Halophilic Bacteria: Diversity and Biotechnological Applications
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Abstract- Hypersaline environments are extreme habitats on the planet and have a diverse microbial population formed by halophilic microorganisms. They are considered to be actual or potential sources for discovery bioactive compounds, compatible solutes including novel and/or extraordinarily enzymes. To date, a number of bioactive compounds for the use in various fields of biotechnology which show assorted biological activities ranging from antioxidant, sunscreen and antibiotic actions have been reported. In addition, some halophilic microorganisms are capable of producing massive amounts of compatible solutes that are useful as stabilizers for biomolecules or stress-protective agents. The present review will impart knowledge and discuss on diversity of halophilic bacteria and their use in various biotechnological applications, including industrial, pharmaceutical, agricultural and environmental aspects. Culture dependent as well as culture independent methods for isolation of halophiles should go hand in hand to provide the insight mechanisms of halophilic adaptation and their future applications.

Keywords: halobacteria, Diversity, biocompounds, biotechnology.

I. INTRODUCTION

Saline habitats are frequently inhabited by an abundance of microbial communities adapted to these ecosystems. Among the microorganisms, the bacteria play a major role as important and dominant in habitants of saline and hypersaline environments [1,2]. Microorganisms that thrive in these environments have been broadly classified into halophilic microorganisms and halotolerant microorganisms. Halophiles are the microorganisms requiring salt for their growth whereas; halotolerant microorganisms are able to grow in the absence as well as in the presence of salt. Halophiles can be further divided into three categories according to their halotolerance, slight halophiles that grow optimally in 3% (w/v) total salt, moderate halophiles with optimal growth at 3- 15% (w/v) salt and extreme halophiles that grow optimally at 25% (w/v) salt [3]. The world of halophilic microorganisms is highly diverse. Halophilic and halotolerant microorganisms are found in all three domains of life: Archaea, Bacteria and Eucarya. Saline and hypersaline environments are found in wide variety of aquatic and terrestrial ecosystems. In terms of marine environment, Morocco has coastline of about 3500 Km. Morocco is know for its rich biodiversity especially in context with halophiles because; it is surrounded by atlantic ocean and mediterranean sea. Marine environment is the the prime reservoir of biological diversity and the marine microorganisms are recognized to be rich sources of novel compounds. Recently, there is accelerated interest in the study of marine halophiles, with the aim of providing the information on microbial diversity and their role in biogeochemical cycling in marine ecosystems and in exploiting their ability to produce novel enzymes and industrially important bioactive substance like biosurfactants, extracellular polymeric substances (EPS), other crucial metabolites/compounds for biotechnological applications.

The above survey shows that thus far the halophilic microorganisms have found relatively
few commercially viable applications. With the exception of β-carotene production by *Dunaliella* and ectoine synthesis using *Halomonas* and other moderately halophilic Bacteria, most other potential applications suggested are no more than ideas only, waiting to be exploited. The list of possible applications presented in the sections below is by no means exhaustive and additional ideas have been presented in the literature, such as, for example, production of liposomes for the cosmetics industry and exploitation of *Halobacterium* gas vesicles in biotechnological processes. Many patents have been issued for these and other applications, but the ideas are still to be implemented in commercial ventures. With all the advantages listed for the use of halophiles in industrial processes, there are disadvantages as well. For mass cultivation of aerobic bacteria, the low solubility of gases in concentrated brines may severely limit oxygen supply to the cultures. Also the aggressive nature of the salts should be taken into account during the construction of reactors with metal parts exposed to the medium. It is possible to build corrosion-resistant bioreactors suitable for high salt media, but their cost is significantly higher than that of conventional fermenters. The tremendous diversity of halophilic microorganisms found in nature is still far from being fully exploited. Approaches derived from genomics and proteomics have opened new possibilities, and genetic systems are now also available for a number of halophiles. The present review summarizes the current diversity of halophilic bacteria and their use in various biotechnological application, including industrial, pharmaceutical, agricultural and environmental aspects. Briefly the mechanisms of adaptation in saline environments and the molecular approaches to access the diversity of halophilics will be discussed.

II. DIVERSITY

Microbial life can be found over a wide range of extreme conditions (salinity, pH, temperature, pressure, light intensity, oxygen and nutrient conditions). Hypersaline environments constitute typical examples of environments with extreme conditions due to their high salinity, exposure to high and low temperatures, low oxygen conditions and in some cases, high pH values. Bacteria and Archaea are the most widely distributed organisms in these environments [4]. The classification of Kushner and Kamekura [5] defines different categories of halophilic microorganisms based on the optimal salt concentration wherein they show optimal growth, and it includes four categories: non-halophilic organisms are defined as those requiring less than 1% NaCl, whereas if they can tolerate high salt concentrations are considered as halotolerant microorganisms. With respect to halophilic microorganisms, the classification distinguishes among slight halophiles (marine bacteria), which grow best in media with 1% to 3% NaCl, moderate halophiles, growing best in media with 3% to 15% NaCl, and extreme halophiles, which show optimal growth in media containing 15% to 30% NaCl. Halophiles have developed two different adaptive strategies to cope with the osmotic pressure induced by the high NaCl concentration of the normal environments they inhabit [6, 7]. The halobacteria and some extremely halophilic bacteria accumulate inorganic ions in the cytoplasm (K+, Na+, Cl−) to balance the osmotic pressure of the medium, and they have developed specific proteins that are stable and active in the presence of salts. In contrast, moderate halophiles accumulate in the cytoplasm high amounts of specific organic osmolytes, which function as osmoprotectants, providing osmotic balance without interfering with the normal metabolism of the cell [8, 9].
Halophilic bacteria are one of the most commonly isolated, reported, studied and characterized microbes amongst halophiles [10]. They exist in various forms of colonies, ranging from pigmented to non-pigmented, according to the salt concentration in the media. They are slow growing compared to non-halophile or normal bacteria. The extremely halophilic bacteria grow extremely slowly [4]. For in vitro culture on agar plates, most require natural brines along with a variety of other nutrients such as fish or milk extracts for their growth and a few of them also require complex nutrients like yeast extract for their survival. During the last few decades there was progress in providing a systematic base for classifying halophilic bacteria, including the use of various phenotypic characteristic tests and analytical apparatus. However, there are now taxonomically emerging groups of halophilic bacteria and their classification has not yet been much developed [11]. Salt characterization for the optimum growth of halophilic bacteria is required prior to classification, but this step is costly, time consuming and is limited to only those cultivable and clonable isolates. A test methodology for halophilic bacteria has been recommended in which the media must be added with salt and the incubation time for subsequent growth is then assessed [12].

The taxonomy of halophilic bacteria was historically based on just a few phenotypic or morphological characters and less attention was paid to the phylogenetics or biochemistry of the organisms [12-15]. Phylogenetic analyses, largely based on the 16S rRNA gene sequence, have revealed that halophilic eubacteria and halophilic archaeobacteria are from different phylogenetic branches. Halophilic bacteria are generally represented by archaeobacteria, which are also comprised of the slightly and moderately halophilic bacteria, but most of these bacteria are eubacteria [16].

In hypersaline environments extremely halophilic bacteria and moderately halophilic bacteria are the important groups that have received most of the attention in recent research [10]. Slightly halophilic bacteria have been reported in studies focused on habitats such as the Dead Sea, GSL, Lake Magadi, Wadi El Natrun and some other extreme hypersaline environments that yield extremophilic bacteria. Slightly halophilic bacteria form a smaller proportion of the population in these environments and typically can not be found in these harsh habitats [17]. The other drawback of characterising slightly halophilic bacteria is their characteristics that resemble the non-halophilic or normal bacteria, and these are typically of a lower interest for research. Due to the lack of sufficient information on the slightly halophilic bacteria, only a description of moderately and extremely halophilic bacteria is provided in this review.

**Extremely halophilic bacteria.** Extremely halophilic bacteria belong to the class *Halobacteria*, family *Halobacteriaceae* and order *Halobacterales* [18]. Fourteen genera of *Halobacteriaceae* family are recognised, namely *Haloarcula, Halobacterium, Halobaculum, Halococcus, Haloferax, Halogeometricum, Halorubrum, Haloterrigena, Natrialba, Natrinema, Natronobacterium, Natronococcus, Natronomonas and Natronorubrum*. They are coccii or rod-shaped and have a number of disk–
to triangle-shaped involutions. They need salt concentration of 1.5 M (~ 8.8%) NaCl for their growth and lack muramic acid but possess peptidoglycan in the cell envelop. The colonies are highlighted by shades of red colour due to the presence of optically active C_{50}-carotenoids. Their intracellular enzymes require high levels (3-5 M) of potassium chloride whilst their cytoplasmic membranes are composed of phytanyl ether lipids. They show a degree of resistance against many antibiotics and occur in hypersaline environments such as salterns, soda lakes and salt lakes [4].

**Moderately halophilic bacteria.** Halophilic bacteria requiring a salt concentration of 0.5 M NaCl for their growth are included in the moderately halophilic bacteria group. Taxonomically, the moderately halophilic bacteria can be divided into the two groups: moderately halophilic eubacteria and moderately halophilic archaeobacteria [19]. Most of the bacterial halophiles are moderately halophilic eubacteria rather than extreme halophiles [7], and are phototrophic or heterotrophic, the latter including Gram-positive and Gram-negative bacteria. Gram-positive moderately halophilic species include *Deleya halophila*, *Desulfovibrio halophilus*, *Desulfoballobium retbaense*, *Flavobacterium halophilum*, *Haloanaerobacter chitinovorans*, *Haloanaerobium praevalens*, *Halobacteroides halobius*, *Halomonas elongate*, *Halomonas eurhinalia*, *Halomonas halodenitrificans*, *Halomonas halodurans*, *Halomonas subglaciescola*, *Paracoccus halodenitrificans*, *Pseudomonas beijerinckii*, *Pseudomonas halophila*, *Spirochaeta halophila*, *Sporohalobacter lortetti*, *Sporohalobacter Marismortui* and *Vibrio costicola*. The Gram-negative moderately halophilic species include *Halobius* spp., *Marinococcus albus*, *Marinococcus halobius* and *Sporosarcina halophila*. Phototrophic moderately halophilic bacteria are *Ectothiorhodospira vacuolata*, *Rhodospirillum salseigens* and *Rhodospirillum salinarum* [19]. Except for the methanogens, all halophilic archaea are considered as extremely Halophilic bacteria [7]. The composition of the membrane-bound cytoplasm of moderately halophilic archaeobacteria is similar to that of extremely halophilic bacteria in having phytanyl ether lipids. They have a unique nutritional intake capacity and use methylotrophic substrates rather than acetate, carbon dioxide and hydrogen. The mode of respiration is strictly anaerobic [20]. The intracellular NaCl concentration is higher than most other bacteria (about 0.6 M or 3.5% NaCl), but this concentration is much lower than that in the extremely halophilic archaeobacteria. Species reported for moderately halophilic archaeobacteria include members of the *Methanohalophilus* genus, e.g. *M. zhilinae*, *M. portucalensis*, *M. mahii*, *M. halophilus* and *M. portucalensis* [21].

**III. MOLECULAR APPROACHES TO ASSESS THE DIVERSITY OF HALOPHILIC BACTERIA**

Molecular phylogeny is a very useful to analyze microbial populations. A number of molecular techniques are known for identifying and analyzing the biodiversity of bacterial strains, such as random amplified polymorphic DNA (RAPD), amplified ribosomal DNA restriction analysis (ARDRA), and 16S rRNA gene analysis. The comparison of 16S rRNA gene sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria [22].
Small- subunit of rDNA gene (16S rDNA) is widely used to establish phylogenetic relationship among bacteria because this gene is universally present, sufficiently small to be easily sequenced and large database for it is available. Furthermore, rDNA that encodes Rdna comprises of highly conserved regions, crucial for structure and function, flanked by highly variable stretch, which varies among various species [23]. Identification and phylogenetic analysis of halophilic bacteria using 16S rDNA genes amplification has been extensively implemented by many authors [24,25]. 16S Rdna primers and probes for specific identification of actinomycetes and especially for streptomyces were designed by Stackebrandt et al., 1991 [26] and Mehling et al., 1995 [27]. ARDRA and 16S rRNA gene sequencing have been applied to characterize many actinobacteria [28-29]. Additionally, there are degenerate primers for genes encoding polyketide synthases (PKS-1 and PKS-2) and non ribosomal peptide synthetases (PKS-1 and PKS-2) and non ribosomal peptide synthetase (NRPS) which are used to screen the biosynthetic potential in terms of natural product drug discovery as identification of these genes provides indirect evidence of potential chemical diversity among the actinobacteria [30-31].

IV. HYPERSALINE HABITATS

Hypersaline habitats can be classified based on the extremity, adaptability and geological and geographical location of the area.

Saline Soil. Soil habitats are heterogeneous and are comprised of a diverse range of minerals and salinity present within the soil at various depths [18]. Soil salinity is much more variable than water salinity [32]. Higher plants that are halotolerant (halophytes) are well adapted to growing in different levels of salinity and play important roles in different ecological nutrient cycles, but the interactions between plants and microbes vary with different saline habitats [33].

A number of bacterial species have been reported to inhabit saline soils, but the dominant species found in such habitats belong to the Bacillus, Pseudomonas, Micrococcus and Alcaligenes genera [7]. In the Alicante, Spain, the rhizosphere and hypersaline soil have been reported to contain 5–10% NaCl. The xerophytic plants present in the area and the salt concentration range allowed the growth of isolated organisms that are not related to the salinity of the soil [16]. Half of the plants were able to grow best in a high- salinity condition (5– 15% NaCl) while the other half grew best in a low-salinity condition (1% NaCl). With respect to the culturable microbes from this environment, agar media plates with 10% NaCl revealed mostly the presence of Gram - positive rod bacteria, while Gram - negative rod bacteria were found to be abundant at NaCl concentrations of 10– 20%. Gram - positive cocci bacteria were found in soil with ≥ 20% NaCl salinity, and were mostly from the Bacillus, Micrococcus, Staphyloccocus, Actinomycetes, Corynebacterium, Planococcus, Arthrobacter and Nocardia genera [7].

Saline Water. Water with salinity of 3% or above is designated as saline water [34]. Brackish water, sea and oceanic water and water from salt lakes and salterns are all considered as saline water. Some of the more famous soil and saline water habitats are discussed below. The Dead Sea is a typical example of the thalassohaline habitat. It is a lake approximately 320 m in depth with a water temperature of 21– 36°C and a salt concentration of 78% NaCl. The pH is slightly low and Na⁺, Cl⁻ and Mg²⁺ are the most abundant ions present [35]. A number of studies have characterized the halophilic microorganisms present in the Dead Sea and those found include the eubacteria, which were aerobes or facultative anaerobes of the
genera *Pseudomonas* [3], *Flavobacterium* [3], *Chromobacterium* [3,19], *Halobacterium* [36], *Halococcus* [37], *Clostridium* [38], *Sporohalobacter* [39] and *Halomonas* [40], plus a novel species *Halobaculum gomorrense* [41]. In addition, filamentous halophilic fungi have also been reported recently from the Dead Sea [42], and so it is clear that it does in fact support life despite the high salinity.

**The Great Salt Lake, USA.** The Great Salt Lake (GSL) is situated in Utah, USA, and is the largest salt lake in the Western hemisphere. This is a thalassohaline lake of moderate depth (~10 m maximum) situated in a salt desert. Unlike the Dead Sea, the GSL has a relatively high pH on the alkaline side, with a high salinity of 33% NaCl [35]. However, the salt concentration has recently changed dramatically due to the activity around the GSL since 1959 [16]. The causeway that has been constructed separates the GSL into three regions (north-east, north-west and south) and prevents the mixing of water, which has resulted in two independent water habitats, the north side and the south side, where the salt concentration of the latter (12% NaCl) is much lower than that of the former (34% NaCl) [35]. The low salinity of the south side is due to a continuous supply of fresh water from the surrounding mountains. The major ions in the lake are Na⁺ and Cl⁻, and the water temperature of the lake is seasonal, ranging from -5 to 35°C [16, 35].

The GSL has a bacterial community which has been classified into three groups. The first is the archaeobacteria and includes members of the Halococcus and Halobacterium genera that are mostly present in the north side of the GSL due to the extreme salinity. Species like *Halorhabdus utahensis* [43] and *Methanohalophilus muhii* [44] are also found in the GSL. The second is the eubacteria, which can be aerobes or facultative anaerobes and includes *Halomonas variabilis* [45], *Pseudomonas halophile* [45], *Chromohalobacter marismortui* [19], *Halobacillus trueperi* and *Halobacillus litoralis* [46]. Finally, the third group is the anaerobic eubacteria, with *Halanaerobium praevvalens* [47] and *Desulfovela halophile* [48] being commonly found.

**The Solar Lake, Egypt.** The Solar Lake, located on the Sinai coast region of the Gulf of Aqaba, is also an extremely hypersaline lake, with a shallow depth of 4-6 m. The solar intensity reaches the bottom of the lake (hence providing the name of the Solar Lake), which has a high rate of evaporation and intense and complex microbial interactions in the sediment as well as in the water [49]. In summer, the water in the lake gets completely oxygenated but in autumn it stratifies. The salinity of the lake rises to 20% NaCl in the summer due to the high rate of evaporation. A gravel bar of 60 m in width separates the lake from the Red Sea. The Lake gets contaminated from the Red Sea and also by occasional showers [16]. The halophilic archaeal community isolated from this lake is dominated by *Halobacteriaceae*, *Methanococcus*, *Methanobacterium*, *Spirochaeta halophile* and *Desulfovibrio halophiles*. The moderately halophilic bacteria found in the lake include *Beggiatoa alba* and *Achromatium vulotans*, a sulphur utilizing bacteria [44]. The normal varieties of halophilic bacteria are not present much in the area and the community also changes due to the continuous variation in the saline conditions of the lake.

**Lakes at Natrun Valley (Wadi El Natrun), Egypt.** The Natrun Valley or Wadi El Natrun is situated below sea level in the arid region of central northern Egypt. The northern region of the valley has eight seasonal hypersaline lakes that are sometimes completely dry. Like the Solar Lake, the lakes at Natrun Valley also have a high rate of evaporation. The water feed is provided from
underground seepage of water from the Great Nile River that touches the area through the burdi swamps (grass swamps) [35]. The salinity near the sediment around the valley varies in the range of 3.1–8.6% NaCl. The Gram - positive bacterium Bacillus halalkaliphilus, a polyextremophilic bacterium that shows alkalophilic and halophilic characteristics, has been isolated from these lakes [50]. This bacterium show a maximum growth in 0.5–3 M (~2.9–17.5%) NaCl and can even tolerate 4 M (~23.4%) NaCl. Some other alkalophilic and phototrophic bacteria have also been reported from the Natrun Valley [35].

Inland saltern of La Mala, Spain. The La Mala area is situated 780 m above sea level with a 2% slope [51]. The area is thalassohaline and the salt bed is created from the saline water near the surface of the soil. The other source of water that feeds the habitat is well water. The chloride concentration fed by the well water is lower than the content of Mg\(^{2+}\), Ca\(^{2+}\) and K\(^+\) ions from sea water. The total salinity found in the well water is 18% NaCl. The main halotolerant species isolated from the La Mala salterns are bacteria, with the major genera being Alteromonas, Flavobacterium, Halomonas, Acinetobacter, Vibrio, Halobacterium and Pseudomonas [52].

In Morocco, halophilic micro flora have been reported from natural hyper saline habitats from the coastal regions of atlantic, Larache, Oualidia and Tarfaya [53, 54]. The bacteria reported from these locations (e.g., Alkalibacillus sp. A1, Virgibacillus sp. V1 and Actinopolyspora sp. AH1) are halo-alkaliphilic in nature, and exhibit optimal growth in the presence of 5–20% NaCl and pH 8–10. In the coastal region of Tarfaya in the south of Morocco, there are two salt water bodies. However, these saltern water bodies have not yet been explored for the presence of halophilic microbes. Therefore, we overlook this study to isolate and characterise the halophilic bacteria and archaeae from this region. Our results reveal the existence of strict halophilic as well as halotolerant microorganisms in the sediment and salt water stream [55].

Salted Food. Fungi, especially yeasts, are common contaminants of salted food [56], along with some halophilic bacteria including Pediococcus halophilus [57], Halobacterium sp. [58], Halococcus sp. [58], Halomonas salina [59], Pseudomonas beijerinckii [10], Halomonas halodenitrificans [10] and Vibrio costicola [60]. The spoiling of food when preserved in salt can occur due to the toxins produced from these microorganisms, for example the aflatoxins from members of the Aspergillus genus.

V. MECANISM OF ADAPTATION IN SALINE ENVIRONMENT

In order to adapt to the saline conditions, halophilic bacteria have developed various strategies to their maintain cell structure and function [60]. There are two main strategies that halophiles have evolved to deal with high salt environments (i) “salt in” strategy and (ii) “compatible- solute” strategy. Bacterial cells maintain internal concentrations that are osmotically equivalent to their external environment. They maintain internal concentrations by accumulating high concentration of KCL. For every three molecules of KCL accumulated, two ATP and hydrolyzed making this strategy more energy efficient that the “compatible solute” strategy. This mechanism is accompagned by certain physiological modifications which are required to protect all the metabolic and regulatory functions (e.g. enzymatic activity, synthesis of cellular components, and structure and function of some organelles) at high salinity [1]. The “salt in” strategy of osmoregulation is adopted by members from archaeabacterial and eubacteria. In the “compatible solute” strategy cells maintain low concentration of salt in their cytoplasm by
balancing osmotic potential with organic, compatible solutes. Compatible solutes include polyols such as glycerol, sugars and their derivatives e.g.; trehalose, sucrose; amino acids and their derivatives e.g. proline, glutamate and quaternary amines such as glycine betaine. Compatible solutes could be synthesized de novo or, if present in the medium, can be taken up by the organisms [61]. Osmoprotectants are defined as exogenously provided organic solutes that enhance bacterial growth in media having high osmolarity. These substances may themselves be compatible solutes, or they may act as precursor molecules that can be enzymatically converted into these compounds. Compatible solutes regulate cells by accumulation of them up to molar concentrations; compatible solutes lower the cytosolic osmotic potential and hence make major contributions to the restoration and maintenance of turgor [62]. Energetically this is an expensive process. This strategy of adaptation is followed by many halophilic eubacteria. In addition to their well studied function as osmoprotectants, compatible solutes also have protein-stabilizing properties that support the correct folding of polypeptides under denaturing conditions both in vitro and in vivo [63]. Besides these strategies, bacteria have evolved some other possible mechanisms to adapt to saline environments by changing the composition of their cell envelope especially the exopolysaccharides. Sandhya et al. [64] reported that Pseudomonas, a halotolerant bacteria could survive under stress condition by producing exopolysaccharide, which protects them from fluctuations in water potential by increasing water retention and maintaining the diffusion of carbon sources in microbial environment. Similarly, Halomonas variabilis and Planococcus rifietoensis were reported to survive under salinity stress by exopolysaccharide production [65]. The chemical composition of cell membranes is also occasionally modified and synthesis pattern of proteins, lipids, fatty acids and peptidoglycan are changed with a moderate increase in salinity.

VI. BIOTECHNOLOGICAL APPLICATIONS

In the recent years, studies on halophilic microorganisms have significantly increased. Halophiles or their products such as enzymes, antimicrobial compounds, exopolysaccharides, biosurfactants etc. finds vital application in diversified fields ranging from industries, pharmaceuticals, food industries, environment and agriculture.

Enzymes. Halophilic bacteria are considered as one of the most important extremophiles, they can be found in saline or hypersaline environments. Enzymes from halophilic bacteria are considered as a novel alternative for use as biocatalysts in different industries. Currently, there are few studies on halophilic enzymes [66]. Due to unique properties, halophilic microorganisms have been explored for their biotechnological potential in different fields [67]. Enzymes produced by halophilic microorganisms offer important opportunities in biotechnological applications such as food processing, environmental bioremediation, biosynthetic processes fermented food, textile, pharmaceutical and leather industries [68]. These enzymes are stable at high salt concentrations, but also can withstand and carry out reactions efficiently under extreme conditions such as high pH values, high or low temperature, low oxygen availability, pressure, and toxic metals [69,70]. Enzymes such as xylanases, dehydrogenase, amylases, proteases, α-amylases and lipases, have been produced by different genera of halophilic bacteria such as Cyanobacteria, Proteobacteria, Firmicutes, Actinobacteria, Spirochaetes Salinisivibrio, Halomonas, Bacillus-Salibacillus, Bacteroidetes, Pseudoalteromonas ruthenica, Bacillus, Halobacillus and Thalassobacillus. These enzymes have been commercialized especially in
the production of polyunsaturated fatty acids, food, biodiesel, baking, feed, chemical and pharmaceutical, paper and pulp, detergent, leather industries, fish sauce and soy sauce preparations, saline waste water, and oil field waste treatment [71-74]. Lipases, proteases and amylases isolated from halophilic bacteria constitute an excellent alternative in the industrial processes due to their stability and versatility [75]. Hydrolases is other enzymes characterized from halophilic bacteria [76]. During the last years, the halophiles have developed of novel enzymes. These enzymes have unique structural features and catalytic power to sustain the metabolic and physiological processes under high salt conditions [77, 78]. Due to stability under high salt concentrations, the demand for enzymes produced by halophiles has increased considerably [79]. Various investigations have reported on production or purification of haloenzymes from halophilic bacteria, resistance of the enzymes toward different organic solvents has been examined [80]. Enzymes produced by halophilic bacteria show interesting properties for use in different biotechnological and industrial applications.

Exopolysaccharides (EPS). Polysaccharides are the most abundant organic material in the world. They are ubiquitous, as they can be recovered from plants, animals, algae and microorganisms in soil, water and atmosphere. The chemical structure of a polysaccharide comprises monomers called monosaccharides (Table I), linked to each other through glycosidic linkages. They can be constituted of one type of monosaccharide, in homopolysaccharides, or by several types, usually up to ten, in heteropolysaccharides. They can display either a regular repeating unit, or random, or block distribution of monosaccharides. Various types of inorganic and organic substituents (sulphates, phosphates, acetates, ethers, amino acids, lactates and pyruvates) can decorate the polysaccharide backbone that in turn can be linear or branched. Arms can show different lengths and include the same monosaccharide types or different ones. Additionally, branches can be distributed in a regular or random way on the backbone. Finally, polysaccharides sizes range between 50 Da up to several thousand KDa.

**Table I**

<table>
<thead>
<tr>
<th>Monosaccharides</th>
<th>Example</th>
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<tbody>
<tr>
<td>Pentoses</td>
<td>arabinose (Ara), ribose (Rib), xylose (Xyl)</td>
</tr>
<tr>
<td>Hexoses</td>
<td>glucose (Glc), galactose (Gal), mannose (Man)</td>
</tr>
<tr>
<td>Deoxy-hexoses</td>
<td>quinovose (Qui), fucose (Fuc), rhamnose (Rha)</td>
</tr>
<tr>
<td>Uronic acids</td>
<td>glucuronic acid (GlcA), galacturonic acid (GalA), mannanuronic acid (ManA)</td>
</tr>
<tr>
<td>Amino sugars</td>
<td>glucosamine (GlcN), galactosamine (GalN), mannosamine (ManN)</td>
</tr>
<tr>
<td>Uncommon sugars</td>
<td>3-deoxy-D-manno-2-octulosonic acid (Kdo), neuraminic acid (Neu)</td>
</tr>
</tbody>
</table>

Polysaccharides are used in several industrial fields, as thickeners, stabilisers and gelling agents in food products and as antitumoral, antioxidant and/or prebiotic in pharmacology [81]. They derived from a variety of sources: bacteria, fungi, algae and plants. Despite all these sources, the world market is dominated by polysaccharides from algae [82], like carrageenans, agar and alginates [83,84] and from lactic acid bacteria, due to the high number of EPSs recovered after the extraction. In the last years, EPSs produced by marine bacteria have been attracted the interest of
several researchers for their unique properties of considerable biotechnological importance and, therefore, of commercial significance (Table II). Currently, despite the vast number and biodiversity of the marine EPSs, these polymers represent only a small fraction of the current polymers market, due to the very low amount of purified polysaccharide obtained. Therefore, to achieve a larger amount to be commercialized it is necessary to proceed with an enhancement of the production. Particularly, microbial polysaccharides production is greatly influenced by fermentation conditions. Indeed, the structure, the composition and the viscosity of EPSs depend on several factors, such as the composition of the culture medium, the sources of carbon and nitrogen and the precursor molecules, the mineral salts, trace elements, the type of the strain and the fermentation conditions such as pH, temperature, oxygen concentration and agitation [85]. In addition, engineering modifications of genes involved in the polysaccharide biosynthesis could also be convenient. During the last years, diverse marine microbial exopolysaccharides turned out to be promising candidates in biotechnology field. They span from the exopolysaccharides displaying biological activity, exploitable in the pharmaceutical and medical industry [86-90], with particular regard to the sulphated polysaccharides [91-93], to the emulsifier EPSs, that find application in the food, pharmaceutical, cosmetic and petroleum industries [94-100]. Furthermore, it is important to consider the applications of the cryoprotectant and anti-freezing EPSs in many industrial fields [101-107].

Several EPS producing halophiles have been reported from coastal areas such as *Vibrio parahaemolyticus*, *Bacillus Licheniformis*, *Salinicoccus sp*. and *Chromohalobacter sp.* [108, 109]. Similary, poly-β-hydroxyalkanoate (PHA), a polymer containing β-hydroxybutyrate and β-hydroxyvalerate units, is accumulated by many prokariotes. It is used for the production of biodegradable plastics. Some halophilic bacteria also produce PHA e.g. *Halomonas compisalis* [110].

### Table II

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Source</th>
<th>Functions and applications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. macleodii sub. Fijiensis</em></td>
<td><em>biovar deepsane HYD 657</em></td>
<td>Cosmetic, keratinocytes protection</td>
<td>[111, 112]</td>
</tr>
<tr>
<td></td>
<td>Deep-sea hydrothermal vent</td>
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<tr>
<td></td>
<td><em>polychaeta annelid</em></td>
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<tr>
<td><em>A. strain HYD-1545</em></td>
<td>Hydrothermal vent <em>polychaeta</em></td>
<td>-</td>
<td>[113]</td>
</tr>
<tr>
<td><em>A. macleodii sub. Fijiensis</em></td>
<td><em>strain ST716</em></td>
<td>Deep-sea hydrothermal vent</td>
<td>[114, 115]</td>
</tr>
<tr>
<td></td>
<td><em>strain</em> ST716</td>
<td>Gel forming</td>
<td></td>
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<tr>
<td><em>A. strain JL2810</em></td>
<td>Sea water</td>
<td>Biosorption of heavy metal</td>
<td>[116, 117]</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Organism</td>
<td>Environment</td>
<td>Activity</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td><em>A. infernus</em> GY785</td>
<td>Hydrothermal vent</td>
<td>Metal recover</td>
<td>[118,119]</td>
</tr>
<tr>
<td><em>A. hispanica</em> F32</td>
<td>Hypersaline inland</td>
<td>-</td>
<td>[120]</td>
</tr>
</tbody>
</table>

**Bacillus**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Environment</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. strain B3-15</em> halophile</td>
<td>Marine hot spring</td>
<td>-</td>
<td>[121, 122]</td>
</tr>
<tr>
<td><em>B. strain B3-72</em> thermophile</td>
<td>Hydrothermal vent</td>
<td>-</td>
<td>[123]</td>
</tr>
<tr>
<td><em>B. strain I-450</em> haloalkaliphile</td>
<td>Mudflats</td>
<td>Gel forming</td>
<td>[124, 125]</td>
</tr>
<tr>
<td><em>Cobetia marina</em> DSMZ 4741</td>
<td>Coast</td>
<td>-</td>
<td>[126]</td>
</tr>
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**Colwellia**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Environment</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. psychrerythraea</em> 34H</td>
<td>Sea sediments, sea ice</td>
<td>Anti-freeze</td>
<td>[127, 128]</td>
</tr>
<tr>
<td><em>C. psychrerythraea</em> 34H</td>
<td>Sea sediments, sea ice</td>
<td>No anti-freeze activity</td>
<td>[129]</td>
</tr>
<tr>
<td><em>Flavobacterium uliginosum MP-35</em></td>
<td>Sea weed</td>
<td>-</td>
<td>[130]</td>
</tr>
</tbody>
</table>

**Geobacillus**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Environment</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. strain 4004</em> thermophile</td>
<td>Sea water</td>
<td>-</td>
<td>[131]</td>
</tr>
<tr>
<td><em>G. tepidamans Y264</em> thermophile</td>
<td>Hot spring</td>
<td>Immunomodulant, anti-viral</td>
<td>[132]</td>
</tr>
<tr>
<td><em>G. thermodenitrificans</em> thermophile</td>
<td>Shallow marine vent</td>
<td>-</td>
<td>[133]</td>
</tr>
<tr>
<td><em>Hahella chejuensis</em> strain 96CJ1035</td>
<td>Marine sediments</td>
<td>Emulsifier</td>
<td>[134, 135]</td>
</tr>
</tbody>
</table>

**Halomonas**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Environment</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. eurihalina</em> F2-7</td>
<td>Dead Sea</td>
<td>Emulsifier</td>
<td>[136, 137]</td>
</tr>
<tr>
<td><em>H. maura</em> halophile</td>
<td>Solar saltern</td>
<td>Viscous, pseudoplastic</td>
<td>[138, 139]</td>
</tr>
<tr>
<td><em>H. sp. OKOH</em> halophile</td>
<td>Bottom sediments</td>
<td>Flocculant</td>
<td>[140]</td>
</tr>
<tr>
<td><em>H. sp AAD6</em> (JCM 15723)</td>
<td>Soil saltern</td>
<td>-</td>
<td>[141, 142]</td>
</tr>
<tr>
<td><em>H. alkaliantarctica</em> strain CRSS</td>
<td>Salt lake</td>
<td>-</td>
<td>[143]</td>
</tr>
</tbody>
</table>

**Hyphomonas**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Environment</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. strain MHS-3</em></td>
<td>Shallow marine sediments</td>
<td>Adhesion</td>
<td>[144-146]</td>
</tr>
<tr>
<td><em>H. strain VP-6</em></td>
<td>Vent region</td>
<td>Adhesion</td>
<td>[147]</td>
</tr>
<tr>
<td><em>Polaribacter sp. SM1127 psychrophile</em></td>
<td>Artic brown alga Laminaria</td>
<td>Cryoprotectant, anti-oxidant</td>
<td>[148]</td>
</tr>
<tr>
<td><strong>Pseudoalteromonas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------------------------</td>
<td>-------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><em>P. strain HYD721</em></td>
<td>Deep-sea hydrothermal vent</td>
<td>-</td>
<td>[149]</td>
</tr>
<tr>
<td><em>P. strain TG12</em></td>
<td>Sea-water</td>
<td>Metal binding</td>
<td>[150]</td>
</tr>
<tr>
<td><em>P. ulvae TC14</em></td>
<td>Marine biofilm</td>
<td>Anti-biofilm</td>
<td>[151]</td>
</tr>
<tr>
<td><em>P. ruthenica</em></td>
<td>Sea-water</td>
<td>Pseudoplastic</td>
<td>[152]</td>
</tr>
<tr>
<td><em>P. sp. strain MD12-642</em></td>
<td>Marine sediments</td>
<td>Viscosity</td>
<td>[153]</td>
</tr>
<tr>
<td><em>P. haloplankits TAC125 psychrophile</em></td>
<td>Antarctic sea water</td>
<td>-</td>
<td>[154]</td>
</tr>
<tr>
<td><em>P. sp. strain SM20310</em></td>
<td>Arctic sea-ice</td>
<td>Cryoprotectant</td>
<td>[155]</td>
</tr>
<tr>
<td><em>P. arctica KOPRI 21653 psychrophile</em></td>
<td>Sea-side sediments</td>
<td>Cryoprotectant</td>
<td>[156]</td>
</tr>
<tr>
<td><em>P. sp. CAM025 psychrophile</em></td>
<td>Particles form Antarctic sea</td>
<td>Adhesion</td>
<td>[157]</td>
</tr>
<tr>
<td><em>P. sp. CAM036 psychrophile</em></td>
<td>Particles from Southern Ocean</td>
<td>-</td>
<td>[157]</td>
</tr>
<tr>
<td><em>P. sp. SM9913 psychrophile</em></td>
<td>Deep-sea sediment</td>
<td>Metal binding</td>
<td>[158]</td>
</tr>
<tr>
<td><em>P. sp. MER144 psychrophile</em></td>
<td>Terra Nova Bay, Ross Sea Antarctic</td>
<td>-</td>
<td>[159]</td>
</tr>
<tr>
<td><strong>Pseudomonas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. sp. NCMB 2021</em></td>
<td>Not reported</td>
<td>metal binding (A)</td>
<td>[160]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adhesion (B)</td>
<td></td>
</tr>
<tr>
<td><em>P. sp. S9 psychrophile</em></td>
<td>Polar basin</td>
<td>Adhesion</td>
<td>[161]</td>
</tr>
<tr>
<td><em>P. sp. WAK1</em></td>
<td>Brown seaweed Undaria pinnatifida</td>
<td>Anti-cancer</td>
<td>[162]</td>
</tr>
<tr>
<td><em>P. stutzeri 273</em></td>
<td>Marine sediments</td>
<td>Anti-biofilm, antioxidant</td>
<td>[163]</td>
</tr>
<tr>
<td><em>P. sp. ID1</em></td>
<td>Marine sediments</td>
<td>Cryoprotectant</td>
<td>[164]</td>
</tr>
<tr>
<td><strong>Rhodococcus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. sp. 33</em></td>
<td>Contaminate site near a chemical plant</td>
<td>-</td>
<td>[165, 166]</td>
</tr>
<tr>
<td><em>R. erythropolis PR4</em></td>
<td>Ocean</td>
<td>Emulsifier</td>
<td>[167]</td>
</tr>
</tbody>
</table>
Biosurfactants. Biosurfactants are one such extracellular amphiphilic compounds produced by Halobacteria especially when grown on hydrophobic substrates. Biosurfactants are a structurally diverse group of surface-active substances produced by microorganisms [177]. All biosurfactants are amphiphiles and consist of a polar (hydrophilic) and a non polar (hydrophobic) group. They are used as emulsifiers, foaming and dispersing agents. In comparison to the chemically synthesized biosurfactants they are considered to be environmental friendly, biodegradable and non-hazardous. They are active at extreme temperatures, pH and salinity and can be produced from industrial wastes and by-products. Because of their potential advantages they are widely used in industrial and medicinal applications. Biosurfactants can also be produced from cheap raw materials like rapeseed oil, potato process effluents, oil refinery waste, cassava flour wastewater, curd whey and distillery waste, sunflower oil etc. [178]. In recent years, much attention has been directed towards biosurfactants due to their broad range of functional properties and diverse synthetic capabilities of microbes. The most significant advantage of a microbial surfactant over chemical surfactant is its ecological acceptance [177]. Biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil. Due to their unique properties and applicaiton, identification of new biosurfactant producing microbes are in great demand. There are reports of biosurfactant production by many halophilic bacteria such as Bacillus circulans, Kocuria marina, Halomonas sp., Planococcus maritimus [179, 180].

Fermented food. The industrial production of fermented products, like soy sauce and fish sauce that uses the degradative properties of halophiles. The fermentation of salty foods such as Chinese fermented beans, salty cod, salted anchovies,
sauerkraut, often involves halobacteria as essential ingredients. Halophiles including *Halobacteria, Halococci* have been isolated from various food sources including fermented foods and sauces, including Kimchi and Thai fish sauce [181]. A culture independent method of isolation of bacteria diversity from Kimchi showed the presence of many halophilic bacteria including lactic acid bacteria [182].

**Probiotics.** Probiotics are live microorganisms thought to be beneficial to the host organism. These are commonly consumed as part of fermented foods with specially added active live cultures. Recently halophilic lactic acid bacteria *Tetragenococcus halophilus* was isolated from soy sauce and showed to possess an immunomodulatory activity that promotes helper type 1 immunity in humans. Thus this strain can be efficiently used as probiotics for humans [183]. Similarly, the use of halophilic *Bacillus sp.* as probiotics for shrimps has been reported from India [184] and also the use of *Tetragenococcus halophilus* from miso has been reported from Japan [185].

**Pharmaceutical applications.** The mechanism of drug resistance among the pathogenic microorganisms lessens the efficacy of available antibiotics and this in turn strengthens the need to search new antibiotics. Marine microbes are continuously explored for production of novel antimicrobial compounds. Bioactive compounds from halophilic bacteria have typical features because of their varied environmental conditions (pH, temperature, salinity, pressure, etc). Among marine microorganisms, halophilic bacteria are recognized as most promising prokaryote for novel bioactive metabolite production. There are several reports of production of antimicrobial metabolites, antimicrobial biosurfactants and anticancer agents such as *Staphylococcus aureus, Bacillus sp.* [186, 187].

**Environmental applications.** The large numbers of contamination sites are often saline to hypersaline and halophiles are prevalent in such environments making their significant utilization in bioremediation of contaminants. The accelerated industrial activities such as mining and metal plating resulting in pollution, due to the release of the high amount of organic and heavy metals into the environments. These toxic compounds are often found in runways and accumulate near seashores. Due to the evaporitic nature of hypersaline environments, heavy metals are frequently found in concentrated brine. As a result, many halophiles have developed tolerance to heavy metals [188, 189]. The use of halophilic bacteria for bioremediation of Cd has been reported by Solanki and Kothari [190]. The textile industry produces a large quantity of polluted wastewater containing azo dyes, phenol and other toxic anions. These effluents are highly saline with typical salt concentration of 15-20%. Recently, a halophilic bacterium *Kocuria rosea* has been reported to decolorize triphenyl methane dyes like malachite green, crystal violet and methyl violet [191]. Similarly, fluorides are prevalent in environment and have cytotoxic effect on humans. A halophilic bacteria *Bacillus flexus* has been reported to reduce fluoride concentration up to 67.45% in contaminated soil [192]. These halophilic microbes play a bioremediative role by transforming these anions and xenobiotics into less toxic forms.

**Agricultural applications.** Soil salinity is a naturally occurring problem in various parts of the world, but the exhaustive use of chemical fertilizers, inadequate cultivation practices, and improper irrigation schemes management have resulted in exacerbated salt concentrations in soil. Salinity is one of the important abiotic stresses that limit the plant growth and crop productivity. In addition, salinity also affects nutrient uptake by
plants. Agriculture under saline conditions already presents major challenges in many countries. Application of halotolerant plant growth promoting rhizobacteria (PGPR) is an important strategy by which cultivation in saline soils can be improved [193]. Many reports have been published stating the beneficial effects of inoculation of halotolerant bacteria on plant growth under salt stress conditions such as Micrococcus sp. on cowpea, Bracybacterium saurashtrense on groundnut etc [194, 195]. Halotolerant bacterial and their role in plant growth promotion under saline condition are mentioned in table III.

### Table III
Role of halophilic microbes in plant growth promotion under the saline environments

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Strain</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas fluorescens</td>
<td>153</td>
<td>Salinity stress, growth</td>
<td>[199]</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>108</td>
<td>Salinity stress, growth</td>
<td>[199]</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>TSAU1</td>
<td>Growth and salinity tolerance</td>
<td>[198]</td>
</tr>
<tr>
<td>Arthrobacter sp.</td>
<td>AS 18</td>
<td>Growth and salt stress</td>
<td>[200]</td>
</tr>
<tr>
<td>Bacillus licheniformis</td>
<td>RS656</td>
<td>Ameliorates salt stress, nutrient uptakes</td>
<td>[201]</td>
</tr>
<tr>
<td>Brevibacterium iodinum</td>
<td>RS16</td>
<td>Ameliorates salt stress, nutrient uptakes</td>
<td>[201]</td>
</tr>
<tr>
<td>Nitricola lacisaponensis</td>
<td>SL 11</td>
<td>Growth salt stress</td>
<td>[200]</td>
</tr>
<tr>
<td>Zhihengliuella alba</td>
<td>RS111</td>
<td>Ameliorates salt stress, nutrient uptakes</td>
<td>[201]</td>
</tr>
<tr>
<td>Metarhizium anisopliae</td>
<td>LHL07</td>
<td>Growth and salt stress</td>
<td>[202]</td>
</tr>
<tr>
<td>Azotobacter chroococcum</td>
<td>C5</td>
<td>Alleviated the saline stress</td>
<td>[203]</td>
</tr>
</tbody>
</table>

#### VII. CONCLUSION AND FUTURE PERSPECTIVES

Both academic and industrial research mainly focuses on marine microorganisms due to its impulsive potential. The importance of halophilic bacteria as potential applications has been recognized in various fields varying from antioxidants, sunscreens, compatible solutes and hydrolytic enzymes. These biomolecules are valuable and show commercial potential for food, pharmaceutical, biomedical, industrial and environment. The availability of these halophilic biomolecules and their advantages in production can be optimized to produce sustainable yields at industrial scale. The recent availability of various complete genome sequences of halophiles together with advances in omics technologies
would further provide new opportunities for exploration, discovery and identification of unique properties and/or novel biomolecules derived from halophiles in the future.

References


[34] De-Dekker, P. 1983. Australian salt lakes: Their history, chemistry, and biota-a review, Hydrobiologia, 105, 231-244.


