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Exopolysaccharides from marine bacteria and their pharmaceutical potential

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Abstract. The marine environment is the largest aquatic ecosystem on Earth, it is often harbored by a great variety of microorganisms responsible for more than 50% of total biomass of prokaryotes in the world. Among these microorganisms, bacteria are one of the important and dominant inhabitants of this ecosystem. They are known to be a potential source of unique and stable biomolecules with high biotechnological interest such as enzymes, exopolymers, pigments, antimicrobial compounds, biosurfactants, and so forth. Many marine bacteria produce exopolysaccharides as a significant strategy for growth, adhering to solid surfaces, and to survive adverse conditions. These exopolysaccharides possess distinctive characteristics, huge structural diversity and various valuable biological properties. Therefore, they have attracted great interest among scientists due to their wide potential applications spanning areas such as health (pharmaceuticals and medicine), industry (cosmetics, textile, dairy etc.), and environment. This review aims to give an overview of current knowledge on EPSs produced by marine bacteria and their potential applications in pharmaceutical field.

Keywords: Exopolysaccharides, Marine bacteria, Antioxidant, Antimicrobial, Anticancer.

1 Introduction

The marine environment is the largest aquatic ecosystem on Earth and it offers an extraordinary microbial diversity responsible for more than 50% of total biomass of prokaryotes in the world. Microorganisms living in marine habitats, such as deep sea and shallow hydrothermal vents, characterized by extreme conditions (high pressure, high concentrations of H2S, heavy metals, low nutrient concentration etc.) have developed bioactive substances which possess a wide variety of properties that may not be found in their terrestrial counterparts. Hence, marine microorganisms are considered as one of the main producers of novel biomolecules with attractive biotechnological potentialities such as enzymes, lipids, biopolymers, compatible solutes, and antibiotics [1,2].

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Polysaccharides are one of the major biopolymers produced by marine bacteria, they are located in the cell wall (lipopolysaccharide, LPS), attached to the cells forming capsules (capsular polysaccharide, CPS), or secreted into the extracellular environment in the form of slime (exopolysaccharide, EPS) [3]. Among those, EPS are of special interest owing to their chemical characteristics, their huge structural diversity and their rheological properties. They serve as natural adhesives to both biological and inert surfaces, in cell-to-cell aggregation processes and in biofilm formation. They also protect cells against desiccation, stress condition and predation by protozoans and viruses. Additionally, microbial EPS play essential in the cells development by allowing the entrapment of nutrients [4,5].

Due to their structural diversity, their biocompatibility, their biodegradability and their wide functional property, bacterial EPS have high potential as biologically active/inert polymeric materials in biomedical fields. Their unique composition of sugars with different types of linkages is the prime cause of their multidimensional bioactivity. These exopolymers are considered as potent antibacterial, antioxidant, anticancer, and antitumor agents with acceptability in nano drug delivery, bioactive compound encapsulation, as prebiotics. They also help in therapeutic diagnosis, drug delivery, wound healing, etc [6,7]. Moreover, EPS molecules can provide an impetus as they are less hazardous than chemically synthetized polymers, and the production processes are environmentally friendly [8].

The aim of this review is to present a summary of the recent developments of EPSs from marine bacteria and their potential utilization in the pharmaceutical industries.

2 Marine Bacteria producing EPS

It has been reported that a large number of marine bacteria species can produce EPSs. They are cyanobacteria, mesophilic bacteria and some strains of extremophiles (thermophiles, psychrophiles). Most of them are Gram-negative in nature, while very few are Gram positive. They are widely distributed in the ocean, and can be found in different samples from seawater, sediment, sea-ice, marine hot springs, hydrothermal vents, salt lakes and marine salterns. Some of them were also isolated from the surface or interior of marine plankton, plant, animal, etc. [9,10]. Members of the genera Bacillus, Geobacillus, Halomonas, Enterobacter, Alteromonas, Pseudomonas, Pseudoalteromonas, Vibrio, Rhodococcus, Shewanella, Exiguobacterium, Kocuria, Marinobacter are reported as the principal EPS producers and have been extensively studied [2]. Table 1 gives a non-exhaustive overview of some EPS-producing marine bacteria and their sources in marine environment.

Microorganisms	Source
Mesophilic marine bacteria	
Aerococcus uriaeequi HZ	Aquaculture water
Alteromonas strain JL2810	Sea water
Alteromonas macleodii 2MM6	Intertidal zone of Halifax, Nova Scotia
Bacillus marinus	Marine sediment
Enterobacter sp. ACD2	Sea water
Exiguobacterium aurantiacum	Lagoon
Enterobacter cloacae MBB8	Sea water
Flavobacterium uliginosum MP-55	Sea weed
Hahella chejuensis 96CJ1035	Marine sediments
Hyphomons sp. MHS-3	Shallow marine sediments
Idiomarina fontislapidosi F23	Lagoon
Idiomarina ramblicola R22	Water-course
Microbacterium aurantiacum FSW-25	Sea water
Pantoea sp. BM39	Seafloor sediments
Pseudoalteromonas TG12	Sea-water
Pseudoalteromonas ulvae TC14	Marine biofilm
Pseudoalteromonas ruthenica	Sea-water
Pseudoalteromonas sp. MD12-642	Marine sediments
Pseudomonas sp. WAK1	Brown seaweed Undaria pinnatifida
Pseudomonas stutzeri 273	Marine sediments
Rhodococcus erythropolis PR4	Ocean
Shewanella colwelliana	Associate bivalve
Vibrio alginolyticus	Sea water
Vibrio *harveji* VB23	Sea water
Zooglea sp. KCCM 100376	Seaweed Undaria
Bacteria from extreme marine environments	
Alteromonas macleodii sub. fijiensis ST716	Deep-sea hydrothermal vent
Alteromonas infernus GY785	Hydrothermal vent
Alteromonas hispanica F32	Hypersaline inland
Bacillus licheni formis	Vulcano Island
Bacillus sp. B3-15	Marine hot spring
Bacillus sp. B3-72	Hydrothermal vent
Bacillus thermoantarcticus	Sea sand in Ischia Island
Colwellia psychrerythraea	Sea ice
Geobacillus tepidamans V264	Hot spring

Table 1. Some examples of EPS-producing marine bacteria

3 Structure of EPS by Marine Bacteria

EPS are high molecular weight polymers that are known by either homopolymeric or heteropolymeric composition (Figure 1). Homopolysaccharides, contain a single type of monosaccharide, either fructose or glucose, they may be unbranched or branched. They are clustered, based upon their chemical composition such as type of linkage and monomeric units present in EPS, into four groups thus; -D-glucans, -D-glucans, fructans, and polygalactans. On the other hand, heteropolysaccharides consist of different osidic residues and usually displaying a regular back- bone structure with a repeating unit. This repeating unit is either linear or branched and may contain monosaccharide such as pentoses (as D-ribose, D-arabinose and D-xylose), hexoses (as D-glucose, D-galactose, D-fructose, D-mannose, D-allose, L-rhamnose and L-fucose), amino

sugars (D-Glucosamine and D-Galactosamine) or uronic acids (D-Glucuronic acids, D-Galacturonic acids). Organic or inorganic substituents such as sulfate, phosphate, acetates, ethers, lactates and pyruvate may also be present [11,12]. The differences between homopolysaccharide and heteropolysaccharide are not only reflected in the chemical nature and linkage bonds but also in synthetic enzymes and site of synthesis [13].

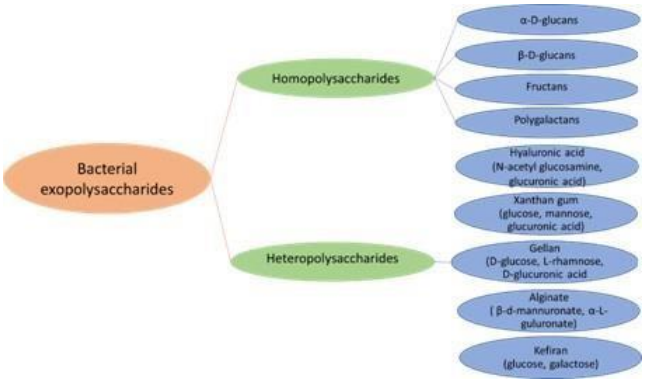

Fig. 1. Classification of bacterial exopolysaccharides.

The majority of EPSs produced by marine bacteria are linear heteropolysaccharides containing three or four different monosaccharides arranged in groups of 10 or less to form repeating units, with an average molecular weight comprised between 100 and 300 kDa (Figure 2). Few of them are neutral macromolecules, but the majority is polyanionic due to the presence of either uronic acids (commonly D-glucuronic, D-galacturonic and D-mannuronic), ketal-linked pyruvate or inorganic residues such as phosphate or sulphate. Bonds between repeating units at the backbone of the polymers are 1,4-- or 1,3--linkages and 1,2-- or 1,6--linkages. The former is characterized by strong rigidity while the latter; more flexible ones [11,13].

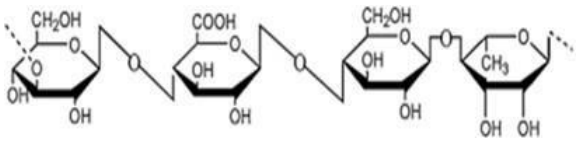

Fig. 2. Gellan structure: repeating units of D-glucose, D-glucuronic acid, D-glucose, and L-rhamnose .

4 Pharmacological applications of exopolysaccharides

Bioactive compounds have been the pillar of disease chemotherapy for as long as 30 years and will be probably utilized as formats for the development of novel compounds with improved biological properties [14]. During the last year, EPSs produced by marine bacteria have been attracted the interest of several researchers for their unique properties and their numerous biological effects. Therefore, they have found outstanding pharmaceutical and medical applications as antioxidative agents, antimicrobial compounds, immunomodulators, antiproliferative compound, conventional pharmaceutical excipients, suspension stabilizer, etc [2,15].

4.1 Characteristics of exopolysaccharides as antioxidant agents

The oxidative stress, occurring from the imbalance between oxidants and antioxidants for the oxidants, is an inevitable and pervasive phenomenon that leads to cell damage and the emergence of numerous diseases (ie, ageing, cancer, diabetes, chronic obstructive pulmonary, cardiovascular diseases and neurodegenerative disorders) [16]. Antioxidants play a significant role in delaying or reducing this oxidative damage of cells. Evidence suggests that synthetic antioxidants are double-edged swords wherefore the requirement for natural antioxidants is increasing globally. So, the exploration of non-toxic, biodegradable and both human and environmental friendly metabolites from microbes have been augmented by the researchers to combat functional abnormalities and dreadful diseases [17]. In this context, EPS from marine bacteria have been shown to possess an interesting antioxidant proprieties. For instance, a marine sedimentary bacterium belonging to the genus Bacillus, produces an EPS in the exponential and stationary development stages when grown in a glucose mineral salt medium. The antioxidant capability of produced EPS was assayed by different assays. The results demonstrated that the EPS delivered had a strong scavenging activity on hydrogen peroxide (81±0.14), DPPH (69±0.72), ABTS (74±0.34), hydroxyl (74±0.49) and superoxide anion (71±0.27) radicals [18]. Priyanka et al [19] isolated a sulfated EPS mainly consisting of glucose and having a molecular weight of 269 kDa from the marine halophilic strain Enterobacter sp. PRIM-26. This EPS displayed an important antioxidant activity with IC50 values for DPPH and hydroxyl radical scavenging activities of 0.44 mg/mL and 0.33 mg/mL, respectively. In a study investigating the bacterial antioxidant EPS isolated from marine Egyptian habitats, EPS from Bacillus circulans was reported to have a strong antioxidant potential, which was 98% against DPPH•. Six other EPS producing marine bacteria belonging to Bacillus and Staphylococcus genera were also discovered, their EPS displayed antioxidant activity ranging from 80 to 97% against DPPH• [20]. Another study [21] found that the exopolysaccharide HMEPS secreted by the marine halophilic bacterium Halolactibacillus miurensis, mainly composed of galactose and glucose, has strong DPPH free radical scavenging activity with IC50 value less than 0.10 mg/mL. HMEPS also has a strong reducing ability, the superoxide radical scavenging capacity was 89.15%

at the concentration of 0.5 mg/mL. Polararibacter sp. SM1127, a flavobacterial strain isolated from the Arctic Ocean produced an EPS with a molecular mass of 220kDa and mainly comprises N-acetyl glucosamine, mannose and glucuronic acid residues bound by heterogeneous linkages. This EPS showed good antioxidant activity, exhibited a protective effect on human skin cells in a low-temperature environment and is safe for oral and external application [22]. In India, an EPS produced by the marine Microbacterium aurantiacum FSW-25 (named EPSMi-25) was found to have 80% DPPH• scavenging capacity at 1 mg/mL. EPSMi-25 is an acidic sugar that exhibits rheological stability similar to xanthan with a molecular weight of 7 × 103 kDa [23]. In 2018, an exopolysaccharide from Aerococcus uriaeequi HZ named EPS-A, was extracted and characterized, it exhibited hydroxyl and superoxide radicals scavenging activities comparable to vitamin C with values of 45.65% at 0.1 mg/mL and 67.31% at 0.25 mg/mL, respectively [24]. Another EPS (BAEPS) produced by the marine Bacillus amyloliquefaciens 3MS 2017, which contains 22.8% sulfate and has a molecular weight of 37.6 kDa, was found to have a strong antioxidant power. The IC50 values of BAEPS on radical scavenging of DPPH and hydrogen peroxide were 0.21 and 30.04 μg/mL, respectively [25]. Recently, a similar study revealed that the marine bacterium Enterobacter cloacae, isolated from the Gulf of Mannar Biosphere, produced an EPS with total antioxidant and hydroxyl radical scavenging activities close to that of gallic acid, a potent antioxidant compound [26]. Collectively, Table 2 gives an overview of some marine bacteria, reported in the last decade, that produce EPS with important antioxidant potential. To understand the structure–function relationship between the reported antioxidant potential and the associated EPS structural features, deep and detailed structural analysis must be performed. It was reported that the basic mechanisms for in vitro antioxidant activity of bacterial EPS is linked to their OH, SH, COOH, PO3H2, C=O, NR2, S, and O functional groups. Particular side chains, such as 1→2, 1→4, or 1→6, can also influence these properties in addition to rhamnose, fucose, or mannan residues. Low molecular weight EPS display better antioxidant properties due to a higher ratio of reducing terminals, which awards them the ability to better accept and/or eliminate free radicals [27, 28].

Table 2. Some antioxidant exopolysaccharides derived from marine bacteria

Marine Bacteria	Average Mw of EPS (kDa)	EPS Concentration	DPPH• Scavenging	ABTS• Scavenging	$O_2^{-\bullet}$ Scavenging	·OH Scavenging	NO Scavenging	H_2O_2 scavenging	Ferric reducing Power	Lipid Peroxidation	Metal chelating Capacity	Ref
Achromobacter piechaudii NRC2	5670	25-400 μg/mL	IC_{50}= 170 μg/mL		IC_{50}= 199.31 μg/mL			IC_{50}= 205.12 μg/mL		IC_{50}=112.41 μg/mL	IC_{50}= 100.80 μg/mL	[29]
Aerococcus uriaeequi	/	100 μg/mL ($O_2^{-\bullet}$) 250 μg/mL ($^{\bullet}OH$)			67.31%	45.65%						[24]
Alteromonas sp. PRIM-21	269	0.25 -1.0 mg/mL	IC_{50} = 0.61 mg/mL		IC_{50} = 0.65 mg/mL							[19]
Bacillus amyloliquefaciens 3MS 2017	37.6	1.0 mg/mL	99.39%	67.44%	91.44%	/	90.18	92.17%	0.005	80.67%	71.14%	[25]
Bacillus alvei	/	20-100 mg/mL	95.83%									[20]
Bacillus anthracis		20-100 mg/mL	81.50%									
Bacillus circulans		20-100 mg/mL	98.10%									
Bacillus insolitus		20-100 mg/mL	85.01 %									
Bacillus licheniformis UD061	/	5–250 mg/L			42.53%	51.06%			0.34			[30]
Bacillus marinus		20-100 mg/mL	83.26%									[20]
Bacillus polymyxa		20-100 mg/mL	84.31%									
Bacillus thuringiensis	/	1.0 mg/mL	79.00 %		75.12 %	/	/	/	/	/	/	[31]
Bacillus licheniformis UD061	/	5–250 mg/L			42.53%	51.06%			0.34			[30]
Bacillus velezensis MF347997	/	/	69%	64 %	71%	74%		81%				[18]
Bacterium polaribacter sp. SM1127	220	10.0 mg/mL	55%		28%	52%						[32]
Edwardsiella tarda	29	8 mg/mL	88%			89%				79%		[33]

Marine Bacteria	Average Mw of EPS (kDa)	EPS Concentration	DPPH• Scavenging	ABTS• Scavenging	$O_2^{-\bullet}$ Scavenging	·OH Scavenging	NO Scavenging	H_2O_2 scavenging	Ferric reducing Power	Lipid Peroxidation	Metal chelating Capacity	Ref
Enterobacter sp. PRIM-26	/	0.25–1.0 mg/mL	IC_{50}=0.44 mg/mL		IC_{50}=0.33 mg/mL							[19]
Halolactibacillus miurensis	/	10 mg/mL (DPPH•, Reducing power); 0.5 mg/mL ($O_2^{-\bullet}$); 3.2 mg/mL (•OH)	84 %		89.15 %	61 %					50%	[21]
Microbacterium aurantiacum FSW-25	7000	1 mg/mL (DPPH•, $O_2^{-\bullet}$), 2.5 mg/mL (•OH), 3.5 mg/mL (Reducing power)	80%		92%	90%			1.7			[23]
Nitratireductor sp. PRIM-24	/	0.25–1.0 mg/mL	IC_{50} = 0.49 mg/mL		None							[19]
Polaribacter sp. SM1127	220	10 mg/mL	55.40%			52.1%						[22]
Pseudomonas PF-6	88300	0–3 mg/mL	IC_{50} = 180 µg/mL		IC_{50} = 149 µg/mL	IC_{50} = 340 µg/mL			0.2–0.8			[34]
Pseudomonas stuzeri 273	190	5–20 µg/mL			IC_{50} = 20 µg/mL	IC_{50} = 60 µg/mL						[35]
Staphylococcus sp.	/	20-100 mg/mL	80%									[20]
Zunongwangia profunda SM-A87	/	10 mg/mL	49%		27%	59%						[36]

4.2 Antimicrobial activity

The rapid emergence of drug-resistant bacteria has led the scientists to search for new antimicrobial compounds from natural sources. Several studies have shown that EPSs derived from marine bacteria showed remarkable antimicrobial activity and serve thus as powerful therapeutics against pathogenic microbes. For example, under in vitro conditions, EPSs produced by Bacillus sp. ors1 and Brachybacterium sp ors2, two marine bacteria isolated from an open ocean aquaculture, displayed substantial antimicrobial activity against the pathogens Lysinibacillus sp., Paenibacillus sp, Escherichia coli, Pseudomonas aeruginosa and Mesorhizobium sp [37].

Aullybux et al [38] isolated eight EPSs from Bacillus, Halomonas, Psychrobacter and Alcaligenes species and screened their activities against a set of seven pathogenic strains (Enterococcus faecalis ATCC 29212, Streptococcus pneumonia ATCC 49619, Streptococcus agalactiae ATCC 27956, Campylobacter jejuni NCTC 11351) and three more clinical strains namely methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa and a species of Acinetobacter. The produced EPSs showed antibacterial activities against a minimum of four pathogenic strains. Another study built up a bacterially produced EPS (cellulose), containing nisin in order to control Listeria monocytogenes in foodstuff. Bacterial EPS was produced by Gluconacetobacter xylinus K3. Nisin (2500 IU/mL) was joined into the polymer matrix. EPS decreased the concentration of L. monocytogenes on foodstuff of around 2 log CFU/g after 14 days of storage [39].

The EPS formed by Enterobacter sp. ACD2 showed a considerable antibacterial effect (inhibition zone ¿ 25 mm) against Staphylococcus aureus and Escherichia coli [40]. EPS from marine Bacillus subtilis SH1 was found to exhibit inhibitory activity against three pathogenic strains consisting of Aeromonas hydrophila, Pseudomonas aeruginosa and Streptococcus faecalis [41]. In another work, EPS produced from marine Pseudomonas mendocina AB1 has been shown to possess antimicrobial activity against different Gram-positive bacteria (Staphylococcus aureus ATCC 6538 and Bacillus subtilus ATCC 33018), Gram-negative bacteria (Pseudomonas aeruginosa ATCC 9027 and Klebsiella pneumoniae ATCC 43816) and fungi (Aspergillus niger ATCC 16404 and Candida albicans ATCC 10231) [42]. Also, EPS formed by a marine Klebsiella sp showed an important antibacterial activity against Staphylococcus aureus and Escherichia coli in both its native and sulfated form [43].

At the present time, there is no definitive mechanism to explain the antibacterial action of the bacterial EPS against Gram-positive and -negative bacteria. Attempts to investigate a potential mechanism to understand this antibacterial activity are continuing. Sivasankar et al [44] suggest that potential inhibitory mechanism of EPS is related to its ability to disrupt the structure of the bacterial cell envelope, especially the peptidoglycan layer. Likewise, Zhou et al [45], propose that in some manner, functional groups in the structure of EPS interact with bacterial cell envelopes to yield antimicrobial activity. Another study reported

that EPS could facilitate accumulation of secondary metabolites in the growth media, which might adversely affect Gram-positive and negative pathogens [46].

4.3 Anticancer activity

Data from individual studies also suggested that marine-derived antioxidant EPS could alleviate the oxidative stress-mediated diseases, such as cancer. Ruiz-Ruiz et al [47] demonstrated that the oversulfated EPS produced by the novel halophilic bacterium Halomonas stenophila strain B100 applied anticancer activity on T cell lines getting from acute lymphoblastic leukemia (ALL). Just tumor cells were vulnerable to apoptosis actuated by the sulfated EPS (B100S), while essential immune system cells were safe. Likewise, the potency of the EPS derived from Pseudoaltermonas sp. S-5 for inhibition of the proliferation of leukemia K562 cells was demonstrated by Chen et al [48].

EPSs formed by marine Bacillus licheniformis and Bacillus subtilis showed a dose-dependent cytotoxic effect against MCF-7 human breast cancer, the cytotoxicity was 67.8% and 65.5% respectively [49,41]. An in vitro study screened EPSs derived from a set of marine bacteria for their anticancer activity against hepatocellular carcinoma cells (HepG2). Results showed that HepG2 cells treated with the isolated EPS showed significant reduction in B-cell lymphocyte/leukemia-2 (BCL-2) gene expression levels which suggest their promising role as pro-apoptotic factors [50]. Another study has also revealed that EPSs, produced by five bacterial strains isolated from marine sediment of the Mediterranean and Red Seas, exhibited high cytotoxicity against HepG2 cells [51]. Similarly, the thermophilic Geobacillus sp. TS3–9, isolated from radioactive radon hot spring, excretes an EPS of high molecular weight that possess the capability to inhibit hepatoma carcinoma cell (HepG2) proliferation [52].

A marine bacterial exopolysaccharide EPS11 was reported to suppress cell adhesion, migration, and invasion in liver cancer cells. Proteomic analysis showed that this EPS induced downregulation of proteins related to the extracellular matrix–receptor interaction signaling pathway [53]. Through in vitro experiments, Abdrabo et al [42] demonstrated that EPS produced by Pseudomonas mendocina AB1 isolated from sea water of Suez Gulf, exhibited antitumor activity by inhibition of A-549, HepG-2, MCF-7 and HCT-116 cell lines at a concentration of 50 g/mL.

BAEPS, an acidic exopolysaccharide derived from Bacillus amyloliquefaciens 3MS 2017 and contained uronic acid and sulfate was found to have potent and selective effect to breast cell cancer MCF7 with a good margin of safety, the death percentage was 65.20% with IC50 = 70 mg/mL. BAEPS decreased counted viable EAC cells and induced non-viable cells [25]. A recent study by Hao et al [54] demonstrated that EPS extracted from Arctic Sea ice Cryptococcus heimaeyensis S20 had antitumoral activity on Non-small cell lung cancer, and had no cytotoxic effect on normal human cells. Other studies have demonstrated that the chemical modification of EPS (such as sulfation, acetylation, carboxymethylation, phosphorylation, sulfonation) affect positively its biological activities. For exemple, Sarilmiser and Oner [55] has shown that periodate oxidation of levan

structure produced by Halomonas smyrnensis AAD6T leads to the increase of aldehyde groups. These latter enhance the anti-cancer activity of EPS over different human cell lines including lung adenocarcinoma (A549), liver hepatocellular carcinoma (HepG2/C3A), gastric adenocarcinoma (AGS), and human breast adenocarcinoma (MCF-7).

According to the literature, mechanisms announced for the anticarcinogenic effects of EPSs incorporates: (1) coordinating hindrance of development of different sorts of malignant cells; (2) immunostimulating activity against tumors in combination with chemotherapy; (3) preventive impact on spreading or relocation of malignancy cells in the body [14].

4.4 Antiviral activity

Diseases caused by viral pathogens have demonstrated the need for new medicines, due to the increasing appearance of resistance to these available treatments. The screening of exopolymers produced by marine bacteria for antiviral activity has yielded a considerable number of active extracts. For instance, Arena et al [56] tested EPS-1, a novel exopolysaccharide produced by Bacillus licheniformis, isolated from a shallow marine hot spring in Italy, for its antiviral and immunomodulatory effects. Results showed that EPS-1 treatment impaired Herpes Simplex Virus type 2 (HSV-2) replication in human peripheral blood mononuclear cells (PBMC) but not in the human amnion-derived cell line (WISH cells). Three years later, the same authors [57] isolated a second novel exopolysaccharide EPS-2 from Geobacillus thermodenitrifican. This EPS was shown to be noncytotoxic to human peripheral blood mononuclear cells (PBMC) and WISH cells at concentration 300 g/ml. The antiviral effect produced by EPS-2 at concentrations of 200 and 300 g/ml was shown to significantly reduce HSV-2 viral titer. It was determined that EPS-2 is able to inhibit HSV-2 replication in PBMCs by up-regulating the expression of proinflammatory cytokines, particularly triggering polarization in favor of the Th1 subset.

Al-Nahas et al [58] found that EPS produced by marine Pseudoalteromonas sp. AM elicited a marked antiviral activity against HSV-I and showed lysis of plasma clots comparable to pentosan sulphuric polyester as a standard. Sulfated derivatives of an EPS from a marine Pseudomonas showed strong antiviral activity against HSV-1. This EPS inhibited the cytopathic effect of HSV-1 at concentration of 0.72g/ml [59].

It is suggested that sulfated-EPS interfere with the absorption and penetration of viruses into host cell and to inhibit various retroviral reverse transcriptases [11].

4.5 Antiviral activity

Glycosaminoglycan heparin is the drug of choice in the prevention and treatment of thromboembolic disorders. Some sulfated forms of EPSs were found to have anticoagulant impacts by repressing thrombin, by initiating against thrombin

III or by expanding the coagulating time. Additionally, these molecules can likewise have an antithrombotic action by blocking thrombin movement, interceded through the heparin cofactor II. Thus, they have been thought to serve as an alternative with enhanced activity [14]. For example, EPS produced by Enterobacter sp. ACD2 showed significant anticoagulation activity as the coagulation time was prolonged more than 24 h. It also showed 100% lysis of both plasma clots, which exceeds the fibrinolytic activity of standard hemoclar [40]. Another EPS delivered from Bacillus subtilis MKU SERB2 exhibited an important anticoagulant activity which was close to that of heparin [60].

An homoexopolysaccharide (constituted of glucose) produced by Pseudoalteromonas sp. strain AM, isolated from Red Sea has been described as a good emulsifier, comparable to natural gums [58]. This polymer showed fibrinolytic activity comparable to a pentosan sulphuric polyester, a fibrinolytic drug.

Jouault et al [61] extracted an exopolysaccharide from Alteromonas infernus, a mesophilic strain found in deep-sea hydrothermal vents. The isolated EPS was sulfated, partially depolymerized by acid hydrolysis and then its anticoagulant activity was evaluated. Results showed that the modified EPS was able to prolong the activated partial thromboplastin time appreciably. Levan, a sulfated EPS, isolated from Halomonas smyrnensis AAD6T, was found to exhibit anticoagulation activity via the intrinsic pathway like heparin in a dose-dependent manner. Exceptionally high heparin equivalent activity of sulfated levan was shown to proceed via thrombin inhibition and above a certain concentration, sulfated levan showed a better inhibitor activity than heparin [62].

Sulfated EPS exert anticoagulant activity through directly inhibiting Xa and IIa factors mediated by antithrombin and heparin cofactor II. Hence, these molecules could provide biochemical entities with suitable functions for obtaining new anticoagulant drugs [63].

4.6 Immunomodulation activity

Immunomodulatory activity is the pharmacological effect to influence the cellular and/or humoral immune system, either through stimulation, suppression, or modulation of the innate or adaptive arms of the immune response. Some EPSs derived from marine bacteria have been reported to be immunomodulatory agent of great interest [15]. An in vitro study found that a selenium-enriched exopolysaccharide (Se-ECZ-EPS-1) derived from marine Enterobacter cloacae Z0206 showed an interesting immunomodulatory potential. Administration of Se-ECZ-EPS-1 to cyclophosphamide (CP)-exposed animals resulted in improvement of cellular and humoral immune responses, it promoted a significant increase of relative spleen and thymus weight compared with CP-treated animals alone. Moreover, Se-ECZ-EPS-1 showed significant recovery in the serum hemolysin concentration [64].

Another study evaluated a novel exopolysaccharide (EPS1-T14) synthesized by an haloalkaliphilic, thermophilic Bacillus licheniformis strain T14, isolated from a shallow hydrothermal vent of Panarea Island (Italy), for its immunomodulatory effects. Results showed high levels of Th1-type cytokines detected in

supernatants of EPS1-T14 treated in human peripheral blood mononuclear cells (PBMC), which increases the immune response. Moreover, this EPS did not cause any decrease in the percentage of PBMC cells at a concentration of 400 µg/mL [65].

A novel EPS TA-1 isolated from the marine bacterium Thermus aquaticus YT-1 displayed immunomodulatory effects in murine macrophage. Treatment of macrophage cell line RAW264.7 with TA-1 resulted in a marked increase in the production of cytokines involved in nonspecific primary defense against infectious agents, especially the tumour necrosis factor (TNF-) and interleukin (IL-6) [66]. Similarly, Bai et al [67] and Chen et al [48] found that the hetero-exopolysaccharide (PEP) of high molecular weight produced by the deep-sea psychrophilic bacterium Pseudoaltermonas sp. S-5 promoted the production of cytokines, in particular TNF- and interleukin (IL-), and it has no cytotoxic effect on the murine macrophage cell line, RAW 264.7.

A new -mannan exopolysaccharide (Sphingobactan) from Arctic Sea ice Sphingobacterium sp. IITKGP-BTPF3 promoted the modulation of RAW 264.7 murine macrophages activity, and the proliferation of splenocytes cells in vitro treatment [68]. An immunomodulatory response was observed in treatments using EPS isolated from moderately halophilic bacteria Halomonas eurihalina. This exopolysaccharide was found to enhance the unspecific proliferation of human lymphocytes in response to the presence of anti-CD3 monoclonal antibody [69]. Biswas et al [70], investigated the effect of EPS (named SUR308) produced by Halomonas xianhensis SUR308 on the viability of human hepatocytes (Huh 7). Results demonstrated that this EPS, at high concentrations, enhances proliferation of Huh 7 cells. Recently, an in vivo study demonstrated that EPS produced by marine Bacillus megaterium DSKPDF CMST3 enhance fish immunity. EPS-supplemented diet significantly enhanced immunological and hematological parameters in fish species Labeo rohita. The immune gene expression (IL10, IL-1, and TGF) were significantly upregulated in lymphoid organs of the fish. Moreover, the dietary supplementation of EPS showed higher survival rates compared with basal diet fed fish against Aeromonas hydrophila infection [71].

4.7 Application of marine bacterial EPS

During the previous three decades, A large number of EPS from marine bacteria have been reported and their composition, structure, biosynthesis and therapeutic properties have been extensively studied. However, only few of these bioactive compounds have been launched in the pharmaceutical market [14]. Among them, EPS from cyanobacteria Spirulina could be considered as the first example used in pharmaceutical industries, it has been used in the treatment of pulmonary metastasis and as an anti-infammatory agent in many drugs [72]. The EPS (namely Deepsane) secreted by Alteromonas macleodii subsp. Fijiensis isolated from Pacific Rise is commercially available under the name of AbyssineTM (patent PCT 94907582-4). It is used for soothing and reducing irritation of sensitive skin against chemical, mechanical and UVB aggression. Likewise, two other

EPS produced by marine bacteria Alteromonas sp and Vibrio sp have found application in the cosmetic pharmacology area. The first one commercially available as Exo-HTM restores hyaluronic acid secretion in aged skin cells, stimulates lipid synthesis, supports the dermo-epidermal junction and induces filaggrin expression in the skin. It also displays action on natural moisturizing factors and lipids synthesis. The second one extracted from a Vibrio sp and commercially available as Exo-TTM stimulates desquamation, differentiation markers for skin regeneration and optimal skin texture [73, 74].

A mixture of glycoproteins and exopolysaccharides produced by Arctic Sea ice Pseudoalteromonas sp is commercially available as SeaCodeTM. It helps reduce the facial wrinkles associated with the aging process by increasing collagen I [74].

The limited industrial development of marine exopolysaccharides is related to the high cost of their production. For example, the production cost of the main marine EPSs commercialized to date can exceed 500 to 1,000 euros/kg, while that of xanthan is around 5 to 10 euros/kg. The solutions to reduce production costs involve the use of inexpensive carbon substrates, such as co-products from industry (food, energy) and the use of a simplified extraction and purification processes while avoiding, as much as possible, the use of organic solvents [75].

5 Conclusion

In recent years, the demand for new therapeutic drugs from natural products have promoted the interest for the production of bioactive molecules from microorganisms. In this context, marine bacteria have proven to be potential source of interesting compounds. Among those, exoploysaccharides are of special interest due to their unique structures, biocompatibility, biodegradability, non-toxic nature and their distinct therapeutic properties. Thus, these molecules can be applied in a wide range of industrial applications in biomedicines. Nevertheless, their production at large scale by means of suitable bioprocess engineering tools, mainly specifically designed bioreactors, is still challenging. Great effort should be made in the near future to scale-up the engineering tools required to produce EPSs from marine bacteria and to explore their pharmaceutical uses at large scale.

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