Marine bacterial extracellular polysaccharides: A review

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1. Introduction

Marine biotechnology has been one of the emerging and key areas of research since 1940s due to diverse functional areas (marine natural products for medicine, marine nutraceuticals, marine bioenergy, marine bioremediation). Moreover, marine organisms have the capacity to produce unique bioactive compounds which can be utilized for human and industries. Bioactive compounds from marine sources have found various implications in the progress of mankind due to their diverse biological properties such as anticancer, antiviral, etc. In addition to bioactive compounds, extracellular polysaccharides from marine organisms have found to be beneficial in various industries.

Due to diverse biological properties and physiological parameters, exopolysaccharides has been found to be implicated in various industries such as food, textile, etc. (Table 1[1]). Significant studies of microbial extracellular polymeric substances (EPS) have been carried out extensively for decades, which further enhances the researches to figure out the mechanism of EPS biosynthesis providing divergent directions to the study of EPS. EPS have been isolated from many natural sources such as soils, fresh water, hydrothermal vents, etc. Researchers focus on isolating EPS producing microorganisms from marine sources as many marine microbes are useful for the human. Thus, this leads to new insights in exploring the EPS production from marine bacteria and other microbes. This review article focuses on the new insights of exopolysaccharides from marine bacteria, along with its chemical properties and applications in the industries.

Table 1

| Various commercial applications of EPS. |
|----------------------------------------|
| EPS | Producer bacteria | Commercial applications |
| Kelcogel/gelrite | Sphingomonas paucimobilis | Gelling, Stabilizing agent in food industry |
| Xanthan | Xanthomonas campestris | Viscofying agent |
| Emulsan | Pseudomonas fluorescens | Emulsifiers |
| Dextran | Streptococcus mutans, Leuconostoc mesenteroides | Purification |
| Curdlan | Agrobacterium and Rhizobium spp. | Antithrombotic |

Adapted and modified from the table made by Madhuri and Prabhakar[1].
2. Classification and chemistry of bacterial EPS

On the basis of structural composition, microbial EPS can be classified into three groups[2] which are depicted in the following Figure 1. Homo-EPS consist of single type of monosaccharide usually α-D-glucans, β-D-glucans, fructans and other polygalactan. Hetero-EPS consist of different types of monosaccharides mainly, D-glucose, D-galactose, L-rhamnose and their derivatives[3,4]. The differences arise between the homopolysaccharides on the basis of their primary structures such as patterns of main chain bonds, molecular weights and branch structures.[4].

Exopolysaccharides consist of linear, branched repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose, mannose, N-acetylglucosamine, N-acetyl galactosamine and rhamnose in variable ratios[6]. In addition to sugar moieties, some non-carbohydrate substituents such as acetate, pyruvate, succinate and phosphate are also present[7]. The typical composition of bacterial EPS is tabulated in Table 2.

3. Possible biosynthesis pathways of marine bacterial EPS

Extracellular polysaccharides biosynthesis is a complex process due to the involvement of various enzymes. Various studies have been carried out to figure out the exact mechanisms of biosynthesis of EPS in bacteria, but mechanisms of biosynthesis of EPS in the marine bacteria have still remained unclear. Generally, the precursor sugars are synthesized and activated inside the cell and the polymerization takes place in the inner cell membrane. Several reviews and studies suggested three possible biosynthesis pathways of EPS in marine bacteria, namely Wzx/Wzy-dependent pathway, ATP-binding cassette (ABC) transporter-dependent pathway, and synthase-dependent pathway (Figure 2).

3.1. Wzx/Wzy-dependent pathway

The Wzx/Wzy-dependent mechanism has been widely studied in Gram-negative bacteria especially for heteropolysaccharide production[10]. Wzx/Wzy-dependent pathway occurs in the cytoplasm with the help of series of the membrane-spanning proteins[11]. The initiation of the pathway occurs through the initiator protein known as phosphoglycosyl transferases which harbours the first osidic residue to the undecaprenyl phosphate resulting in the sugar repeat units. These units are then polymerized by Wzy protein, namely, polymerase, through a putative catch and release mechanism[14]. Wzy mediated polymerization is carried out at the negative terminus end of the growing chain and the length of the repeating unit is controlled by another protein known as Wzz.

3.2. ABC transporter-dependent pathway

ABC transporter-dependent pathway of EPS biosynthesis is quite similar to the Wzx/Wzy-dependent pathway since the initiation of EPS biosynthesis takes place with the action of various phosphoglycosyl transferases in the cytoplasm[15]. The only difference between the Wzx/Wzy-dependent pathway and the ABC transporter-dependent pathway is the export of sugar linked moieties from the cytoplasm to the periplasm of the inner membrane. Export of sugar linked repeated moieties occurs due to the ABC transporter proteins spanning the inner membrane and the periplasmic polysaccharide co-polymerase and outer membrane polysaccharide export families[16-18]. Polysaccharides produced through this pathway carry a conserved glycolipid at the reducing end composed of phosphatidylglycerol and poly-2-keto-3-deoxyoctulosonic acid linker[14].

3.3. Synthase-dependent pathway

The unique feature of synthase-dependent pathway is the secretion of the complete polymer strands across the membranes and the cell wall which is independent of a flippase[14]. The polymerization as
well as the translocation process is performed by a single synthase protein[11]. Synthase-dependent pathways are often utilized for the assembly of homo-polymers requiring only one type of sugar precursor.

Most of the enzymatic steps for exopolysaccharide precursor biosynthesis occur inside the cell while the polymerization/secretion is localized in the cell envelope. The genes involved in the different biosynthesis pathways encode various types of glycosyl transferases, polymerizing and branching enzymes, as well as enzymes responsible for addition of substituents or modifications of sugar moieties[14]. The genes encoding these enzymes can be found in most of the EPS producing microbes clustered within the genome or on large plasmids[11]. EPS biosynthesis gene clusters are often located on plasmids[19,20].

4. EPS producing marine bacteria

Marine bacteria, such as *Bacillus, Halomonas, Planococcus, Enterobacter, Alteromonas, Pseudoalteromonas, Vibrio, Rhodococcus, etc.*, are the primary EPS producers and have been extensively studied till date[21]. Most of the EPS producing marine bacteria are Gram-negative in nature, while very few are Gram-positive. It was observed that marine bacterium *Saccharophagus degradans* (*S. degradans*) produced EPS in high amounts from several carbohydrates sources including starch and xylose. Thus, the production of EPS from *S. degradans* was enhanced by nutritional limitation[22].

*Vibrio furnissii* strain VB0S3 was isolated and characterized from coastal regions of Goa and showed to produce highest EPS in batch cultures during the late exponential growth.
phase[23]. Planococcus maitiensis Anita I was isolated from the coastal sea water area of Bhavnagar, India[9]. This bacterium was able to produce an EPS which can be further used for bioremediation, enhanced oil recovery and cosmetic applications. Enterobacter cloacae, isolated from marine sediments in India produced an acidic EPS that showed excellent emulsifying properties as comparable to other commercial gums[24]. EPS production by Pseudoalteromonas CAM025 and CAM036, isolated from Antarctica sea water and sea ice were described[25]. Marine bacteria such as Halomonas maura, Halomonas ventosae, and Halomonas alkaliartartica were isolated and evaluated for the EPS production[26-28]. Some exopolysaccharide producing marine bacteria have been tabulated in Table 3.

Table 3

| Marine bacteria                      | Sources                      | References |
|--------------------------------------|------------------------------|------------|
| Planococcus maitiensis Anita I       | Coastal sea water of Bhavnagar District, India | [19]       |
| S. degradans                         | -                            | [22]       |
| Vibrio furnissi strain V8053         | Coastal region of Goa        | [23]       |
| Enterobacter cloacae                 | Marine sediments             | [24]       |
| Halomonas spp.                       | -                            | [26]       |
| Halomonas antarcetica               | -                            | [27]       |
| Halomonas ventosae                   | -                            | [27]       |
| Alteromonas haloplankts KMM 156     | -                            | [29]       |
| Alteromonas inferns (A. inferns)     | Deep sea hydrothermal vent   | [30,31]    |
| Alteromonas nucleoidi 2MM6           | Intertidal zone of Halifax, Nova Scotia | [32]       |
| Bacillus licheniformis (B. licheniformis) | Volcano island               | [33,34]    |
| Bacillus marinus                     | Marine sediment              | [35]       |
| Bacillus strain B3-15                | Shallow water, marine hot spring | [36]       |
| Bacillus strain B3-72                | Shallow vent                 | [37]       |
| Bacillus thermarctica               | Ischia island                | [37]       |
| Geobacillus sp.                      | -                            | [37]       |
| Desulfotibrio sp. strain Indl        | Indonesian coast             | [38]       |
| Flavobacterium uliginosum            | -                            | [39]       |
| Halella chejuensis                   | -                            | [40]       |
| Pantoea sp. BM39                     | Seafloor sediments           | [41]       |
| Pseudoalteromonas atlantica         |                              | [42,43]    |
| Pseudoalteromonas sp. strain 59      | Marine sediment              | [44-46]    |
| Pseudomonas sp strain NCMB 2021      | Madiyn fletche Halifax, Nova Scotia | [47]       |
| Rhodococcus erythropolis PR4         | -                            | [48]       |
| Shewanella colwelliana               | Eastern oyster               | [49]       |
| Vibrio alginolyticus                 | Marine fouling material      | [50]       |
| Vibrio paraheautemicus               | Marine water                 | [51]       |
| Zunongwangia profundia SM-A87       |                              | [52]       |

5. Potential applications of marine bacterial EPS

Bacterial EPS have been implicated in industries such as pharmaceutical, biomedical, food, bioremediation and so on due to their stringent physical and chemical parameters. The applications of EPS in industries are mainly determined by their physical and chemical properties[19]. Rheological, emulsifying, solidifying properties of bacterial exopolysaccharides have been the key properties for the diverse applications. In addition to EPS extracted from terrestrial bacteria, marine bacteria have found their role in the pharmaceutical and biomedical industries (Table 4).

Table 4

| Marine bacteria | Biotechnological applications |
|-----------------|------------------------------|
| A. inferns strain GY785 | Anticoagulant activity, increased the viability and proliferation of chondrocytes, cartilage tissue engineering |
| Alteromonas nucleoidi subsp. fijiensis | Thickening agent in food industry, detoxification of waste water, bone healing, treatment of cardiovascular diseases, protection of sensitive skin against chemical, mechanical and UVB aggressions |
| B. licheniformis B3-15 | Antiviral activity |
| Bacillus thermodienericus strain B3-72 | Immunomodulatory and antiviral activity |
| Geobacillus sp. strain 4004 | Pharmaceutical applications |
| Paracoccus zeaxanthinifaciens subsp. payriae | Bioremediation of toxic metals |
| Polaribacter sp. SM1127 | Food, cosmetic, pharmaceutical, biomedical |
| Pseudoalteromonas strain 721 | Gelling properties |
| Pseudoalteromonas strain CAM025 | Cryoprotection |
| Pseudoalteromonas strain CAM036 | Trace metal binding |
| Pseudoalteromonas strain SM9913 | Flocculation behaviour and biosorption capacity |
| Vibrio diabolicus strain HE800 | Bone regeneration |

Adapted and modified from researches of Poli et al.[34] and Donato et al.[53].
5.2. Environmental applications

It has been found that the EPS produced by marine bacteria have strong affinity for heavy metals and thus EPS can be used for bioremediation of heavy metals from the environment. It was found that the strong interaction of EPS produced by Alteromonas sp. strain 1644 between divalent and monovalent cations[56,61]. The EPS produced by A. infernus also showed a very strong affinity for lead, cadmium, and zinc[62].

6. Recent advances

*In vitro* studies conducted in Italy found that the EPS1-T14 produced by marine bacterium, *B. licheniformis* T-14 inhibited the biofilm formation of clinical isolates *Escherichia coli* 463, *Klebsiella pneumoniae* 2659, *Pseudomonas aeruginosa* 445 and *Staphylococcus aureus* 210 to a considerable extent dependent on the dosage and concentrations of the EPS1-T14[63]. According to them, this antibiofilm activity of EPS1-T14 was due to the surfactant properties of EPS1-T14 which could influence bacterial cell surface hydrophobicity and thereby interfere with the initial adhesion step, which was essential for the biofilm formation. Authors also suggested that the presence of fructose and fucose in the EPS1-T14 could interfere with the surface lectins of various bacteria such as *Pseudomonas aeruginosa*, thereby interfering with the assembly of adhesions in the cell wall. Thus, EPS1-T14 could be fascinating anti-adhesive drug in medical and non-medical prospects, which needs further researches and studies.

7. Conclusion

Due to the rheological, emulsifying and solidifying properties of exopolysaccharide, it has become one of the most fascinating fields of research in terms of marine science. Bone regeneration activity of some EPS has facilitated enormous researches in order to explore the pros and cons of the EPS as bone regeneration agent. Antitumor and antiulcer activities of marine EPS can be further explored in terms of its mechanisms and other aspects. These various activities of EPS have facilitated the enormous findings to figure out the enhanced production of these EPS by modifying the organisms using genetic engineering principles. Thus, EPS can be more fascinating as the EPS from marine sources is an emerging field.

Conflicts of interest

We declare that we have no conflict of interest.

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References

[1] Madhuri KV, Prabhakar KV. Microbial exopolysaccharides: biosynthesis and potential applications. *Orient J Chem* 2014; 30(3): 1401-10.

[2] Sutherland IW. Microbial exopolysaccharides – structural subtleties and their consequences. *Pure Appl Chem* 1997; 69(9): 1911-7.

[3] Mayo B, Aleksandrzak-Piekarczyk T, Fernández M, Kowalczyk M, Álvarez-Martin P, Bardowski J. Updates in the metabolism of lactic acid bacteria. In: Mozzi F, Raya RR, Vignolo GM. *Biotechnology of lactic acid bacteria*. Iowa: Blackwell Publishing; 2010, p. 3-33.

[4] Harutoshi T. Exopolysaccharides of lactic acid bacteria for food and colon health applications. Rijeka: InTechOpen; 2013. [Online] Available from: http://cdn.intechopen.com/pdfs-wm/42337.pdf [Accessed on 14th June, 2016]

[5] Flemming HC, Wingender J. The biofilm matrix, *Nut Rev Microbiol* 2010; 8: 623-33.

[6] Patel S, Majumder A, Goyal A. Potentials of exopolysaccharides from lactic acid bacteria, *Indian J Microbiol* 2012; 52(1): 3-12.

[7] Ates O. Systems biology of microbial exopolysaccharides production, *Front Bioeng Biotechnol* 2015; 3: 200.

[8] Kenne L, Lindberg B. Bacterial polysaccharides. In: Aspinall GO, editor. *The polysaccharides*. New York: Academic; 1983, p. 287-363.

[9] Laurienzo P. Marine polysaccharides in pharmaceutical applications: an overview, *Mar Drugs* 2010; 8: 2435-65.

[10] Delbarre-Ladrat C, Sinquin C, Lebellenger L, Zykwinska A, Colliec-Jouault S. Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycans-like molecules. *Front Chem* 2014; 2: 85.

[11] Rehm BH. Bacterial polymers: biosynthesis, modifications and applications. *Nat Rev Microbiol* 2010; 8: 578-92.

[12] De Vuyst L, De Vin F, Vaningelgem F, Degeest B. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *Int Dairy J* 2001; 11: 687-707.

[13] Whitfield C. Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annu Rev Biochem* 2006; 75: 39-68.

[14] Schmid J, Sieber V, Rehm B. Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies. *Front Microbiol*.
Arias S, del Moral A, Ferrer MR, Tallon R, Quesada E, Béjar V, Mauran, an exopolysaccharide produced by the halophilic bacterium *Halonomas maura*, with a novel composition and interesting properties for biotechnology. *Extremophiles* 2003; 7(4): 319-26.

Mata JA, Béjar V, Llamas I, Arias S, Bressollier P, Tallon R, et al. Exopolysaccharides produced by the recently described halophilic bacteria *Halonomas ventosae* and *Halonomas anticariensis*. *Res Microbiol* 2006; 157(9): 827-35.

Polli A, Espósito E, Orlando P, Lama L, Giordano A, de Appolonia F, et al. *Halonomas alkaliantarctica* sp. nov., isolated from saline lake Cape Russell in Antarctica, an alkalophilic moderately halophilic, exopolysaccharide-producing bacterium. *Syst Appl Microbiol* 2007; 30(1): 31-8.

Gorshkova RP, Nazarenko EL, Zubkov VA, Ivanova EP, Ovodov IuS, Shashkov AS, et al. [Structure of the repeating link of the acid polysaccharide of *Alteromonas haloplanktis* KMM 156]. *Bioorg Khim* 1993; 19(3): 327-36. Russian.

Raguénès GH, Peres A, Ruimy R, Pignet P, Christen R, Loaec M, et al. *Alteromonas infernus* sp. nov., a new polysaccharide-producing bacterium isolated from a deep-sea hydrothermal vent. *J Appl Microbiol* 1997; 82(4): 422-30.

Roger O, Kervarec N, Ratiskol J, Colliere-Jouault S, Chevolot L. Structural studies of the main exopolysaccharide produced by the deep-sea bacterium *Alteromonas infernus*. *Carbohydr Res* 2004; 339(14): 2371-80.

Nazarenko EL, Zubkov VA, Shashkov AS, Knirel’ IuA, Gorshkova RP, Ivanova EP, et al. [Structure of the repeating unit of acid polysaccharide from *Alteromonas maculeoti* 2MM6]. *Bioorg Khim* 1993; 19(7): 740-51.

Arena A, Maugeri TL, Pavone B, Iannello D, Gugliandolo C, Bisignano G. Antiviral and immunoregulatory effect of a novel exopolysaccharide from a marine thermotolerant *Bacillus licheniformis*. *Int Immunopharmacol* 2006; 6: 8-13.

Polli A, Anzelm G, Nicolaus B. Bacterial exopolysaccharides from extreme marine habitats: production, characterization, and biological activities. *Mar Drugs* 2010; 8(6): 1779-802.

El Sayed OH, El Sayed AM, Salem HM, Mahmoud MG, Asker MS, Mohamed SS. Isolation, characterization and biological activities of exopolysaccharide produced by *Bacillus marinus*. *Pharm Chem* 2015; 7(2): 200-8.

Maugeri TL, Gugliandolo C, Caccamo D, Pancio A, Lama L, Gambacorta A, et al. A halophilic thermotolerant *Bacillus* isolated from a marine hot spring able to produce a new exopolysaccharide. *Biotechnol Lett* 2002; 24: 515-9.

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

Zinkevich V, Bogdarina I, Kang H, Hill MAW, Tapper R, Beech IB. Characterization of exopolymers produced by different isolates of marine sulphate reducing bacteria. *Int Biodeterior Biodegradation*
Bartlett DH, Wright ME, Silverman M. Variable expression of extracellular polysaccharide in the marine bacterium *Pseudomonas atlantica* is controlled by genome rearrangement. *Proc Natl Acad Sci USA* 1988; 85: 3923-7.

Hoskins DL, Stanecy SE, Decho AW. Utilization of algal and bacterial extracellular polymeric secretions by the deposit feeding brittlestar *Amphipholis gracillima* (Echinodermata). *Mar Ecol Prog Ser* 2003; 247: 93-101.

Wrangstadh M, Conway PL, Kjellebery S. The production and release of an extracellular polysaccharide during starvation of a marine *Pseudomonas* sp. and the effect thereof on adhesion. *Arch Microbiol* 1986; 145: 220-7.

Wrangstadh M, Conway PL, Kjellebery S. The role of an extracellular polysaccharide produced by the marine *Pseudomonas* sp. S9 in cellular detachment during starvation. *Can J Microbiol* 1989; 35: 309-12.

Christensen BE, Kjoebakken J, Smidsrød O. Partial chemical and physical characterization of two extracellular polysaccharides produced by marine, periphytic *Pseudomonas* sp. strain NCMB 2021. *Appl Environ Microbiol* 1985; 50(4): 837-45.

Urui M, Yoshizaki H, Anzai H, Ogihara J, Iwabuchi N, Harayama S, et al. Structural analysis of mucoid, an acidic extracellular polysaccharide produced by a pristine-assimilating marine bacterium, *Rhodococcus erythropolis* PR4. *Carbohydr Res* 2007; 342: 927-32.

Sledjeski DD, Weiner RM. Production and characterization of monoclonal antibodies specific for *Shewanella colwelliana* exopolysaccharide. *Appl Environ Microbiol* 1993; 59(5): 1565-72.

Muralidharan J, Jayachandran S. Physicochemical analysis of the exopolysaccharides produced by a marine biofouling bacterium, *Vibrio alginolyticus*. *Process Biochem* 2003; 38(6): 841-7.

Enos-Berriage JL, McCarter LL. Relation of capsular polysaccharide production and colonial cell organization to colony morphology in *Vibrio parahaemolyticus*. *J Bacteriol* 2000; 182(9): 5513-20.

Liu SB, Qiao LP, He HL, Zhang Q, Chen XL, Zhou WZ, et al. Optimization of fermentation conditions and rheological properties of exopolysaccharide produced by deep-sea bacterium *Zunowangia profunda* SM-A87. *PLoS One* 2011; 6(11): e26825.

Donato PD, Poli A, Taurisano V, Abbamondi GR, Nicolaus B, 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. *J Mar Sci Eng* 2016; doi: 10.3390/jmse4020034.

Arena A. Exopolysaccharides from marine thermophilic bacilli induce a Th1 cytokine profile in human PBMC. *Clin Microbiol Infect* 2004; 10: 366.

Guezennecc J, Pignet P, Lijour Y, Gentic E, Ratiskol J, Colliec-Jouault S. Sulfation and depolymerization of a bacterial exopolysaccharide of hydrothermal origin. *Carbohydr Polym* 1998; 37: 19-24.

Zhenming C, Yan F. Exopolysaccharides from marine bacteria. *J Ocean Univ China* 2005; 4(1): 67-74.

Zanchetta P, Lagarde N, Guezennecc J. A new bone-healing material: a hyaluronic acid-like bacterial exopolysaccharide. *Calcif Tissue Int* 2003; 72: 74-9.

Senni K, Pereira J, Gueniche F, Delbarre-Ladrat C, Sinquin C, Ratiskol J, et al. Marine polysaccharides: a source of bioactive molecules for cell therapy and tissue engineering. *Mar Drugs* 2011; 9: 1664-81.

Matsuda M, Yamori T, Naitoh M, Okutani K. Structural revision of sulfated polysaccharide B-1 isolated from a marine *Pseudomonas* species and its cytotoxic activity against human cancer cell lines. *Mar Biotechnol (NY)* 2003; 5: 13-9.

Fedorov SN, Ermakova SP, Zvyagintseva TN, Stonik VA. Anticancer and cancer preventive properties of marine polysaccharides: some results and prospects. *Mar Drugs* 2013; 11: 4876-901.

Bozzi L, Milas M, Rinaudo M. Solution and gel rheology of a new bone-healing material: a hyaluronic acid-like bacterial exopolysaccharide. *Calcif Tissue Int* 2003; 72: 74-9.

Shah V, Ray A, Garg N, Madanwar D. Characterization of the extracellular polysaccharide produced by a marine cyanobacterium, *Cyanotoce* sp. ATCC51142, and its exploitation toward metal removal from solutions. *Carr Microbiol* 2000; 40: 274-8.

Spanò A, Laganà P, Visalli G, Mauger TL, Gugliandolo C. *In vitro* antibiofilm activity of an exopolysaccharide from the marine thermophilic *Bacillus licheniformis* T14. *Carr Microbiol* 2016; 72(5): 518-28.