International Journal of Research in BioSciences
Vol. 6 Issue 4, pp. (20-32), October 2017
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Marine bacterial exopolysaccharides [EPSs] from extreme environments and their biotechnological applications

Prashakha J. Shukla, Neelam M. Nathani, *Bharti P. Dave
Department of Life sciences,
Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar-364001 (Gujarat), INDIA

(Received June 24, 2016, Accepted December 15, 2016)

Abstract

Marine bacteria are habituated to survive in extreme environments like hot, ice cold and saline niches, polluted as well as acidic and alkaline conditions, pressure fluctuations, heavy metal accumulation, pressure, nutrient availability and CO_2, which are typically considered inhospitable for life. For survival in these extreme conditions, marine bacteria have developed new adaptive strategies. Exopolysaccharides [EPSs] are one of the most important secondary metabolites which are secreted externally to the cell surface for growth promotion, protection as barrier against predator, adhering to solid surfaces. They survive under adverse conditions like pH, temperature, salinity and nutrient starvation. Recently, the focus of researchers is towards marine bacteria that produced structurally diverse EPSs. Marine derived EPSs have reported copious biotechnological applications such as, detoxification of heavy metals, bioremediation of pollutants, immune-modulator agents, anticoagulants and cosmetic industry.

Keywords: Extreme marine environments, Marine bacteria, Exopolysaccharides [EPSs], biotechnological applications.

Introduction

Research in marine microbial biodiversity is increasing because of the marine microbes' ability to survive in extreme conditions. Marine organisms can withstand various conditions such as alkaline, hyper saline, fluctuation in temperature, pH, pressure and nutrient availability[1]. Biological and physicochemical systems of marine organisms undergo alterations with changes in the above listed conditions[2-5]. Extreme environments exert a driving force on bacteria for selection of new adaptive strategies leading to natural synthesis of novel bioactive compounds[6,7]. These compounds are secondary metabolites which are mainly produced by marine bacteria and also referred to as natural bioactive compounds. The first bioactive compound was an "antibiotic" produced by a marine actinomycete *Chainia purpurogena* in 1975[8]. Bioactive natural products with unique characteristics such as "anti-tumor and anti-cancer" properties were obtained from marine microbial diversity, sponges and Algae[9] From 1997 to 2008, 659 bioactive compounds were isolated from five major marine bacterial phyla viz., *Bacteroidetes, Firmicutes, Proteobacteria*, *Cyanobacteria* and *Actinobacteria*[10]

Extracellular polymeric substances are one of the most important bioactive compounds which are secreted outside the cell, produced by many species of Gram positive and Gram negative Bacteria, Algae, Fungi and some Archaea[11]. Extracellular polymeric substances include macromolecules, such as polysaccharides [carbohydrates], proteins, nucleic acids and lipids[12]. Extracellular polymeric substances in microbes generally represent 40-95% of polysaccharide component[13]. In 1972, for the very first time Sutherland used the term exopolysaccharides [EPS] for microbial polysaccharides[11].

EPSs are high molecular weight [HMW] carbohydrate polymers which are valuable component of extracellular polymeric material. EPSs are important and easily available carbon reservoirs for marine organisms in extreme environment, helping the microbial communities to survive under extreme conditions such as high or low temperature, salinity, nutrient availability, pH, pressure and presence of CO_2 or O_2[14]. Marine EPSs exhibit differences in molecular weight as well as in heteropolymeric composition. EPSs are generally obtained in 2 types from terrestrial as well as from marine biota: [i] covalently attached to surface /capsular type [ii] loosely attached /slime type[15,16].

Synthesis of EPSs are initiated inside the cell and then exported in the external environment. Maximum EPSs are produced during stationary phase and under nutrient limiting conditions[17-19]. The first EPS with antibacterial characteristic was produced by *Pseudoalteromonas tunicata*, which formed a biofilm on the surface of eukaryotic organisms[15,16,20,21]. EPSs of marine bacterial provenance are pervasive in nature and have unique properties. These biopolymers have significant applications in the field of glycochemistry and glycobiology in the form of Glycosaminoglycans [GAGs] and are also being extensively used for the design and preparation of therapeutic drugs[22]. Biofilm formation mainly depends on the EPSs, proteins, DNA and lipids, several studies conclude that EPSs mediated biofilm formation is closely associated with the quorum sensing [QS] systems for the production of autoinducers[23]. These QS mechanisms have been very well studied in the marine strain of *Vibrio fischeri* with two signling molecules *ain* and *lux*[24].

Present review focuses on the EPSs produced by marine bacteria with major focus towards their biotechnological applications viz., biodetoxification of metals, QS, biomedical field and biodegradation/remediation of pollutants.

Marine microbes derived EPSs: Production, structure and biosynthesis

Structure of EPSs

Marine microbes are being highly explored because of their high diversity, complex physiological and metabolic systems. Changes in environmental conditions affect the marine microbes for production of novel biomolecules. EPSs, significantly important biomolecules are often produced in response to environmental disturbances such as change in salinity, pH, nutrient requirement, osmotic stress and temperature[25,26]. The composition and structure of EPSs generally depends on the conditions of environments and ecological niche. Structural changes in EPSs have been reported in *Pseudomonas* NCIB 11264, when grown in different N, P, C sources and in stationary phase with suboptimal growth conditions[27]. A bacterium belonging to the *Alteromonas* sp. isolated from deep sea hydrothermal vent was observed to produce the highest amount of EPSs during initiation of the stationary phase and in oligotrophic conditions[28].

Most bacteria use carbohydrates as sole source of carbon and energy while amino acids or other ammonium salts as the sole source of nitrogen. Production of EPSs also relies on the type of carbon and nitrogen substrates used. Marine bacterium *Hahella chejuensis* belonging to phylum *Proteobacteria*, isolated from the sediment sample of Marado, Cheju Island, Korea has been reported to synthesize copious amount of EPSs when grown in sucrose containing medium[29]. Few other examples of EPSs producing marine microbes include psychrotolerant strain of *Pseudoalteromonas* sp*. Bsi20310* from sea-ice of Antarctic[30]. *Bacillus licheniformis* strain B3-15 originated from shallow marine vent also produced a novel EPS used as counteractive agent for immune disorder caused by Herpes virus[31]. Apart from these varied implications of marine derived EPSs, the impact of various growth conditions on the production of EPSs by marine microbes is being explored by many groups of scientists since quite a time. Maximum production of EPSs was also observed during the stationary phase in marine bacterial strains of *Alteromonas* 1644 isolated from deep sea hydrothermal vents[28], strain 4004 belonging to *Geobacillus* genus cultured from sea sand in Ischia island, Italy[32] and B3-15 a new strain of *Bacillus licheniformis,* isolated from marine hot spring volcano island[33] whereas, *Pseudoalteromonas antarctica* strain NF3, isolated from a glacial marine sludge of the South Shetland Island in Antarctica produced maximum EPSs during its exponential growth phase[30]. Strain of *Alteromonas macleodii* also has been similarly reported for maximum EPSs production in stationary phase [6000 mg/L at 60 h of incubation][15,34]. The use of some detergents such as Tween 40, Tween 80, 3-[[3-cholamidopropyl] dimethyl ammonio]-1-hydroxypropane-sulfonate [CHAPS] and Triton X has been proved to enhance the production of EPSs[35]. Similarly, in our recent study aimed at screening and isolation of EPSs producing marine microbes from Bhavnagar coast, Gujarat, India. We screened several bacteria for production of EPSs wherein *Terribacillus* sp. PS1 was the observed as one of the most potential EPSs producing marine isolate (unpublished data).

EPSs mainly occur in 2 forms: homopolysaccharides and heteropolysaccharides. The main monosaccharide component commonly found in marine EPSs are [1] pentoses like D-Arabinose, D-Ribose, D-Xylose [2] hexoses like D-Glucose, D-Galactose, D-Mannose, D-Allose, L-Rhamnose, L-Fucose [3] amino sugars like D-Glucosamine and D-Galactosamine [4] uronic acids like D-Glucuronic acids, D-Galacturonic acids and [5] other organic or inorganic components such as sulfate, phosphate and acetic acid. Molecular weight of EPSs ranges from 1×10^5 to 3×10^5 Da[14] and as per recent report marine EPSs ranges from 4 to 15 kDa[84]. Presence of uronic acids, ketal-linked pyruvate and inorganic ions such as phosphate or sulfate endows polyanionic nature to EPSs whereas some EPSs are neutral in nature. The monosaccharides have either 1,4-β- or 1,3-β- linkages which gives strong rigidity to these EPSs. The physical properties of EPSs are deeply affected by the way monosaccharides are arranged[36]. The marine bacterium *Vibrio diabolicus* isolated from a Pompei worm tube secreted EPSs that had similar amounts of glucuronic acid and hexosamine. It is a hyaluronic acid-like polymer (Figure 1)[37].

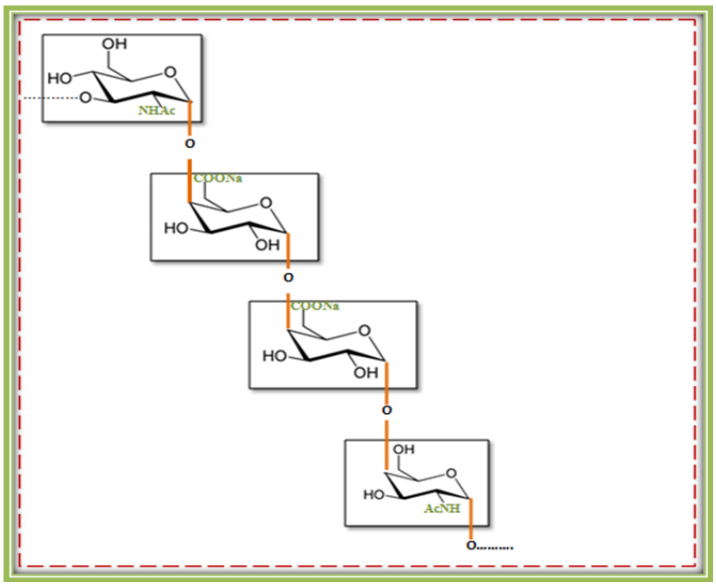

Figure 1: Chemical structure of marine bacterial EPSs produced by *Vibrio diabolicus*

A marine bacterium *Alteromonas infernus* was reported to secrete branched acidic heteropolysaccharide EPS having high molecular weight. Its monosaccharide repeating units composed of galacturonic, glucuronic acid, galactose, glucose substituted with sulfate group[38]. *Alteromonas* strain 1644 possessed the ability to produce two different kinds of EPS, and this same picularity in EPSs production were also observed in strain of *pseudomonas* sp. These EPSs differ in their viscosity and concentration of ions so the separation was difficult due to its gelling nature[39]. The tertiary structure and characteristics of EPSs mainly depends on the presence of hydroxyl and carboxyl groups. Microbial exopolymers are available either in dissolved form or as aggregates in a gel-like slime matrix. The intramolecular interactions for biofilm formation are of three types, dispersion forces, electrostatic interactions and hydrogen bonding[40]. Figure 2 indicates the EPSs structure of psychrotolerant strain *Pseudoalteromonas* sp. SM9913 isolated from deep sea. On the basis of obtained data the EPSs contains linear simple arrangement of α-1,6 glucose with elevated degree of acetylation[41].

Biosynthesis of EPSs

Biosynthesis of EPSs are initiated when carbon substrate is available as precursor to the cell. as Synthesis is initiated at the cytoplasmic membrane by using lipid as a carrier molecule. Most of the enzymatic steps for synthesis of EPSs occur inside the cell whereas polymerization and secretion is carried out in the cell envelope[42,43]. Dextran and levan are examples of extracellularly synthesized polysaccharides. Biosynthesis of bacterial EPSs occurs through four general mechanisms:-

1. Wzx/Wzy-dependent pathway
2. ABC transporter-dependent pathway
3. Synthase-dependent pathway and
4. Extracellular synthesis by use of a single sucrase protein.

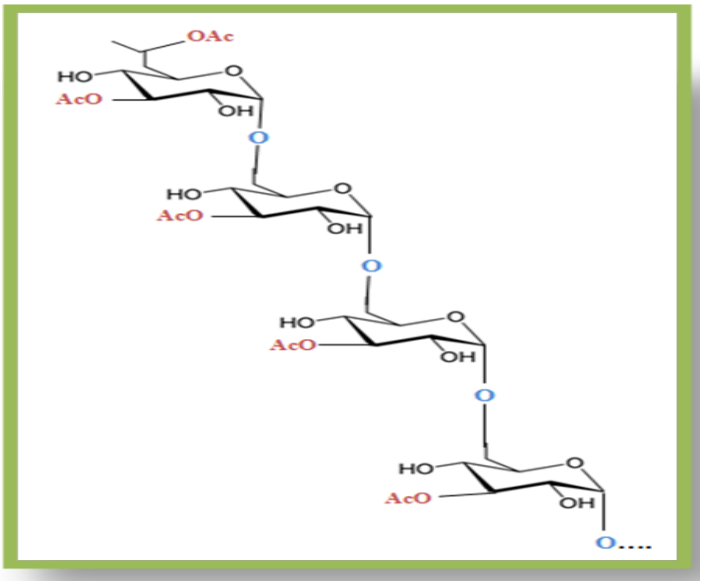

Figure 2: Chemical structure of marine bacterial EPSs produced by *Pseudoalteromonas* sp. SM9913

Wzx/Wzy-dependent pathway

In this mechanism, each individual repeating unit is linked by diphosphate anchor at inner membrane,that is assembled by several glycosyltransferases [GT's] and translocated across the cytoplasmic membrane by the protein Wzx which is also called flippase. Polymerization occurs in the periplasmic space with the help of Wzy protein, before they are exported to the surface of the cells. Polymerized repeating units are then transported from the periplasm to the surface of cell by polysaccharide co-polymerase [PCP] and the outer membrane polysaccharide export [OPX] proteins. Most of the carbohydrate polymers are assembled by the Wzx/Wzy – dependent pathway due to their highly diverse sugar patterns [4-5 sugars] and therefore is termed as heteropolymers. This pathway uses two types of enzymes: flippase [Wzx] and polymerase [Wzy] (Figure 3)[42,43].

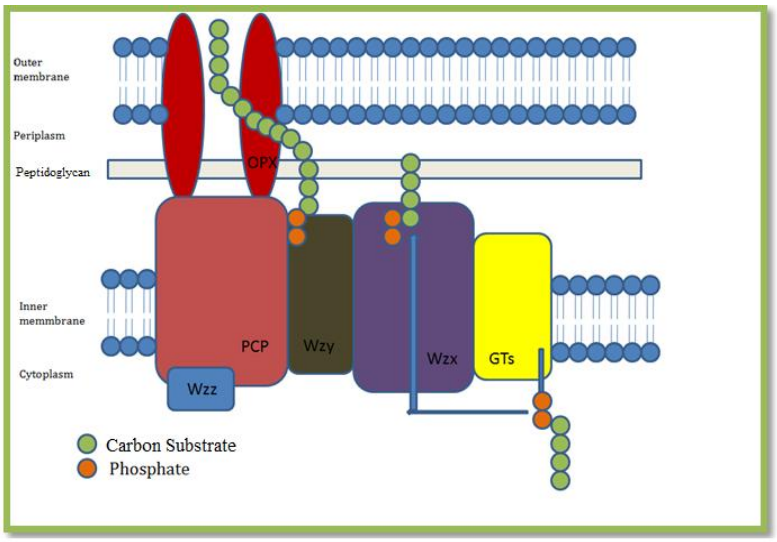

Figure 3: Wzy-dependent pathway (Modified from Schmid et al., 2015)

ABC transporter-dependent pathway

EPSs in this mechanism synthesized through the ABC-transporter-dependent pathway where the capsular polysaccharide is assembled by the action of glycosyltransferases [GT's] located in inner cytoplasmic membrane. This mechanism represents tripartite efflux pump like complex composed of ABC- transporters at the inner membrane, and periplasmatic proteins, PCP and OPX. These two proteins are closely similar to OPX and PCP proteins involved in the Wzx/Wzy- dependent pathway. CPSs produced via these mechanisms contain conserved glycolipid at the reducing terminus

composed of phosphatidylglycerol and a poly-2-keto-3-deoxyoctulosonic acid [Kdo] linker. This is one of the major difference between of the Wzx/Wzy and the ABC dependent pathways (Figure 4)[42,43].

Synthase-dependent pathway

In the synthase-dependent pathway, polymerization and transport is carried out by the synthase complex, which is complex protein envelope made up of multiple subunits and the tetratricopeptide repeat [TPR] proteins. Polymerization as well as the translocation process is performed by a single synthase protein (Figure 5)[42,43].

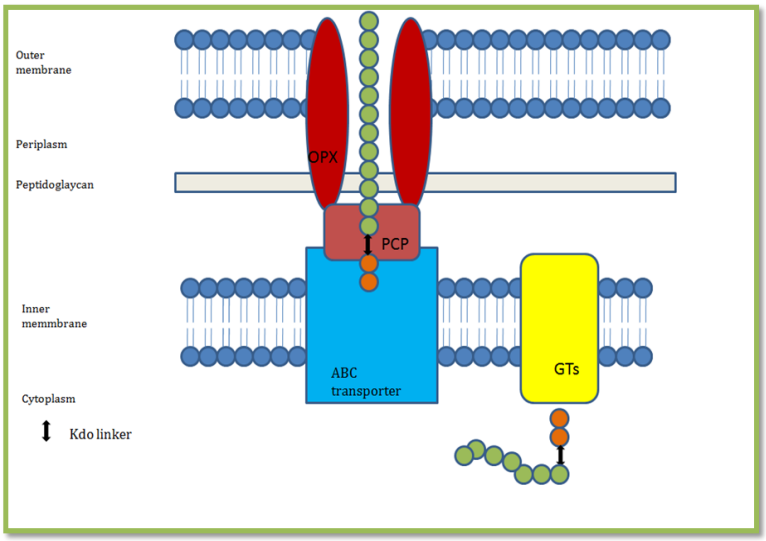

Figure 4: ABC transporter-dependent pathway (Modified from Schmid et al., 2015)

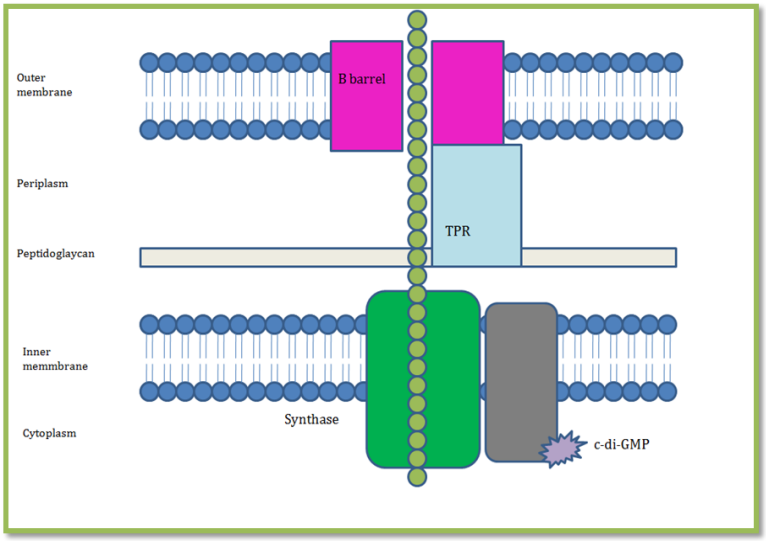

Figure 5: Synthase-dependent pathway Modified from Schmid et al., 2015)

Biotechnological Applications of EPSs produced by marine bacteria

Significance of EPSs

Capsular exopolysaccharides and proteins play a significant role in bacterial adhesion to surfaces. The initial attachment can be reversible but at the final stage of EPSs synthesis, the attachment is irreversible. Bacteria may reversibly attach on a surface for using surface associated nutrients[44]. A biochemical interaction between the organisms and the surrounding environments is made by production of EPSs. Secretion of exoenzymes is important in cycling of organic and inorganic material. This hydrated layer increases the cellular uptake of small molecules for energy and biomass[18,45]. EPSs form a biofilm which helps the organisms for nutrient transportation and horizontal

gene transfer[46]. Changes in physical environmental conditions affect the stability of EPSs[47] Capsular type of EPSs are more protective for organisms against toxic substances in the water column[48].

Biotechnological Applications of EPSs

Hot springs and hydrothermal vents offer unique source of EPSs producing bacterial diversity. *Pseudoalteromonas* strain 721 produced EPSs containing an octasaccharide repeating unit with two side chains[49]. Another EPSs exhibited gel formation and visco elastic nature at increasing temperature. This gelling property has been applied in various fields of biotechnology. *Alteromonas macleodii* is an aerobic, mesophilic bacterial strain from North Fiji basin[50], produced EPSs exhibiting metal-binding capacity up to 316 mg Pb[II]/g biopolymer[51]. Proposed applications for human welfare using this biopolymer include water treatment and removal of heavy metal pollutants. Moreover, the xanthan produced by *Alteromonas macleodii* finds application as a food-thickening agent[52]. In addition, it has bone healing tendency as it showed ability to promote the adhesion of rat calvaria osteoblastic cells in vivo and is also used in treatment of cardiovascular diseases as suggested by Colliec *et al*[52]. Some marine thermophilic bacilli strains are reported to produce novel EPSs. *Bacillus licheniformis* B3-15 cultivated from hot marine shallow vents, Vulcano island of Italy produced novel EPSs with antiviral activities. *B. licheniformis* T4 is also isolated from the Panarea island of Italy produced EPSs with fructo-fucan polymer structure showed significant cytotoxic activity[53,54].

Psychrotolerant strain of *Pseudoalteromonas* sp. SM9913 which was isolated from deep-sea sediments of the Bohai Gulf, China gave EPSs yield of 5.25 g/L at 10-30°C[41]. The produced EPSs comprised of α-[1→6] glucose linkages with MW [4 × 10^4 Da], this yield was relatively copious when compared with the data reported for the EPSs produced by other types of bacteria. The former mentioned EPSs was investigated for its bioflocculant behavior and biosorption capacity[55]. The two bacterial strains *Pseudoalteromonas* sp. CAM025 and CAM036 produced mucoid colony on marine agar medium when supplemented with glucose[56]. Novel EPSs of *Colwellia psychrerythraea* belonging to the phylum Gamma Proteobacteria were used as Cryoprotectant[57]. EPSs with unique rheological properties were obtained from isolate *Zunongwangia profunda* SM-A87 from the Southern Okinawa deep-sea sediment samples and this EPSs was ecologically used in organic nitrogen degradation[58].

The first archaeal EPSs reported were produced by the halophilic archaea *Haloferax mediterranei*, isolated from the Mediterranean Sea[59,60]. The halophilic strain of archaea produced thick layer surrounding the cells under laboratory conditions with unique rheological properties exhibiting a pseudo plastic behavior of the EPSs. The extreme salt tolerant EPSs producing bacteria are valuable candidates for the microbial enhanced oil recovery processes, especially in oil deposits with high salinity concentrations. A novel exopolysaccharide, called EPS-R, has been reported to be produced by a halophilic bacterium *Hahella chejuensis*, isolated from marine sediment from Marado, Cheju Island, Republic of Korea. This EPS-R showed specific emulsifying capacity higher than that observed in commercial EPSs such as gellan gum, sodium alginate or xanthan gum. Halophilic archaea belonging to the genera *Haloarcula, Haloferax*, *Halococcus*, *Halobacteriu and Natronococcus* are significantly researched for EPSs production with unique properties. Species of the halophilic bacterial genus *Halomonas* are commonly found as EPSs producers. Some species of *Halomonas such as H. eurihalina, H. maura, H. anticariensis* and *H. ventosae* are mostly novel EPSs producers[61-63]. EPSs from *H. Maura* and *H. eurihalina* showed unique emulsifying activity with pseudo-plastic behavior[64]. EPSs of *H. Maura* used as immunomodulator in biomedical field[65-67]. EPSs are renewable resources which are also applied as anti-corrosive agents for coating[68]. EPSs act as natural bio flocculant and well replace the chemical flocculants. Bacterium *Bacillus cereus* SK has been reported for its significant bioflocculant activity[69]. EPSs of *Corynebacterium glutamicum* and *Bacillus* sp. As-101 also exhibited noticeable flocculation activity[70,71]. Bioflocculant is used in waste water treatment as an ecofriendly process for environment. EPSs producing *Pseudomonas* sp. and *Staphylococcus* sp. were found to possess applicable bioflocculant properties and a novel bioflocculant MM1 was introduced from the EPSs produced by the above said bacteria. Similarly, combination of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6 and a consortium of *Oceanobacillus* sp., *Halobacillus* sp. have been applied in the industrial waste water and river water treatment due to the bioflocculant properties of EPSs[72-75]. Marine derived EPSs is also applies in Anti-Metastatic treatment as recently reported by Heymann et al., 2016[84.]

EPSs mainly form gel-metrix and this film forming bacterial biopolymer is applicable in various fields. In below table, the summary of above mentioned applications is discussed with some recent reports for EPSs producing marine bacteria, their site of isolation and their biotechnological applications.

Table 1: Biotechnological applications of EPSs produced by marine bacteria

Name of marine bacteria	Site of isolation	Application	References
Pseudoalteromonas strain 721	Hot springs and hydrothermal vents	gelling properties	49
Alteromonas macleodii	North Fiji basin	metal-binding capacity, food-thickening agent	50,51,52
Bacillus licheniformis B3-15	hot marine shallow vents, Italy	antiviral activities	53
B. licheniformis T4	Panarea island	cytotoxic activity	54
Pseudoalteromonas sp. SM9913	Deep-sea	bioflocculant	55,56
Pseudoalteromona sp. CAM025 and CAM036	Bohai Gulf, China	behavior and biosorption ability	
Zunongwangia profunda SM-A87	Southern Okinawa deep-sea	organic nitrogen degradation	58
Haloferax mediterranei	Mediterranean Sea	microbial enhanced oil recovery	59,60
Hahella chejuensis	Marado, Cheju Island, Republic of Korea	emulsifying capacity	61-63
Halomonas Maura	Marado, Cheju Island, Republic of Korea	immunomodulator	65-67
Bacillus licheniformis, Geobacillus thermodenitrificans	marine hot spring of Vulcano Island, Italy	immunomodulatory agent	76
Pseudomonas aeruginosa JP11	Marine environment	Bio-detoxification of cadmium metal	77
Alteromonas macleodii subsp. *Fijiensis,* strain HYD657	East Pacific Rise system (deep sea)	Protecting agent against UV radiation, chemical and other reagents	78
Colwellia psychrerythraea strain 34H	Arctic sediment	Cryoprotectant	57
Polaribacter sp. SM1127	Arctic water sample	Cosmetics and pharmaceutical fields	80
Pseudomonas sp. ID1	Antarctica sample	Cryoprotectant agent and emulsifier	23,81
Cyanothece ATCC 51142	As a gift from London professor to paper auther's	Biodetoxification of lead, cadmium and zinc	82
Vibrio diabolicus, strain HE800	deep sea hydrothermal vent in East Pacific Rise system	Regenerating capacity for skin and bone healing, Protein delivery	79,83

Conclusion

EPSs produced from marine bacterial diversity are ubiquitous in the extreme environment where they are important for microbial survival. The main significant functions ascribed to EPSs are of a protective nature which functions as protective barrier against changing pH, temperature and salinity as compared to the optimum range of the particular bacteria. In oligotrophic condition, EPS layer is also important in nutrient transportation and their precise roles are dependent on the ecological niches in which the marine diversity exists. They could assist the microbial communities by creating a boundary between the bacterial cell and its immediate environment. Several EPSs produced by marine bacteria from extreme habitats show promising characteristics for biotechnological applications. By examining their various structure and chemical-physical characteristics, it is possible

to gain insight into their commercial implication and they are employed in several fields like industries ranging from pharmaceutical to food-processing fields, through to the detoxification capability of polluted areas from petrochemical oils and also biodegradation aspect. Considering that the microbial biodiversity of marine ecosystems is unexplored yet and the unprecedented advances in molecular techniques to study the less explored microbial population, it can be reasonable to hypothesize that the identification and characterization of new marine diversity will provide wide opportunities for new industrial and medical level applications as well as to develop eco-friendly environmental preservation strategies.

Acknowledgment

PJS is thankful to Department of Science & Technology, New-Delhi 110016 for financial support as DST-INSPIRE FELLOWSHIP (IF140644). NMN is thankful to SERB DST for support in the in the form of NPDF (PDF/2016/000190).

Conflicts of interest
The authors declare no conflicts of interest.

References

1. Rampelotto P.H., Extremophiles and extreme environments. Life, 3(3): 482-485 **(2013)**

2. MacLeod R.A., The question of the existence of specific marine bacteria. Bacteriol. Rev, 29(1): 9 **(1965)**

3. DeLong E.F and Yayanos A.A., Properties of the glucose transport system in some deep-sea bacteria. Appl. Environ. Microbiol, 53(3): 527-532 **(1987)**

4. Wai S.N., Mizunoe Y. and Yoshida S.I., How *Vibrio cholerae* survive during starvation. FEMS Microbiol. Lett., 180(2): 123-131 **(1999)**

5. Bowers K.J., Mesbah N.M. and Wiegel J., Biodiversity of poly-extremophilic Bacteria: Does combining the extremes of high salt, alkaline pH and elevated temperature approach a physico-chemical boundary for life? Saline systems, 5(1): 9 **(2009)**

6. Jensen P.R. and Fenical W., Marine bacterial diversity as a resource for novel microbial products. J. Ind. Microbiol. Biotechnol., *17*(5):346-351 **(1996)**

7. De Carvalho C.C. and Fernandez P., Production of metabolites as bacterial responses to the marine environment. Mar. Drugs, 8(3): 705-727 **(2010)**

8. Okazaki T., Kitahara T. and Okami Y., Studies on marine microorganisms .4. New antibiotic Ss-228 Y produced by *Chainia* sp isolated from shallow sea mud. J. Antibiot., 28: 176–184 **(1975)**

9. Proksch P., Edrada R. and Ebel R., Drugs from the seas-current status and microbiological implications. Appl. Microbiol. Biotechnol, 59(2): 125-134 **(2002)**

10. Williams P.G., Panning for chemical gold: marine bacteria as a source of new therapeutics. Trends biotechnol, 27(1): 45-52 **(2009)**

11. Sutherland I.W., Bacterial exopolysaccharides. Adv. Microbiol. Phy, 8: 143–213 **(1972)**

12. Wingender J., Neu T.R. and Flemming H.C., What are bacterial extracellular polymeric substances? In Microbial extracellular polymeric substances. Springer Berlin Heidelberg,1-19 **(1999)**

13. Flemming H.C. and Wingender J., Relevance of microbial extracellular polymeric substances (EPSs)-Part I: Structural and ecological aspects. Wat. Sci. Tech., 43(6): 1-8 **(2001)**

14. Poli A., Anzelmo G. and Nicolaus B., Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. Mar. drugs, 8(6): 1779-1802 **(2010)**

15. Sutherland I.W., Biosynthesis of microbial exopolysaccharides. Adv. Microbiol. Phy, 123: 79–150 **(1982)**

16. Decho A.W., Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. In Oceanography and Marine Biology: an Annual Review Barnes, M., Ed., Aberdeen Univ. Press: Aberdeen, UK, 73-153 **(1990)**

17. Harder W. and Dijkhuizen L., Physiological-responses to nutrient limitation. Annu. Rev. Microbiol., 37: 1–23 **(1983)**

18. Manca M.C.L.L., Improta R., Esposito A., Gambacorta A. and Nicolaus B., Chemical composition of two exopolysaccharides from *Bacillus thermoantarcticus*. Appl. Environ. Microbiol, 62:3265–3269 **(1996)**

19. Van der Merwe P., Lannuzel D., Nichols C.A.M., Meiners K., Heil P., Norman L., Thomas D.N. and Bowie A.R., Biogeochemical observations during the winter-spring transition in East Antarctic sea ice: evidence of iron and exopolysaccharide controls. Mar. Chem.,115: 163–175 **(2009)**

20. Debnath M., Paul A.K. and Bisen P.S., Natural bioactive compounds and biotechnological potential of marine bacteria. Current Pharmacy and Biotechnology, 8: 253–260 **(2007)**

21. Egan S., Thomas T., Holmström C. and Kjelleberg S., Phylogenetic relationship and antifouling activity of bacterial epiphytes from the marine alga *Ulva lactuca*. Environ. Microbiol., 2(3): 343-347 **(2000)**

22. Delbarre-Ladrat C., Sinquin C., Lebellenger L., Zykwinska A. and Colliec-Jouault S., Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycan-like molecules. Frontier in chemistry, 2 **(2014)**

23. Di Donato P., Poli A., Taurisano V., Abbamondi G.R., Nicolaus B. and Tommonaro G., Recent advances in the study of marine microbial biofilm: from the involvement of quorum sensing in its production up to biotechnological application of the polysaccharide fractions. Journal of Marine Sci. and Engg., 4(2): 34 **(2016)**

24. Lupp C. and Ruby E.G., *Vibrio fischeri* uses two quorum-sensing systems for the regulation of early and late colonization factors. J. bacteriol, 187(11): 3620-3629 **(2005)**

25. Krembs C., Eicken H., Junge K. and Deming J.W., High concentrations of exopolymeric substances in Arctic winter sea ice: Implications for the polar ocean carbon cycle and cryoprotection of diatoms. Deep. Sea. Res, 49: 2163-2181 **(2004)**

26. Junge K., Eicken H. and Deming J.W., Bacterial activity at 2 to 20^{0}C in Arctic wintertime sea ice. Appl. Environ. Microbiol., 70: 550- 557 **(2004)**

27. Williams A. and Wimpenny J., Exopolysaccharide production by *Pseudomonas* NCIB ll264 grown in continuous culture. J. Gen. Microbiol., 104: 47-57 **(1978)**

28. Samain E., Milas M., Bozzi L., Dubreucq G. and Rinaudo M., Simultaneous production of two different gel-forming exopolysaccharides by an *Alteromonas* strain originating from deep sea hydrothermal vents. Carbohydr. Polymr., 34: 235–241 **(1997)**

29. Ko S.H., Lee H.S., Park S.H. and Lee H.K., Optimal conditions for the production of exopolysaccharide by marine microorganism *Hahella chejuensis*. Biotechnol. Bioprocess. Engg., 5(3): 181-185 **(2000)**

30. Bozal N., Tudela E., Rossello-Mora R., Lalucat J. and Guinea J., *Pseudoalteromonas antarctica* sp. nov., isolated from an Antarctic coastal environment. Int. J. Syst. Evol. Microbiol, 47(2): 345-351 **(1997)**

31. Spanò A. and Adriana A., Bacterial Exopolysaccharide of Shallow Marine Vent Origin as Agent in Counteracting Immune Disorders Induced by Herpes Virus. J. Immunoassay. Immunochem, 251-260 **(2016)**

32. Moriello V.S., Lama L., Poli A., Gugliandolo C., Maugeri T.L., Gambacorta A. and Nicolaus B., Production of exopolysaccharides from a thermophilic microorganism isolated from a marine hot spring in flegrean areas. J. Ind. Microbiol. Biotechnol., 30(2): 95-101 **(2003)**

33. Maugeri T.L., Gugliandolo C., Caccamo D., Panico A., Lama L., Gambacorta A. and Nicolaus B., A halophilic thermotolerant *Bacillus* isolated from a marine hot spring able to produce a new exopolysaccharide. Biotechnol. Lett., 24(7): 515-519 **(2002)**

34. Morin A., Screening of polysaccharide-producing microorganisms, factors influencing the production and recovery of microbial polysaccharides. Polysaccharides–Structural Diversity and Functional Versatility , New York: Marcel Dekker Inc. 275-296 **(1998)**

35. Lee H.K., Chun J., Moon E.J., Ko S.H., Lee D.S., Lee H.S. and Bae K.S., *Hahella chejuensis* gen. nov., sp. nov., an extracellular-polysaccharide-producing marine bacterium. Int. J. Syst. Evol. Microbiol., 51: 661–666 **(2001)**

36. Vanhooren P.T. and Vandamme E.J., Biosynthesis, physiological role, use and fermentation process characteristics of bacterial exopolysaccharides. Recent research developments in fermentation & bioengineering, 1: 253–299 **(1998)**

37. Rougeaux H., Kervarec N., Pichon R. and Guezennec J., Structure of the exopolysaccharide of *Vibrio diabolicus* isolated from a deep-sea hydrothermal vent. Carbohydr. Res, 322: 40–45 **(1999)**

38. Roger O., Kervarec N., Ratisko J., Colliec-Jouault S. and Chevolot L., Structural studies of the main exopolysaccharide produced by the deep-sea bacterium *Alteromonas infernus*. Carbohydr. Res., 339: 2371–2380 **(2004)**

39. Christense B.E., Kjosbakken J. and Smithrod O., Partial chemical and physical characterization of two extracellular polysaccharides produced by a marine, periphytic *Pseudomonas* sp. Strain NCMB 2021. Appl. Environ. Microbiol., 50: 837–845 **(1985)**

40. Mayer C., Moritz R., Kirschner C., Borchard W., Maibaum R., Wingender J., and Flemming H.C., The role of intermolecular interactions: studies on model systems for bacterial biofilms. Int. J. Biol. Macromolec., 26(1): 3-16 **(1999)**

41. Qin G., Zhu L., Chen X., Wang P.G., and Zhang Y., Structural characterization and ecological roles of a novel exopolysaccharide from the deep-sea psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913. Microbiol., 153(5): 1566-1572 **(2007)**

42. Parkar D., Jadhav R. and Pimpliskar M., Marine bacterial extracellular polysaccharides: A review. J. Coast. Life Med, 5(1): 29-35 **(2017)**

43. Schmid J., Sieber V. and Rehm B., Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies. Front. Microbiol, 6: 496 **(2015)**

44. Wolfaardt G.M., Lawrence J.R. and Korber D.R., Function of EPS. In Microbial extracellular polymeric substances Springer Berlin Heidelberg. 170-200 **(1999)**

45. Decho A.W. and Herndl G.J., Microbial activities and the transformation of organic matter within mucilaginous material. Sci. Total. Environ, 165(1): 33-42 **(1995)**

46. Caron D.A., Grazing of attached bacteria by heterotrophic microflagellates. Microbiol Ecol, 13: 203–21 **(1987)**

47. Boyle C.D. and Reade A.E., Characterization of two extracellular polysaccharides from marine bacteria. Appl. Environ. microbiol, 46(2): 392-399 **(1983)**

48. Nicolaus B., Kambourova M. and Oner E.T. Exopolysaccharides from extremophiles: from fundamentals to biotechnology. Environ. Technol., 31(10): 1145-58 **(2010)**

49. Rougeaux H., Guezennec J., Carlson R.W., Kervarec N., Pichon R. and Talaga P., Structural determination of the exopolysaccharide of *Pseudoalteromonas* strain HYD 721 isolated from a deep-sea hydrothermal vent. Carbohydr. Res., 315: 273–285 **(1999)**

50. Rougeaux H., Talaga P., Carlson R.W. and Guerzennec J., Structural studies of an exopolysaccharide produced by *Alteromonas macleodii* subsp. *fijiensis* originating from a deepsea hydrothermal vent. Carbohydr. Res., 312: 53–59 **(1998)**

51. Loaec M., Olier R. and Guezennec J., Chelating properties of bacterial exopolysaccharides from deep-sea hydrothermal vents. Carbohydr. Polymr., 35: 65–70 **(1998)**

52. Jouault S.C., Chevolot L., Helley D., Ratiskol J., Bros A., Sinquin C. and Fischer A.M., Characterization, chemical modifications and in vitro anticoagulant properties of an exopolysaccharide produced by *Alteromonas infernus*. Biochim. Biophys. Acta., 1528(2-3): 141-151 **(2001)**

53. Spanò A., Gugliandolo C., Lentini V., Maugeri T.L., Anzelmo G., Poli A. and Nicolaus B., A novel EPS-producing strain of *Bacillus licheniformis* isolated from a shallow vent off Panarea Island (Italy). Curr. Microbiol., 67(1):21-9 **(2013)**

54. Finore I., Di Donato P., Mastascusa V., Nicolaus B. and Poli A., Fermentation technologies for the optimization of marine microbial exopolysaccharide production. Mar. Drugs, 12(5): 3005-24 **(2014)**

55. Li W.W., Zhou W.Z., Zhang Y.Z., Wang J. and Zhu X.B., Flocculation behavior and mechanism of an exopolysaccharide from the deep-sea psychrophilic bacterium *Pseudoalteromonas* sp. SM9913. Bioresour. technol, 99(15):6893-6899 **(2008)**

56. Mancuso Nichols C.A., Garon S., Bowman J.P., Raguénès G. and Guézennec J., Production of exopolysaccharides by Antarctic marine bacterial isolates. J. Appl. Microbiol. 96(5): 1057–1066 **(2004)**

57. Marx J.G., Carpenter S.D. and Deming J.W., Production of cryoprotectant extracellular polysaccharide substances (EPS) by the marine psychrophilic bacterium *Colwellia psychrerythraea* strain 34H under extreme conditions. Can. J. Microbiol, 55(1): 63-72 **(2009)**

58. Liu S.B., Qiao L.P., He H.L., Zhang Q., Chen X.L., Zhou W.Z., Zhou B.C. and Zhang Y.Z., Optimization of fermentation conditions and rheological properties of exopolysaccharide produced by deep-sea bacterium *Zunongwangia profunda* SM-A87. PLoS One, 6(11): e26825 **(2011)**

59. Anton J., Meseguer I. and Rodriguez-Valera F., Production of an extracellular polysaccharide by *Haloferax mediterranei*. Appl. Environ. Microbiol., 10: 2381–2386 **(1988)**

60. Parolis H., Parolis L.A.S., Boán .IF., Rodríguez-Valera F., Widmalm G., Manca Mc., Jansson P.E. and Sutherland I.W., The structure of the exopolysaccharide produced by the Halophilic Archaeon *Haloferax mediterranei* strain R4 (ATCC 33500). Carbohydr. Res, 295: 147-156 **(1996)**

61. Bouchotroch S., Quesada E., del Moral A., Llamas I. and Béjar V., *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. Int. J. Syst. Evol. Microbiol, 51(5): 1625-1632 **(2001)**

62. Mata J.A., Béjar V., Llamas I., Arias S., Bressollier P., Tallon R., Urdaci M.C. and Quesada E., Exopolysaccharides produced by the recently described halophilic bacteria *Halomonas ventosae* and *Halomonas anticariensis*. Res. Microbiol, 157(9): 827-35 **(2006)**

63. Biswas J. and Paul A.K., Production of extracellular polymeric substances by Halophilic bacteria of solar salterns. Chin. J. Biol., 2014 **(2014)**

64. Calvo C., Martínez-Checa F., Toledo F., Porcel J. and Quesada E., Characteristics of bioemulsifiers synthesised in crude oil media by *Halomonas eurihalina* and their effectiveness in the isolation of bacteria able to grow in the presence of hydrocarbons. Appl. Microbiol. Biotechnol., 60(3): 347-51 **(2002)**

65. Arias S., Del Moral A., Ferrer M.R., Tallon R., Quesada E. and Bejar V., Mauran, an exopolysaccharide produced by the halophilic bacterium *Halomonas maura*, with a novel composition and interesting properties for biotechnology. Extremophiles 7(4): 319-326 **(2003)**

66. Llamas I., Moral A.D., Martínez-Checa F., Arco Y., Arias S. and Quesada E., *Halomonas maura* is a physiologically versatile bacterium of both ecological and biotechnological interest. Antonie van Leeuwenhoek, 89(3): 395-403 **(2006)**

67. Oren A., Industrial and environmental applications of halophilic microorganisms. Environ. Technol, 31(8-9): 825-834 **(2010)**

68. Scheerder J., Breur R., Slaghek T., Holtman W., Vennik M. and Ferrari G., Exopolysaccharides (EPS) as anti-corrosive additives for coatings. Progress in Organic Coating, 75(3): 224-30 **(2012)**

69. Busi S., Karuganti S., Rajkumari J., Paramanandham P. and Pattnaik S., Sludge settling and algal flocculating activity of extracellular polymeric substance (EPS) derived from *bacillus cereus* SK. Wat. Environ. J, 31(1): 97-104 **(2017)**

70. He N., Li Y. and Chen J., Production of a novel polygalacturonic acid bioflocculant REA-11 by *Corynebacterium glutamicum*. Bioresour. Technol, 94(1): 99-105 **(2004)**

71. Salehizadeh H., Vossoughi M. and Alemzadeh I., Some investigations on bioflocculant producing bacteria. Biochem. Eng. J, 5(1): 39-44 **(2000)**

72. Zhang Z.Q., Bo L., Xia S.Q., Wang X.J. and Yang A.M., Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. J. Environ. Sci, 19(6): 667-73 **(2007)**

73. Wang L., Ma F., Qu Y., Sun D., Li A., Guo J. and Yu B., Characterization of a compound bioflocculant produced by mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6. World. J. Microbio. Biotechnol., 27(11): 2559-65 **(2011)**

74. Cosa S. and Okoh A., Bioflocculant production by a consortium of two bacterial species and its potential application in industrial wastewater and river water treatment. Pol. J. Environ., 23: 689-96 **(2014)**

75. Vijayendra S.V.N. and Shamala T.R., Film forming microbial biopolymers for commercial applications—a review. Crit. Rev. Biotechnol., **(2014)**

76. Arena A., Pavone B., Gugliandolo C., Maugeri T. L. and Bisignano G. Exopolysaccharides from marine thermophilic bacilli induce a Th1 cytokine profile in human PBMC. Clin. Microbiol. Infect, 10: 366 **(2004)**

77. Raj R., Dalei K., Chakraborty J. and Das S., Extracellular polymeric substances of a marine bacterium mediated synthesis of CdS nanoparticles for removal of cadmium from aqueous solution. J. Colloid Interface Sci., 462: 166-175 **(2016)**

78. Lelchat F., Cozien J., Le Costaouec T., Brandilly C., Schmitt S., Baudoux A.C. and Boisset C., Exopolysaccharide biosynthesis and biodegradation by a marine hydrothermal *Alteromonas* sp. strain. Appl. Microbiol. biotechnol, 99(6): 2637-2647 **(2015)**

79. Senni K., Pereira J., Gueniche F., Delbarre-Ladrat C., Sinquin C., Ratiskol J. and Colliec-Jouault S., Marine polysaccharides: A source of bioactive molecules for cell therapy and tissue engineering. Mar. Drugs, 9(9): 1664-1681 **(2011)**

80. Sun M.L., Zhao F., Shi M., Zhang X.Y., Zhou B.C., Zhang Y.Z. and Chen X.L., Characterization and biotechnological potential analysis of a new exopolysaccharide from the arctic marine bacterium *Polaribacter* sp. SM1127. Sci, rep, 5: 18435 **(2015)**

81. Carrión O., Delgado L. and Mercade E., New emulsifying and cryoprotective exopolysaccharide from Antarctic *Pseudomonas* sp. ID1. Carbohydr. Polymr., 117:1028-1034 **(2015)**

82. Shah V., Ray A., Garg N. and Madamwar D. Characterization of the extracellular polysaccharide produced by a marine *cyanobacterium, Cyanothece* sp. ATCC 51142, and its exploitation toward metal removal from solutions. Curr. microbiol, 40(4): 274-278 **(2000)**

83. Zykwinska A., Marquis M., Sinquin C., Cuenot S. and Colliec-Jouault S., Assembly of HE800 exopolysaccharide produced by a deep-sea hydrothermal bacterium into microgels for protein delivery applications. Carbohydr. polymr, 142: 213-221 **(2016)**

84. Heymann D., Ruiz-Velasco C., Chesneau J., Ratiskol J., Sinquin C. and Colliec-Jouault S. Anti-Metastatic Properties of a Marine Bacterial Exopolysaccharide-Based Derivative Designed to Mimic Glycosaminoglycans. Molecules, 21(3): 309 **(2016)**