Application of Halophilic Bacteria in Medicine

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ABSTRACT

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

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

I. INTRODUCTION

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

Extremophiles are organisms that can only thrive in an environment that would kill most of many other species. These extremophiles divided into five groups depending on where they live: acidophiles, alkalophiles, halophiles, thermophiles, and psychrophiles. Halophiles are extremophiles that can endure conditions with high salt concentrations. These microorganisms live in the saltiest places on earth, like flats, evaporation ponds, hypersaline lakes, and tidal marine settings. Halophilic bacteria can counteract the osmotic pressure of their surroundings and withstand salts' denaturing effects. These organisms are classified as mild (2–5 percent NaCl), moderate (5–15 percent NaCl), or extreme halophiles depending on their halotolerance or even the salt concentration at which they survive (15-30 percent NaCl). Halophilic microorganisms employ a variety of methods to survive in high-salinity- surroundings. For example, they produce suitable solutes in their cells or have transporters that enable them to endure such extreme conditions. These bacteria were found to include osmoregulatory solutes like proline, potassium ion, ectoine, betaine and glutamate.Some halophiles manufacture acidic proteins that can work in environments with a lot of salt. They have been isolated from various salinity settings, ranging from saturating salinities to natural brines and hypersaline lakes. Eukarya, Archaea, & bacteria are included in the diverse halophilic community. The Bacteria and Archaea, including Salinibacter, *Halobacterium Halomonas,* are the most frequently seen halophiles. One of the recognized excellent source of carotenoids in nature is the halophilic Eukarya, like Dunaliella salina. Halophiles may survive in conditions that range in salt concentration from hypersaline (0.6 M) to saturated (> 5 M NaCl). To react to a salt-stress condition, they have evolved a variety of molecular and cellular mechanisms. A common'salt-in strategy' halophilic Archaea and Bacteria. The most well-known mechanism is the so-called referred to as the'salt-out strategy' or 'organic-osmolyte mechanism,' this mechanism allows for osmotic adaptation by excluding salts and/or synthesising de novo compatible solutes.. Archaea and Bacteria both use this strategy. Furthermore, the molecular basis of protein halotolerance and adaptation of halophilic enzymes to high salinity is increased protein charges and hydrophobicity. Halophiles have been regarded for biotechnological applications over the last few decades. Diverse halophile response mechanisms under high-salinity conditions result in the production of a variety of valuable biomolecules. Halophiles are also known to be major sources of stable enzymes that function in extremely high salinity, a condition that causes denaturation and aggregation in most conventional proteins. For example, the -amylase isolated from Haloarcula sp. works best at 4.3 M salt at 50°C and is stable in benzene, toluene, and chloroform. Furthermore, halophiles are thought to be potential sources for the discovery of bioactive compounds, compatible solutes, novel enzymes, and other potential biotechnological applications. At the moment, only two industrial processes that use halophilic microorganisms are in operation: carotenoid production by the halophilic alga Dunaliella and ectoine production by the halophilic bacterium Halomonas elongate. There are numerous bioactive compounds derived from halophiles that exhibit various biological activities ranging from antioxidant, sunscreen, and antibiotic actions.. Several studies show that some halophiles can synthesise large amounts of compatible solutes like glycine betaine and ectoine. These small organic molecules can act as biomolecule stabilisers or stress protectants. A wide range of microorganisms live in hypersaline environments, and they are often found in extremely high community densities. The most common are coloured by the pigment carotenoid and others; halophilic microorganisms can be found without the use of a microscope in saltern crystallizer brines and other salt lakes with saturating or near-saturating salt concentrations. Halophilic microorganisms live in a diverse environment. Archaea, Bacteria, and Eucarya have all been found to be salt tolerant up to saturation. Aerobic and anaerobic halophiles, as well as heterotrophic, phototrophic, and chemoautotrophic types, are capable of converting a wide variety of substrates. Some anaerobic halophilic thermoalkaliphilic bacteria are "polyextremophilic" halophiles, adapted to grow in a combination of extreme measures such like alkaline and salt conditions (a popular combination),low pH and salt (relatively uncommon), or high temperatures and salt.

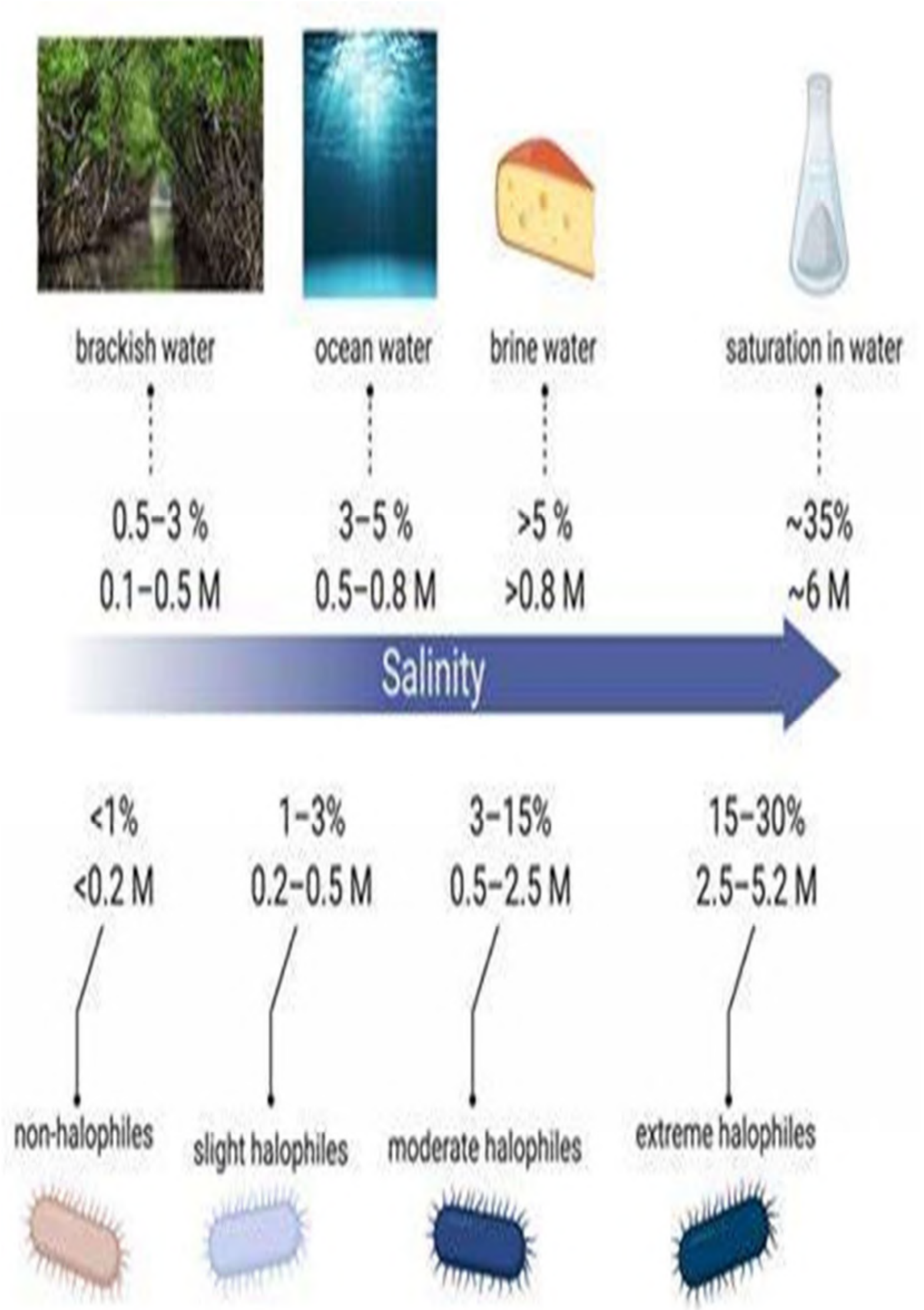

Figure 1: Halophiles classification based on salt concentration

II. MECHANISMS FOR SALINE TOLERANCE

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

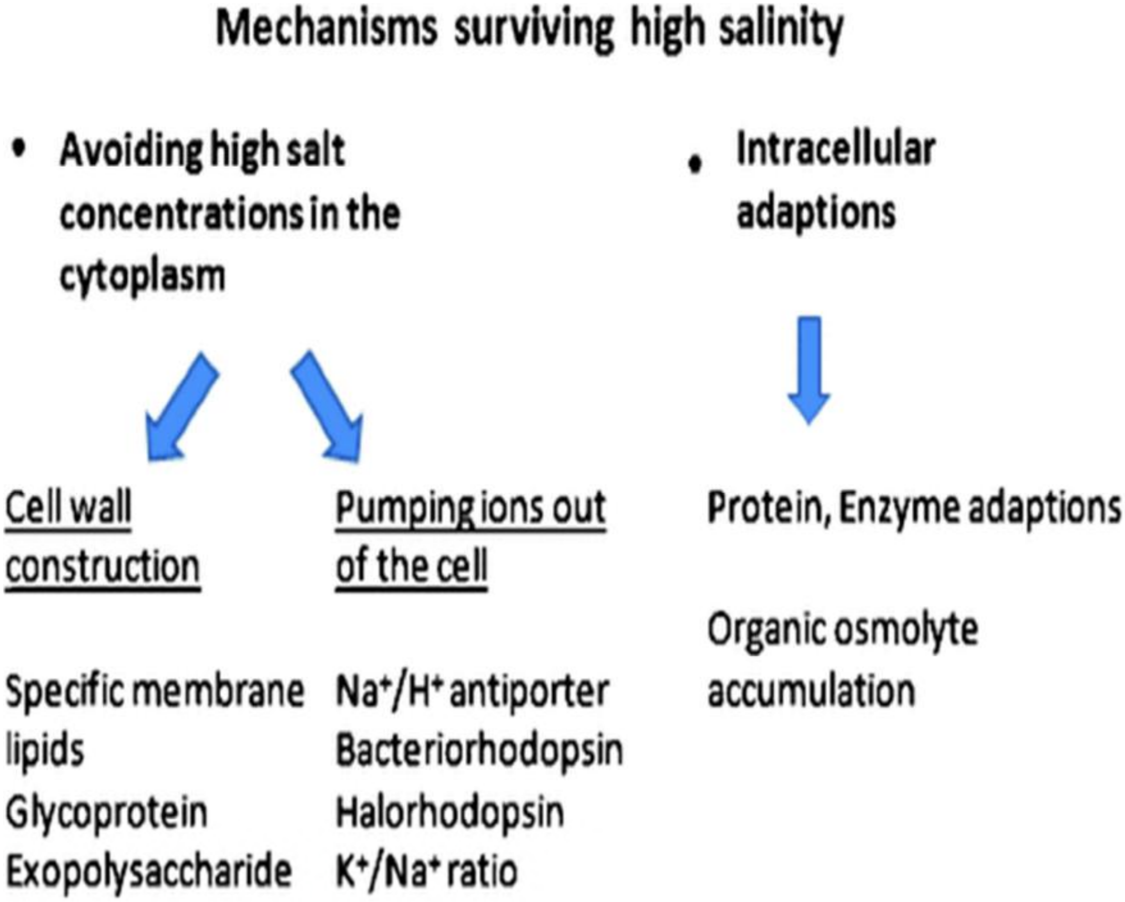

Figure 2: High salinity survival mechanism

Halophiles are organisms that include archaea, bacteria, and eukarya and are defined by their salinity demand, halophilic "loving salt." Natural microbial communities of hypersaline ecosystems all over the world are made up of halophilic microorganisms. For growth and metabolism, they need sodium ions. Temperature, pH, and growth medium all influence tolerance parameters and salt requirements. Halophiles adapt to and are limited by specific environmental factors in this way. Polyextremophiles are microorganisms that can thrive and survive in a variety of environments range of extreme environmental conditions. The halophilic microorganism can also be an alkaliphile, designated as a haloalkaliphile, and can grow optimally or very well at pH values above 9.0, but not at the near neutral pH value of 6.5. The general characteristics of halophilic microorganisms include low nutritional requirements, resistance to high salt concentrations, and the ability to balance the osmotic pressure of the environment. They have a wide range of physiological characteristics, including aerobic, anaerobic, phototrophic, heterotrophic and chemoautotrophic organisms. Halophilic microorganisms live in a variety of ecosystems with salinities higher than seawater, i.e., 3.5 percent sodium chloride, ranging from salt lakes, hypersaline soils to springs and other naturally occurring coastal saline habitats. Human intervention has resulted in other known habitats such as oil fields, brines, salted foods, saltern ponds and tanneries.

HALOPHILIC ARCHAEA AND BACTERIA: EVOLUTION,GENOMICS AND TAXONOMY

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

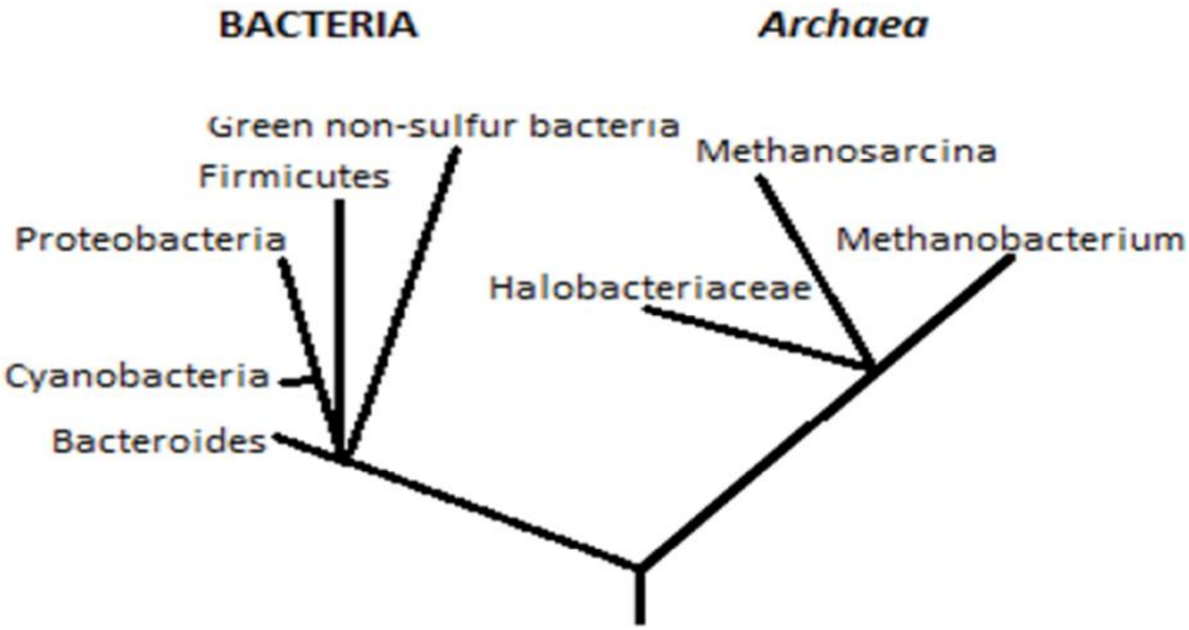

Figure 3: The distribution of halophilic Bacteria and Archaea on focus on small subunit rRNA gene sequences, the universal phylogenetic tree of life

Haloarcula marismortui, Natronomonas pharaonis,aloquadratum,walsbyi, Halorubrum,lacusprofundi, Halomicrobium,mukohataei,Halorhabdus, utahensis,Halogeometricu m,borinquense,Haloterrigena,turkmenica, and Haloferax,volcanii were included among the recently completed genomes. Among those on the list are a haloalkaliphilic species, a cold-adapted species, a species adapted to life in low-Na+-high-Mg2+ environments, and isolates with exciting cell morphologies. These genomes range in size from 2.6 to 5.4 Mb. The presence of large megaplasmids and minichromosomes, as well as core acidic proteomes, are among the conserved properties of haloarchaeal genomes. The genome diversity of Haloquadratum populations in a single saltern crystallizer pond has been compared, as have comparisons between populations in similar environments in different geographic locations. Haloquadratum walsbyi's pan-genome is at least 40 times larger than the genome of the type strain, and genomic microdiversity is very high in an extremely simple and relatively constant environment. Sequencing of new isolates' genomes are becoming easier & less expensive, and it will most likely become routine in the near future. This discovery will undoubtedly have far-reaching implications for halophile taxonomy until comparative taxonomy can be used.

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

III. VIRUSES: HALOPHILIC

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

Predators and protozoa are absent in saltern crystallizer ponds so there are high numbers of prokaryotes and virus-like particles are present. The salterns have stable & predictable species of Archaea and viruses, which are distinct from the "Kill-the-winner" behaviour, as expected in such an environment, with rapid cycling of viral predators and microbial taxa. The distribution of microbial taxa and viral taxa in the salterns near San Diego, according to a metagenomic analysis of the viruses, the occurrence of viral genotypes and microbial strains has remained stable over time, but with strong dynamic fluctuations. At the fine level, populations of both individual strains and viral genotypes fluctuate in a" kill- the-winner" manner. Virus action may also have an impact on the spread of highly halophilic bacteria like Salinibacter (Bacteroidetes). A viral metagenome from a saltern pond, where Salinibacter represents about 15% of the prokaryotic group. Bioinformatic analysis (G+C content and dinucleotide frequency analysis) suggests that approximately 24% of the recovered viral sequences are Salinibacter phages. Phages that infect Salinibacter seem to to be more active in the habitat than phages that infect Haloquadratum, which may explain why Haloquadratum outnumbers Salinibacter in every environment to support their development.

IV FUNGI: HALOPHILIC

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

V DIVERSITY

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

In salt saturated ponds around the world, the unicellular alga Dunaliella salina (Chlorophyceae, Eucarya), the square archaeon Haloquadratum walsbyi (Halobacteriales, Euryarchaeota), and Salinibacter ruber can be found (Bacteroidetes, Bacteria). Halophiles are found in a variety of phyla within the domain Bacteria, most notably the Cyanobacteria, Gammaproteobacteria and Bacteroides. Large number of different types of cyanobacteria that grow at high salt concentrations but not at the highest levels found in saturated brines. The Halomonadaceae family is probably the best known group of halophilic or highly halotolerant Bacteria (Gammaproteobacteria). Halophilic organisms are frequently related to organisms in the aerobic Firmicutes and a number of other phyla that have little salt tolerance and requirement. Halanaerobiaceae and Halobacteriodaceae included, order Halanaerobiales , they are anaerobic halophilic, & fermentative bacteria. The red aerobic S. ruber (Bacteroidetes) is a new addition to the group of real halophiles and is now acknowledged as a significant contributor to the Archaea of the Halobacteriaceae-dominated biota of salt lakes and salterns close to salt saturation. This organism is phylogenetically related to the Firmicutes and the Mollicutes. It lives by fermentation or denitrification. It is the sole recognised member of the family Haloplasmataceae and order

Haloplasmatales. As evidenced by the brine shrimp Artemia, which has been discovered at salt concentrations as high as 150–200 g/l and occasionally greater, eukarotic life is also feasible at high salt concentrations.

The green algae in the genus Dunaliella, some of which appear orange rather than green due to significant b-carotene buildup, are without a doubt the most well-studied halophilic eukaryotes. It is vital to the life of heterotrophic communities in salt lakes because it is the only or principal producer when the salt content is high. The existence and importance of heterotrophic halophilic eukaryotic microorganisms have only lately come to light. In the salt lake,various types of ciliate protozoa and flagellate are present up to sodium chloride saturation, and culture-independent studies show that many more are still unknown. Over 40 phylotypes of protists were discovered using 18S rRNA studies. Fungi, in hypersaline ecosystems all over the world, including salterns and salt lakes like the Dead Sea, black yeasts and other melanized kinds such as Hortaea, Trimmatostroma, Candida, Pichia, Yarrowia, and others play a crucial role. It is unknown how common this type is or whether it is part of a larger group of organisms that have yet to be identified.

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

A. Halophiles Metabiolc Diversity

Given the significant evolutionary diversity of creatures found at high salt concentrations, the halophile world is as diverse as one could anticipate in terms of the techniques employed to extract energy and carbon for growth. However, certain processes that have been studied in low-salt environments seem to break down at salt concentrations greater than 100–150 g/l. Aerobic methane oxidation, autotrophic nitrification, methanogenesis from hydrogen and carbon dioxide or acetate, are some examples

B. Oxygenic and Anoxygenic Phototrophs

The diversity of phototrophs thriving at high salt concentrations is somewhat limited, yet oxygenic photosynthesis continues until salt saturation. The sea water phytoplankton cant live in concentrated brines. The only two major families of phototrophs that function at the highest salt concentrations are cyanobacteria and green unicellular algae of the genus Dunaliella, despite the fact that some species of diatoms can be found at salt concentrations of up to 100–150 g/l. Plankton and shallow benthic biofilm sheets are both popular places to find cyanobacteria in salt lakes and saltern evaporation ponds. These include filamentous and unicellular organisms (Aphanothece halophytica and kindred forms) (e.g., Microcoleus chthonoplastes, Halospirulina tapeticola, Phormidium spp., and others). Nitrogen-fixing microbes were never found in salt concentrations greater than 70 g/l. At high salt concentration that is above 200-250 g/l nitrogen fixing cyanobacteria are never found and only oxygenic phototrophs are found. By using sulphide as an electron donor at high salt concentration the process of anoxygenic photosynthesis is taking place. Purple layers of anoxygenic phototrophs are common in hypersaline microbial mats dominated by cyanobacteria in the upper aerobic layers. Phototrophic sulphur microbes are those that have intracellular sulphur as elemental form as well as those that excrete sulphur . Some species can tolerate high pH values and high salinity. Some photoheterotrophic Halophiles and purple bacteria like Rhodovibrio salinarum, R. sodomense, and Rhodothalassium salexigens, can withstand a salt concentration of 200-240 g/l. A entirely new sort of anoxygenic phototrophic life that thrives at the highest salinities uses the light energy absorbed by retinal-based pigments like bacteriorhodopsin and related pigments like xanthorhodopsin. The main retinal pigment of Halobacterium and certain related halophilic Archaea, bacteriorhodopsin, can promote anaerobic growth in the light in Halobacterium salinarum. Photoautotrophy does not occur in this group because organic substrates are used as carbon sources. Light can be used as an additional energy source in the phylogenetically unrelated but physiologically very similar Salinibacter. Its proton pumping retinal protein, xanthorhodopsin, harvests light using the membrane carotenoid salinixanthin.

C. Aerobic Heterotrophs

Aerobic heterotrophs are prevalent in all three domains of life, and they make up the majority of the halophilic and halotolerant microorganisms known. In environments with salt concentrations of up to 200–250 g/l, bacteria are primarily responsible for heterotrophic activity, and some species are particularly adaptable in terms of the range of salt concentrations tolerated and the variety of resources used for growing. The list of organic substrates that support growth at high salt concentrations is shorter than that known for freshwater environments, but it includes several hydrocarbons and aromatic compounds. At high salinities, groups such as the Halomonadaceae cease to exist, and aerobic degradation of organic compounds is assumed by two groups of prokaryotes: the Halobacteriaceae (Archaea) and Salinibacter (Bacteria). The halophilic Archaea can grow to saturating salt concentrations, by oxidizing simple sugars and amino acids as limited substrates. It is ineffective to employ hydrocarbons and aromatic chemicals. We previously believed that the only aerobic heterotrophs capable of functioning at 200–250 g/l of salt were members of the Halobacteriaceae; however, Salinibacter has now been found to be a significant part of the biota in saltern crystallizer ponds. Salinibacter is not very versatile in terms of growth substrates.

D. Anaerobic Respiration

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

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

E. Methanogens and Homoacetogens

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

F. Fermentation

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

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

G. Methanotrophs and Chemoautotrophs

Although some aerobic chemoautotrophic systems seem to be restricted to low salinities, others seem to be able to function at extremely high salt concentrations. At high salt concentrations, there is no information on the autotrophic oxidation of nitrite to nitrate. Reduced sulphur compounds undergo chemoautotrophic oxidation at even higher salt concentrations. Thiosulfate, elemental sulphur, and tetrathionate can all be oxidised by Halothiobacillus halophilus at concentrations up to 240 g/l salt, with 50 to 60 g/l being ideal. The highly halophilic Thiohalorhabdus denitrificans develops between 11 and 290 g/l NaCl, with an optimum concentration of 175 g/l. Using nitrate as an electron acceptor, it can also grow anaerobically as a chemoautotroph. The organisms Thiohalospira halophila, T. alkaliphila, and Thioalkalivibrio halophilus may thrive at pH values of 8 to 9 and above as well as in solutions containing up to 5 M of $Na+$. Arsenic is biogeochemically cycled in the alkaline hypersaline habitat by chemoautotrophic oxidation of As(III) to As(V) and anaerobic reduction of As(V) (arsenate) to As(III) (V). Gammaproteobacterium Alkalilimnicola ehrlichii can grow heterotrophically or as a chemoautotroph on arsenite with oxygen or nitrate as the electron acceptor at salt concentrations up to 190 g/l (optimum 30 g/l). Anaerobic sediments can create methane up to very high salt concentrations. Methane-oxidizing bacteria have been identified from salt lakes, including "Methylomicrobium modestohalophilum," however their ideal salt concentration for growth is only approximately 20 g/l.

VII. HABITAT

Hypersaline environments favour microbial species with a diverse order and environment. The fraction of found microbes is small, even with recent advances in molecular science techniques, and further studies are required. Halophiles are found in a variety of habitats, including the world's most toxic environments. These extremeophiles have acquired unique abilities for surviving in the natural world thanks to this deviation from the usual environmental circumstances. The diversity of halophiles is largely a result of their capacity to survive in all hypersaline biotopes. Due to both manmade and natural global change, these hypersaline settings are becoming more prevalent. Halophiles can be found in a variety of habitats, including soda lakes, thalassohaline and athalassohaline ecosystems, the Dead Sea, carbonate springs, salt lakes, alkaline soils, and many others. These environments vary in terms of their nature and makeup. Thalassohaline habitats are created when seawater evaporates, increasing the amount of sodium and chloride ions and their pH level to a slightly alkaline state. The ionic composition of the athalassohaline environment differs from that of sea water because it contains salts in non-marine proportions. As a result of NaCl precipitation, high concentrations of K+ and Mg+ salts are left in sea water. The most productive habitats are soda lakes, where pH levels close to 11 encourage the growth of salt-loving bacteria. Due to their difficulty in exploration, soda lakes have only recently drawn the attention of microbiologists. The concentration of monovalent cations in the Dead Sea is lower than that of divalent cations. It supports dense microbial blooms because it is the largest hypersaline environment

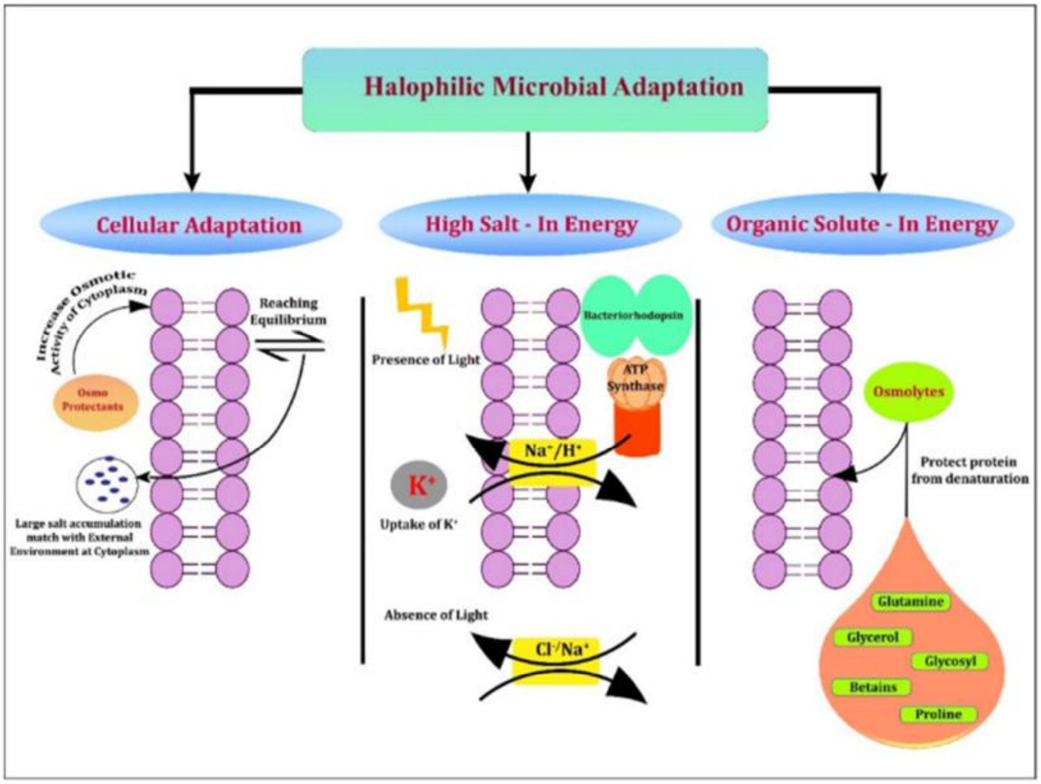

Figure: 4 Common halophilic microbial adaptation strategies include cellular adaptation, high salt-in energy, and organic solute-in energy.

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

Osmotically comparable cytoplasmic contents to the surrounding media are essential. A cell will rapidly lose water via its water-permeable cell membrane if its internal osmotic pressure is lower than that of the salt solution around it. The osmotic pressure inside the cell must be greater than that of its medium in order to sustain turgor (in all cells with the possible exception of the very halophilic Archaea of the family Halobacteriaceae). Numerous strategies for osmotic stabilisation of halophilic bacteria have been developed by nature. One thing that all halophiles have in common is that sodium is constantly kept as far away from the cytoplasm as possible by strong, energy-intensive sodium pumps (in prokaryotes, these pumps are typically based on Na+ /H+ antiporter systems). However, depending on the type of microbe, different substances can be used to maintain osmotic balance in place of sodium chloride. One method is to build up potassium and chloride ions to levels that are similar to the ionic concentrations of the medium. To stop water loss from the cell by osmosis, the second and much more typical technique is to greatly restrict ions and collect tiny electrically neutral organic solutes (also known as "compatible solutes"). Due to the requirement that all cytoplasmic enzymes and processes operate in the presence of molar amounts of KCl, the first technique (salt-in to balance "salt-out") calls for considerable modifications to the intracellular apparatus. High salt levels also decrease the solubility of proteins and enzymes. As a result, the proteome as a whole has to be altered. A distinctive characteristic of halophilic proteins is their extremely acidic nature, with a preponderance of acidic amino acids (glutamate, aspartate) over basic amino acids. Halophilic proteins are produced by microorganisms that collect KCl for osmotic equilibrium (lysine, arginine). These proteins are very negatively charged compared to their non-halophilic counterparts. Additionally, the proportion of hydrophobic amino acids in halophilic proteins is minimal. These protein modifications necessitate the presence of molar quantities of salts at all times in order to maintain the proteins in their native and active states; dilution of the salt results in unfolding and denaturation. Because of this, "salt-in" strategy-using microorganisms are typically obligate halophiles with limited flexibility in the range of salt concentrations that allow growth, and they are thus suited to living at as steady and extremely high salinity as possible. The "salt-in" approach to osmotic adaptation has the advantage of requiring less energy than manufacture of molar quantities of organic osmotic solutes, even though the latter method also expels sodium ions.

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

The genome of the similarly fermentative anaerobic thermophile Halothermothrix orenii was not discovered to have an acidic proteome. The process of osmotic adaptation of this fascinating polyextremophilic bacterium is yet unknown because no genes for any of the known organic osmotic solutes have been found. In the microbial world, the buildup of organic osmotic solutes is a considerably more prevalent osmotic adaptation method. The strategy's drawback is the high energy cost of making the solutes, but its advantage is that it is highly flexible and can be adjusted to a wide range of salt concentrations. In response to ambient salinity, the cell can simply alter the intracellular solute concentration because solutes typically have little impact on protein structure and enzyme activity. The quantity of energy required for de novo biosynthesis can be significantly reduced when suitable solutes are found in the medium and can frequently be taken up from the outside. Examples include polyols like glycerol and others, simple sugars like sucrose and trehalose, a number of amino acid derivatives such glycine betaine and ectoine, among others. The bulk are uncharged or zwitterionic at physiological pH. Glycerol is utilised by various yeasts and fungi as well as the unicellular algae Dunaliella. The chemical must have a very low cytoplasmic membrane permeability in order to function as an osmotic solute. Since glycerol is very permeable to most membranes, it has never been discovered in prokaryotes as an osmotic solution. As prokaryotic oxygenic phototrophs, cyanobacteria utilise glucosylglycerol, glycine betaine, or sugars (trehalose, sucrose), with the latter substance being favoured by the most halotolerant species. Numerous bacteria can accumulate glycine betaine from the medium and use it to stabilise the osmotic pressure, but only a few prokaryotes can synthesise the substance. The amino acid metabolic intermediary ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) and its hydroxy derivative, produced from aspartate-b-semialdehyde, are more frequently encountered suitable solutes. A single organism frequently contains mixtures of osmotic solutes; for instance, the cells of the Gammaproteobacterium Halorhodospira may contain glycine betaine, ectoine, and trehalose. As was already mentioned, KCl is used by the Archaea of the Halobacteriaceae to stabilise osmotic pressure. But 2-sulfotrehalose, an organic osmotic solute, was found in some haloalkaliphilic individuals, where it collaborates with KCl to keep the osmotic equilibrium. The osmotic solutes that methanogenic Archaea appear to utilise include glycine betaine, b-glutamine, b-glutamate, and Ne-acetyl-b-lysine.

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

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

X. PHYSIOLOGY

a) The amount of internal ions.

In order to thrive in extremely saline environments, halophiles regulate their internal ion concentration in a way that they prevents their cytoplasm from being impacted by external osmotic conditions. The goal of osmotic balance is achieved by internal salt buildup.

b) Metals tolerance

Numerous halophilic organisms from the Archaea and Bacteria domains have been found to be metal-tolerant. Diverse halophilic fresh and cultured strains showed disparate reactions to arsenic, cadmium, chromium, and copper. It was discovered that Acinetobacter strains were the most metal sensitive, whereas Flavobacterium strains were the most metal tolerant. Variations in salinity had an immediate effect on halophile tolerance. When the amount of yeast extracts is decreased, metal tolerance in cultivated strains is also decreased.

c) Osmoadaptation

Halophiles, which are ubiquitous in nature and live in high salinity environments, utilize efficient osmotic techniques to handle fluctuating salt concentrations. Many different strategies have evolved in different halophile taxa to help them survive in toxic surroundings. Halophiles typically rely on suitable solutes and sodium pumps based on the Na+/H+ antiporter system to sustain their internal osmotic environment. Recently, a number of these suitable solutes with significant biotechnological applications have been found. Depending on the environment and the type of microorganism, halophiles utilise different types of suitable solutes. Polyols, sugars, and derivatives of amino acids are examples of two main families of compatible solutes that are frequently employed to balance osmotic pressure. Due to the lack of very sophisticated molecular tools at this time, the complicated nature of these suitable solutes still has to be thoroughly researched. Bacteria and archaea both utilise several compatible solutes. Anionic solutes produced from bacterial carbohydrate sources are predominantly used by Archaea. Polyol phosphodiesters are additionally used favourably by Archaea. The most frequent compatible solutes that enable bacteria to maintain osmotic equilibrium are a-glutamate, proline, and derivatives of a-amino acids. Due to certain energy considerations, absorption of suitable solutes like glycine betaine, ecotine, glycerol, and others is favoured over production. Every Archaea and bacterial species possesses at least one transporter for glycine betaine, the most popular suitable solute. For instance, the glycine betaine transporters Ota and Otb in Methanosarcina mazei include three subunits each. Through transcriptional research, it was found that glycine betaine transportation starts when Ota is expressed but stops when it is removed.

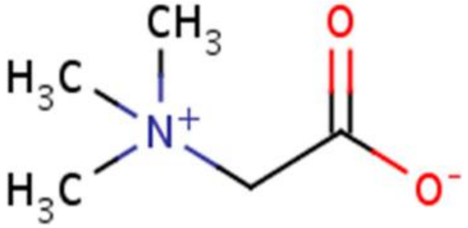

Figure 5: Glycine betaine

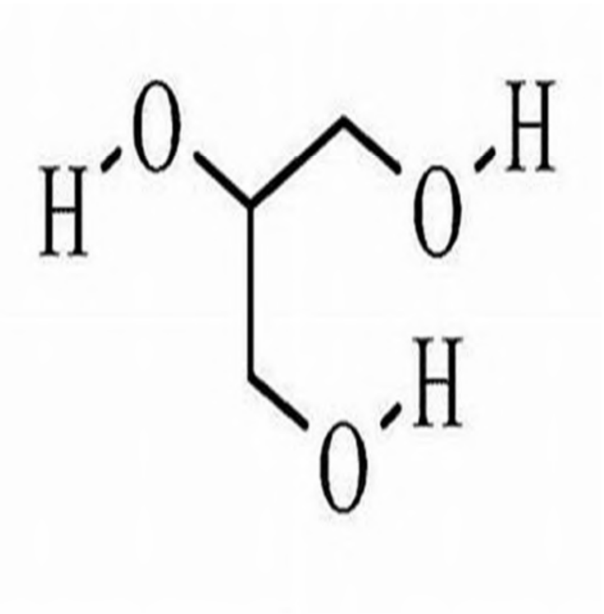

Figure 6:Glycerol

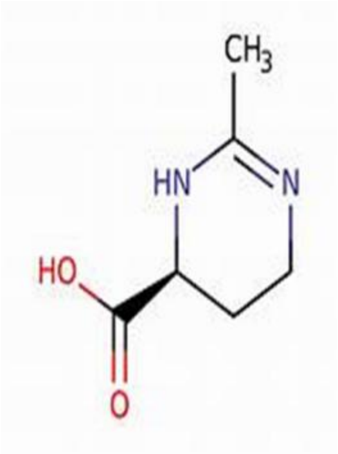

Figure 7:Ecotine

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

XI. HALOPHILES : SIGNIFICANT CHARACTERISTICS

A. Enzymes

Catalysts like enzymes are useful in a variety of industries, including metal recovery, leather processing, food, detergent formulation, and others. Extracellular enzymes in particular, which are extremophile enzymes because they can survive and catalyse reactions under unusual habitats, have been found to have significant applications in industries.

B. Nucleases

M. varians subsp. halophilus has been found to have a nuclease (nuclease H) that is both DNase and RNase active. Another halophilic nuclease was also generated by Bacillus halophilus (an exonuclease that releases 5 -mononucleotides from both DNA and RNA).

C. Amylases

Acinetobacter, Halobacterium salinarum, M. varians subsp. Halophilus, N. halobia, Natronococcus amylolyticus, Halomonas meridiana, and Haloferax mediterranei were used to purify and characterise a few -amylases. The enzyme from Haloarcula sp. S-1 was highly resistant to organic solvents. At varying Na+/Mg 2+ concentration ratios, the impact of ionic strength on amylase activity has also been documented.

D. Proteases

Proteases produced by halophilic microorganisms have novel applications because they are highly stable at saturated salt concentrations or because they can withstand organic solvents. An extracellular serine protease that Halobacterium salinarum makes has the potential to be employed in peptide synthesis. The other serine proteases were isolated from Natrial bamagadii, Natronococcu soccultus, and Natronomonas pharaonis. Other proteases from Natrial baasiatica, Haloferax mediterranei, and other halophilic isolates were purified and characterised. Several studies have been conducted in recent years to characterise extracellular proteases from the Salinivibrio genus, Pseudoalteromonas sp. strain CP76, Natrialba magadii, Halobacterium mediterranei, and Bacillus clausii. The haloalkaliphilic archaeon Natronomonas pharaonis also had a chymotrypsinogen B-like protease.

E. Lipases

One of the most crucial hydrolytic enzymes, lipase has uses in both the pharmaceutical and agricultural sectors. It has been reported that moderately or very halophilic microbes such as Salinivibrio sp., Natronococcus sp., haloarchaeal strains, and others produce lipase that is also stable at high temperatures.

F. Cellulose-Degrading Enzymes

Halocella cellulolytica, an obligate anaerobic organism, can use cellulose as its sole carbon source. In a different investigation, a large number of Archaea that are very halophilic and consume cellulose were found. Extracellular hydrolytic enzymes of subterranean rock salt halophilic microorganisms havethe presence of cellulose.

G. Pigments

Microorganisms that are halophilic are a rich source of natural compounds. One of the natural substances responsible for the red, orange, yellow and purple colors observed in a variety of plants, animals, and microbes is carotenoid pigment. Carotenoid pigments are particularly abundant in hypersaline environments. The reddish and orange colour of hypersaline habitats is a result of the presence of pigmented microorganisms such the -carotene-rich Dunaliella, the bacterioruberin-producing Haloarchaea, and the halophilic Salinibacter ruber. These organisms also create the carotenoid salinixanthin. Halorubrum sp. TBZ126, an extremely halophilic archaeon isolated from Urmia Lake, has been identified as a potential carotenoids producer. Melanin is a nearly ubiquitous pigment with enormous application potential in cosmetics, agriculture and pharmaceutical industries (photoprotection and mosquitocidal activity isolated from Streptomycete). On potato dextrose agar, the halophilic black yeast Hortaea werneckii developed a dark pigment that was diffusible. Additionally, it prevented the growth of potential pathogens, with action seen in Vibrio parahaemolyticus and Salmonella typhi.

H . Antibiotic Resistance

Antibiotic resistance develops through an evolutionary process based on the selection of organisms with improved tolerance to previously fatal antibiotic dosages. Different antibiotics such as penicillin, erythromycin, and gentamycin, which were previously successful treatments are no longer as effective due to bacteria's increased resistance. The halophilic bacteria Halobactericeae, Halomonas elongate, and Bacillus cereus SIU1,they are of various regions, were discovered to be resistant to antibiotics.

H. Antimicrobial Activity

At different bacterial biodiversity most actinobacterial isolates can produce antibacterial and antifungal metabolites. Antibacterial and antifungal activity of halophiles have vast actions against pathogens. Marine environments may be a source of new antimicrobial and antifungal agents, according to the results of antibacterial and antifungal experiments on halophiles (protein crude extract). In the presence of pathogenic bacteria and yeasts, Microbacterium oxydans and Streptomyces fradiae showed anibactrial activity.

I. Anticancer Compounds

Bioactive compounds, commonly referred to as natural products, are anticancer medications made by living things. Microorganisms are a great alternative to plant cells for producing anticancer natural products because of their diversity, ease of manipulation, and capacity for physiological screening to find new antitumor natural products, despite the fact that previously and well-established anticancer natural products were obtained from plant cells. Bacterial metabolites have been regarded as the most conventional strategy against cancer cell viability, despite the fact that bacterial cells have other experimental communication techniques with tumour cells than metabolites. Extremophiles are gaining more focus as a potential new source of innovative biomolecules. In hypersaline conditions, halophilic and halotolerant microorganisms are recognised as viable sources of anticancer compounds with few side effects. The use of halophilic microorganism metabolites in the treatment of cancer has been the subject of several investigations in recent years.

XII HALOPHILES' PRODUCING STRAINS OF NOVEL ANTIMICROBIALS

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

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

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

The inhibitory activity of Actinobacteria are the cause of decreased activity of humen pathogens. With Actinobacteria heterotrophic bacteria are present in soils in saline and hypersaline environments at less extreme conditions. Nocardiopsis and Streptomyces species are the primary metabolite producers and they are the most frequently reported organisams. Some bacterial strains produces novobiocin. Some pocess antibacterial activity against different Gram positive and Gram negative bacteria. Some have antifungal and cytotoxic activity. Halophiles have a number of distinctive and advantageous qualities like production of substrates with low cost for industrial biotechnology, and because of these distinctive properties, they constitute a crucial instrument for competitive and affordable production that industrial biotechnology cannot avoid using them. Alkaliphilic, or able to develop and thrive in hostile environments, many halophiles are particularly tolerant of high salt salinities. Halophiles take advantage of the high salt concentration for growth and survival because a conventional cell will experience plasmolysis when exposed to high salt concentrations. They have the ability to endure salt and water stress and adapt to hostile settings. Halophiles, especially extreme halophiles, thrive in brines due to their abundance and capacity to exclude nonhalophilic pollutants, creating the perfect environment for numerous biotechnological transformations. Halophiles can get their energy from a number of different places. They can employ less costlier and more renewable substrates, such kitchen and agricultural waste, in substitute of costly raw materials. Due to their halophilic (salt-loving) nature, they can also consume recycled seawater during bioprocessing, which lowers the need for freshwater. Equipment made of inexpensive materials, such as ceramics, plastics, or carbon steels, instead of the pricey stainless steel, can be utilised during fermentation since the medium has a high salt concentration, which lowers the high cost of equipment maintenance (stainless steel). Unsterile and continuous bioprocessing of halophiles can also save energy for intensive sterilisation and increase process efficiency. Halophilic production causes less environmental damage because it is bio-based, and bio-based products are biodegradable. There for it contributes less environmental pollution.

XIV. HALOPHILE GENETIC MANIPULATION

Genetic manipulation is critical for improving halophile performance for industrial applications. It takes a lot of effort to change halophiles so that they express foreign genes by giving them a variety of genetic tools. These currently accessible genetic tools must be improved, nevertheless, as they have some limitations. Many of them have improved accelerated cell growth, high cell density, downstream separation simplification, enlarged space for more inclusion body accumulation (increased cell size for PHA granules), and production yield and reduced bioproduction costs by using synthetic biology and genetic modification technologies such as recombineering, gene knockout, gene overexpression, and others.Fu et al. (2014) used an efficient markerless gene knockout procedure to develop a genetic manipulation method for Halomonas TD01. They increased PHB and PHBV production by deleting the prpC gene, which encodes 2-methylcitrate synthase, and the phaZ gene, which encodes PHA depolymerase, as well as overexpressing the udhA gene, which encodes soluble pyridine nucleotide transhydrogenase. The deletion of the prpC gene in Halomonas TD01 increased the conversion efficiency of propionic acid to 3-hydroxyvalerate (3HV) monomer fraction from 10% to nearly 100% in random PHBV copolymers of 3-hydroxybutyrate (3HB) and 3HV, resulting in cell growth to accumulate 70% PHBV in dry weight (CDW) consisting of 12 mol percent 3HV from 0.5 g/L propionic acid in glucose mineral medium. Furthermore, manipulation of PHA granule-associated proteins leads to an increase in PHA granule size, allowing for more natural separation. Yue et al. (2014) improved the PHA synthesis ability of Halomonas campaniensis LS21 by constructing an overexpression plasmid (pBBR1MCS1-oriC-Pporin-phaCABLS) of PHA synthesis genes containing genes of PHA synthase phaC, Recently, overexpression of recombinant, leaderless thioesterase I (TesA) from Escherichia coli and cyanobacteria increased free fatty acid production. coli. For biofuel production, a TesA homolog from the moderately halophilic bacterium Chromohalobacter salexigens was identified, cloned, and recombinantly expressed in E. coli strains BL21 and M15. Additionally, the alsS gene from Bacillus subtilis, as well as the ilvC and ilvD genes from Corynebacterium glutamicum, were overexpressed to produce KIV (2- Ketoisovalerate) for isobutanol production. By creating a Halomonas elongata transformatant (H. elongata/pHS15N-lysC) overexpressing lysC and contrasting it to a control strain carrying an empty vector (H. elongata/pHS15N), the researchers were able to boost ectoine synthesis from glucose. After 4 hours of cultivation, H. elongata/pHS15N produced 174 mmol/kg FW of ectoine and 0.31 mol of ectoine/mol of glucose, while H. elongata/pHS15N-lysC produced 207 mmol/kg FW of ectoine and 0.39 mol of ectoine/mol of glucose. Ectoine production from glucose was 1.2-fold greater in H. elongata/pHS15N-lysC than in the control, and the yield was marginally higher. This suggests that lysC overexpression enhances ectoine production from glucose.

XV. HALOPHILE PHARMACEUTICAL APPLICATIONS AND FUTURE PROSPECTS

The pharmaceutical and healthcare sectors are anticipated to use halophilic microbial products extensively. Proteolytic enzymes are used to create pharmaceutical compounds. Bioactive chemicals are important and understudied sources of bioactivities like antibacterial, antiviral and anti-tumor agents and are produced by a variety of halophilic bacteria. Marine cyanobacteria have drawn a lot of interest as a potent group in the creation of medications like curacin and cryptophycin, both of which are presently undergoing clinical studies. Studies on the antioxidant and anticancer effects of peptide compounds produced from marine diatoms have also been conducted. Halophile biosurfactants are becoming more and more common in vaccines, immunomodulators, dermal fibroblast stimulators, antiviral, antibacterial, antifungal, anticancer, and antiviral treatments. Halophilic bacteria need to produce novel medicines, anti-oxidants, sunscreens, suitable solutes, and hydrolytic enzymes from uncharted territories in order to reach a tipping point in the future. Brominated analogues exhibit 32-fold increased activity against MRSA and 16–64–fold increased activity against P. aeruginosa and E. coli, respectively, as a result of recent developments in the incorporation of halogenated compounds into peptoids (oligomers of N-substituted glycines), which improve antimicrobial efficacy against multi-drug-resistant pathogens. Future halogenated medications might be more effective against microorganisms that are resistant to them. OMIC technologies offer tremendous promise for the future identification of novel biomolecules originating from halophiles due to the recent findings of halophilic bacteria, even in terrestrial habitats.

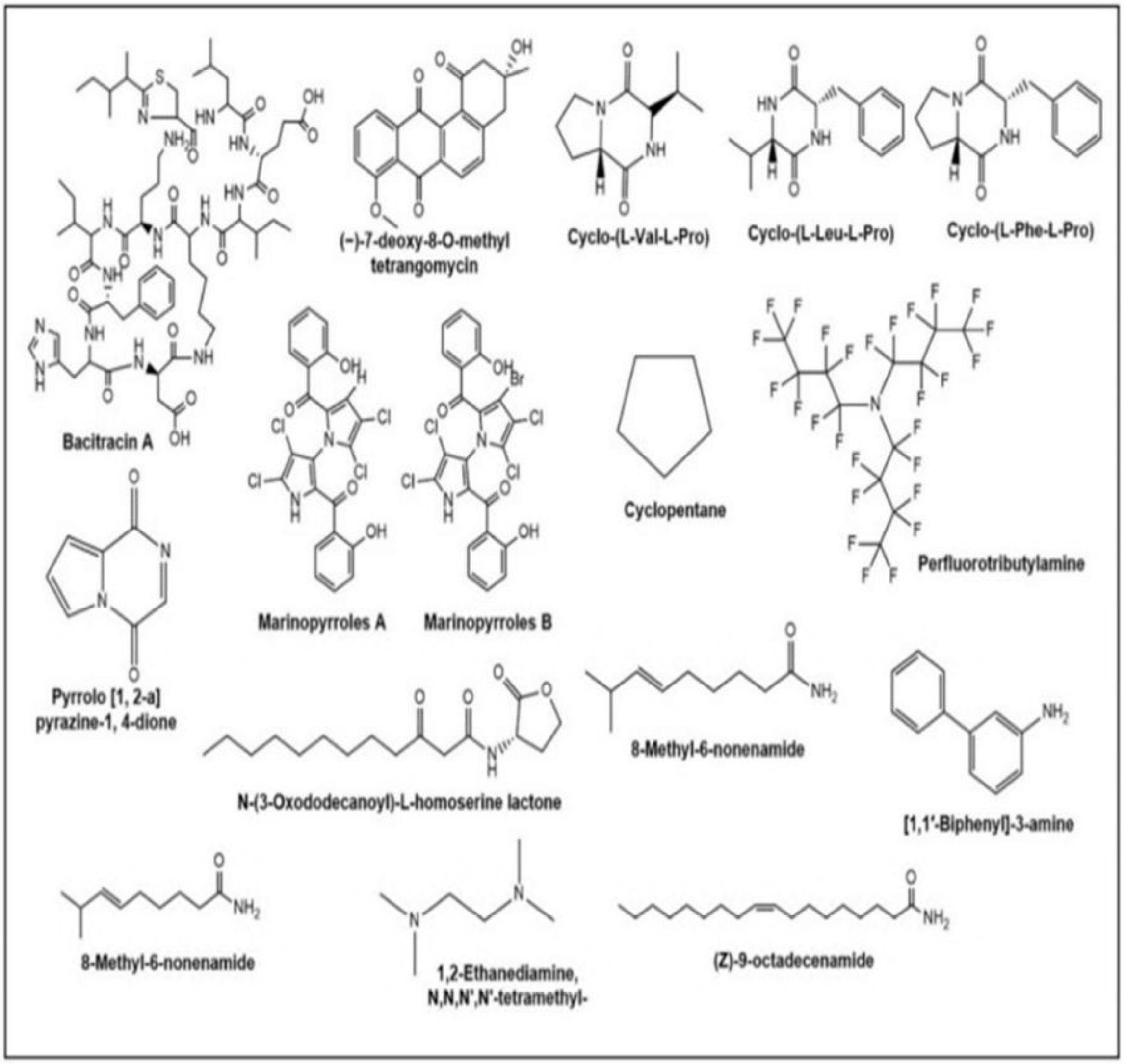

Figure 8: Antimicrobial substances produced by halophilic microorganisms are their chemical structure.

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

CONCLUSION

Halophiles are extremophiles that can live in high salt concentration. This habitat is a great source of various bioactive compounds with high economic value in chemical, pharmaceutical, agricultural, industrial, and biotechnological applications. Future industrial and biotechnological activities could benefit greatly from the diversity of microorganisms. Novel biomolecules in halophilic ecosystems are primarily motivated by the human pathogens that are resistant to medication. Antibacterial activity in Haloarchaeon has not been discovered. Isolation of new and more halophilic microbes are required to combat with drug resistant human pathogens. Bacillus halophilic representatives and the dominant actinomycete biomolecules have already been shown to be effective against human drug-resistant infections. Halophilic microorganism enzymes have not yet been shown to be effective against human infections. Clinical investigations should concentrate more on halophile-produced antimicrobials, nevertheless, as it is unknown how halo-antimicrobials work against organisms that are drug-resistant. Thus, it is feasible that many more members of this incredibly diverse collection of bacteria will eventually be included in the biotechnological applications of halophiles or genes produced from them.

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