Source and Structure of Exopolysaccharides

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Marine microorganisms, as important members of marine organisms, can produce numerous specific active substances in an extreme environment in low temperature, high salt, high pressure, and oligotrophic conditions. They have gained scientific interest due to their potential applications in the pharmaceutical, cosmetic and food industries, for environmental remediation, and astrobiology. The monosaccharide composition, high molecular weight, hydrophobicity and polycharged characteristics of marine Exopolysaccharides (EPS) are involved in their cryoprotective effect, water-holding capacity, and good thermostability. The source and structure of exopolysaccharides are summarized.

exopolysaccharides microorganisms marine environment

1. The Source of Marine Exopolysaccharides

1.1. Exopolysaccharides Produced by General Marine Environmental Microorganisms

Among the several types of aquatic microorganisms, marine microorganisms account for half of the production of organic matter on earth [1]. Up to now, many common marine strains which can produce EPS were identified, such as *Pseudoalteromonas* species [2][3], *Bacillus* species [4], *Alteromonas* species [5], and the *Vibrio* species [6]. Several EPS with various structural features and biological activities have been isolated from those common marine strains, which are shown in **Table 1** [7][8][9][10][11][12][13][14][15][16][17]. For example, an EPS, produced by a novel probiotic *Pediococcus pentosaceus* M41, was isolated from a marine source [7]. An exopolysaccharide EPS273 from marine bacterium *P. stutzeri* 273 could inhibit biofilm formation and disrupt the established biofilms of *P. aeruginosa* PAO1, indicating that EPS273 had a promising prospect in combating bacterial biofilm-associated infection [16]. A *bacterium Bacillus thuringiensis* RSK CAS4 was isolated from the ascidian *Didemnum granulatum,* in which the condition of producing EPS was optimized by the response surface method [9]. An EPS-producing strain FSW-25, assigned to the genus *Microbacterium*, was isolated from the Rasthakaadu beach, Kanyakumari, which could produce a large quantity of EPS [17]. Recently, a strain of *Bacillus cereus* was isolated from the Saudi Red Sea coast. EPSR3 was a major fraction of the EPS from this marine strain, which showed antioxidant, antitumor, and anti-inflammatory activities. These biological activities of EPSR3 may be attributed to its content of uronic acids [18].

Table 1. Information of some EPS obtained from marine bacteria in the last decade.

Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
Pantoea sp. *YU16-S3*	EPS-S3	Ethanol precipitation extraction and purification by Sephacryl S500-HR column	Glc, Gal, GalNAc, GalN (1.9:1:0.4:0.02)	1.75 × 10^5	Promotion of Wound Healing	[19]
Pediococcus pentosaceus M41	EPS-M41	Culture centrifugal extraction and purification by ultra-filtration	Ara, Man, Glc, Gal (1.2:1.8:15.1:1.0)	6.8 × 10^5	Antioxidant, Anticancer	[7]
Bacillus cereus KMS3-1	EPS	Culture centrifugal extraction and purification by dialysis	Man, Glc, Xyl, Rha (73.51:17.87:2.18:6.49)		Waste-water treatment	[8]
Oceanobacillus iheyensis	EPS	Ethanol precipitation and purification by dialysis	Man, Glc, Ara (47.78:29.71:22.46)	2.14 × 10^6	Anti-biofilm	[10]
Bacillus thuringiensis RSK CAS4	EPS	Culture centrifugal extraction and purification by Sepharose 4-LB Fast Flow column	Fuc, Gal, Xyl, Glc, Rha, Man (43.8:20:17.8:7.2:7.1:4.1)		Antioxidant, Anticancer	[9]
Pseudoalteromonas, MD12-642	EPS	Culture centrifugal extraction and purification by ultra-filtration	GalA, GlcA, Rha, GlcN (41–42:25–26:16–22:12–16)	>1.0 × 10^6		[11]
Bacillus sp. H5	EPS5SH	Aqueous extraction and purification by GPC	Man, GlcN, Glc, Gal (1.00:0.02:0.07:0.02)	8.9 × 10^4	Immunomodulatory activity	[12]
Alteromonas sp. *JL2810*	EPS	Ethanol precipitation extraction and	GalA, Man, Rha (1:1:1)	>1.67 × 10^5		[13]

Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
		purification by DEAE column				
Pseudoalteromonas sp. YU16-DR3A	EPS-DR3A	Culture centrifugal extraction and purification by dialysis	Fuc, Erythrotetrose, Glc, Rib (6.7:1.0:1.5:1.0)	2×10^4	Antioxidant	[14]
Enterobacter sp. *ACD2*	EPS	Culture centrifugal extraction	Glc, Gal, Fuc, GlcA (25:25:40:10)		Antibacterial	[15]
P. stutzeri 273	EPS273	Culture centrifugal extraction and purification by GPC	GlcN, Rha, Glc (35.4:28.6:27.2)	1.9×10^5	Antibiofilm, Anti-Infection	[16]
Microbacterim FSW-25	EPS Mi25	Culture centrifugal extraction and purification by dialysis	Glc, Man, Fuc, GlcA	7.0×10^6	Antioxidant	[17]
Bacillus cereus	EPSR3	Culture centrifugal extraction	Glc, GalA, Arb (2.0: 0.8: 1.0)		Antioxidant, Antitumor, Anti-inflammatory activities	[18]
Vibrio sp. QY101	A101	Ethanol precipitation extraction and purification by GPC	GlcA, GalA, Rha, GlcN (21.47:23.05:23.90:12.15)	5.46×10^3	Antibacterial	[20]
Halolactibacillus miurensis	EPS	Culture centrifugal extraction and purification by Sepharose 4-LB Fast Flow column	Gal, Glc (61.87:25.17)		Antioxidant	[21]
Halomonas saliphila LCB169T	hsEPS	Ethanol precipitation, anion-exchange and gel-filtration chromatography	Man, Glc, Ara, Xyl, Gal, Fuc (81.22:15.83:1.47:0.59:0.55:0.35)	5.133×10^4	Emulsifying activity	[22]

Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
C.psychrerythraea 34H	EPS	Culture centrifugal extraction and purification by QFF column	QuiN, GalA (1:2)		Antifreeze	[23]
Issachenkonii [20] [21]	SM20310	Ethanol precipitation extraction and purification by DEAE column	Rha, Xyl, Man, Gal, Glc, GalNAc, GlcNAc (2.1:0.9:71.7:9.0:10.7:1.5:4.0)	>2.0 × 10^6	Anti-freeze	[24]
Halomonas sp. 2E1	EPS2E1	Culture centrifugal extraction and purification by DEAE column and Sephadex G75 column	Man, Glc (3.76:1)	4.7 × 10^4	Immunomodulatory activity [22]	[25]
Sphingobacterium sp. *IITKGP-BTPF3*	Sphingobatan	Culture centrifugal extraction and purification by DEAE column	Man	>2 × 10^6	Immunomodulatory activity	[26]
[34] [35] *Pseudoaltermonas* sp.	PEP	Culture centrifugal extraction and purification by dialysis and GPC	Glc, Gal, Man (4.8:50.9:44.3)	3.97 × 10^5	Anticancer	[27]
Polaribacter sp.	SM1127 EPS	Ethanol precipitation extraction and purification by Sepharose column	Rha, Fuc, GlcA, Man, Gal, Glc, GlcNAc (0.8:7.4:21.4:23.4:17.3:1.6:28.0) [36]	2.2 × 10^5	Promotion of Wound Healing, Prevention of Frostbite Injury, Antioxidant	[28][29]
Aeribacillus pallidus 418	EPS1, EPS2	Culture centrifugal extraction and purification by Sepharose DEAE CL-6B column	[37] Man, Glc, GalN, GlcN, Gal, Rib (69.3:11.2:6.3:5.4:4.7:2.9); Man, Gal, Glc, GalN, GlcN, Rib, Ara (33.9:17.9:15.5:11.7:8.1:5.3:4.9)	7 × 10^5; [38] >1 × 10^6		[30]

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ulted in Glc:Man:Gal:GalN:GalA:GlcA, with a relative molar ratio of 1:0.9:0.2:0.1:0.1:0.01 [39]. Now, several EPS isolated from psychrophilic bacteria in the Arctic and Antarctic marine environment have been reported, which have a potential application in the cryopreservation, food, and biomedical industries [23][24][25][26][27][28][29][40]. The source, structural, and biological information of these EPS are shown in **Table 1**.

1.3. Exopolysaccharides from Marine Hot Spring Microorganisms

Over the past decade, lots of microbes from marine hot springs have been reported, most of which produce special EPS to protect themselves from extreme conditions. These thermophilic microorganisms are classified as thermophiles growing at 55 °C~80 °C and hyperthermophiles growing above 80 °C. The thermophilic microorganisms contain multiple genera, such as *Aeribacillus, Anoxybacillus, Brevibacillus, and Geobacillus* [30]. The EPS produced by thermophilic bacteria usually have a high molecular weight with good emulsifying properties, leading to great potential application in the food and cosmetics industries [31][32][33][41][42]. Four thermophilic aerobic

Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
Rhodobacter johrii CDR-SL 7Cii	EPS RH-7 [30]	Ethanol precipitation and purification by dialysis	Glc, GlcA, Rha, Gal (3:1.5:0.25:0.25)	2×10^6	Emulsifying activity	[31]
Alteromonas ininus	GY785	Culture centrifugal extraction and purification by ultra-filtration	Rha, Fuc, Man, Gal, Glc, GalA, GlcA (0.2:0.1:0.4: 3.6:4.7:1.0:2.0) [31]	2.0×10^6		[32][33]

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2. The Structural Characteristics of Marine Exopolysaccharides

2.1. Structural Characterization Methods of Marine Microbial Exopolysaccharides

The marine microbial EPS have high structural diversity and complexity. To evaluate their structure, the monosaccharide composition, molecular weight, and glycosidic linkage need to be determined. Before the structural analysis, a homogeneous EPS should be obtained to remove the influence of other salt, pigment, and protein impurities. At present, the commonly used purification methods of EPS include ethanol precipitation, ultrafiltration, ion-exchange chromatography, and gel chromatography. The methods of SDS-PAGE and DOC-PAGE with Alcian blue staining are very useful to detect the presence of EPS [43]. The monosaccharide composition of EPS has been determined by a variety of methods, including acid hydrolysis followed by appropriate derivatization and gas chromatography (GC); pre-column derivatization with high-performance liquid chromatography (HPLC); and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [44][45]. The molecular weight can be determined by high-performance gel-permeation chromatography (HPGPC), combined with differential detector (ID) or multi-angle laser light scattering (MALS) [46]. Furthermore, through the data of the Fourier-Transform infrared spectroscopy (FTIR), methylation analysis, and nuclear magnetic resonance (NMR), the glycosidic bond-linking types and main functional groups can be obtained [23][47][48][49].

Besides these classical chemical procedures, new and powerful tools, such as zeta potential and particle-size analyzer, attenuated total reflectance Fourier-Transform infra-red spectroscopy (ATR-FTIR), differential scanning calorimetry (DSC), scanning electron microscope (SEM), atomic force microscopy (AFM), circular dichroism spectrum (CD), small-angle neutron scattering (SANS), and X-ray diffraction (XRD) techniques have been applied to investigate the surface morphology and physical properties of EPS [7][8][42][50][51][52][53]. For example, the physicochemical properties and rheological properties of an EPS-M41, produced by a novel probiotic *Pediococcus pentosaceus* M41 isolated from a marine source, were evaluated in detail. The average molecular weight of this EPS was determined to be 682.07 kDa by HPGPC method. The EPS-M41 consisted of Ara, Man, Glc, and Gal with a molar ratio of 1.2:1.8:15.1:1.0 by the GC method. The structure of the EPS-M41 was proposed as →3) α-D-Glc (1→2) β-D-Man (1→2) α-D-Glc (1→6) α-D-Glc (1→4) α-D-Glc (1→4) α-D-Gal (1→), with Ara linked at the terminals by FTIR and NMR analysis. The SEM analysis showed that the EPS-M41 possessed a unique compact, stiff and layer-like structure. The particle and zeta charges analyses exhibited that the EPS-M41 had a size diameter of

446.8 nm and a zeta potential of -176.54 mV. The DSC thermogram exhibited that the EPS-M41 had a higher melting point, indicating its resistance to the thermal processes [7]. Another example, the ATR-FTIR technique, was used to observe the movement of the -SH, -PO4, and -NH functional groups in the EPS from *Pseudomonas pseudoalcaligenes* NP103, and confirmed their involvement in the Pb (II) binding. The results emphasized the potential importance of *P. pseudoalcaligenes* NP103 EPS as a biosorbent for the removal of Pb (II) from the contaminated sites [42].

2.2. Examples of Marine Microbial Exopolysaccharides in the Last Decade

Several reviews have summarized the culture and fermentation conditions, distribution, biosynthesis, and biotechnological production of microbial EPS from marine sources [36][40][41][54][55][56][57][58][59]. In the past decade, with the development of separation and identification technology, numbers of new marine microorganisms have been identified. By optimizing the culture conditions, novel EPS with new biological activities have been discovered. Here, a variety of EPS obtained from marine microorganisms, including bacteria, fungi, and microalgae, in the last decade are summarized in **Table 1**, **Table 2** and **Table 3**. It will give people more useful information of the structure–activity relationship of the marine EPS through the analysis of their origin, monosaccharide composition, molecular weight, and bioactivities.

Table 2. Information of some EPS obtained from marine fungi in the last decade.

Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
Aureobasidium melanogenum SCAU-266	AUM-1	Alcohol precipitation and further purified through DEAE-column	Glc, Man, Gal (97.30:1.9:0.08)	6.0 × 10^3	Immunomodulatory activity	[49]
Aspergillus Terreus	YSS	Culture centrifugal extraction and purification by QFF column	Glc, Man (8.6:1.0)	1.86 × 10^4	Antioxidant	[60]
Fusarium oxysporum	Fw-1	Culture centrifugal extraction and purification by QFF column	Gal, Glc, Man (1.33:1.33:1.00)	6.12 × 10^4	Antioxidant	[61]
Alternaria sp.	AS2-1	Culture centrifugal extraction and purification by QFF column	Man, Glc, Gal (1.00:0.67:0.35)	2.74 × 10^4	Anticancer, Antioxidant	[62]

Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
Aspergillus versicolor	AWP	Culture centrifugal extractionand purification by QFF column	Glc, Man (8.6:1.0)	5×10^7		[63]
Aspergillus versicolor	LCJ-5-4	Culture centrifugal extractionand purification by QFF column	Glc, Man (1.7:1.0)	7×10^3	Antioxidant	[64]
Penicillium solitum	GW-12	Ethanol precipitation, anion-exchange and size exclusion chromatography	Man	1.13×10^4		[65]

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Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
Porphyridium sordidum	EPS	Cold aqueous centrifugal extraction and purification by dialysis	Fuc, Rha, Ara, Gal, Glc, Xyl, GlcA (1.93:0.36:0.36:48.28: 19.01:28.2:0.76)		Antibacterial	[67]
Porphyridium marinum	EPS-0C, EPS-2C, EPS-5C	Culture centrifugal extraction, ultra-filtration and High-Pressure Homogenizer	Xyl, Gal, Glc, Fuc, Ara, GlcA (44–47:25–29:19–20:1:1–2:4–5)	1.4×10^6 5.5×10^5 5.5×10^5	Antibacterial, Anti-biofilm, Anticancer	[68]
Flintiella sanguinaria	EPS	Culture centrifugal extraction and purification by ultra-filtration	Xyl, Gal, GlcA, Rha, Glc, Ara (47:21:14:10:6:2)	1.5×10^6		[69]
Cyanothece sp. CCY 0110	Cyanoflan	Cold aqueous extraction and	Man, Glc, uronic acid, Gal, Xyl, Rha, Fuc, Ara (20:20:18:10:9:9:8:6)	$>1 \times 10^6$		[50]

Source	Name	Preparation Method	Monosaccharides Composition	Mw (Da)	Bioactivity	References
		purification by dialysis				
Chlamydonas reinhardtii	EPS	Culture centrifugal extraction	GalA, Rib, Rha, Ara, Gal, Glc, Xyl	2.25 × 10^5	Antioxidant	[70]
Nostoc carneum	EPS	Culture centrifugal extraction	Xyl, Glc (4.3:2.1)		Antioxidant	[71]
Nostoc sp.	EPS	Culture centrifugal extraction and purification by DEAE column	Uronic acid, Rha, Fuc, Ara, Xyl, Man, Gal, Glc (25.0:0.2:0.8:18.6:15.3:19.1:1.3:19.7)	2.37 × 10^5	Antitussive, Immunomodulatory activity	[72][73]
Tetraselmis suecica	EPS	Cold aqueous extractionand purification by dialysis	Ara, Rib, Man, GalA, Gal, Glc, GlcA (5.23:0.83:6.64:0.1:25.27:35.46:21.47) [76]		Antioxidant, Anticancer	[74]
Leptolyngbya sp.	EPS	Culture centrifugal extraction	Man, Ara, Glc, Rha, uronic acid (35:24:15:2:8)		Antioxidant	[75]

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from *Colwellia psychrerythraea* 34H was fullly characterized to have a repeating unit, composed of a N-acetyl-quinovosamine (QuiN) unit and two galacturonic acid residues both decorated with alanine amino acids, which had a significant cryoprotective effect. By NMR and computational analysis, the pseudo-helicoidal structure of this EPS may block the local tetrahedral order of the water molecules in the first hydration shell, and could inhibit the ice recrystallization [23]. A novel anionic EPS, named as FSW-25, was produced by marine *Microbacterium aurantiacum.* FSW-25 was a high molecular-weight heteropolysaccharide with a high uronic acid content. It had good antioxidant potential when compared with xanthan, which might be due to the presence of sulphate and its higher uronic content [17]. In addition, the monosaccharide composition of the EPS and other residues could play an essential role in thermostability. For example, the high thermal stability of EPS1-T14 produced by *Bacillus licheniformis* was mainly attributed to the fucose content [42][77]. The role of the monosaccharides' composition in the thermal stability of the marine bacteria EPS has to be further investigated.

Compared with the diversity and complexity of the marine bacteria EPS, these EPS isolated from marine fungi exhibit less significant diversity in the monosaccharide composition (**Table 2**). The monosaccharide compositions show that these marine fungal EPS are mainly composed of neutral monosaccharide, including Glc, Man, and Gal with a different molar ratio. Generally, these EPS have antioxidant activity. Mao et al. completed relatively systematic studies on the structure and activity screening of marine fungal EPS. Several of the EPS are isolated and fullly characterized from *Aspergillus versicolor*, *Aspergillus Terreus, Fusarium oxysporum,* and *Hansfordia sinuosae* (**Table 2**). Recently, a novel EPS (AUM-1) with immunomodulatory activity was obtained from the marine *Aureobasidium melanogenum* SCAU-266. The AUM-1 with a molecular weight of 8000 Da had a main monosaccharide of Glc (97.30%), whose structure possessed a potential backbone of α-D-Glcp-(1→2)-α-D-Man*p*-

(1→4)-α-D-Glc*p*-(1→6)-(-(SO_3^-)-4-α-D-Glc*p*-(1→6)-1-β-D-Glc*p*-1→2)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-1→6)-α-D-Glc*p*-1→4)-α-D-Glc*p*-6→1)-[α-D-Glc*p*-4]$_{26}$→1)-α-D-Glc*p* [49]. The possible structure of these marine fungal EPS mentioned in **Table 2** are shown in **Figure 1**.

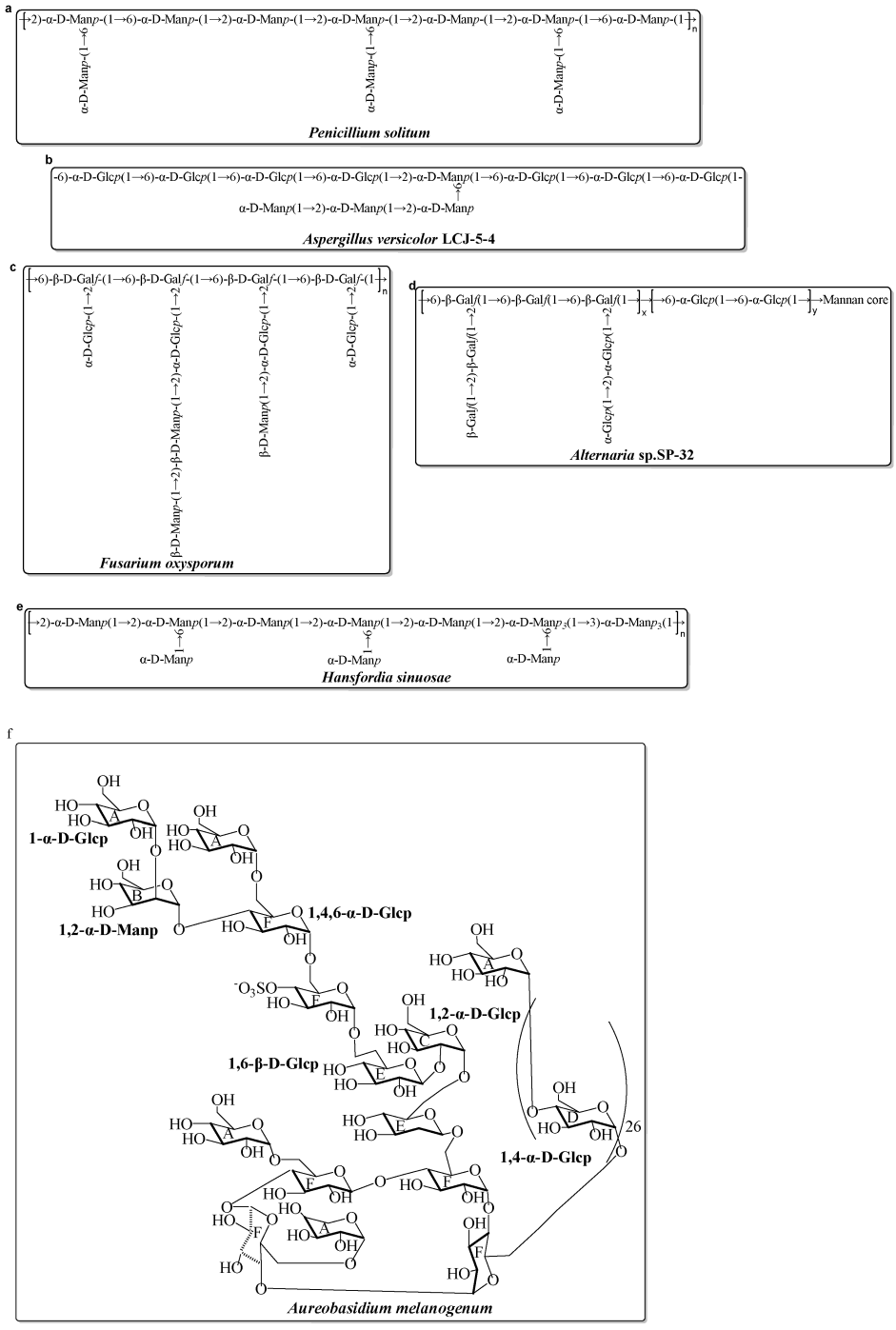

Figure 1. Structures of several EPS, isolated from marine fungi in the last decade [49][61][62][63][64][65][66]. (**a**) EPS from *Penicillium solitum*; (**b**) EPS from *Aspergillus versicolor*; (**c**) EPS from *Fusarium oxysporum*; (**d**) EPS from *Alternaria* sp.; (**e**) EPS from *Hansfordia sinuosae*; (**f**) EPS from *Aureobasidium melanogenum*.

Besides the marine bacteria and fungi, marine microalgae and cyanobacteria are other important resources to produce EPS. Information of some of the EPS obtained from marine microalgae and cyanobacteria in the last

decade are shown in **Table 3**. These EPS usually have a complex monosaccharide composition with uronic acid and sulfate groups, with various biological activities such as antioxidant, antiviral, antifungal, antibacterial, anti-ageing, anticancer, and immunomodulatory activities [78][79]. Recently, Esqueda et al. systematically explored the diversity of 11 microalgae strains belonging to the proteorhodophytina subphylum for EPS production. Regarding the compositions, some of the common features were highlighted, such as the presence of Xyl, Gal, Glc, and GlcA in all of the compositions, but with different amounts depending on the samples. In addition, the existence of sulfate groups in EPS from those microalgae strains were much more different [80]. The EPS from *Chlorella sorokiniana* had anticoagulant and antioxidant activities. The sulfate content and their binding site, monosaccharide composition, and glycoside bond were involved in its bioactivity [81]. Cyanoflan, a cyanobacterial-sulfated EPS, was characterized in terms of its morphology, structural composition, and rheological and emulsifying properties. The glycosidic linkage analysis revealed that this EPS had a highly branched complex structure with a large number of sugar residues, including Man, Glc, uronic acids, Gal, Rha, Xyl, Fuc, and Ara with a molar ratio of 20:20:18:10:9:9:8:6. The high molecular weight (>1 MDa) and entangled structure was consistent with its high apparent viscosity in aqueous solutions and high emulsifying activity [50]. The EPS from the cyanobacterium *Nostoc carneum* was a type of polyanionic polysaccharide that contained uronic acid and sulfate groups [71]. Another EPS from *Tetraselmis suecica* (Kylin) with antioxidant and anticancer activities also had a high amount of uronic acid [74]. The acid groups played important roles in the antioxidant activity of the marine EPS.

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