as macromolecules stabilizers, biopolymers, and biofertilizers .Halophilic bacteria and extremely halophilic aerobic archaea, also known as haloarchaea, play a significant role in the industry with a large number of applications like fermented food products, cosmetics, preservatives, manufacturing of bioplastics, photoelectric devices, artificial retinas, holograms, biosensors, etc. .In this review, we focus on the biomolecules described as antimicrobial or anticancer compounds produced by halophilic bacteria, archaea, or fungi and discuss current and future perspectives in this field.

Antimicrobial Compounds the current situation of antibiotic resistance propagation poses a global threat to public health. Over the past decades, antibiotics have saved millions of lives, but their misuse has led to the emergence of multi-drug resistant bacteria (MDR), reducing or nullifying their effectiveness. Recently, the continuous increase in antibiotic resistance is reaching critical levels, which implies an increase in morbidity in the healthy population and an imminent risk for hospitalized patients .In fact, the main cause of death of inpatients are attributable to complications due to MDR infections .Preventing the return to the pre-antibiotic era is one of the main challenges for science. The urgent need to introduce new effective antimicrobial therapies is leading to the exploitation of all possible Mar. Drugs 2020, 18, 33 3 of 33 natural and sustainable resources, including extreme environments as a promising resource for new antibiotic discovery. Halocin was the term coined for substances secreted by several members of the genus Halobacterium capable of causing death and lysis of the surrounding microbiota. Halocins are the proteins and antimicrobial peptides (AMPs) produced by haloarchaea .Despite the ecological and environmental role of several halocins, their action against human pathogens has been less studied. In the fight against time, the clinical significance of halophilic microorganisms is minorly reported and the antimicrobial action against the most important risk group of human pathogens ESKAPE: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, still remains as a potential. According to the data inferred, the antagonistic action identified and the production of bioactive compounds by halophilic microorganisms are derived from bacteria, archaea, and fungi. In the chronology of AMPS discovery, several authors have gone beyond the primary screenings deciphering the chemical structure of the molecules in bacteria ,while the vast majority of inhibitory studies are solely limited to the activity .

Bacteria Members of the phylum Actinobacteria are mainly responsible for the inhibitory activity against human pathogens with clinical significance. As in non-extreme environments, in saline and hypersaline environments heterotrophic bacteria are also present in soils, being Actinobacteria frequently isolated from solar salterns, mangroves, and seafloor sediments .The most frequent producers of metabolites reported come from species of the genus Nocardiopsis and Streptomyces, hence constituting the main producers of bioactive compounds. In fact, members of the genus Streptomyces are widely recognized as fruitful producers of natural compounds .The chemical elucidation of molecules known from halophilic members of Nocardiopsis are: (i) pyrrolo (1,2-A (pyrazine-1,4-dione, hexahydro-3-[2-methylpropyl]-) and Actinomycin C2, two compounds produced by the haloalkaliphilic strain Nocardiopsis sp. AJ1, isolated from saline soil of Kovalam solar salterns in India; (ii) Angucyclines and Angucyclinones are produced by Nocardiopsis sp. HR-4, isolated from a salt lake soil in Algerian Sahara, the new natural compound was established as 7-deoxy-8-O-methyltetrangomycin, which is also effective against Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 43300; (iii) Borrelidin C and D are produced by Nocardiopsis sp. HYJ128, isolated from topsoil saltern in Jeungdo, Jeollanamdo, Republic of Korea, exhibited antimicrobial action against Salmonella enterica ATCC 14028; (iv) Quinoline alkaloid (4-oxo-1,4-dihydroquinoline-3-carboxamide) was identified as a new natural product from Nocardiopsis terrae YIM 90022 isolated from saline soils in China. The antibacterial activity of the quinolone was reported in S. aureus, B. subtilis and E. coli; the quinolone has also antifungal activity against the pathogenic fungi, as it was observed against Pyricularia oryzae. Another five known compounds were also produced by N. terrae YIM 90022; (v) new p-terphenyls: p-terphenyl 1 and a novel p-terphenyl derivative bearing a benzothiazole moiety are produced by halophilic actinomycete Nocardiopsis gilva YIM 90087, isolated from a hypersaline soil Xinjiang, China. Furthermore, of the antimicrobial activity against clinical strains, these compounds exhibit antifungal activity against species of Fusarium, Trichophyton, Aspergillus, Candida, and Pyricularia. Known molecules like p-terphenyl 2, novobiocin, cyclodipeptides, and aromatic acids are also produced by N. gilva YIM 90087, which is considered as a new source for novobiocin .Regarding the metabolites produced by members of the genus Streptomyces, only a low number of strains has been isolated from hypersaline environments; however, members of this genus are frequently isolated from marine deep or coastal sediments where the salinity is higher than that of seawater. Among the molecules identified are: (i) 1-hydroxy-1-norresistomycin, this quinone-related antibiotic was extracted from Streptomyces chibaensis AUBN1/7, isolated from marine sediment samples of the Bay of Bengal, India. This compound exhibited antibacterial activities against Gram-positive and Gram-negative bacteria, besides of a potent in vitro cytotoxic activity against cell lines HMO2 (gastric Mar. Drugs 2020, 18, 33 4 of 33 adenocarcinoma) and HePG2 (hepatic carcinoma); (ii) Himalomycin A and Himalomycin B, two new anthracycline antibiotics produced by Streptomyces sp. strain B692, isolated from sandy sediment of a coastal site of Mauritius (Indian Ocean). In addition, known metabolites like rabelomycin, fridamycin D, N benzylacetamide, and N-(20 -phenylethyl) acetamide were also produced by Streptomyces sp. strain B692; (iii) 7-demethoxy rapamycin was produced by a moderately halophilic strain Streptomyces hygroscopicus BDUS 49, isolated from seashore of Bigeum Island, South West coast of South Korea; the molecule displayed a broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria. Antifungal and cytotoxic action was also identified on this strain; (iv) Streptomonomicin (STM) is an antibiotic lasso peptide from Streptomonospora alba YIM 90003, isolated from a soil sample in Xinjiang province, China. STM is active against several Gram-positive bacteria, in particular species of Bacillus, Listeria, Enterococcus, Mycobacterium and Staphylococcus. Despite that STM has an inhibitory action against a wide panel of Gram-positive pathogens, the activity against fungi and Gram-negative bacteria was not evidenced. In addition to the mentioned genera of Actinobacteria (Nocardiopsis and Streptomyces), recognized as the more prolific producers of natural substances, other halophilic species belonging to different genera have also been described as producers of molecules like: (i) cyclic antimicrobial lipopeptides: Gramicidin S and four cyclic dipeptides (CDPs), named cyclo(l-4-OH-Pro-l-Leu), cyclo(l-Tyr-l-Pro), cyclo(l-Phe-l-Pro), and cyclo(l-Leu-l-Pro), were extracted from Paludifilum halophilum strain SMBg3, which constitute a new genus of the family Thermoactinomycetaceae, isolated from superficial sediment collected from Sfax marine solar saltern in Tunisia. These CDPs possess an inhibitory effect against the plant pathogen Agrobacterium tumefaciens and the human pathogens Staphylococcus aureus, Salmonella enterica, Escherichia coli, and Pseudomonas aeruginosa; (ii) A semi synthetic derivative N-(4-aminocyclooctyl)-3,5-dinitrobenzamide, obtained from the precursor of the novel natural product cyclooctane-1,4-diamine and a known compound 3-([1H-indol-6-yl] methyl) hexahydropyrrolo [1,2-a] pyrazine-1,4-dione were obtained from Pseudonocardia endophytica VUK-10, isolated from sediment of Nizampatnam mangrove ecosystem in Bay of Bengal, India. The new compound, semi synthetic derivative N-(4-aminocyclooctyl)-3,5-dinitrobenzamide showed a strong antimicrobial and antifungal activity against Streptococcus mutans, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger. Significant anticancer activities at nanomolar concentrations were also observed in carcinoma cell lines MDA-MB-231 (breast), HeLa (cervical), OAW-42 (ovarian), and MCF-7 (breast) reported as resistant to cancer drugs .In minor grade, other halophilic bacteria not belonging to the phylum Actinobacteria produce antimicrobial compounds, as for example halophilic strains of the genus Vibrio, like Vibrio sp. A1SM3-36-8, isolated from Colombian solar salterns, which produces 13-cis-docosenamide with special antimicrobial action against Methicillin-resistant Staphylococcus aureus (MRSA) and cytotoxic activity against cervical adenocarcinoma (SiHa) and lung carcinoma (A-549) .Within this genus, Vibrio parahaemolyticus strain B2 is recognized by producing Vibrindole A, and was also effective against Staphylococcus aureus .Finally, Bacillus sp. BS3 and Halomonas salifodinae MPM-TC showed antimicrobial action against Pseudomonas aeruginosa. Both strains were isolated from solar salterns in Thamaraikulam, Tamil Nadu, India. In the case of Halomonas salifodinae MPM-TC, besides of the inhibition of bacterial growth also exhibits an antiviral action against the White Spot Syndrome Virus (WSSV) in the white shrimp Fenneropenaeus indicus. The effect suppressor of the virus and the boosting of immune system of the shrimps make of the extracted compound a feasible alternative to commercially banned antibiotics and excellent candidate to develop new antiviral drugs against shrimp viruses such as WSSV. A genome-mining study conducted on 2699 genomes across the three domains of life demonstrated the widespread distribution of non-ribosomal peptide synthetase (NRPSs) and modular polyketide synthase (PKSs) biosynthetic pathways. Among 31 phyla of bacteria inferred, Actinobacteria is the most representative exhibiting the presence of 1225 gene clusters between NRPS, PKS and hybrids from a total of the 271 genomes studied. It was observed that Salinispora arenicola CNS-205 and Salinispora tropica CNB-440 harbor PKS and NRPS gene clusters, respectively.

several properties that make them unique and preferable for industrial biotechnology. With the advantage of these unique properties, halophiles become a useful tool for competitive and low-cost production that industrial biotechnology cannot overlook .Many halophiles are alkaliphilic and can grow and survive in hostile environments, especially high salt salinities. As a typical cell will undergo plasmolysis upon exposure to high salt concentration, halophiles to their advantage, require the high salt concentration for growth and survival. They are capable of employing adaptive mechanisms to survive in hostile environments and to overcome salt and water stress. The ability of halophiles, especially extreme halophiles to flourish in brines, provide ideal conditions for carrying out many biotechnological transformations, due to their great abundance and exclusion of nonhalophilic contaminants. Halophiles also can use a variety of energy sources. They can use less expensive and sustainable substrates such as agricultural waste and kitchen waste instead of expensive raw materials as substrates. Their halophilic nature (salt-loving) also enables them to use seawater, which can be recycled during bioprocessing, thereby reducing the high consumption of freshwater. Due to high salt concentration medium during fermentation, equipment made of low-cost materials such as ceramics, plastics, or carbon steels instead of the expensive stainless steel can be used to reduce the high cost of maintenance of equipment (stainless steel) .Halophiles can also undergo unsterile and continuous bioprocessing, which saves energy for intensive sterilization and increases process efficiency. Halophilic production contributes to less environmental pollution as production is bio-based, and bio-based products are biodegradable .

XVI. Genetic manipulation of halophiles

Genetic manipulation is crucial to improving the performance of halophiles for industrial applications. Researchers have made efforts in modifying halophiles to express foreign genes by developing various genetic tools for them.However, further development of these available genetic tools is needed as they suffer some disadvantages. Many researchers have used synthetic biology and genetic modification technologies including recombineering, gene knockout, gene overexpression, etc., to improve accelerated cell growth, high cell density, simplification of downstream separation, enlarged space for more inclusion body accumulation (increased cell size for PHA granules), and to improve production yield and reduce the cost of bioproduction. Fu et al. (2014) developed a genetic manipulation method for Halomonas TD01 based on efficient markerless gene knockout procedure. They improved PHB and PHBV production via deleting prpC gene encoding 2-methylcitrate synthase and PHA depolymerase gene phaZ and also overexpressing udhA gene encoding soluble pyridine nucleotide transhydrogenase. The deletion of prpC gene in Halomonas TD01 significantly increased the conversion efficiency of propionic acid to 3- hydroxyvalerate (3HV) monomer fraction in random PHBV copolymers of 3-hydroxybutyrate (3HB) and 3HV from 10% to almost 100%, and this resulted in the growth of cells to accumulate 70% PHBV in dry weight (CDW) consisting of 12 mol% 3HV from 0.5 g/L propionic acid in glucose mineral medium. Also, it is perceptible that the manipulation of PHA granule-associated proteins leads to an increase in PHA granule size, allowing for more natural separation .Yue et al. (2014) also improved the PHA synthesis ability of Halomonas campaniensis LS21, constructing an overexpression plasmid (pBBR1MCS1-oriC-Pporin-phaCABLS) of PHA synthesis genes containing genes of PHA synthase phaC, β-ketothiolase phaA, NADPH-dependent acetoacetyl-CoA reductase phaB, consisting of a native porin promoter and a native OriC from the host chromosome. Recently production of free fatty acids in Escherichia coli and cyanobacteria has increased following overexpression of recombinant, leaderless thioesterase I (TesA) from E. coli. A homolog of TesA from the moderately halophilic bacterium Chromohalobacter salexigens was identified, cloned, and recombinantly expressed in E. coli strain BL21 and M15 for biofuel production.Also, the alsS gene from Bacillus subtilis together with ilvC and ilvD genes from Corynebacterium glutamicum were overexpressed to generate KIV (2- Ketoisovalerate) for isobutanol production.The enhanced ectoine production from glucose by constructing a lysC-overexpressing Halomonas elongata transformatant (H. elongata/pHS15N-lysC) using a strain carrying an empty vector (H. elongata/pHS15N) as a control. After 4 h of cultivation, H. elongata/pHS15N-lysC produced 207 mmol/kg FW of ectoine and 0.39 mol of ectoine/mol of glucose whereas H. elongata/pHS15N produced 174 mmol/kg FW of ectoine and 0.31 mol of ectoine/mol of glucose. Production of ectoine by H. elongata/pHS15N-lysC was 1.2-fold higher than that of the control, and the yield was slightly increased, indicating that overexpression of lysC enhances ectoine production from glucose.

XVII. Applications and Future Perspectives of Halophiles as Pharmaceuticals

Halophilic microbial products are predicted to have significant uses in the pharmaceutical sector and healthcare .Proteolytic enzymes are used to produce pharmaceutical products .According to bioactive compounds, diverse halophilic bacteria are employed to produce bioactive compounds, which are significant and understudied sources of bioactivities, such as antiviral, antibacterial, and anti-tumour agents.Marine cyanobacteria have gained a lot of attention as a powerful group in the creation of pharmaceuticals such as cryptophycin and curacin, which are currently in clinical trials .Peptide molecules from marine diatoms also have been explored with respect to their antioxidant and anticancer properties .Biosurfactants from halophiles are receiving more attention for antioxidants, antiviral antibacterial, antifungal, anticancer, antiviral, anti-adhesive, immunomodulator, stimulating dermal fibroblasts, gene therapy, and vaccines .Halophilic bacteria must reach a tipping point in the future by manufacturing various novel drugs, antioxidants, sunscreens, compatible solutes, and hydrolytic enzymes from unexplored regions. Recent advances in the incorporation of halogenated compounds into peptoids (oligomers of N-substituted glycines) improve antimicrobial efficacy against multi-drug-resistant pathogens, with brominated analogues showing 32-fold increased activity against MRSA and 16-64-fold increased activity against *P. aeruginosa* and *E. coli* .In the future, halogenated drugs may have increased action against drug-resistant bacteria .OMIC technologies present new potential for the discovery of exclusive properties and/or novel biomolecules derived from halophiles in the future as a result of recent findings of halophilic bacteria, even from terrestrial environments .

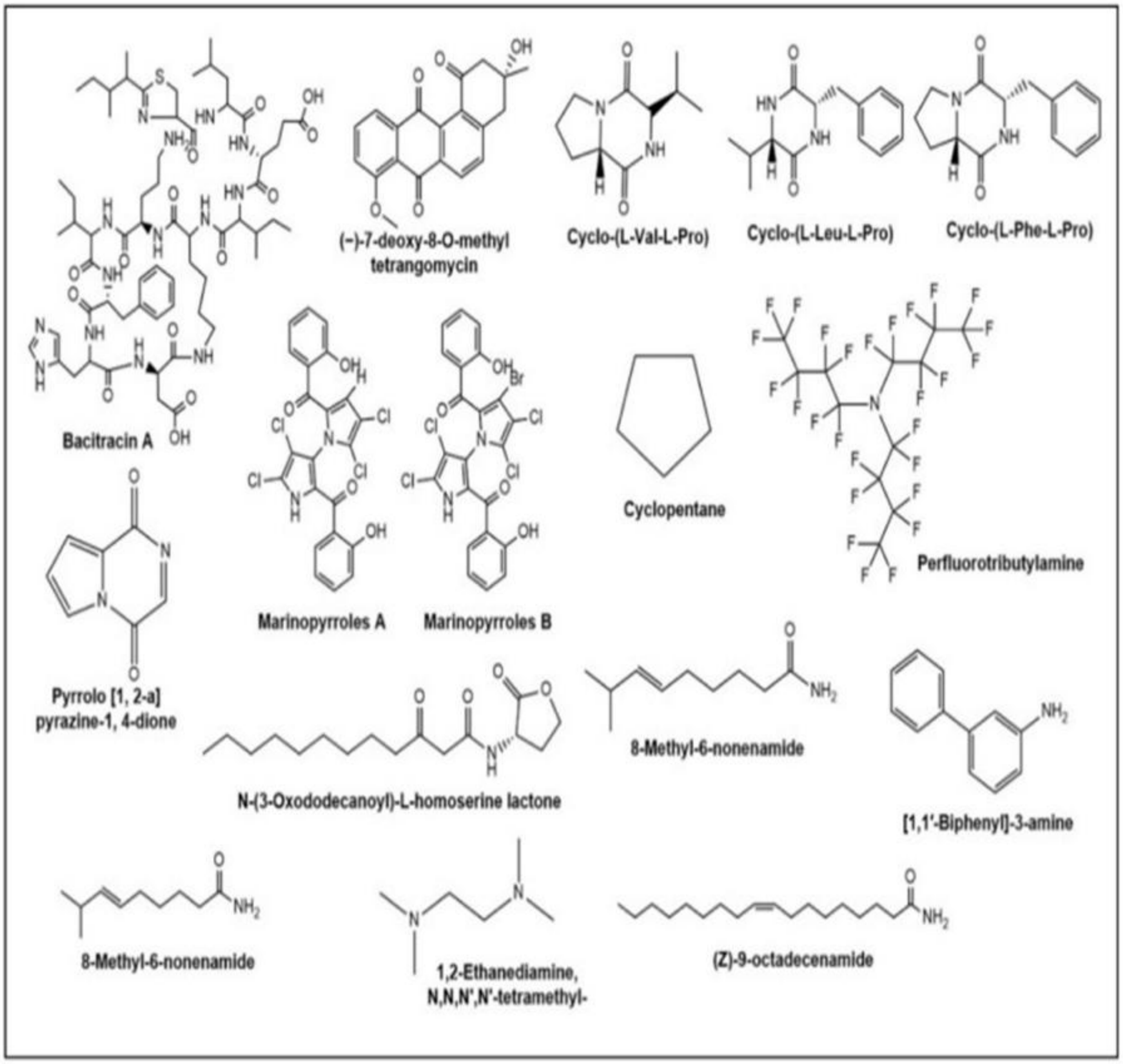

Figure 8: The structure of different antimicrobial compounds produced by halophilic microbes.

Further research is needed to report on how halophilic microorganisms evolved during the early phases of evolution of life on earth, as well as how they diversified and spread around the world. Their biotechnological potency for generating compatible solutes, biopolymers, and other molecules is of industrial interest. To fully realise their clinical potential, additional research must focus on their physical organisation and modes of action, allowing physicians to forecast which molecule could produce the desired medicinal effect.

CONCLUSION

Comparative studies of micro flora in the extreme environments results in better understanding of the ecosystem and can benefit in designing the applications. Hyper saline environments represent a valuable source of different bioactive compounds with great economical potential in industrial, agricultural,chemical, pharmaceuticals and biotechnological applications. The microbial diversity can prove to be a valuable future resource in various industrial and biotechnological process. Researchers focusing on halophilic ecosystems in their search for novel biomoleculesvare mostly motivated by the threat of drug-resistant human pathogens.No Haloarchaeon has been found to show antibacterial action. More new compound extraction from more halophilic microbial genera is needed to combat human pathogenic drug-resistant microbes. Halophilic representatives of Bacillus and the dominating actinomycete biomolecules have already been demonstrated to be effective against human drug-resistant infections. There is no benign report yet for the enzymes from halophilic microbes against human pathogens. However, clinical trials should focus more on antimicrobials produced from halophiles because knowledge on the mode of action of halo-antimicrobials against drug-resistant organisms is lacking.It is thus possible that in the future the biotechnological application of halophiles, or of genes derived from them, will extend to many more members of this extremely diverse group of microbes.

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