

# Diversity and production of extracellular polysaccharide by halophilic microorganisms

## Abstract

Halophilic microorganisms derived from diverse thalassohaline and athalassohaline environments including marine estuaries, saline and soda lakes, inland solar salterns and acidic habitats are categorized as slight-, moderate- and extreme halophiles according to their NaCl requirements. Taxonomic studies with culturable diversities of halophiles revealed that they belong to both Archaea and Bacteria representing the families Halobacteriaceae, Methanosarcinaceae and the class Gammaproteobacteria. As adaptive strategies against harsh salt stresses, majority of halophiles often synthesize and accumulate extracellular polysaccharides (EPS) which differ significantly in terms of their physical, chemical and material properties. So far the novelty in structure and functions of exopolysaccharides are concerned, producer strains belonging to the genera *Halomonas* and *Haloferax* have attracted the main attention. However, EPS producing strains belonging to the genera *Idiomarina*, *Salipiger* and *Alteromonas* are not uncommon. Through process optimization and metabolic regulation a number of potent halophilic strains have been found to produce copious amount of EPS indicating its commercial viability. Moreover, the significance of production, physico-chemical and biological properties along with the possible applications of halophilic EPS in industry and biotechnology have also been highlighted.

**Keywords:** halophiles, archaea, bacteria, thalassohaline environment, athalassohaline environment, solar salterns, compatible solutes, extracellular polysaccharides

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Jhuma Biswas, Paul AK

Department of Botany, University of Calcutta, India

**Correspondence:** Paul AK, Department of Botany, University of Calcutta, West Bengal, Kolkata, India, Tel 91-033-2461-5445/4959/5277/4711, Fax 0332461-4849, Email amalk\_paul@yahoo.co.in

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## Introduction

Halophiles, the salt-loving microorganisms are distinguished by their characteristic requirement of high salt concentration for growth and have evolved physiological and genetic features to survive in hypersaline environments. In addition, factors like temperature, pH, availability of oxygen and nutrients, as well as solar radiations plays an important role in determining the growth of halophiles. Ever since the time of Larsen<sup>1</sup> and Kushner,<sup>2</sup> these organisms, depending on their salt dependence and tolerance have been distinguished as slight halophiles, moderate halophiles, and extreme halophiles.<sup>3</sup> Most of the halophiles are inhabitants of hypersaline waters and soils, salt or salt deposits and salted products<sup>4,5</sup> Multi-pond solar salterns, representing typical thalassohaline water systems with salinities ranging from seawater salinity to halite saturation have been correlated with the changes in microbial community densities.<sup>6</sup> Likewise, aerobic, anaerobic and facultative anaerobic microbes belonging to domains Archaea and Bacteria have been recovered from the athalassohaline waters of the Dead Sea, Great Salt Lake, hypersaline lakes in Antarctica, Lake Magadi etc.<sup>7</sup>

The halophiles follow two different strategies to cope with the osmotic stress exerted by the saline environment. Halophilic Archaea maintain an osmotic balance of their cytoplasm with the hypersaline environment by accumulating high concentrations of inorganic ions in the cytoplasm (salt in cytoplasm strategy). In contrast, halophilic or halotolerant bacteria adapt themselves by accumulating high concentrations of various organic osmotic solutes (compatible solutes).<sup>4</sup> Apart from these, the deleterious hypersaline environment forced the halophiles to produce a variety of biomolecules, pigments, biosurfactants, proteins, extracellular polysaccharides (EPS) and intracellular polyester polyhydroxyalkanoates, which have attracted the attention of microbial biotechnologists.<sup>8</sup>

Production of extracellular polysaccharides by halophilic Archaea and Bacteria has been reported by several workers and the members of the genus *Halomonas* have been identified as the most potential producers.<sup>9-11</sup> While the chemistry, structure and functions of microbial extracellular polymeric substances in general have been highlighted with special emphasis on microbial ecology, medicine, dairy industry, formation of biofilms, and environmental biotechnology; their role in the remediation of heavy metals, toxic compounds and dyes from the anthropogenic environments could not be ruled out.<sup>12</sup> Survey of literature have clearly indicated that the information pertaining to the production and characterization of extracellular polysaccharides by the wide variety of halophilic microorganisms isolated from hypersaline environments are inadequate.<sup>9,13</sup> However, there is an increasing demand for the production of extracellular polysaccharides by the halophiles with properties better than those of the existing ones. The present review is aimed at to explore the diversity of halophilic microorganisms from saline and hypersaline ecosystems and also to evaluate their potential for production of extracellular polysaccharides with special emphasis on their significance, regulation, properties and applications.

## Diversity of halophiles

The culturable diversity of halophilic microorganisms from hypersaline environments includes both the extremely halophilic Archaea and moderately halophilic Bacteria. Halophilic archaea represented by the family *Halobacteriaceae* is currently comprised of 47 genera and 165 species and *Methanosarcinaceae* which includes 4 genera and 7 species.<sup>14,15</sup> While the members of *Halobacteriaceae* are aerobic or facultatively anaerobic and generally red-pigmented ones, the methanogens obtain their energy form methylated amines under anaerobic conditions.<sup>14</sup> Moderate or extremely halophilic bacteria isolated from diverse environments are currently represented by a

large number of species included under the phyla of *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Spirochaetes*, *Bacteroidetes*, *Thermotogae*, *Cyanobacteria* and *Tenericutes*.<sup>15</sup> Members belonging to these phyla constitute a heterogeneous assemblage of microorganisms with diverse physio-biochemical activities and morphological variations.<sup>15</sup> The class *Gammaproteobacteria* of *Proteobacteria* contains the largest number of moderately halophilic genera and the members of the family Halomonadaceae represented the best studied and most important genera.<sup>15,16</sup>

Quesada et al.<sup>17</sup> following the conventional plate count method, analyzed the hypersaline soils of multi-pond salterns near the Mediterranean coast in Alicante, Spain and reported the predominance of Gram-negative halophiles belonging to the genera of *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Flavobacterium* and *Acinetobacter*. Gram-positive rods and cocci were assigned to the genera *Bacillus*, *Nesterenkonia*, *Arthrobacter*, *Marinococcus*, *Staphylococcus*, *Corynebacterium*, *Brevibacterium*, *Nocardia* and *Actinomyces*. Subsequently, species assigned to the genera *Planococcus* and *Sporosarcina* were also added to the list.<sup>18</sup> Garabito et al.<sup>19</sup> also isolated 71 halotolerant Gram-positive endospore forming rods from saline soils and sediments of salterns located in different parts of Spain and tentatively assigned them to the genus *Bacillus*. Numerical taxonomic studies have been conducted on inland, athalassohaline salterns near Granada, Spain<sup>20</sup> and Chile<sup>21</sup> and the isolated strains were assigned to moderately halophilic genera *Halomonas*, *Vibrio*, *Alteromonas* and *Acinetobacter*. In a more selective diversity study, Ghozlan et al.<sup>22</sup> isolated 90 Gram-positive and Gram-negative moderately halophilic bacterial strains from coastal solar salterns, salt marshes and salt lakes of Alexandria, Egypt. The Gram-negative isolates belonged to the genera *Pseudoalteromonas*, *Flavobacterium*, *Chromohalobacter*, *Halomonas* and *Salegenibacter* while, the Gram-positive strains were included in the genera *Halobacillus*, *Salinicoccus*, *Staphylococcus* and *Tetragenococcus*. They concluded that greater diversity occurred in the inlet and lower salinity ponds.

Yeon et al.<sup>23</sup> studied the culturable diversity of moderately halophilic, organotrophic bacteria from solar salterns of Taean-Gun, Chungnam Province, Korea following RFLP analysis of PCR amplified 16S rDNA and phylogenetic analysis of the partial 16S rDNA sequences. Based on these, 64 strains were segregated into genera like *Vibrio*, *Pseudoalteromonas*, *Halomonas*, *Alteromonas* and *Idiomarina*. Litchfield et al.<sup>24</sup> studied the seasonal changes of halophilic community present in saltern at Eilat, Israel, as well as Cargill Solar Salt Plant in Newark, California by using traditional and molecular techniques. Recently, Mutlu and Guven<sup>25,26</sup> employed a combination of denaturing gradient gel electrophoresis of 16S rRNA gene fragments of PCR amplified DNA extracted from the water samples of the saltern and 16S rRNA gene library analysis to identify the bacterial diversity of Camalti solar saltern in Turkey and explored a total of 42 isolates, which belonged to the genera *Halobacillus*, *Virgibacillus* and *Halomonas*.

The diversity analysis of bacteria and archaea present in El Golea's sebkha of Algerian Sahara<sup>27</sup> has led to the isolation of 471 strains belonging to 31 different genera of halophilic bacteria and archaea. The bacterial genera include *Vibrio*, *Pseudomonas*, *Staphylococcus*, *Pasteurella*, *Streptococcus*, *Salmonella*, *Shigella*, and *Escherichia*. However, only 52 isolates belonged to the halophilic aerobic archaea which were placed in the genera *Halobacterium*, *Halococcus*, *Natronobacterium*, *Haloferax*, *Natronococcus*, *Haloarcula* and *Natrinema*.

The occurrence of red pigmented haloarchaeal communities has been documented from the north arm of the Great Salt Lake,<sup>28</sup> the Dead Sea<sup>29</sup> and hypersaline alkaline lake, Lake Magadi.<sup>30</sup> Haloarchaea isolated from coastal salt-marsh sediments able to grow at lower salinities have also been reported.<sup>31</sup> Birbir and Sesal<sup>32</sup> studied the extreme halophilic bacterial communities of Sereflikochisar salt lakes of Turkey by using conventional biochemical features, while Birbir et al.<sup>33</sup> characterized extremophilic communities in Tuzkoy salt mine and the adjacent Kaldirim and Kayacik salterns using positive PCR amplification and denaturing gradient gel electrophoresis (DGGE) analysis of DNA sequences which revealed the phylogenetic inclusion of the isolated strains within the genera *Halobacterium*, *Haloarcula*, *Natrinema* and *Halorubrum*. Elvi et al.<sup>34</sup> isolated extremely halophilic strains from the Ayvalik saltern in the north-eastern part of Turkey. Similarly, Enache et al.<sup>35</sup> isolated extreme halophiles from Telega salt lake, of Romania and identified them as members of the genus *Haloferax*.

Bacterial and archaeal aerobic communities were recovered from sediments of El-Djerid salt lake in Tunisia.<sup>36</sup> By using phenotypic and phylogenetic approaches, the authors found that the members of the domain Bacteria belonged to *Salicola*, *Pontibacillus*, *Halomonas*, *Marinococcus* and *Halobacillus*, whereas, the only member of domain Archaea was represented by *Halorubrum*. Recently, Kim et al.<sup>37</sup> analyzed the hypersaline sediment of Death Valley National Park and documented the availability of the genera *Halorubrum*, *Halococcus*, *Haloarcula*, *Halorhabdus* and *Halobacterium*. Studies on halophilic diversity from hypersaline environments of India are not uncommon. Dave and Desai<sup>38</sup> isolated halophiles from marine salterns of Bhavnagar, Gujarat, India and majority of them belonged to Archaeal domain. Presence of Gram-positive alkalitolerant moderate halophiles of the genera *Bacillus*, *Micrococcus*, *Planococcus*, *Vagococcus* as well as Gram-negative representatives of *Paracoccus*, *Halomonas* and *Providencia* were recorded from Alkaline Lonar Lake of Maharashtra, India.<sup>39</sup> An elaborate account of the community structure of the halophilic archaea at initial and crystallization stage of salt production in solar salterns of Goa was documented by Mani et al.<sup>40</sup> The isolates obtained during the pre-salt harvesting phase, belonged to *Halococcus* spp. while, at salt harvesting phase, *Halorubrum*, *Haloarcula*, *Haloferax* and *Halococcus* were predominant.

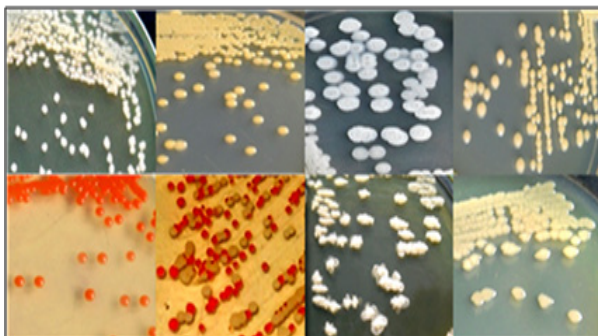
Surve et al.<sup>41,42</sup> in their survey of moderate halophiles reported the presence of *Virgibacillus pantothenticus*, *Bacillus atrophaeus*, *Corynebacterium diphtheria* and *Idiomarina zobellii* from salt pans of Goa using FAME and 16S rDNA sequence analysis. Recently, Sardar and Pathak<sup>43</sup> investigated the halophilic microbiota of solar salterns of Mumbai, India and recorded *Halorubrum*, *Haloferax* and *Halobacterium* as the extremely halophilic genera while, moderate halophilic members belonged to *Halomonas*, *Halobacillus*, *Pseudomonas*, *Salicola* and *Halovibrio*.

Diversity of halophiles in solar salterns of Tamilnadu, India revealed the presence of representatives of the family Halobacteriaceae which were dominated by members of the genera *Haloferax*, *Halorubrum*, *Haloarcula*, *Halobacterium* and *Halogeometricum*<sup>44</sup> while, that of Kovalam salt pans in Kanyakumari belonged to *Staphylococcus*, *Halobacillus*, *Halococcus*, *Natronobacterium* and *Halobacterium*.<sup>45</sup> The halophilic bacterial diversity along the coastal regions of Karnataka, India showed the predominance of the genera *Virgibacillus*, *Halobacillus*, *Salinibacillus*, *Nesterenkonia*, *Pontibacillus* and *Staphylococcus*.<sup>46</sup> Similarly, moderately halophilic aerobic bacteria belonging to the genera *Halomonas*, *Salinicoccus*,

*Bacillus*, *Aidingimonas*, *Alteromonas*, and *Chromohalobacter* were isolated by Biswas and Paul<sup>47</sup> from multi-pond solar salterns along the coasts of Gujarat (Figure 1), Orissa, and West Bengal, India. Colony morphology of some of the representative members are shown in Figure 2.



**Figure 1** Typical multi-pond solar salterns at Jogrinar located in Kachchh districts of Gujarat, India.



**Figure 2** Variations in colony morphology of halophilic bacteria isolated from soil and water samples of multi-pond solar salterns distributed along the coasts of India.

## Production of extracellular polysaccharides (EPS) by halophiles

Ever since the discovery of “dextran”, the first microbial polysaccharide in 1880, continued search for novel polysaccharides from microbial resources have resulted in the discovery of a number of extracellular polymers. Some of them have been commercially accepted, while others are at various stages of development. Production of extracellular polysaccharides by halophilic bacteria highlighting their properties, distribution and possible applications has been done over the last couple of years. The main EPS producers so far reported are represented by members of the family Halomonadaceae and Alteromonadaceae. Members of the genus *Halomonas*, the most common moderately halophilic bacteria, have been identified as potential EPS producers synthesizing polymers of diverse physico-chemical properties.<sup>9-11</sup>

The first study on EPS production by members belonging to the genus *Halomonas* was made by Quesada et al.<sup>48</sup> They have optimized the cultural parameters of EPS production by *H. eurihalina* and recorded a maximum production of 2.8 g/L. Along with pseudoplastic behavior, the purified sulfated EPS also showed an unusual property to jellify at acidic pH. Moreover, the sulfate content of the EPS and cations affected the rheology of the EPS. Bejar et al.<sup>49</sup> also established similar

rheological behavior of EPS isolated from strains of *H. eurihalina*. However, substrate specific emulsifying activity of the sulfated EPS in presence of hydrocarbon and oil was established by Calvo et al.<sup>50</sup> EPS produced by *H. eurihalina* in hydrocarbon supplemented medium showed enhanced emulsifying activity, but reduced viscosity. This was possibly due to a change in chemical composition of the EPS produced in hydrocarbon and oil supplemented media.<sup>40-51</sup>

*Halomonas maura* was first introduced as EPS producing isolate by Bouchotroch et al.<sup>9, 52</sup> during isolation and screening of EPS producing moderate halophiles. Later, Arias et al.<sup>53</sup> isolated an anionic, sulfated heteropolysaccharide, mauran from *H. maura* S-30, which under optimized cultural condition produced 3.8 g/L of highly viscous EPS. Mauran displayed pseudoplastic and thixotropic rheological properties.

Under optimum cultural conditions EPS production by *H. ventosae* and *H. anticariensis*<sup>54</sup> was 0.28 g/L and 0.49 g/L respectively. Though, the production was comparatively low, the polysaccharides exhibited high capacity of metal binding. Moreover, these exopolymers showed emulsifying activity to many hydrophobic substrates possibly due to their high protein content and low viscosity. Extracellular polysaccharide production by *H. almeriensis*<sup>11, 55</sup> have been optimized and revealed a growth associated production of 1.7 g/L of EPS. The sulfated EPS so produced was capable of emulsifying several hydrophobic substrates and binding of metal ions. More recently, Amjres et al.<sup>56</sup> characterized an extracellular polysaccharide, haloglycan produced by *H. stenophila* HK30. Under optimized cultural conditions, the strain produced 3.89 g/L of haloglycan which was highly viscous and capable of emulsifying different hydrocarbons.

*Halomonas* strain CRSS isolated from salt sediments of Antarctica produced 2.9 g of EPS per g dry cells.<sup>57</sup> Mannan and xylomannan were obtained when cells were grown on complex media. However, in presence of acetate, a fructo-glucan was produced. *Halomonas* sp. AAD6 isolated from Camalti saltern area in Turkey was found to produce high levels of levan in the sucrose containing medium and yielded 1.84 g/L of levan.<sup>58</sup> Besides these, *Halomonas* sp. V3a<sup>59</sup> was able to produce an EPS as potential biosurfactant, while *Halomonas* sp. strain TG39<sup>60</sup> produced an EPS with high uronic acid content and possessed specific binding capacity for Ca, Si, Fe, Mg, Mn and Al. EPS derived from members of the family *Alteromonadaceae* were low in viscosity and was capable of emulsifying hydrocarbons and binding of heavy metals.<sup>61</sup> *Salipiger mucosus* A3<sup>T</sup> belonging to *Alphaproteobacteria*, produced a fucose containing EPS (1.7 g/L) which showed solution properties similar to EPS of most halophilic strains.<sup>62</sup>

Optimization, isolation, and characterization of EPS produced by *H. xianhensis* SUR308 were studied elaborately.<sup>63, 64</sup> Under optimum cultural conditions of 2.5% NaCl, 3% glucose, 0.5% casein hydrolysate, the strain produced 7.87 g/L of EPS (Figure 3)<sup>65</sup> which showed antioxidant and emulsifying activity against hydrocarbons as well as oils. However, a higher yield of EPS (12.98 g/L) was successfully obtained with mutants of this strain.<sup>66</sup> Massive amounts of EPS are also excreted by members of the haloarchaeal genera *Haloferax*, *Haloarcula*, *Halococcus*, *Natronococcus* and *Halobacterium*. Anton et al.<sup>67</sup> were the first to report the production of EPS by an archaeobacterium, *Haloferax mediterranei* (ATCC 33500). The structure of the neutral extracellular polysaccharides of *Haloferax gibbonsii* (ATCC 33959) has been determined by Paramonov et al.<sup>68</sup> while, Parolis et al.<sup>69</sup> elucidated the structure of

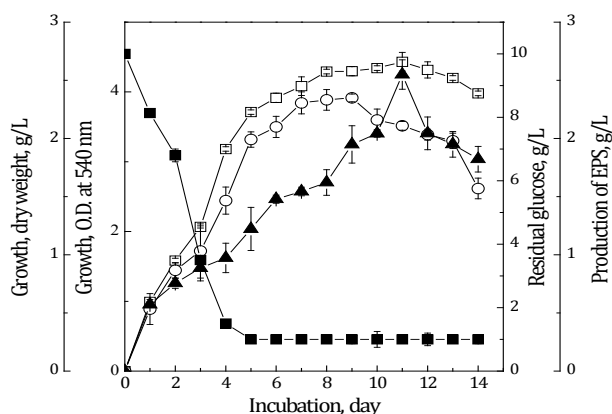
a linear, acidic exopolysaccharide from *Haloferax denitrificans*. In a screening program, Nicolaus et al.<sup>70</sup> isolated three obligatory halophilic microorganisms (T5, T6, and T7) from an unexplored site in Tunisia. These strains produced sulfated extracellular polysaccharides

in a minimal medium containing glucose as sole carbon source with EPS yields ranging from 35 to 370 mg/L. A comparative account of EPS production by moderate and extreme halophiles so far reported is presented in Table 1.

**Table 1** Production of extracellular polymeric substances by moderate and extremely halophilic microorganisms

Microorganism	Medium	Carbon source and nacl conc. (%)	Phase of maximum eps production	EPS production	References
<b>Moderate Halophile</b>					
<i>Alteromonas hispanica</i> F32T	MY Medium	Galactose; 7.5% NaCl	Stationary phase	1.0 g/L	Mata et al. <sup>61</sup>
<i>Halomonas alkaliantarctica</i> strain CRSS	Medium B	Maltose; 7.5% NaCl	ND	2.9 g/g	Poli et al. <sup>57</sup>
<i>H. alkaliphila</i>	Medium 2	1% Glucose; 10% NaCl	ND	ND	Romano et al. <sup>71</sup>
<i>H. almeriensis</i> MST	MY Medium	1% Glucose; 7.5% NaCl	Stationary phase	1.7 g/L	Llamas et al. <sup>11</sup>
<i>H. anticariensis</i>	MY Medium	1% Glucose; 7.5% NaCl	Stationary phase	0.3-0.5 g/L	Mata et al. <sup>54</sup>
<i>H. eurihalina</i> F2-7	MY Medium	1% Glucose; 7.5% NaCl	Stationary phase	1.4 g/L	Quesada et al. <sup>48</sup>
<i>H. eurihalina</i> AI-12T				2.8 g/L	
<i>H. maura</i> S-30	MY Medium	1% Glucose; 2.5% NaCl	Stationary phase	3.8 g/L	Arias et al. <sup>53</sup>
<i>H. rifensis</i>	MY Medium	1% Glucose; 7.5% NaCl	ND	ND	Amjres et al. <sup>72</sup>
<i>H. smyrensis</i>	Medium B	Glucose; 10% NaCl	Stationary phase	ND	Poli et al. <sup>73</sup>
<i>H. stenophila</i>	MY Medium	1% Glucose; 5% NaCl	Stationary phase	3.89 g/L	Llamas et al. <sup>74</sup>
<i>H. ventosae</i> AI-12T	MY Medium	1% Glucose; 7.5% NaCl	Stationary phase	0.28 g/L	Mata et al. <sup>54</sup>
<i>H. ventosae</i> AI-16				0.30 g/L	
<i>Halomonas</i> sp.AAD6	CD Medium	5% sucrose; 13.5% NaCl	Exponential phase	1.073 g/L	Poli et al. <sup>58</sup>
<i>H. xianhensis</i> SUR308	MY Medium	3% glucose, 2.5% NaCl	Stationary phase	7.87 g/L	Biswas et al. <sup>65</sup>
<i>Idiomarina fontislapidosi</i> F-23T	MY Medium	Glucose; 7.5% NaCl	Stationary phase	1.4 g/L	Mata et al. <sup>61</sup>
<i>I. rambicola</i> R-22T	MY Medium	Glucose; 7.5% NaCl	Stationary phase	1.5 g/L	Mata et al. <sup>61</sup>
<i>Palleronia marisminoris</i>	MY Medium	1% Glucose; 5% NaCl	Stationary phase	ND	Martinez-Checa et al. <sup>75</sup>
<i>Salipiger mucosus</i> A3T	MY Medium	1% Glucose	Stationary phase	1.2 g/L	Llamas et al. <sup>52</sup>
<b>Extreme Halophile</b>					
<i>Haloarcula japonica</i>	Minimal Medium	Glucose	Stationary phase	35-350 mg/L	Nicolaus et al. <sup>70</sup>
<i>Haloferax denitrificans</i>	Nd	Glucose	ND	ND	Parolis et al. <sup>69</sup>
<i>H. gibbonsii</i>	Nd	Glucose	ND	ND	Paramonov et al. <sup>68</sup>
<i>H. mediterranei</i>	Minimal Medium	Glucose/ yeast extract	Stationary phase	3 mg/mL	Anton et al. <sup>67</sup>
<i>Halopiger aswanensis</i>	SG Medium	Na-citrate	ND	ND	Hezayen et al. <sup>76</sup>

"MY Medium", Malt extract-Yeast extract medium; "SG medium", Sehgal and Gibbons medium; "CD medium", Chemically defined medium; ND= Not documented.



**Figure 3** Time course of growth (O.D. at 540 nm (□), Dry weight in g/L (○), glucose utilization (■) and production of EPS (▲) by *H. xianhensis* SUR308 (Adopted from Biswas et al.).<sup>63</sup>

## Significance of EPS production

The extracellular polysaccharides in general play a wide variety of biological functions including prevention of desiccation, protection from environmental stresses, adherence to surfaces, pathogenesis and symbiosis.<sup>77</sup> In addition EPS can sequester nutrient materials from the surrounding environment,<sup>78</sup> facilitates biofilm formation and prevent access of antimicrobial agents into the biofilms.<sup>79</sup> The EPS molecules are regarded as the major factor influencing the microbial adhesion process. EPS molecules strengthen the interactions between the microorganisms and as a result they determine the cell aggregates formation process on the solid surface.<sup>80</sup> Parker et al.<sup>81</sup> established that removal of exopolysaccharides from the cells of *Bacillus* spp. decreased their attachment to stainless steel surfaces.

Bacteria living within EPS are believed to be about 1000 times more resistant to antibacterial compounds than planktonic cells.<sup>82</sup> Sauer and Camper<sup>83</sup> confirmed that increasing hydrophilic value of

bacterial cell surface often restrict the penetration of antimicrobial agents with hydrophobic character. The functional groups of exopolysaccharides react with antimicrobial agents and prevent the diffusion process to cytoplasm. However, it is recognized that killing properties of antibiotics are increased when all possible binding sites in the EPS matrix are saturated.

## Mechanisms and regulation of EPS production

Over the last couple of years significant progress has been made in understanding the biochemical and genetic mechanisms and regulation of different classes of EPS production by wide diversity of bacterial species. With the exception of a few, majority of bacterial EPS are synthesized intracellularly and secreted to the extracellular environment. In general, regulation of such intercellular biosynthesis of EPS is determined by various physiological and metabolic parameters of the producer cells. These include the availability of sugar precursors, energy for building the repeating units, expression of enzymes for polymerization and transportation of building units across the membrane.<sup>84</sup> In addition, several factors such as medium composition, culture age, type of carbon and nitrogen sources, carbon to nitrogen ratio, pH, temperature and aeration have definite impact on EPS production. Cellular adaptation to limiting factors, such as stress conditions, osmolarity of the medium, ammonium and phosphate availability also influence EPS biosynthesis in a coordinated fashion. Recently, Ates<sup>85</sup> has analyzed the application of omics technology and system biology tools in understanding the microbial EPS biosynthesis mechanism and regulation and pointed out that the general mechanisms of bacterial polysaccharide production involve Wzx/Wzy-dependent pathway, the ATP binding cassette (ABC) transporter-dependent pathway and the synthase-dependent pathway, while the extracellular synthesis is accomplished by the use of a single sucrose protein. On the contrary, information related to the genetics of microbial EPS biosynthesis particularly the identification of genes involved in the assembly of repeat units, polymerization, transportation and regulation are known only for the biosynthesis of xanthan, levan and dextran, however, genetic data pertaining to EPS biosynthesis by halophilic microorganisms is scanty.<sup>86</sup>

Mauran, the EPS produced by *Halomonas maura* S-30 is similar to xanthan and has interesting functional properties that make it suitable for use in food and pharmaceutical industries and biotechnology. Analysis of genes involved in mauran production was conducted by Arco and coworkers.<sup>87</sup> They identified three conserved genes, *epsA*, *epsB* and *epsC*, and demonstrated their role in the assembly and translocation of mauran. A *wzx* homologue, *epsJ*, was also found which indicates that mauran is formed by a Wzy-dependent polymerization system. This EPS-gene cluster reaches maximum activity during stationary phase, in the presence of high salt concentrations (5% w/v).

Levan, a long linear homopolymeric EPS of  $\beta$  (2-6)-linked fructose residues is produced from sucrose-based substrates by a halophilic bacterium *Halomonas smyrnensis* AAD6T.<sup>88</sup> However, there is very limited information available about the mechanisms involved in the biosynthesis of levan<sup>88</sup> and there is no report about a systematic approach to analyze levan production by *H. smyrnensis* AAD6T. Following this, systems-based approaches were applied to improve the levan production capacity of *H. smyrnensis* AAD6T. Mannitol as an effective stimulatory factor for levan production has also been analyzed systematically.<sup>89</sup> Draft genome sequence analysis by Sogutcu et al.<sup>90</sup> identified several genes related to EPS biosynthesis, including

the genes for levansucrase and ExoD. More recently, whole-genome analysis of *H. smyrnensis* AAD6T by Diken et al.<sup>91</sup> revealed Hs\_SacB gene encoding the extracellular levan sucrose which catalyzes levan synthesis from sucrose-based substrates by transfructosylation<sup>86</sup> and bears striking similarities with levansucrases from *Pseudomonas* strains.

## Halophilic eps: properties and applications

Among the halophilic bacteria, the main EPS producers belong to members of *Halomonas*, *Alteromonas* and *Idiomarina* and the polymers produced by them are characterized by distinct physical and biological properties for exploitation in biotechnological, industrial and environmental purposes. Most of the EPS produced by halophiles characteristically form both high and low viscous solutions. EPS produced by *H. maura* form highly viscous solution (800 cP) while that of *H. eurihalina* jellifying at acidic pH have been identified as viscofier and gelling agent respectively in food industries.<sup>48,53</sup> In general EPS obtained from most of the species of *Halomonas*<sup>9,49,92</sup> also possess pseudoplastic, thixotropic and shear thinning rheological properties. Moreover, EPS produced by *H. xianhensis* SUR308 was stable over a number of different stress conditions and the viscosity of the polysaccharide solution remains unaltered at extreme pH, temperature and high concentrations of salts.<sup>63</sup> Emulsification efficiency of EPS produced by different strains varies considerably. EPS produced by *H. maura*, *H. almeriensis*, *H. xianhensis* and *Salipiger mucosus* were able to emulsify hydrocarbons, crude oils, mineral oils, hexadecane, tetradecane, octane and many others.<sup>63,92</sup> Moreover, the highest anionic activity of EPS produced by *Halomonas* sp. TG39 was correlated with 100% emulsifying capacity of hexadecane.<sup>60</sup>

Antioxidant activity of extracellular polysaccharides derived from halophilic strains is not common but recently, the extracellular polysaccharide of *H. xianhensis* SUR308 has been shown to exert 43 to 72% DPPH radical scavenging activity at concentrations ranging from 0.06 to 1 mg/mL.<sup>63</sup> The sulphated EPS from *H. eurihalina* H2-7 enhanced the unspecific proliferation of human lymphocytes in response to the presence of the anti-CD3 monoclonal antibody,<sup>93</sup> while that of *H. stenophila* (B100 and N12T)<sup>74</sup> blocked the growth of human T-lymphocyte tumours.<sup>94</sup> The halophilic EPS has the property of removing toxic heavy metals and synthetic dyes present in anthropogenic environment. The EPS from *Halomonas* sp. TG39 was capable of removing methylene blue at the rate of 464 mg/g of EPS<sup>60</sup> while removal of polycyclic aromatic hydrocarbons such as naphthalene, phenanthrene, fluoranthene, and pyrene was recorded by an EPS producing strain *H. eurihalina* H-28.<sup>95,96</sup>

## Conclusion

From the above survey it is apparent that studies on the diversity of halophilic microorganisms from natural environments have received a momentum in the recent past, but the tremendous diversity of halophiles are far from being explored and exploited. Production of exopolysaccharides by a number of potent moderate and extremely halophilic Archaea and Bacteria has been optimized under laboratory conditions and found to produce the polymers in copious amounts. Such findings have generated interests for potential applications and exploration in a commercially viable manner. It may also be mentioned that mass cultivation of halophiles for EPS production and overcoming constraints of process development and bioreactor designing and construction for halophiles will help in the commercialization of the process. Finally, as an outcome,

this neglected group of microorganisms, the halophiles will find respectable position in the world of biotechnology.

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## Conflict of interest

Authors have declared that no competing interests exist.

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