

## Marine Microbial Polysaccharides: Environmental Role and Applications (An Overview)

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**M**ARINE habitat represents about 70% of our planet. Microbial populations including bacteria, fungi and algae represent a base of food pyramids in this environment. The exudates of microbial cells (like polysaccharide) in the marine environment play an important role in energy cycling between the surface and the bottom in the sea by aggregation of small molecules causing them to sink. So it plays an important role in carbon sequestration and re-emission through what called biologic pump. Otherwise, microbial polysaccharides help the microbial cell itself to colonize hydrophobic surfaces forming biofilms and tolerate drastic conditions like heat, salinity and cold stresses. Marine microbial polysaccharides are characterized by unique properties making them a good source of bioactive agents that can be used in many fields as anti-tumor, antiviral, antioxidant, anticoagulant, food and feed applications. This current review will highlight the role of polysaccharides in the marine environment, its advantages and applications.

**Keywords:** Marine habitat, Polysaccharide, Biologic pump, bacteria

### Introduction

Exopolysaccharides (EPSs) produced by bacteria and eukaryotic phyto-plankton represent a major fraction of marine dissolved organic matter (DOM) by the biosynthesis and release into surrounding water (Santschi *et al.* 1999). It constitutes a large fraction of the reduced carbon reservoir in the ocean. In the marine environment, bacterial EPSs are essential for the production of aggregates, adhesion to or colonization of surfaces, and the formation of biofilms or sequestration of nutrients; EPSs thus provide protection for bacterial and ecosystem stability (Chin *et al.* 20005; Nichols *et al.* 2005; Lynch *et al.* 2017). The attention to EPSs has increased considerably recently, as they are used for many commercial applications in different industrial fields (Zannini *et al.* 2016). Compared to other bioactive constituents, polysaccharides from natural sources are found to be effective, non-toxic substances with a wide variety of pharmacological activities, such as immunomodulating agent, anti-tumor, anti-inflammatory and antioxidant

(Ananthi *et al.* 2010; Liu *et al.* 2012). There are still good prospects, however, for developing new polysaccharides with better properties than those of the existing polymers because of the wide diversity offered by microorganisms. Most research is aimed at identifying EPSs producing extremophiles with the idea that as these microorganisms survive environmental extremes of desiccation, temperature, pressure, salinity and acidity, it is to be expected that their EPSs will also have some unique properties to adapt to such extreme conditions (Poli *et al.* 2009).

Hyper-saline ecosystems can be highly productive, often containing dense populations of prokaryotes and microalgae (Oren 2002). Such environments may harbor unusual halophiles and halo-tolerant microorganisms of biotechnological interest. There are number of reports at EPSs production by moderately halophilic species e.g., *Halomonas eurihalina*, *Halomonas maura*, *Halomonas ventosae*, *Halomonas anticariensis*, *Alteromonas hispanice*, *Idiomarina rambicola*

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and *Idiomarina fontisalpitosi*. Polymers produced by these bacteria show potential applications as viscosifying, jellying, emulsifying and metal binding compounds. Sulfated EPSs also provide interesting applications in the pharmaceutical industry for antitumor (Inoue *et al.* 1988), antiviral (Zheng *et al.* 2006; Oren 2010) and anticoagulant (Nishino *et al.* 1989; Sutherland 1990) properties.

Therefore, the role of polysaccharide in the marine environment and its advantages as well as their applications will be presented in this review.

## 2. Exopolysaccharides identification

Exopolysaccharides (EPSs) are long chain biopolymers composed of repeating units of sugar moieties connected via glycosidic linkages (Mehta *et al.* 2014). Polysaccharides can be distinguished by their osidic composition: homo-polysaccharides, which contain a single type of monosaccharide, and hetero-polysaccharides composed of different osidic residues and usually displaying a regular backbone structure with a repeating unit. This repeating unit may be linear or branched and may contain up to 10 monomers as well as organic or inorganic substituents such as phosphate, sulfate, and lactic, succinic, acetic and pyruvic acids (Delbarre-ladrat *et al.* 2014)

The chemical structure including monosaccharide composition and repeating unit sequence as well as non-carbohydrate substituents is species-specific (Decho 1990) and may vary, most of the time, with production, culture conditions and the physiological state of the organism. The linkages most commonly found between monomers are  $\beta$ -1,4 or  $\beta$ -1,3 giving a more rigid backbone vs.  $\alpha$ -1,2 and  $\alpha$ -1,6 for more flexible zones. The overall physical properties of polysaccharides are also influenced by the monosaccharide composition, the osidic sequence and the network formed by the single polymer chains (Poli *et al.* 2010). These polymers are high-molecular weight macromolecules usually above  $10^6$  g mol<sup>-1</sup>.

### Importance of polysaccharides to microbial cells

#### Adaptation in hostile environments

EPSs are high molecular weight polymers secreted by microorganisms into the surrounding environment. These macromolecules can be found as in capsular material or as dispersed slime with no association to any particular cell

(Sutherland 1982). They act like an adhesive favoring interactions and cellular associations among microorganisms. In this manner, EPSs create micro-environments within which the transfer of genes and metabolites is very common, so providing a way for microorganisms to ensure their survival in nutrient-starved environments (Sutherland 2001). Another important role concerns the protective function they provide against high or low temperature and salinity or against possible predators (Krüger *et al.* 2008; Nichols *et al.* 2005). The production of polysaccharides allows the survival of cells by their interactions with ions such as heavy metals. EPSs produced by marine prokaryotes protect them against antibacterial compounds. EPSs are also able to concentrate charged organic molecules and inorganic ions (Maugeri *et al.* 2002).

#### Biofilm formation

Bacterial colonization on non-living surfaces such as suspended particles, metal surfaces and concrete or on living surfaces like sea weeds is thought to be one of the microbial survival strategies because it provides microorganisms with important advantages, including (i) increased access to nutrients, (ii) protection against toxins and antibiotics, (iii) maintenance of extracellular enzyme activities and (iv) shelter from predation (Dang and Lovell 2000). During colonization on particular surfaces, bacteria overproduce extracellular polymeric substances (EPS) (Geesey and White 1990). These EPS, especially EPSs, are the materials that construct the biofilm matrix, serving as a multipurpose functional element for adhesion, immobilization of cells on the colonized surface, protection, recognition and facilitating spatial arrangement of different species within the biofilm (Allison *et al.* 1998).

#### Dispersal agent during starvation

Marine habitat is characterized by the diversity of nutrient sources and availability (Alldredge and Hartwig 1986). In the case of low availability of nutrients (starvation) most microbes undergo different mechanisms to survive. In addition to the production of starvation-specific proteins (including proteolytic enzymes), which enable bacterial cell to scavenge nutrients (Albertson *et al.* 1990; Marden *et al.* 1987), further functions of starvation-induced survival mechanisms may include alteration of the ability of cell to adhere to hydrophobic surfaces causing it to disperse

in the environment and scavenge nutrients (Dawson *et al.* 1981; Hermansson *et al.* 1987). For example *Pseudomonas sp* strain S9 produces an extracellular polysaccharide (EPS) during the initial phase of starvation making the cells less adhesive to animate hydrophobic surfaces (Wrangstadh *et al.* 1986) and causing detachment from these surfaces.

#### *Role of polysaccharides in marine environments*

##### *Formation of aggregates*

Particle aggregation leading to production of marine detritus has been attributed to physico-chemical flocculation (Kranck and Milligan 1980). However, a duplication of these experiments with killed- bacterial controls indicates that presence of active bacteria is necessary for aggregate formation (Biddanda 1985). The formation of polysaccharide particles is an important pathway to convert dissolved into particulate organic carbon during phytoplankton blooms, and can be described in terms of aggregation kinetics. Transparent exopolymer particles (TEP) were first described by Alldredge *et al.* (1993) as a class of large, discrete, transparent particles in sea water and diatom cultures formed by dissolved exopolymers (formed mainly from exopolysaccharide) exudates by phytoplankton and bacteria. The existence of these particulates has far reaching implication for food web, structure, microbial process, carbon cycling and particulate flux in the ocean by its important role in aggregate formation.

A transmission electron microscope study of laboratory-produced marine aggregates showed bacterial extracellular polysaccharide processes to be responsible for aggregate formation. It also appears that similar extracellular material is responsible for attachment of bacteria to particles of decomposing seaweed (Biddanda 1986). The aggregates formed in this process sink transporting energy sources and other nutrients to the bottom and so complete the missing ring in the energy transfer between the surface and the bottom (Engel *et al.* 2004). Coagulation of single particles into rapidly settling aggregates as so called biological bump (Asper *et al.* 1992; Fig. 1). Engel (2002) found a relationship between  $\text{CO}_2$  concentration and the production of TEP, with TEP production being linearly related to theoretical  $\text{CO}_2$  uptake rates. This process resembles the mechanism involved in wastewater treatment plants where the treatment mainly depends on the formation of floc which sinks in the final settling tank forming sludge.

##### *Scavenging metal ions*

Some observations have demonstrated that nano-scale fibrils rich in acid polysaccharides form a major component of the population of colloidal particles in aquatic systems. These nanoparticles form a matrix for the formation of larger aggregates and can scavenge metal ions from the surrounding water (Santschi *et al.* 1999)

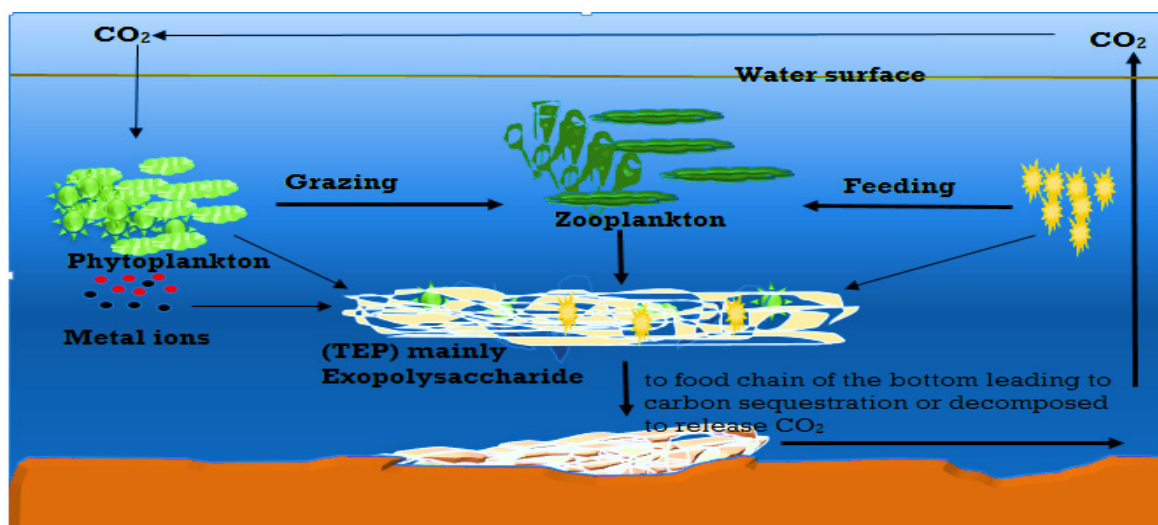


Fig. 1. Organic carbon formed by phytoplankton and incorporated in the food chain of the surface is aggregated by exopolysaccharide and scavenge some metal ions then sink to the bottom (biologic pump)

### Advantages of marine polysaccharides

Materials found in the marine environment are of great interest because the chemical and biological diversity found in this environment is almost uncountable and continuously growing with the research in deeper waters. Moreover, there is a lower risk of these materials causing illnesses to humans (Silva *et al.* 2012). It is obvious that the various extreme marine habitats (deep-sea hydrothermal vents, cold seeps, coastal hot springs, Polar Regions, hypersaline ponds, etc.) should represent a huge source of unknown and uncultivated bacteria. Many microbial EPSs produced by such extreme bacteria have unique properties. The bacteria must adopt special metabolic pathways to survive in extreme conditions, and so have better capacity to produce special bioactive compounds, including EPSs, than any other microorganisms (Laurienzo 2010). Halophilic bacteria are just such extremophiles and the properties of their extracellular polysaccharides seem to offer numerous applications in various

fields of industry (Margesin and Schinner 2001). More importantly, marine microbes tend to have significant osmotic tolerance leading to their capability for polysaccharide production at higher sugar concentration, which is very much desired for developing of an economically feasible process for polysaccharide production (Mehta *et al.* 2014). The marine microbial polysaccharides also characterized by containing glucuronic acid, galacturonic acid, amino sugars, and pyruvate which make them gain potential applications in fields such as pharmaceuticals, food additives, and industrial waste treatments (Wang *et al.* 2012).

### Producing polysaccharides from marine microorganisms

#### Polysaccharides from marine bacteria

Bacterial growth is often accompanied by the production of EPSs (Table 1), which have important ecological and physiological functions. Studies on the presence of bacteria in hypersaline environments appeared as early as 1914 (Pierce 1914).

**TABLE 1. Examples of polysaccharides produced by some marine bacteria and their applications**

Strain	Poly-saccharide	Structure	Application	Reference
<i>Alteromonas infernus</i>	GY785	monosulfated monosaccharide composed of three uronic acids (two glucuronic acids and one galacturonic acid) and six neutral hexoses (four glucoses and two galactoses)	Driving efficient mesenchymal stem cell chondrogenesis for cartilage repair	Roger <i>et al.</i> (2004) Senni <i>et al.</i> (2011)
<i>Halomonas</i> sp. AAD6	Levan	$\beta$ (2-6)-linked fructose	High biocompatibility and affinity with both cancerous and non-cancerous cell lines.	Küçükaşık <i>et al.</i> (2011)
<i>Halomonas salmeriensis</i> M8 <sup>T</sup>		Composed of two fractions, one of $6.3 \times 10^6$ and another of $1.5 \times 10^4$ Daltons. The high-molecular-weight fraction contains mannose (72% w/w), glucose (27.5% w/w) and rhamnose (0.5% w/w). The low-molecular-weight fraction contained mannose (70% w/w) and glucose (30% w/w).	-Emulsifying agent -bio-detoxifier	Diken <i>et al.</i> (2015)
<i>Haloterrigena turkmenica</i> halophilic archaeon		Composed of two main fractions of 801.7 and 206.0 kDa. It was a sulfated heteropolysaccharide containing glucose, galactose, glucosamine, galactosamine, and glucuronic acid	-emulsifying activity -moisture-retention ability	Squillaci <i>et al.</i> (2016)
<i>Halomonas maura</i>	Mauran	Molecular weight $4.7 \cdot 10^6$ Da. Contain % mannose, 34.8; galactose, 14; glucose, 29.3; glucuronic acid, 21.9. This EPS also contains approximately 1.3% w/w phosphate. sulfate content of 6.5% w/w	high capacity for binding lead and other cations	Arias <i>et al.</i> (2003)
A halophilic, thermotolerant <i>Bacillus</i> strain (B3-15)	Fretion 2	Mannopyranosidic Configuration with Molecular weight 600 KDa	-----	Maugeri <i>et al.</i> (2002)
<i>Halomonas myrmensis</i> AAD6T	levan	$\beta$ (2-6)-linked fructose	foods, feeds, cosmetics, pharmaceutical	Diken <i>et al.</i> (2015)
<i>Alteromonas macleodii</i>	-----	-----	capability of synthesizing biocompatible metal nanoparticle.	Mehta <i>et al.</i> (2014)
<i>Salipiger mucosus</i> strain A3T		a heteropolysaccharide with a molecular mass of 250 kDa and its components are glucose (19.7%, w/w), mannose (34%, w/w), galactose (32.9%, w/w) and fucose (13.4%, w/w)	Emulsifying activities	Llomas <i>et al.</i> (2010)

### *Polysaccharides from marine fungi*

For a long time, halotolerant and halophilic fungi have been known exclusively as contaminants of food preserved with high concentrations of either salt or sugar. They were first reported in 2000 to be active inhabitants of hypersaline environments, when they were found in man-made solar salterns in Slovenia (Gunde-Cimerman *et al.*, 2000). Studies of fungal populations in hypersaline environments have revealed the high diversity of fungal species, most of which do not require salt for growth, and have

their growth optimum in the absence of salt. The dominant fungal group in the hypersaline waters of salterns is the melanized polymorphic black yeast (Plemenitaš *et al.* 2014). Fungi often produce extracellular polysaccharides that are secreted into the growth media or remain tightly attached to the cell surface (Seviour *et al.* 1992). Research on extracellular polysaccharides from marine fungi was attempted for providing polysaccharide with novel functions and structures (Table 2; Chen *et al.* 2009; Sun *et al.* 2011).

**TABLE 2. Examples of polysaccharide produced by some marine fungi and their application**

Strain	Poly-saccharide	Structure	Application	Reference
<i>Phoma herbarum</i> YS4108	YCP (acronym of Yancheng polysaccharide)	It has a backbone of $\alpha$ -1,4-D-glucan with a lower proportion of $\alpha$ -1,6-linked glucopyranosyl and glucuronic acid residues as nonreducing terminals. Molecular weight of $2.4 \times 10^6$	1- Macrophage receptors 2-antitumor potential 3-Immunomodulatory Effects on T Cells and Dendritic Cells	Chen <i>et al.</i> (2009, 2014) Yang <i>et al.</i> (2005)
<i>Aspergillus versicolor</i>	AWP	Consists of glucose and mannose in a molar ratio of 8.6:1.0, and its average molecular weight is estimated to be 500kDa		Chen <i>et al.</i> (2013a)
<i>Fusarium oxysporum</i>	Fw-1	Consists of galactose, glucose, and mannose in a molar ratio of 1.33:1.33:1.00, its molecular weight is about 61.2 kDa. The structure of Fw-1 contains a backbone of (1 $\rightarrow$ 6)-linked $\beta$ -D-galactofuranose residues with multiple side chains.	antioxidant activity	Chen <i>et al.</i> (2015)
<i>Penicillium griseofulvum</i>	Ps1-1	Ps1-1 is a galactomannan with a molecular weight of about 20 kDa, and a molar ratio of mannose and glucose of 1.1:1.0.		Chen <i>et al.</i> (2013b)
<i>Penicillium</i> sp. F23-2	PS1-1, PS1-2 and PS2-1	PS1-1, PS1-2 and PS2-1 primarily consisted of mannose with variable amounts of glucose and galactose,	Antioxidant	Sun <i>et al.</i> (2009)

### *Polysaccharides from marine algae*

Sulfated polysaccharides can be found in different algae species in the marine environment. These polysaccharides don't have equivalents in terrestrial plants and resemble the chemical and biological properties of mammalian glycosaminoglycans. Because of this, they are receiving growing interest for application in health-related fields (Silva *et al.* 2012) (Table 3).

### *Application of marine polysaccharides*

Marine derived polysaccharides have been shown to have a variety of bioactivities such as antitumor, antiviral, anticoagulant, antioxidant, immuno-inflammatory effects

and other medicinal properties. The studies on the antiviral activities of marine natural products, especially marine polysaccharides, are attracting more and more attention all over the world. Marine-derived polysaccharides and their lower molecular weight oligosaccharide derivatives have been shown to possess a variety of antiviral activities (Wang *et al.* 2012)

### *Modification of polysaccharide from marine source*

Polysaccharides are a promising group of antioxidative compound. Some of them have been accepted to be one of the important candidates for the development of effective and non-toxic medicines with stronger free radical scavenging

and antioxidant actions. It was reported that the antioxidant activity of some compounds be relative to their polyhydroxyl group and this activity can be enhanced by chemical substitution (Yang *et al.*, 2005). Depending on the final purpose of their utilization, natural properties of

polysaccharides can be enhanced by structural modifications. Many polysaccharides derivatives such as degraded and semi-synthetic products, obtained by chemical modifications, demonstrate anticancer and cancer preventive properties (Table 4).

**TABLE 2. Examples of polysaccharide produced by some marine fungi and their application**

Strain	Poly-saccharide	Structure	Application	Reference
<i>Phoma herbarum</i> YS4108	YCP (acronym of Yancheng polysaccharide)	It has a backbone of $\alpha$ -1,4-D-glucan with a lower proportion of $\alpha$ -1,6-linked glucopyranosyl and glucuronic acid residues as nonreducing terminals. Molecular weight of $2.4 \times 10^6$	1- Macrophage receptors 2-antitumor potential 3-Immunomodulatory Effects on T Cells and Dendritic Cells	Chen <i>et al.</i> (2009, 2014) Yang <i>et al.</i> (2005)
<i>Aspergillus versicolor</i>	AWP	Consists of glucose and mannose in a molar ratio of 8.6:1.0, and its average molecular weight is estimated to be 500kDa		Chen <i>et al.</i> (2013a)
<i>Fusarium oxysporum</i>	Fw-1	Consists of galactose, glucose, and mannose in a molar ratio of 1.33:1.33:1.00, its molecular weight is about 61.2 kDa. The structure of Fw-1 contains a backbone of (1 $\rightarrow$ 6)-linked $\beta$ -D-galactofuranose residues with multiple side chains.	antioxidant activity	Chen <i>et al.</i> (2015)
<i>Penicillium griseofulvum</i>	Ps1-1	Ps1-1 is a galactomannan with a molecular weight of about 20 kDa, and a molar ratio of mannose and glucose of 1.1:1.0.		Chen <i>et al.</i> (2013b)
<i>Penicillium</i> sp. F23-2	PS1-1, PS1-2 and PS2-1	PS1-1, PS1-2 and PS2-1 primarily consisted of mannose with variable amounts of glucose and galactose.	Antioxidant	Sun <i>et al.</i> (2009)

**TABLE 3. Examples of polysaccharides produced by some marine algae and their applications**

Strain	Poly-saccharide	Structure	Application	Reference
Brown algae (Phaeophyceae)	Fucoidans	$\alpha$ -L-fucans, galactofucans, fucomannouronans and other intermediate structures	1-anticancer and cancer preventive agent 2- protective agent against heavy metal toxicity	Fedorov <i>et al.</i> (2013) Berteau and Mulloy (2003)
<i>Gyrodinium impudicum</i> strain KG03	p-KG03 Sulphated	Molecular weight of $1.87 \times 10^7$ , and was characterized as a homopolysaccharide of galactose with uronic acid (2.96% wt/wt) and sulfate groups (10.32% wt/wt).	Anti-encephalomyocarditis virus (EMCV)	Yim <i>et al.</i> (2004)
<i>Pavlova viridis</i>	P <sub>0</sub> Sulphated	Rha, D-Fru, Glu, Man Molecular weight 3645 KDa with $\beta$ -pyranose and $\alpha$ -pyranose configurations	Antioxidant	Sun <i>et al.</i> (2014)
<i>Sarcinochrysis marina</i>	S <sub>0</sub> Sulphated	D-Fru, Glu, Man Molecular weight 387 KDa with $\beta$ -pyranose and $\alpha$ -pyranose configurations	Antioxidant	Sun <i>et al.</i> (2014)
<i>Porphyra</i> sp.	Porphyran	sulfate ester and 3, 6-anhydrogalactose whose arrangement is similar to agarose	Antiallergic, antioxidant, antiviral, antifatigue, metallic adsorption ability, prebiotic, antibacterial, anticoagulant, antiviral, microphage promotion action, hypocholesterolemic, prebiotic activity.	Zhang <i>et al.</i> (2009)

**TABLE 4. Examples of modification processes of marine microbial exopolysaccharides (EPSs) and their applications**

Strain	EPS	Modification	Application	Reference
<i>Alteromonas infernus</i>	GY785	One step sulphation in ionic liquid producing highly sulphated derivatives (GY785 DRS)	-----	Chopin <i>et al.</i> (2015)
<i>Pavlovaviridis</i>	P0	Degradation: leading to increase sulphated content	Antioxidant	Sun <i>et al.</i> (2014)
<i>brown seaweed Saccharina japonica</i>	Alginate	Depolymerisation by subcritical water hydrolysis (SWH)	Antioxidant	Meillisa <i>et al.</i> (2015)
<i>Sarcinochrysis marina</i>	S0	Degradation(leading to increase sulphated content)	Antioxidant	Sun <i>et al.</i> (2014)
<i>Alteromonas macleodii</i> sub sp. <i>Fijiensis</i> biovardeepsane		Free-radical depolymerization with metallic catalyts	-----	Petit <i>et al.</i> (2006)
<i>Keissleriella</i> sp.	YCP	Sulphation producing 3 derivatives (YCP-SL, YCP-SM and YCP-SH)	Anticoagulant activity and antiplatelet aggregation	Han <i>et al.</i> (2005)
<i>Phomaherbarum</i> YS 4108	YCP	Chemical sulphation resulted in sulfated derivatives YCP-S1 and YCP-S2	Antioxidant	Yang <i>et al.</i> (2005)

## Conclusion

Microorganisms play an important role in element recycling and ecological balance especially in marine environment (oceans and seas) which occupies the largest area in the earth. Polysaccharides produced by such microorganisms are one of the most important exudates responsible for the survival in marine environment through different strategies such as food storage, biofilm formation and acting as dispersing agent during starvation. Also, polysacchrides consider a key player in carbon sequestration and stabilization of metal ions through what is called biologic pump responsible for the aggregation of small particles of the surface causing it to sink to the bottom. The exudates of microorganisms (mainly exopolysaccharide) are considered the amblers of this pump. On the other hand these exudates present a renewable pool for materials used in industrial and pharmaceutical applications.

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