Polysaccharides
DOI 10.1007/978-3-319-03751-6_47-1

Bioactivity and Applications of Polysaccharides from Marine Microalgae

Maria Filomena de Jesus Raposo, Alcina Maria Miranda Bernardo de Morais and Rui Manuel Santos Costa de Morais*
CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Porto, Portugal

Abstract

Marine microorganisms have been under research for the last decades, as sources of different biocompounds, each with various applications. Polysaccharides (**PSs**) are among these chemicals being produced and released by marine microalgae. These are very heterogeneous, including cyanobacteria and eukaryotic microalgae from several divisions/phyla, each of which with different characteristics. The **PSs**, sulfated or not, that they produce have already proved to be promising agents in various fields, such as food, feed, pharmaceutical, and biomedical. They can also be applied in wastewater and/or soil treatment and in some engineering areas, as naval engineering.

After a brief introduction on the general types of biopolymers produced by marine microalgae and cyanobacteria, this chapter starts by presenting the species of these microorganisms and the types of **PSs** they produce, as well as the respective chemical composition; goes into the production of **PSs** and the effect of specific compounds; and focuses on the physicochemical properties of these **PSs** and their composition and structure, approaching the rheological properties relevant for their functions and behavior. The bioactivity of **PSs** and their applications are, next, presented, including therapeutic applications based on their antiviral and antibacterial activities, antioxidant properties, anti-inflammatory and immunomodulatory characteristics, antitumoral activity, and antilipidemic and antiglycemic properties, among others. The potential use of **PSs** from marine microalgae as it is or incorporated in health foods is also considered. The mechanisms behind their antiviral and antibacterial activities are explained. Toxicological and safety issues are also disclosed, and there is a brief mention of the bioavailability of **PSs** from microalgae. The chapter ends by listing some preclinical studies with this type of polymers.

Keywords

Marine microalgae; Polysaccharides; Sulfate (exo)polysaccharides; Health foods; Bioactivity-Antioxidant; Antiviral; Antitumoral; Immunomodulators; Toxicity

Abbreviations

arab Arabinose
CaSp Calcium spirulan
CB Cyanobacterium(a)

*Email: rcmorais@porto.ucp.pt

DOI 10.1007/978-3-319-03751-6_47-1

EC_{50}	The molar concentration of a drug that produces 50 % of the maximum possible response for that drug
ED_{50}	In vitro or in vivo dose of drug that produces 50 % of its maximum response or effect
fru	Fructose
fuc	Fucose
GAG	Glycosaminoglycan
gal	Galactose
galAc	Galacturonic acid
glcAc	Glucuronic acid
glc	Glucose
IC_{50}	The molar concentration of a drug which produces 50 % of its maximum possible inhibition
ID_{50}	In vitro or in vivo dose of a drug that causes 50 % of the maximum possible inhibition for that drug
man	Mannose
MW	Molecular weight
NaSp	Sodium spirulan
NO	Nitric oxide
PS	Polysaccharide
rham	Rhamnose
sEPS	Sulfated exopolysaccharide
sPS	Sulfated polysaccharide
xyl	xylose

1 Introduction

Polysaccharides (**PSs**) and oligosaccharides can be synthesized by microorganisms; some of them are even secreted out into the environment (or culture medium), from which they are easily extracted. **PSs** have been studied for a long time due to their characteristics, especially their conformation, which is reflected in their chemical behavior and, therefore, in their wide range of applications. However, the knowledge on their complete composition and structure is still taking the first steps, and despite the applications that might appear after a solid understanding of their structure and conformation, these applications could even be extended to the medicine field. As a matter of fact, there is some difficulty on studying these polymer chains because of the diversity and distribution of simple sugars (mono-, di-, and oligosaccharides) along the **PSs** chains and whether these chains are linear or ramified. Therefore, the analysis of the **PSs** structures and, consequently, their applications is a real challenging task. Thus, the analysis of these polymer chains has been limited to oligosaccharides obtained by hydrolysis of high molecular polymers and to *X-ray* diffraction studies of **PSs** gels (Eteshola et al. 1998).

Among the **PSs** produced by microalgae and cyanobacteria (thereafter referred to as microalgae), especially marine species, only the **sPS** from *Gyrodinium impudicum* is a homopolymer of **gal** (Yim et al. 2007) and perhaps a cell wall **PS** from *Chlorella vulgaris*, a *β*-(1,3)-glucan (Nomoto et al. 1983), composed of D-**glc**; the **PSs** from all the other marine unicellular algae are heteropolymers, mostly constituted of **xyl**, **gal**, and **glc** in different proportions, but some other neutral sugars can also be constituents of the **PS**, as it is the case of **rham**, **man,** or **fuc**, and also some

DOI 10.1007/978-3-319-03751-6_47-1

Table 1 Marine species of microalgae producing **PS**s

Microalgae/ cyanobacteria	Group	Type of polysaccharide	Main neutral sugars	References
Microalgae				
Cylindrotheca closterium	Diatoms	**sPS**	xyl, glc, man, rham	Staats et al. 1999; Pletikapic et al. 2011
Navicula salinarum		**sPS**	glc, xyl, gal, man	Staats et al. 1999
Phaeodactylum tricornutum		**sEPS**	glc, man, xyl, rham	Guzman et al. 2003; Ford and Percival 1965a, b
Haslea ostrearia				
Nitzschia closterium		**EPS**		Rincé et al. 1999
Skeletonema costatum		**EPS**		Penna et al. 1999
Chaetoceros sp.		**EPS**		
Amphora sp.		**EPS**		Chen et al. 2011
Chlorella stigmatophora	Chlorophytes	**sPS**	glc, xyl, **fuc**	Guzman et al. 2003
C. autotrophica		**sPS**		Guzmán-Murillo and Ascenci 2000
C. vulgaris		**PS** β-(1,3)-glucan	rham, gal, arab 2-*O*-methyl-rham glc	Ogawa et al. 1997, 1999; Nomoto et al. 1983
Dunaliella salina		**EPS**	gal, glc, xyl, fru	Mishra et al. 2011
Ankistrodesmus angustus		**EPS**		Chen et al., 2011
Botryococcus braunii		**EPS**	gal, **fuc**, glc, rham	Allard et al. 1987 Allard and Casadeval 1990
Tetraselmis sp.	Prasinophyte	**sPS**		Guzmán-Murillo and Ascencio 2000
Isochrysis sp.	Prymnesiophyte/ haptophyte	**sPS**		Guzmán-Murillo and Ascencio 2000
Porphyridium sp.	Rhodophytes	**sPS**	xyl, gal, glc	Geresh and Arad 1991; Dubinsky et al. 1990; Arad 1988
P. cruentum		**sPS**	xyl, gal, glc,	Garcia et al. 1996;
P. purpureum		**sPS**	3-*O*-methyl-xyl	Kieras 1972; Raposo
Rhodella reticulata		**sPS**	xyl, rham	et al. 2014; Gloaguen
R. maculata			3-*O*-methyl- rham	et al. 2004; Geresh et
			4-*O*-methyl-gal	al. 2002a; Dubinsky et
			xyl, gal, glc	al. 1992
			3-*O*-methyl-xyl	Radonic et al. 2010
				Geresh and Arad 1991; Dubinsky et al. 1992
				Evans et al. 1974; Fareed and Percival 1977
Cochlodinium polykrikoides	Dinoflagellates	**sPS**	man, gal, glc	Hasui et al. 1995
Gyrodinium impudicum		**sPS**	gal	Yim et al. 2007

(*continued*)

DOI 10.1007/978-3-319-03751-6_47-1

Table 1 (continued)

Microalgae/ cyanobacteria	Group	Type of polysaccharide	Main neutral sugars	References
Cyanobacteria *Aphanothece halophytica*	Cyanophytes	**EPS**	glc, **fuc**, man, arab	Li et al. 2001
Arthrospira platensis		**EPS** s-Spirulan	gal, xyl, glc, fru rham, **fuc**, glc 3-*O*-methyl-rham	Radonic et al. 2010; Hayashi et al. 1996b; Martinez et al. 2005
Anabaena, *Gloeothece*, *Nostoc Aphanocapsa*, *Phormidium*, *Synechocystis*, *Cyanothece*		**sPS**		Hayashi et al. 1996b; Senni et al. 2011; Lee et al. 2000 Senni et al. 2011

Adapted from Raposo et al. (2013)

methyl sugars (Table 1). But in spite of this similarity in monosaccharide composition, the types of sugar themselves and the glycosidic bonds between each molecule are two of the characteristics that establish all the differences between the properties of **PSs** found in microalgae. Either the composition of monosaccharides and their distribution or the percentage in sulfate and uronic acids greatly determines the rheological behavior of the **PSs**, whose aqueous solutions can be highly viscous, as it happens with the **EPSs** from the marine red microalgae, or they may not present any apparent viscosity, as it is the case of the glucuronorhamnan from *C. vulgaris*.

2 Marine Sources

In the last decades the interest in products that have a marine origin, mainly seaweeds and microalgae, and also in the compounds they produce is growing rapidly. Nevertheless, microalgae have an advantage over macroalgae: they are easy to grow and manipulate, and harvesting does not depend on the climate or season. Marine microalgae do not need much for culturing: a simple medium of seawater, with a source of nitrogen, phosphate, iron, magnesium, and some minor salts, is the only requirement to produce them. Their culture can be easily controlled and, hence, the properties and physicochemical characteristics of the biocompounds they produce, such as the polysaccharides, can be maintained all over different cultures.

2.1 Marine Unicellular Algae Producing PSs

Some marine/brackish species are already produced commercially, as it is the case of *Arthrospira* (*Spirulina*) *platensis*, *Dunaliella salina*, *Isochrysis galbana*, *Nannochloropsis salina*, *Phaeodactylum tricornutum*, and *Porphyridium cruentum* (Fig. 1), either for their biomass and/or extracts or the compounds they produce. In addition, many other species are known to produce and secrete out **PSs** into the culture medium (Table 1), **EPSs**, which can be, or not, sulfated polysaccharides (**sPSs**) these **EPSs** show properties that go from application as antiviral agents to inclusion in health foods. But these marine microalgae are so diverse (Table 1) that it seems useful to locate their taxonomic positions and present some of their characteristics.

All diatoms belong to the class Bacillariophyceae, which includes organisms with round cells (Centrophycidae) and organisms with elongated cells (Pennatophycidae). *Chaetoceros* and

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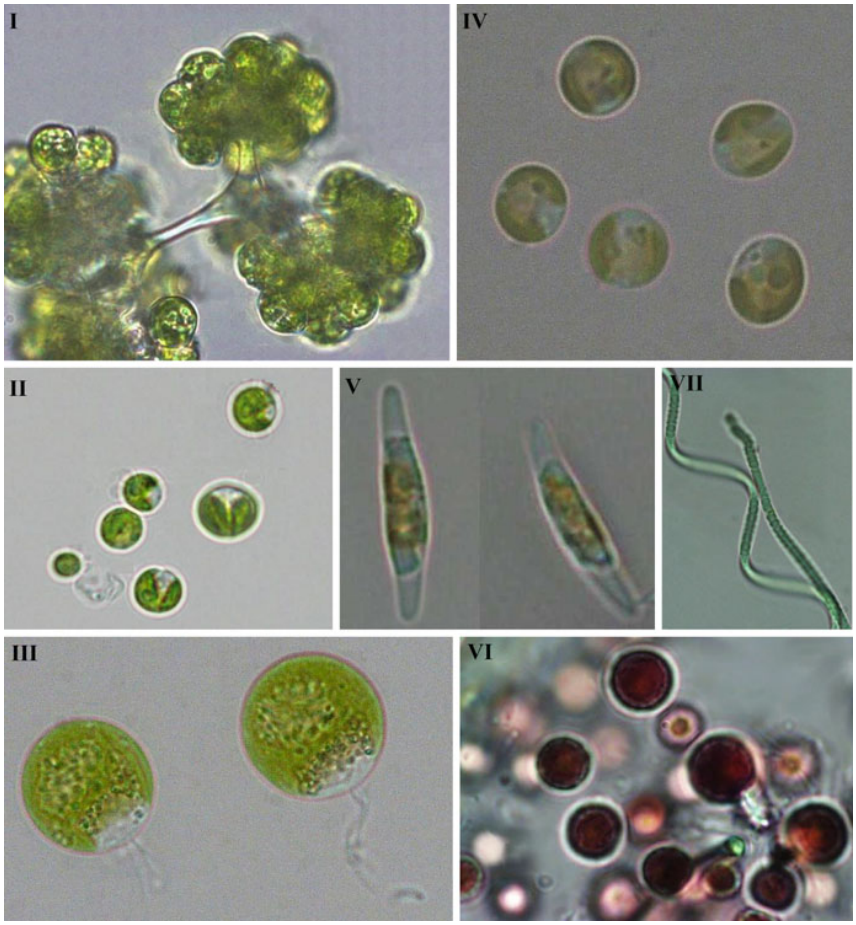

Fig. 1 Some of the microalgae cited in this chapter: **I**, *Botryococcus braunii*; **II**, *Chlorella vulgaris*; **III**, *Dunaliella salina*; **IV**, *Isochrysis galbana*; **V**, *Phaeodactylum tricornutum*; **VI**, *Porphyridium cruentum*; **VII**, *Arthrospira platensis*

Skeletonema belong to the first group; *Amphora*, *Cylindrotheca*, *Haslea*, *Navicula*, *Nitzschia,* and *Phaeodactylum* (Fig. 1-V) are included in the second group. The main characteristic of these unicellular organisms is the presence of a silicate ornamented two-piece frustule surrounding the protoplast. Their brownish color comes from the large quantities of xanthophylls (fucoxanthin, diatoxanthin, diadinoxanthin, neoxanthin), but they also possess chlorophylls a and c and α- and *β*-carotenes. Their main reserves are lipids, leucosin (or chrysolaminarin, a *β*-(1,3)-linked and *β*-(1,6)-linked glucose polymer) being the second main reserve.

Isochrysis (Fig. 1-IV) is a flagellated organism belonging to the class Prymnesiophyceae (or Haptophyceae). These golden-colored unicellular algae also have chlorophylls a and c, *β*-carotene, and xantophylls fucoxanthin, diatoxanthin, diadinoxanthin, and echinenone. They usually present two flagella and one smooth haptonema (hence the name of the class). Their main reserve compound is leucosin. Prymnesiophyceae and Bacillariophyceae are two classes of the phylum Chromophyta.

Another diverse group of algae is phylum Chlorophyta. As it happens with the plants, the microalgae members of Chlorophyta are green in color due to the high quantities of chlorophylls a and b. But α- and *β*-carotene and xantophylls (neoxanthin, lutein, violaxanthin, and zeaxanthin) are also present. Their reserve is mainly starch. This is a very diverse group, including macro- and microalgae. Only two classes are referred to in this chapter: Prasinophyceae, to which *Tetraselmis*

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Table 2 Percentage of sulfate, protein, and uronic acids in polysaccharides from different marine microalgae

Microalgae/ cyanobacteria	Sulfate (%)	Protein (%)	Uronic acids (%)	References
Microalgae				
Porphyridium sp.	4–14.6	1–5.5	7.8–18	Geresh and Arad 1991; Sun 2010; Arad et al. 1985, Gloaguen et al. 2004; Raposo et al. 2014
Rhodella sp.	8	6	5–7.8	Geresh and Arad 1991; Badel et al. 2011a
B. braunii			24	Fernandes et al. 1989
C. stigmatophora	7.8–9.4		3.7–9.0	Guzman et al. 2003
C. vulgaris	–	–	14	Ogawa et al. 1999
P. tricornutum	7.5–13.3		1.4–6.3	Guzman et al. 2003
C. closterium	0–10.9	7.7–9.2	4.8–21.0	Staats et al. 1999
N. salinarum	6.3–11.5	0.5–4.9	7.7–8.0	Staats et al. 1999
C. polykrikoides	7–8		(a) presence	Hasui et al. 1995
G. impudicum	10.3		2.9	Yim et al. 2007
Cyanobacteria				
A. platensis	5–20	6	7–14.4	Lee et al. 2000; Trabelsi et al. 2009
(s-Spirulan)	3.24–5.7		15–16.5	Hayashi et al. 1996b; Lee et al. 1998, 2000
A. halophytica	–	–	14	Li et al. 2001

Adapted from Raposo et al. (2013)

belongs, and Chlorophyceae, the latter includes *Chlorella* (Fig. 1-II), *Ankistrodesmus*, and *Botryococcus braunii* (Fig. 1-I), all of them being Chlorococcales.

Porphyridium and *Rhodella* are two genera from the phylum Rhodophyta. This is the group that includes red macro- and microalgae. The main photosynthetic pigments are chlorophylls a and d, but their red color is associated mostly to the phycobiliproteins phycocyanin, allophycocyanin, and phycoerythrin. Lutein is the main xanthophyll. *Porphyridium* belongs to the class Porphyridiophyceae, order Porphyridiales, and family Porphyridiaceae; *Rhodella* is included in the class Rhodellophyceae, order Rhodellales, and family *Rhodellaceae*. However, there are still some organisms with different scientific names, such as *Dixionella grisea*, *Rhodella reticulata*, and *Porphyridium purpureum* and *P. cruentum* (Fig. 1-VI).

Both *Cochlodinium* and *Gyrodinium* are dinoflagellates that belong to the phylum Pyrrophyta, class Dinophyceae, and order Gymnodiniales. The pigments that characterize dinoflagellates are chlorophylls a and c2, β-carotene, and xantophylls piridinine, dinoxanthin, diadinoxanthin, diatoxanthin, and neodinoxanthin; fucocyanin is the main pigment responsible for the brownish color of pyrrophytes; starch and lipidic droplets are the main reserve substances. Dinoflagellates are very particular organisms, many of them produce highly toxic compounds (dinotoxins), the most known being saxitoxins and gonyautoxins (paralytic shellfish toxins or PST), two groups of carbamate alkaloid neurotoxins, brevetoxins (another group of neurotoxic shellfish toxins or NST), and the diarrheic shellfish toxin okadaic acid (Camacho et al. 2007; Wang 2008). These toxins affect all marine organisms' and also humans' lifes as seafood consumers. The red toxic tides are due to a high accumulation (or bloom) of flagellated dinophyceae, the red color coming from the remarkable accumulation of carotene.

Cyanophyta is a group of prokaryotic organisms that is most of the times studied along with microalgae (eukaryotic organisms). Cyanophytes are unicellular, solitary, or colonial organisms. This phylum includes a class, Cyanophyceae, with either filamentous or nonfilamentous structures,

distinction of subclasses being based on hormogonia formation. *Aphanocapsa*, *Aphanothece*, *Cyanothece*, *Gloeothece*, and *Synechocystis* do not form hormogonia and, therefore, they are included in the subclass Coccogonophycidae, order Chroococcales; *Anabaena*, *Arthrospira* (Fig. 1-VII), *Nostoc*, and *Phormidium* belong to the subclass Hormogonophycidae, order Nostocales/Oscillatoriales, whose filaments are not ramified or, if they present branches, these are false. Only one chlorophyll (chlorophyll a) and phycocyanin are their main pigments, but they also contain carotenes and phycoerythrin (Table 2).

2.2 Production of the Polysaccharides: Influence of Specific Compounds

The production of **EPSs** and their composition depend on the algal species; on the strain; on the composition and nutrient status of the culture medium, namely, the N source (Banerjee et al. 2002), the N/P ratio, and the deficiency in silicon (for diatoms); and on the culture growth phase. Some microalgae produce large amounts of **EPSs** during the stationary phase, but some others increase the yield and continue to release even during the exponential phase of growth (Ramus and Robins 1975), when synthesis of biocompounds is more active, or even during both the growth phases, depending on the culture conditions (Penna et al. 1999).

Glyoxylate is one of the compounds that can positively influence the production of **EPSs** (Bergman 1986). In *A. cylindrica*, *C. capsulata*, and *Scenedesmus obliquus*, the addition of glyoxylate to the culture medium enhanced the yield of **EPSs**. One explanation could be the metabolization of glyoxylate into serine via glycine, a process associated to photorespiration. Glyoxylate, thus, induced some changes in the metabolism of carbon, increasing its relative yield and, therefore, increasing intracellular **PSs** and the release of soluble **EPSs** (Bergman 1986; De Philippis et al. 1996; Liu et al. 2010). However, in some microalgae, the **PS** is secreted out into the culture medium only when N metabolism is not affected. As a matter of fact, after being exposed for a short period to glyoxylate, the concentration of **EPSs** produced by *C. capsulata* increased by 43 % (De Pilippis et al. 1996). Nevertheless, nitrogen starvation had also proved to induce an overproduction of carbohydrates, via an alternative pathway, with the consequent release of **PSs**, not only in other cyanobacteria but also in microalgae (de Phillipis et al. 1993; Arad et al. 1992). In addition to glyoxylate, some other substances can interfere with the production and release of **PSs**. For example, an increase of **EPS** can be induced by a magnesium shortage (de Phillipis et al. 1991; Raposo et al. 2014) or by higher ion concentrations (Raposo et al. 2014), depending on the culture medium and species of microalga. The ratio N/P and a deficiency in silicon also influence the production and release of **PSs** – while a high ratio N/P induces an increase in the **EPS** from *N. closterium*, *S. costatum* and *Chaetoceros* produce high quantities of **EPS** under low N/P ratios (Penna et al. 1999).

3 Biochemical Composition and Physical Properties

Carbohydrates represent the major group of compounds synthesized by microalgae and include some of the substances under research for the last decades because of their physicochemical and biological properties and promising applications, even in medicine. But it is well known that the biological activities of **PSs** are closely related to the chemical composition and structure of the polymers, these factors being also the reason for their physicochemical behavior. Some of the characteristics that must be taken into account are their molecular weight, as large molecules are difficult to transfer across membranes in order to carry out their specific functions, and their sulfate and uronic acid content (or other constituents that can give the polymers their anionic and acidic

DOI 10.1007/978-3-319-03751-6_47-1

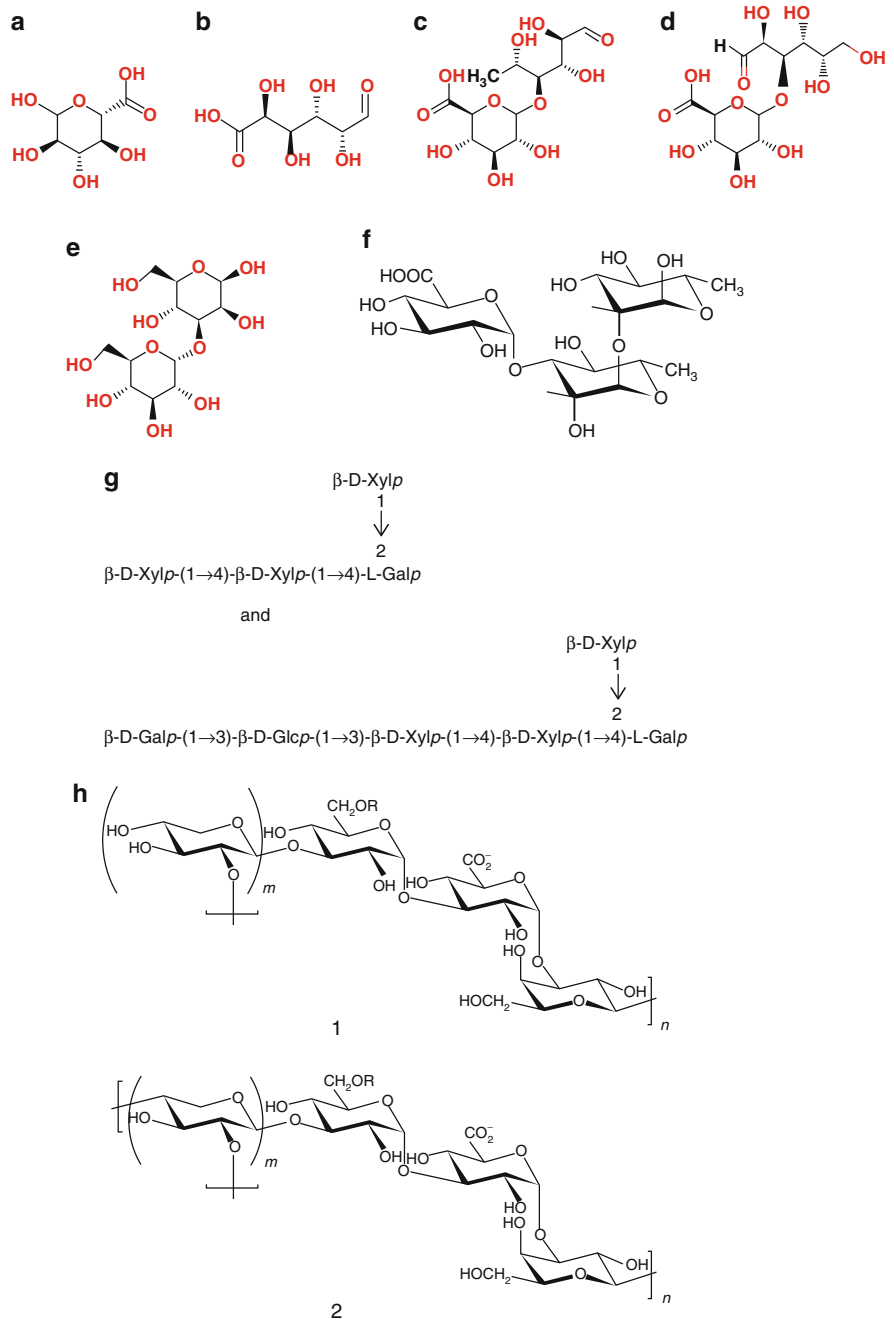

Fig. 2 Some of the components already identified for the **PSs** produced by marine microalgae: (**a**) D-glucuronic acid; (**b**) D-galacturonic acid (http://www.chemspider.com; accessed on 07-04-14); (**c**) glucuronyl-**rham** (or glucuronosyl-(1,4)-L-**rham)**, (**d**) 3-*O*-α-D-glucopyranuronosyl-L-**gal,** and (**e**) *O*-D-glucopyranosyl-(1,3)-*O*-D-mannopyranose are aldobiuronic acids found in the acidic **PSs** produced by microalgae (**c** and **e** http://www.chemspider.com; **d** adapted from http://www.chemspider.com and http://www.ebi.ac.uk; accessed on 07-04-14); (**f**) α-D-glucuronosyl-α-L-rhamnosyl-α-L-**rham**, an acidic trisaccharide found in *Chlorella* (Ogawa et al. 1999); (**g**) oligosaccharides I and II from *Porphyridium* (Gloaguen et al. 2004); (**h**) models 1 or 2 for the possible acidic repeating unit in polysaccharide II, from *Porphyridium* sp., according to Geresh et al. (2009); R = H, SO_2O, terminal **gal** or terminal **xyl**, m = 2 or 3

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properties), as these components seem to have great influence on their biological activity and applications. The number of monosaccharides, the type of linkages and distribution in the molecule, the conformation and type of chains (linear or ramified), and the existence of some other chemical groups (such as amino acids, proteins, or nucleic acids) that can be (non)covalently linked to the **PS** chains are other features that are worthy to be evaluated, along with the rheological properties and resistance to digestion, either acidic or enzymatic.

The composition of the **PSs** may differ due to the method used for the extraction and hydrolysis. Sometimes a single strong step of acidic hydrolysis is used; some other times the sugar profile is obtained by means of a multistep hydrolysis associated to an ion exchange fractionation (Dubinsky et al. 1992). The fractionation and centrifugation can help to obtain different polymers, which can be separated from the initial **PS**, as they have different sedimentation coefficients (Kieras and Chapman 1976). In *Porphyridium*, for example, one of the fractions obtained by the elution with urea showed to be xylan, **xyl** and **glcAc** being the main constituents (75 % and 13 %, respectively) (Geresh et al. 1992). In *R. reticulata*, a similar fraction was obtained by the same technique, **xyl** and **glcAc** also being the predominant constituents (Dubinsky et al. 1992). Cleavage of the **PS** molecule can also be attained by enzymatic action by **PS**-lyases (EC4.2.2.-) and **PS**-hydrolases (EC3.2.1.-), endo- and exoenzymes, but the process can be time-consuming (Badel et al. 2011b) due to all the techniques that have to be employed. These researchers, however, developed a new promising method by adapting the Biofilm Ring Test® (or BRT®) technique used to degrade the **PS**. They applied the BRT in microplate assays and associated the BRT® to the biofilm index (BFI), which corrects some of the discrepancies between images of the former technique, before and after the magnetic treatment of particles (Badel et al. 2011b).

3.1 Structure

Within the group of **PSs**, not only the intracellular and the cell wall **PSs** but also and mainly the exo- or extracellular polysaccharides (**EPS**) will be focused in this work. Among all these polymers, only the **sPS** of *G. impudicum* is a homopolymer of **gal** (Yim et al. 2007), a galactan, and perhaps the cell wall **PS** of *C. vulgaris*, a *β*-(1,3)-glucan, composed of **glc**; the **EPSs** from all the other marine microalgae are heteropolymers of **gal**, **xyl**, and **glc** in different proportions. Other sugars can also be constituents of the **PSs**, such as **rham**, **fuc**, **fru**, and some unusual methyl sugars. The types of glycosidic linkages are described only for some of the **PSs** produced by microalgae. This is the case of the **EPS** from *A. halophytica* – most of the linkages are 1,3-type (1,3-linked **glc**, 1,3-linked **fuc**, 1,3-linked **arab**, 1,3-linked **glcAc**), but 1-linked **glc** and 1-linked **glcAc** (Fig. 2a) can also be found, as well as 1,2,4-linked **man** and 1,3,6-linked **man** (Li et al. 2001). In the **CaSp** of *A. platensis*, the linkages and monosaccharides of the backbone structure are usually 1,3-linked **rham** and 1,2-linked 3-*O*-methyl-**rham** (acofriose) (Lee et al. 1998). 2,3-di-*O*-methyl-**rham** and 3-*O*-methyl-**xyl** are the monosaccharides in the nonreducing end. Besides D-**xyl**, D-**glc,** and L- and D-**gal**, the main neutral sugars, the **EPS** from *P. cruentum* has also small amounts of 3-*O*-methyl-**xyl**, 3-*O*- and 4-*O*-methyl-**gal**, and 2-*O*-methyl-**glcAc** (Percival and Foyle 1979). This type of monosaccharide is also part of the glucurono-rhamnoglycan, or glucuronorhamnan (White and Barber 1972; Ogawa et al. 1999) of *Chlorella*, as 2-*O*-methyl-L-**rham** and 3-*O*-methyl-L-**rham** (or acofriose) (Ogawa et al. 1997); these methylated **rham** sugars seem to appear only in some green algae. 2-*O*-methyl-L-**rham** was firstly reported by Ogawa et al. (1997) to be part of the **PS** of *Chlorella*, but, in fact, it was also identified in *A. platensis* (Collins and Munasinghe 1987). Despite seeming to be a characteristic of *Chlorella* (Ogawa et al. 1997), 3-*O*-methyl-L-**rham** was also identified in *B. braunii* and *A. platensis* (Lee et al. 1998; Collins and Munasinghe 1987). *B. braunii* also present other less common methyl sugars: 3-*O*-methyl-**fuc** and 6-*O*-methyl-hexose besides **fuc** (Banerjee et al. 2002). Besides **rham**

DOI 10.1007/978-3-319-03751-6_47-1

(52.3 %), the main neutral sugar, and other minor monosaccharides (**fuc**, **glc**, **arab**), **CaSp**, another **PS** of *A. platensis*, presents small amounts of 2,3-di-*O*-methyl-**rham** and 3-*O*-methyl-**xyl** (Lee et al. 1998). The positions of sulfate in the exocellular glycan of *P. cruentum* were identified (Archibald et al. 1981) in the **glc** and **gal** residues, as D-galactopyranose 6-sulfate, D-glucopyranose 6-sulfate, and D-galactopyranose 3-sulfate.

If the composition of monosaccharides is indicated for most of the **PSs** by several researchers, further information for higher levels of organization of the polymers is scarce and only for a couple of microalgae. Only some di- and oligosaccharides are described, some of them are characteristic for the microalgae from which the **PS** was obtained. White and Barber (1972) and Ogawa et al. (1998, 1999) advanced in the structure of the glucuronorhamnan of *Chlorella* with the identification of the disaccharide 3-*O*-α-D-glucopyranuronosyl-L-rhamnopyranose (or glucoronosyl-**rham**) (Ogawa et al. 1998) (Fig. 2c) and the acidic trisaccharide α-D-glucopyranuronosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranose (or glucoronosyl-rhamnosyl-**rham**, $C_{18}H_{30}O_{15}$, Ogawa et al. 1999) (Fig. 2f), whose molecular weight was found to be 73 kDa, and with the determination of the respective structures. Besides the aldobiuronic acid 3-*O*-(α-D-glucopyranosyluronic acid)-L-galactopyranose (Fig. 2d), two different heterosaccharides were found to be part of the structure of the **EPS** from *P. cruentum*. These two oligosaccharides were identified after digestion of the **EPS** (Pignolet et al. 2013), whose complete structure was established by Gloaguen et al. (2004) (Fig. 2g, h), who also found the monosaccharide composition and distribution, their absolute configuration in the molecules, and that the oligosaccharide 1 was part of the oligosaccharide 2. Hence, they found the unusual presence of both L-and D-**gal** isomers in the **EPS** from *P. cruentum*, a common characteristic of the red algae **PS**, besides having confirmed the presence of the aldobiuronic acid. **EPS** from *P. cruentum* can, eventually, present two other aldobiuronic acids (3-*O*-(2-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactopyranose and (3-*O*-(2-*O*-methyl-α-D-glucopyranosyluronic acid)-D-glucopyranose) (Heany-Kieras and Chapman 1976), but signals of OMe groups should be confirmed (Geresh et al. 1990). This acid-type was also found in other species of *Porphyridium* and in *Rhodella reticulata* (Geresh et al. 1990). However, a lot of work is still necessary in order to determine whether these oligosaccharides are repeating building blocks of the **EPS** and whether there is any kind of alternate distribution. Another microalga, whose **sPSs** (**CaSp** and **NaSp**) have an aldobiuronic acid-type disaccharide, the *O*-hexunorosyl-**rham**, and *O*-rhamnosyl-acofriose, another disaccharide, is *A. platensis*. But as far as we know, the "hexose" is still waiting to be identified. Further, Lee et al. (2000) referred the structure as consisting alternately of a uronic acid and a **rham**. The *O*-rhamnosyl-acofriose, *O*-rhamnosyl-(3-*O*-methyl-**rham**), is the other repeating di-unit identified as being part of **CaSp** and **NaSp** (Lee et al. 2000; Kaji et al. 2004) constituted by alternating molecules of **rham** and acofriose (Lee et al. 2000). Besides these disaccharide units, an oligosaccharide is also part of the **CaSp** – the trisaccharide *O*-rhamnosyl-acofriosyl-**rham** (Lee et al. 2000). A tetrasaccharide was also identified: it is composed of two units of an aldobiuronic acid; in other words, two uronic acids alternating with two **rham** monosaccharides make up that tetrasaccharide. The di-unit uronic acid-**rham** can eventually be repeated some more times along the **CaSp** glycan, forming some more repeated units of the aldobiuronic acid-type disaccharide (Lee et al. 2000).

Despite being almost "forgotten," some knowledge on the structure of the **PS** produced by *P. tricornutum* came into light some decades ago (Ford and Percival 1965a, b). At that time, these researchers found that the **PS** from this diatom is a hetero-, ramified polymer, one of the products obtained by acid hydrolysis being a sulfated glucuronomannan, composed of β-(1,3)-linked **man**. The aldotriuronic acid *O*-D-glucopyranosyluronic acid-(1,3)-*O*-D-mannopyranosyl-(1,2)-*O*-D-mannopyranose (Fig. 2e) was found to be a constituent of the side chains. As a matter of fact, an

DOI 10.1007/978-3-319-03751-6_47-1

aldobiuronic acid was also identified as being *O*-D-glucopyranosyluronic acid-(1,3)-D-mannopyranose. Another derived product, a glucan, seems to be the other constituent of the crude **PS**, comprising of β-(1,3)-linked **glc** units.

3.2 Rheology

In order to fully understand the several uses and applications of (**E**)**PSs**, the physicochemical characteristics must be taken into account. Rheological properties and the molecular weight are some of the most important parameters as they seem to be relevant to their functions and behavior.

Most **PSs**, especially **EPSs**, are polymers of high molecular weight (220–2.9 × 10^3 kDa; Kaji et al. 2004; Hayashi et al. 1996b; Hasui et al. 1995; Li et al. 2001; Pignolet et al. 2013; Mishra et al. 2011), negatively charged (anionic) and sulfated. Sulfated half-ester groups and uronic acids (mostly **glcAc** and **galAc**), along with the carboxyl groups, are responsible for the acidic and anionic characteristic of **PSs**.

Unfortunately, information on the rheological properties and behavior is scarce and described only for *A. platensis* and a couple of red marine unicellular algae.

Qualitatively, solutions of **sPSs** from red microalgae, such as *Porphyridium* and *Rhodella*, are characterized by their pseudoplastic properties (Sun 2010; Geresh and Arad 1991) and thixotropic characteristics (i.e., exhibit a stable form at rest but become more fluid when under some agitation) (Eteshola et al. 1998) these characteristics can be evaluated quantitatively by their viscosity, elasticity, shear rate, shear strain, and shear stress (www.vilastic.com). It is well known that hydrocolloid solutions of **sPSs** produced by red microalgae and the cyanobacterium *A. platensis* present a non-Newtonian behavior, as their viscosity depends negatively on the shear strain rate, i.e., decreases with the increase of this last parameter (Raposo et al. 2014; Geresh et al. 2000, 2002a; Eteshola et al. 1998; Badel et al. 2011a; Ginzberg et al. 2008), thus showing to be a pseudoplastic compound (Geresh and Arad 1991) with a strong shear-thinning behavior (Eteshola et al. 1998). However, Sun et al. (2009b) showed that fragments of **EPSs** from *Porphyridium* have a different rheological behavior, depending on the degree of degradation, sometimes exhibiting even the typical characteristics of Newtonian fluids. In addition, elasticity, viscosity, and intrinsic viscosity decrease when high temperatures (>90 °C) are applied in the drying process of the **EPS**, as these high temperatures cause significant modifications in the conformation of the polymer chains (Ginzberg et al. 2008). Another reason supporting the idea of **EPS** from *Porphyridium* having weak-gel characteristics is the fact that the elasticity (G') values are higher than the viscosity (G") ones (Raposo et al. 2014) after small deforming oscillatory forces are applied to the **EPS**, which was previously dried at temperatures below 140 °C (Geresh et al. 2002a; Ginzberg et al. 2008). These properties prevailed also when the polysaccharide was obtained from cultures grown under different concentrations of sulfate (Raposo et al. 2014). The decrease in viscosity with the application of higher shear rates was suggested to be related to the dissociation of the strong hydrogen bonds that exist between polymer chains (Ginzberg et al. 2008). In this study, Ginzberg and coworkers described well the influence of several factors on conformational modifications and also highlighted the effects caused by drying on the interactions between the polymer and its non-covalently linked glycoprotein. Raposo et al. (2014) also found that the viscosity of the **EPS** from *P. cruentum* decreases with the increase of $MgSO_4$ concentration in the culture medium. The reason for this behavior can be associated with the decrease in the dimensions of the chains as a result of the intrachain electrostatic repulsions. An explanation of the similar behavior of the **EPS** was given by Eteshola et al. (1998) when NaCl, at different concentrations, was added to the aqueous solutions of **EPSs**. Another mechanism that can explain the gel-forming characteristic of polymers might be associated with hydrophobic and ionic forces, as it was referred for the anionic colloids of *Amphora*,

DOI 10.1007/978-3-319-03751-6_47-1

P. tricornutum (diatoms), and *Ankistrodesmus* (chlorophyte) (Chen et al. 2011). Hence, some of these extracellular polymers behave as fluid-dynamic polymers, as it is the case of the **EPS** from *Porphyridium*, giving place to highly viscous solutions at very low concentrations, in a wide range of pH and temperatures, showing rheological properties similar to the ones of industrial **PSs** (Arad and Levy-Ontman 2010; Patel et al. 2013). Viscosity indexes, or the degree of polymerization, also seem to be closely associated with the culture growth temperature, since solutions with similar concentrations of the polymer presented higher viscosities when the cultures had been grown at the optimum temperature (Lupi et al. 1991). Therefore, growth of the culture at the optimum temperature might induce polymers with a higher polymerization degree.

Further, Eteshola and coworkers (1998) presented a fairly complete study on the rheology of the **EPS** produced by the red microalgae, including X-ray diffraction techniques, referring as well the viscoelastic properties of the **EPS** by using dynamic mechanical spectra. They observed, as well, an increase in the G' modulus for temperatures above 60 °C, suggesting that heat promoted polymer self-association in an aqueous solution.

However, as far as we know, some **PSs** only jellify when dropped into $FeCl_3$ solutions, and some others are not viscous at all. In fact, the somewhat turbid solutions of the glucuronorhamnan produced by *C. vulgaris* do not have the ability to form gels, and hence the solutions do not show any viscosity (Ogawa et al. 1999), and the calcium-rich **PS** produced by *A. halophytica* has the capacity to jellify when some drops of the **EPS** aqueous solution (1 % w/v) are put in contact with $FeCl_3$ solutions (0.05 M), the jellified beads formed being stable for over 1 month (Li et al. 2001).

Furthermore, the rheological behavior of the **EPS** released by both *C. capsulata* and *P. cruentum* seems to be similar, the viscosity decreasing with the increase of the shear rate. Also, there were no significant differences between the **EPS** behaviors when the microalgae were subjected to different culture media (De Philippis et al. 1996; Raposo et al. 2014). This might indicate that the molecular weights of **EPSs** are stable, showing no major differences no matter the changes to the growth media and conditions, even under nutrient starvation (Gasljevic et al. 2008; Geresh and Arad 1991).

The high molecular weight is also an important requirement for the **PSs** to be good drag-reducing polymers, as polymers with higher molecular weights are usually more efficient as drag-reducing agents (Gasljevic et al. 2008). Therefore, **EPSs** from microalgae are promising candidates, as their **PSs** have similar, if not higher, molecular weights than industrial polymers, as xanthan gum, for example.

4 Bioactivity and Applications

Below is a list of some potential applications of the polysaccharides produced by microalgae:

- Drugs or nutraceutical carriers in the pharmaceutical industry, to slow and control the release of the substances; for bacterial vaccines, to improve nonspecific immunity (Mishra et al. 2011);
- Thickeners and gelling agents in food industries, to improve quality and texture (Mishra et al. 2011);
- In soils and water treatment, to act as nutrient carriers to fertilize soils (Raposo and Morais 2011); to improve the aggregation of soils and sand particles (Paterson 1989; Sutherland et al. 1998), influencing the stability and cohesiveness of sediments and improving water holding capacity of soils (Mishra et al. 2011); to act as soil conditioners (Kroen and Rayburn 1984; Metting and Rayburn 1983) and to improve sludge settling and dewatering (Subramanian et al. 2010); to be used in wastewater treatment (Raposo et al. 2010), in detoxification acting as metal chelators due

DOI 10.1007/978-3-319-03751-6_47-1

Table 3 Antiviral applications of **EPS** from marine microalgae (*in* Raposo et al. 2013)

Microalgae/ cyanobacteria	Virus strain	Family/group of virus	Cell lines	EC50/ED50 (μg/mL)	References
A. platensis; *A. maxima*	Vaccinia virus VACV and VACV-GFP; ectromelia virus (ECTV); HSV-1, HSV-2, human cytomegalovirus (HCMV), measles virus, mumps virus, HIV-1, Flu-A	*Orthopoxvirus*/Poxviridae; *Simplexvirus*/Herpesviridae; *Morbillivirus*/ Paramyxoviridae; *Rubulavirus*/ Paramyxoviridae; *Lentivirus*/Retroviridae; *Influenza virus*/ Orthomyxoviridae	HEp-2 and Vero C1008; HeLa, HEL, Vero, MDCK, MT-4 (HIV-1)	0.78; 69; 0.92–16.5; 8.3–41; 17–39; 23–92; 2.3–11.4; 9.4–230	Radonic et al. 2010; Hayashi et al. 1996a, b; Hernandez-Corona et al. 2002
Porphyridium sp.	Herpes simplex virus HSV-1 and HSV-2; varicella zoster virus (VZV); murine sarcoma virus (MuSV-124) and MuSV/ MuLV (murine leukemia virus)	*Simplexvirus*/Herpesviridae; *Varicellovirus*/ Herpesviridae; *Gammaretrovirus*/ Retroviridae (type VI)	NIH/3T3	1–5 (in vivo, 100); 0.7; 10 and 5 (RT_{50})	Huleihel et al. 2001, 2002; Talyshinsky et al. 2002
P. cruentum	Hepatitis B virus (HBV); viral hemorrhagic septicemia virus (VHSV); African swine fever virus (ASFV), vaccinia virus (VACV), vesicular stomatitis virus (VSV)	*Orthohepadnavirus*/ Hepadnaviridae; *Novirhabdovirus*/ Rhabdoviridae; *Asfarvirus*/ Asfarviridae; *Orthopoxvirus*/Poxviridae; *Vesiculovirus*/ Rhabdoviridae	HEL	20, 200 (exocellular extracts); 12–56; 20–45	Huang et al. 2005; Fabregas et al. 1999; Raposo et al. 2014; Vieira and Morais 2008
P. purpureum	Vaccinia virus VACV and VACV-GFP, ectromelia virus (ECTV)	*Orthopoxvirus*/Poxviridae	HEp-2, Vero C1008	0.65	Radonic et al. 2010
R. reticulata	Herpes simplex virus HSV-1 and HSV-2, varicella zoster virus (VZV), murine sarcoma virus (MuSV-124), and MuSV/ MuLV (murine leukemia virus)	*Simplexvirus*/Herpesviridae; *Varicellovirus*/ Herpesviridae; *Gammaretrovirus*/ Retroviridae (type VI)	NIH/3T3	10–20; 8; 150 and 50 (RT_{50})	Huleihel et al. 2001; Talyshinsky et al. 2002
G. impudicum	Encephalomyocarditis virus; influenza A virus (Flu-A)	*Cardiovirus*/Picornaviridae; Orthomyxoviridae	MDCK	0.19–0.48	Yim et al. 2004
C. polykrikoides	Flu-A and Flu-B, respiratory syncytial virus types A (RSV-A) and B (RSV-B), HIV-1, HSV-1, parainfluenza virus type 2 (PFluV-2)	Orthomyxoviridae; *Pneumovirus*/ Paramyxoviridae; Retroviridae; Herpesviridae; *Rubulavirus*/ Paramyxoviridae	MDCK, Hep-2, MT-4, HMV-2	0.45–1.1 and 7.1–8.3; 2.0–3.0 and 0.8; 1.7; 4.52–21.6; 0.8–25.3	Hasui et al. 1995

EC_{50}/ED_{50} is the concentration/dose at which 50 % of the population exhibit a response after being exposed to a certain compound

to the presence of uronic acids (Kaplan et al. 1987), and in bioremediation to remove toxic metals from polluted waters (Otero and Vincenzini, 2003); and to act as growth promoters for crops (Pignolet et al. 2013).

4.1 Therapeutical Applications

Antiviral, antibacterial, anti-inflammatory, immunomodulatory, antilipidemic, antiglycemic, anti-adhesive, antioxidant and free radical scavenging, and prevention and treatment of tumors are some of the therapeutical applications of **PSs**.

4.1.1 Antiviral Activity

Many studies have already highlighted that the polysaccharides released (or not) into the culture medium by some marine microalgae present antiviral bioactivity against different kinds of viruses, either mammalian or otherwise (Table 3); the interest increased after some experiments were conducted on HIV (Hayashi et al. 1996a; Hasui et al. 1995). Radonic et al. (2010) and Chen et al. (2010) have recently reviewed the antiviral effects of several **sPS** on different host cell lines. To date, **PSs** from *Arthrospira* and *Porphyridium* were the most studied anionic sulfonated polymers, exhibiting antiviral activity against a wide range of viruses, including *Herpes simplex* and *Varicella zoster* viruses (HSV AND VZV), human cytomegalovirus (HCMV), measles, mumps and Flu-A viruses, and *vaccinia virus*, a variola-related virus. In fact, the **PS** TK-V3 from *A. platensis* and **EPS** from *P. purpureum* proved to be active against *Vaccinia* and *Ectromelia orthopoxvirus* infection; in studies conducted with HEp-2 and Vero C1008 cells, the $\mathbf{IC}_{50}$ is significantly lower (0.78 and 0.65 μg/ml, respectively) than the response to dextran sulfate (1.24 μg/ml). Despite being slightly toxic, **PSs** can be safely applied for in vivo experiments, as they were also effective *in ovo*, by decreasing the VACV replication (Radonic et al. 2010). Besides, the **EPS** from *P. cruentum* has also demonstrated a significant inhibition against *Vesicular stomatitis* virus proliferation on HEL cells (Raposo et al. 2014), the response being higher for the **EPS** isolated from the culture medium enriched with 104 mM in sulfate. As a matter of fact, the antiviral activity of the **EPS** from *P. cruentum* depends not only on the culture medium, algal strains, and cell lines used for testing but also on the methodology and the degree of sulfation and uronic acid content of the **EPS** (Raposo et al. 2014; Huleihel et al. 2001, 2002). These acidic compounds, along with the half-ester sulfate groups and the carboxyl groups of the polymer, contribute to their anionic characteristics, making the **EPS** of *Porphyridium* a good agent to be used against viruses (Raposo et al. 2014). **CaSp**, an intracellular polysaccharide produced by *A. platensis*, inhibited the replication of several viruses in vitro by inhibiting the penetration of the virus into the different host cells used (Hayashi et al. 1996a, b). The experiments conducted by Hayashi and coworkers (1996b) also confirmed the importance of sulfate groups on the antiviral activity of **CaSp** from *A. platensis*, activity proved by studying the effect of calcium-free-Spirulan and another compound derived from **CaSp**, without sulfate, on the replication of HSV-1 and on the cytotoxicity in HeLa cells, the latter compounds showing a higher toxicity and a significantly lower antiviral capacity. However, the molecular configuration due to the chelation of the calcium ion with the sulfate groups might have a crucial role in the antiviral properties, as no antiviral effect was verified when the calcium-free compound was used despite the presence of sulfate groups (Hayashi et al. 1996b). Other factors that are correlated to the inhibition of viral infection are the size of the molecules and the degree of sulfation (Ghosh et al. 2009); the composition of monosaccharides and the diversity of the linkage types also determine the specificity of **sPSs** and influence their functional properties. That is why tests for antiviral capacity should be carried out with a variety of virus and isolates, as wide as possible.

DOI 10.1007/978-3-319-03751-6_47-1

Unlike what happens with most of **sPSs**, the **PS** from *C. polykrikoides* showed to be effective against influenza virus type B (Flu-B), among other different viruses, including that of HIV-1. This sulfated polymer that does not contain proteins, aminoacids, or nucleic acids showed an inhibitory effect higher than that of dextran sulfate on Flu-B and MSLV, with no cytotoxic effects against the different cell lines used for testing concentrations up to 100 μg/ml (Hasui et al. 1995).

The antiviral activity is probably the most studied quality exhibited by sulfated polysaccharides of marine microalgae, especially the one produced by *Porphyridium*. The mechanisms for this activity are not yet completely understood. As happens with heparin, the anionic nature of **sPS** makes it a good candidate to protect against viruses. Several mechanisms have been proposed. Hayashi and colleagues (1996a, b) noted that **sPS** inhibited infection by different viruses through inhibiting the penetration of viral particles into host cells. But other mechanisms can also be involved, such as the inhibition of attachment/adsorption, or even replication during the early phases of the virus cycle (Martinez et al. 2005; Kim et al. 2012), without any toxicity to the host cells (Hasui et al. 1995).

4.1.2 Antibacterial Activity

The **PS** from *A. platensis* has antibacterial properties, the activity depending on the solvent used to extract the polymer. While water and methanolic extracts showed antimicrobial properties on both Gram-positive and Gram-negative bacteria, methanolic **EPS** extracts show a wider capacity to inhibit the growth of bacteria than aqueous extracts. However, methanolic extracts presented only a bacteriostatic effect against the strain NCIMB8166 of *Micrococcus luteus*, needing an MBC/MIC ratio >4, i.e., the minimum inhibitory concentrations (MIC) of **EPS** is considerably lower than the concentration needed to kill the organisms (MBC, minimum bactericidal concentrations) (Challouf et al. 2011). Ethanolic and some other solvent extracts did not show any antimicrobial activity. In fact, the **EPS** did not exhibit any activity against *E. coli* (strain ATCC25922) and *S. aureus* (ATCC25923). A similar explanation can be applied to the results obtained by Raposo et al. (2014), who tested the antimicrobial activity of the **EPS** from *P. cruentum* and reported that their ethanolic extracts did not show a significant inhibition on the growth of bacteria *E. coli* and *S. aureus*. Perhaps the bioactive portions of the molecules have different affinities to the solvents used, being highly influenced by mutual interactions (Basedow et al. 1980), and ethanol might not be adequate for the extracts to maintain the antibacterial active principle/ingredient of the **EPS**. Nevertheless, the ethanolic extract of the **EPS** from *P. cruentum* showed some activity against *S. enteritidis* (Raposo et al. 2014).

4.1.3 Antioxidant Activity and Free Radical Scavenging

As photoautotrophs, microalgae are highly exposed to oxidative and radical stresses, therefore accumulating effective antioxidative scavenger complexes to protect their own cells from free radicals (Pulz and Gross 2004). Oxidation of lipids by reactive oxygen species (ROS), like hydroxyl radicals, hydrogen peroxide, and superoxide anion, can affect the safety of pharmaceuticals and also decrease the nutritional quality of foods. Sulfated **PSs** produced and secreted out by marine microalgae may act not only as dietary fiber (Dvir et al. 2009), but have also showed the capacity to prevent the accumulation and the activity of free radicals and reactive chemical species, therefore acting as a protective system against these oxidative and radical stress agents (Table 4).

It was already demonstrated that the **sPS** from *Porphyridium* exhibited antioxidant activity against the autoxidation of linoleic acid and inhibited oxidative damage to 3T3 cells that might be caused by $FeSO_4$ (Tannin-Spitz et al. 2005). These researchers also proved that the bioactivity was dose dependent, correlating positively with the sulfate content of the **sPS**, and mentioned the possibility of the glycoprotein to contribute to the antioxidant properties. They even suggested

DOI 10.1007/978-3-319-03751-6_47-1

Table 4 Applications, other than antiviral uses, of **EPS** from marine microalgae (*in* Raposo et al. 2013)

Microalgae/ cyanobacteria	Applications	Cells/animals used for in vitro/in vivo studies	References
Porphyridium	Health foods, nutraceutical, and functional foods	Rats	Dvir et al. 2000, 2009
Rhodella, *Porphyridium*	Antioxidant and free radical scavenging	3T3; mouse liver homogenates and erythrocyte hemolysates, sarcoma 180 cells/mice	Sun 2010; Chen et al. 2010; Tannin-Spitz et al. 2005; Sun et al. 2009b
Porphyridium, *P. cruentum*; *R. reticulata*	Antilipidemic, antiglycemic	Rats/mice, chickens	Dvir et al. 2009; Arad 1999; Ginzberg et al. 2000; Huang et al. 2006
Porphyridium, *Chlorella stigmatophora*, *Phaeodactylum tricornutum*	Anti-inflammatory and immunomodulatory	Polymorphonuclear leukocytes/human dermal microvascular endothelial cells, humans; rabbits and sheep (bone joints); mice macrophages/mice and rats	Guzman et al. 2003; Sun 2010; Matsui et al. 2003; Arad and Atar 2007
Porphyridium, *R. reticulata*, *Gyrodinium impudicum*, *A. platensis*	Prevention of tumor cell growth	FD early myeloid cell line, 24-1 and EL-4T-lymphoma cell lines; Graffi myeloid cells; rats	Senni et al. 2011; Geresh et al. 2002b; Gardeva et al. 2009; Shopen-Katz et al. 2000
Phaeodactylum, *Tetraselmis*	Anti-adhesive	HeLa S3/sand bass culture cells	Guzmán-Murillo and Ascencio 2000; Dade et al. 1990
Porphyridium	Biolubricant (for bone joints)		Arad and Atar 2007; Arad et al. 2006
Porphyridium, *R. reticulata*	Ion exchanger		Lupescu et al. 1991
P. cruentum, *R. reticulata*, *R. maculata*	Drag reducers		Gasljevic et al. 2008; Ramus et al. 1989

that the antioxidant activity of this polymer relied on its ability to act as a free radical scavenger. Despite the various applications suggested for the **EPSs** from different species/strains of marine microalgae, when the biological activity involves crossing the cellular membrane of cells, the high molecular weight of the polymers can be a drawback to pursue their properties. This feature was confirmed by Sun et al. (2009b). These researchers submitted the **EPS** from *P. cruentum* to microwave, and the **EPS**-derived products (6.55, 60.66, and 256.2 kDa) showed different levels of antioxidant activity, a lower molecular weight (6.55 kDa) being a requisite for a stronger activity either by scavenging hydroxyl, superoxide anion, and $DPPH^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl radical) free radicals or by inhibiting the (per)oxidation of lipids induced by $FeSO_4$ and ascorbic acid, thus giving better protection to mouse cells and tissues against oxidative damage. They found that the antioxidant ability is dose dependent; the same is true in relation to the inhibition of oxidation damage of both liver cells and tissue. The free radical scavenging of some of the **EPS** fragments was significantly higher at the same, or even lower, concentration than that reported for vitamin C (Xing et al. 2005). But strangely, they found no scavenging activity and no inhibition of oxidative damage in cells and tissues for the crude high molecular **sPS** from *Porphyridium cruentum* (Sun et al. 2009b).

DOI 10.1007/978-3-319-03751-6_47-1

The sulfated exopolysaccharide from *Rhodella reticulata* also has antioxidant activity, the effects being dose dependent (Chen et al. 2010). Unlike what happened with the **sPS** from *Porphyridium* (Sun et al. 2009b), crude **sPS** from *Rhodella* exhibited higher antioxidant properties than the polysaccharide-modified samples, these demonstrating lower radical scavenging activity (Chen et al. 2010). These researchers found that all the different samples of **sPS** from *R. reticulata* had a stronger ability than α-tocopherol against superoxide anion radical scavenging, the crude polysaccharide being twice as strong as α-tocopherol.

Besides the antibacterial properties, the methanolic extracts of **EPS** from *A. platensis* also exhibit a moderate antioxidant capacity (TEAC=0.27 mg/mL) using Trolox, a common antioxidant substance, while the ethanolic extracts presented lower antioxidant activity (Challouf et al. 2011). According to Mendiola et al. (2007) and Sun et al. (2009b), uronic acid contents are directly related to the radical scavenging properties of **PSs**, but other factors also seem to have influence on the antioxidant capacity, namely, low molecular weights (Chen et al. 2008; Sun et al. 2009a), and the structure and conformation of the polymer (Tao et al. 2007). The antioxidant properties of these **PSs** might be exerted by improving the activity of antioxidant enzymes, scavenging free radicals, and/or inhibiting lipid (per)oxidation (Sun et al. 2009a).

4.1.4 Anti-inflammatory and Immunomodulatory Properties

Polysaccharides from marine microalgae, like *Porphyridium*, *Phaeodactylum*, and *C. stigmatophora*, had already demonstrated to have pharmacological properties, such as anti-inflammatory activity and as immunomodulatory agents (Table 4). The **sPS** from both *C. stigmatophora* and *P. tricornutum* demonstrated a significant anti-inflammatory activity against paw edema induced by carrageenan injected as a sterile saline solution (0.9 %), with $\mathbf{IC}_{50}$ values of 2.25 and 2.92 mg/kg for *C. stigmatophora* and *P. tricornutum*, respectively, compared to the anti-inflammatory indomethacin, with an $\mathbf{IC}_{50}$ of 8.50 mg/kg. The anti-inflammatory efficacy was tested in vivo, by intraperitoneally injecting the crude **PS** in female rats and mice, and in vitro, the phagocytic activity being evaluated in macrophages from mice (Guzman et al. 2003). The direct stimulatory effect of *P. tricornutum* on immune cells was evidenced by the positive phagocytic activity tested either in vitro or in vivo, and the activity of the extract of **sPS** from *C. stigmatophora* showed immunosuppressant effects (Guzman et al. 2003). As reported for the polysaccharide from *Ulva rigida*, a green seaweed (Leiro et al. 2007), the **sPS** p-KG03 from the marine dinoflagellate *G. impudicum* also activates the production of nitric oxide and immunostimulates the production of cytokines in macrophages (Bae et al. 2006). On the other hand, inhibition of leukocyte migration seems to be related to the anti-inflammatory activity of the polysaccharides (Matsui et al. 2003). As leukocyte movement to the site of injury contributes to additional cytokine release and to the production of nitric oxide, therapeutics has to be effective against this over-inflammation. In fact, the **sPS** from *Porphyridium* seems to be a good candidate for this role as it inhibited the movement and adhesion of polymorphonuclear leukocytes in vitro and inhibited the development of erythema in vivo as well (Matsui et al. 2003).

Besides inhibiting tissue oxidative damage, **EPS** from *P. cruentum* can be used to inhibit the biomembrane peroxidation as well (Sun et al. 2009b) and to enhance in vitro immunomodulatory activity (Sun et al. 2012). These researchers have also explained the mechanism/pathway that is most probably involved in the immune response enhancement by **EPS** from *P. cruentum* – the stimulation of macrophages. They found that low molecular fractions of **EPS** can stimulate the proliferation of macrophages and the production of NO (nitric oxide). NO is a signaling free radical gas molecule that can be synthesized by phagocytes (monocytes, macrophages, and neutrophils) and is involved in the human immune system response. When studying the effects of **EPS**-derived

products, Sun (2010) showed that **EPS** from *Porphyridium* presented immunostimulating activity in mice with S180 tumors by increasing both spleen and thymus index and also spleen lymphocyte index. Sulfated **PS**-derived products, with lower molecular weight, can also improve the production of NO in mouse macrophages. In his Ph.D. Thesis, Sun referred to the fact that sulfate content has a positive correlation with the immunomodulatory system. Furthermore, Namikoshi (1996) noted that **sPS** can stimulate the immune system by triggering cells and humor stimulation. This shows the capacity of marine unicellular algae **sPS** to directly stimulate the immune system.

Spirulan is a **GAG**-like **PS** (Senni et al. 2011). This means that spirulan from *A. platensis*, for example, is recognized as having similar properties as glycosaminoglycans (GAG), present in all animals. **GAG**s are sulfated (or not) **PSs** composed of disaccharide repeating units, a uronic acid or a neutral monosaccharide, and an amino sugar (Senni et al. 2011) with anticoagulant properties, such as heparin and hyaluronic acid. By interacting with a vast range of proteins involved in many human body physiological and pathological responses, GAGs show several bioactivities associated either to inflammatory processes or to tissue repair (Gandhi and Mancera 2008; Mulloy and Linhardt 2001). Some sulfated **GAG**s can be covalently linked to proteins (proteoglycans); this is the case of the **sPS** from red marine unicellular algae *Porphyridium*, which has also some protein moieties non-covalently linked, but shows anti-inflammatory properties.

On the other hand, immunomodulators are response modifier biocompounds that present an enhancement or suppression of the immune responses, depending on a wide range of factors, such as dose, way, and time of administration, but also on the site of activity and the respective mechanism of action (Tzianabos 2000). β-(1,3)-Glucans, such as that of *C. vulgaris* (Nomoto et al. 1983), have already proved to exhibit several biological properties, including prevention of some infections and antitumor activity (Bleicher and Mackin 1995; Nomoto et al. 1983). These polymers stimulate the functional activity of macrophages (Burgaleta et al. 1978) and the proliferation of monocytes and macrophages, presenting also potent hematopoietic properties (Patchen and Lotzova 1980; Riggi and DiLuzio 1961).

4.1.5 Activity Against Tumors and Vascular Muscle Cell Proliferation

Sometimes, after suffering some kind of damage, vascular endothelial cells are not sufficiently repaired by their own cell type; there can be an invasion of platelets and/or macrophages or other blood cell types, which secrete cytokines and growth factors that can increase the proliferation of vascular smooth muscle cells, causing a hyperplasia of the arterial *intimae*. This atherosclerosis is one of the main causes of myocardium and cerebral infarction.

Some **PSs** that are used as anticoagulants have also some inhibitory activity against the proliferation of vascular smooth muscle cells. This is the case of heparin (Clowes and Clowes, 1987) and heparin sulfate (Kaji et al. 2004) and the sulfated fucoidan from some seaweeds (Vischer and Buddecke 1991). Nevertheless, spirulan (either Na- or Ca-) from *A. platensis* is a more potent inhibitor of cell proliferation, as it was demonstrated by Kaji and coworkers (2004) on bovine arterial smooth muscle cells. These researchers also demonstrated that it is not enough to be composed of sulfate for the **PSs** to show inhibitory activity against cell growth: while both **NaSp** and **CaSp** inhibited the proliferation of vascular smooth muscle cells, as it happened with heparin and heparin sulfate, the desulfated equivalent compounds did not show this effect. And, as depolymerized compounds (**PS**-derived products with lower molecular weights) of **NaSp** and **CaSp** maintained the inhibitory capacity against the proliferation of arterial smooth muscle cells, with MW $\geq$14,700, this suggests that spirulan (especially **NaSp**) is a particular polymer with a specific structural sequence and conformation maintained by the linkage of Na^+ to the sulfate groups, keeping, therefore, that strong inhibitory activity (Kaji et al. 2004); the effect is dose and

DOI 10.1007/978-3-319-03751-6_47-1

time dependent. Furthermore, depolymerized **NaSp** inhibited the growth of vascular smooth muscle cells without inhibiting the growth of vascular endothelial cells (Kaji et al. 2002, 2004).

Spirulan is also capable of inhibiting pulmonary metastasis in humans and to prevent the adhesion and proliferation of tumor cells (Senni et al. 2011).

Other **sPSs** have also antiproliferative activity in cancer cell lines (in vitro) and inhibitory activity against tumor growth (in vivo). The **sPS** p-KG03 from *G. impudicum* is one of these polymers that prevents and suppresses tumor cell growth either in vitro or in vivo by activating NO production and by stimulating the innate immune system, increasing the production of cytokines interleukin-1 (or IL-1), IL-6, and THF-α in macrophages (Bae et al. 2006; Namikoshi 1996; Yim et al. 2005). This **PS** has immunostimulating properties in vivo as well (Yim et al. 2005).

Another candidate with potential to be used as an antitumor agent is the *β*-(1,3)-glucan from *C. vulgaris*, besides being considered an active immunostimulator (Laroche and Michaud 2007). Low molecular weight fragments (6.53–1,002 kDa) of the **sPS** from *P. cruentum* are also good immunostimulators as they all inhibited in vivo S180 tumors implanted in the peritoneal cavity of mice models by inhibiting the tumor cell proliferation and the growth of the tumor, increasing the spleen and thymus indexes and the number of spleen lymphocytes as well, enhancing the immune system in this way (Sun et al. 2012). However, nonmodified or higher molecular weight fragments of the same **sPS** showed no inhibition of tumor cell growth (Geresh et al. 2002b; Sun 2010). Nonetheless, in a recent study, Gardeva et al. (2009) reported the strong antitumor activity exhibited by the polysaccharide of *P. cruentum*. This sulfated polymer strongly inhibited Graffi myeloid tumor proliferation in vitro and in vivo, the activity being dose dependent, and the survival time of hamsters was increased by 10–16 days. Gardeva and coworkers (2009) also suggested that the antitumor activity could be related to the immunostimulating properties of the polymer. Therefore, it can be concluded that the reinforcement of the immune system induced by the **sPSs** is probably the main mechanism against tumor growth and respective effects (Sun et al. 2012; Zhou et al. 2004). However, other mechanisms, such as changes in the biochemical characteristics of the cell membrane, inducing tumor cell differentiation and apoptosis, and regulation of the cell signaling pathways, can also be involved (Zhou et al. 2004). Besides these mechanisms, the antimetastatic properties may be associated to the capacity of blocking the interactions between cancer cells and the basement membrane or inhibiting the adhesion of tumor cells to the substrates.

In addition, some years ago, it has already been demonstrated that high molecular weight oversulfated **EPSs** from *Porphyridium* inhibited neoplastic mammalian cell growth and that the biomass of this marine microalga could prevent the proliferation of colon cancer in rats (Geresh et al. 2002b; Shopen-Katz et al. 2000).

4.1.6 Antilipidemic and Antiglycemic Properties

Sulfated **PSs** from seaweeds and marine animal origin are potent inhibitors of human pancreatic cholesterol esterase, an enzyme that promotes its absorption at the intestinal level (Laurienzo 2010). These inhibitory effects are enhanced by higher molecular weights and degree of sulfation, as well as by the presence of 3-sulfate in the monosugar molecule (Laurienzo 2010). And most of the **PSs** from marine microalgae are naturally and highly sulfated with high molecular weights, making them non-readily absorbable and thus enabling them to be used as anticholesterolemic agents.

However, this area of research has not been sufficiently explored in what concerns microalgae (Table 4). When Ginzberg and coworkers (2000) fed chickens with biomass containing **EPS** from *Porphyridium*, they verified that cholesterol decreased either in serum or egg yolk of chickens, the fatty acid profile was modified, and the carotenoid content in the egg yolk was improved as well. Furthermore, in rats fed with *Porphyridium* and *R. reticulata* biomass, which **PSs** contain dietary

DOI 10.1007/978-3-319-03751-6_47-1

fibers, there was a decrease in serum cholesterol and triglycerides; hepatic cholesterol levels were also improved and the levels of VLDL considerably lowered with no toxic effects noticed in the animals (Dvir et al. 1995, 2000, 2009). Also, either the biomass of *Rhodella* or the **sPSs** from *Porphyridium* were able to lower the levels of insulin and/or glucose in diabetic rodents (Dvir et al. 1995; Huang et al. 2006), causing no modifications in the pancreatic island cells and no fibrosis or hemorrhagic necrosis in cells (Huang et al. 2006).

These experiments suggest the strong potential of sulfated polysaccharides from unicellular algae to be used as hypolipidemic and hypoglycemic agents, but they are also promising substances in reducing coronary heart disease due to their hypocholesterolemic effects (Dvir et al. 2000, 2009).

Mechanisms focusing on the role of dietary fibers in lowering cholesterol are not yet completely understood, but Oakenfull (2001) proposed that it could be related to the increase in the viscosity of intestinal contents, which have influence on nutrient absorption, micelle formation, and decreasing of lipid absorption. The decrease in serum cholesterol levels and the increase in bile excretion, caused by the disruption of the enteropathic circulation of bile acids, were suggested as another possible explanation (Glore et al. 1994; Marlett 2001).

4.2 Other Biological Activities

4.2.1 Anticoagulant and Antithrombotic

There are several studies on the anticoagulant properties of the **PSs** isolated from seaweeds, presented in a recent review by Wijesekara and coworkers (2011). Carrageenans, for example, are **sPSs** that show potent anticoagulant activity, inhibiting platelet aggregation as well, probably due to the antithrombotic capacity, which, in turn, is associated to a high sulfate content (Prajapati et al. 2014). However, there are only a few references to microalgae. On one hand, it was stated that the anticoagulant activity is associated to the high sulfate content of the **PS**, which is a characteristic of most of the **PSs** with marine microalgae origin. But, this feature could be an inconvenience when considering their use for the treatment of virus-induced diseases, for example, as an anti-inflammatory. On the other hand, Hasui and colleagues (1995) found no anticoagulant activity in the **sPS** of *C. polykrikoides* in spite of the high contents in sulfate of this **PS**. This suggests that the anticoagulant properties of polysaccharides may not only depend on the percentage of sulfate residues but rather on the distribution/position of sulfate groups and, probably, on the configuration of the polymer chains (Ginzberg et al. 2008; Pereira et al. (2002). Spirulan from *A. platensis* is one of the marine microalgae **PS** that strongly interferes with blood coagulation-fibrinolytic system and exhibits antithrombogenic properties (Hayakawa et al. 1996, 2000). Both **NaSp** and **CaSp** enhance the antithrombin activity of heparin cofactor II and the production of the tissue-type plasminogen activator in human fetal lung fibroblasts (Hayakawa et al. 1997), and, in addition, **NaSp** still enhances the secretion of urokinase-type plasminogen activator and inhibits the secretion of the plasminogen activator inhibitor type 1, sulfate being essential for these properties (Yamamoto et al. 2003). Therefore, spirulan is a promising antithrombotic agent in clot breakdown, but some care should be taken in relation to hemorrhagic strokes.

4.2.2 Biolubricant

This is one of the lesser known applications for **sPSs**, and very little has been published on this issue (Table 4). Nevertheless, the **sEPS** of *Porphyridium* has already shown good lubrication capacity due to its rheological properties (Arad and Weinstein 2003). Arad and coworkers (2006) have compared the lubricating properties of **sPSs** to the most used hydrogel lubricant, hyaluronic acid. They simulated efforts of joints, during both walking and running, and found a better quality of the **EPS** from *Porphyridium*. The explanation for these properties is associated to **EPS** rheological

characteristics as they showed to be stable than most lubricants at higher temperatures, the viscosity of the latter decreasing along with a decrease of lubricity. A 1 % **PS** solution presented the best friction properties under high loads, and its viscosity did not suffer any significant change when incubated with hyaluronidase, with standing degradation by this enzyme (Arad et al. 2006). This experiment shows the potential of the **sPS** from *Porphyridium* to be an excellent candidate to substitute hyaluronic acid as a biolubricant. Another promising application could be as a substance to be part of a joint-lubricating solution, as it was demonstrated by injecting the **EPS** from *Porphyridium* into the joints of rabbits' knees (Arad and Atar 2007), thus mitigating degenerative joint disorders caused by arthritis.

4.2.3 Anti-adhesive

Sulfated **PSs** from marine microalgae revealed the ability to block the adhesion of pathogenic microorganisms, suggesting the hypothesis to be used in anti-adhesive therapeutics. In fact, several **sPSs** presented a higher inhibition of the adherence of both *Helicobacter pylori* to HeLa S3 cell line and three fish pathogens to spotted sand bass gills, gut, and skin cultured cells (Guzmán-Murillo and Ascencio 2000) (Table 4).

Infection by microorganisms appears usually after binding to the cell membrane. Carbohydrates were already demonstrated as recognition sites for decades (Ofek et al. 1978), heparan sulfate glycosaminoglycan being one of those receptors in the host cells (Ascencio et al. 1993). These researchers suggested that this interaction could be associated to the net charge and molecular stereochemistry of the polymer.

4.3 Health Foods, Nutraceuticals, Functional Foods

Nowadays, the implications of specific diets on health assume a relevant role in developed countries, and the pursuit for equilibrated diets, supported by considerable epidemiological evidences, is a major issue for the scientific community and consumer in general, who more and more look for natural food products. In this context, microalgae have great potential to be used in food and feed preparation due to their rich composition, including high protein content with balanced amino acid pattern, carotenoids, fatty acids, vitamins, polysaccharides, sterols, phycobilins, and other biologically active compounds (Gouveia et al. 2008). The commercial production of microalgae for human nutrition is already in practice, and they find many applications either as nutritional supplements, for instance, in the form of tablets and pills, or as functional foods, incorporated in food products, such as pastas and cookies. The health-promoting effects associated with microalgal biomass are probably related to several effects due to their phytochemical constituents.

Some **PSs** from microalgae may by themselves be of interest for industrial and commercial applications. **PSs** can find applications in the food industry as emulsifying and gelling agents and as flocculant and hydrating agents, emulsifiers, stabilizers, and thickening agents, i.e., food additives (Bernal and Llamas 2012), like agar E406, alginates E400-404, and carrageenan E407. Because of the presence of peptide/protein moieties and deoxysugars, such as rhamnose and fucose, some **PSs** from marine cyanobacteria and unicellular algae show a significant hydrophobic behavior, conferring them emulsifying characteristics (Flaibani et al. 1989; Shepherd et al. 1995). In addition, some **PSs** include fucose as a constituent, this deoxysugar being of high value in the chemical synthesis of flavoring agents (Lupi et al. 1991). The **sPS** from marine microalgae could also be used as nutraceuticals due to their fiber content, their ability of acid binding and cation exchange, and their property of fecal bulking, and they are also good candidates as prebiotics (Ciferri 1983) and in some cases with a strong bioactive potential as hypolipidemic and hypoglycemic agents (Gonzalez de Rivera et al. 1993; Dvir et al. 2000, 2009) similar to polysaccharides from seaweeds (O'Sullivan

et al. 2010). The **PSs** from microalgae alone or in combination with other compounds have also great potential to be used in edible films and coatings of foods other than carriers of flavors, colorants, spices, and nutraceuticals (Marceliano 2009). **PSs** from microalgae also have the potential to be used in low-fat or fat-free food products, as fat replacers in mayonnaises (Franco et al. 1998; Raymundo et al. 1998), and salad dressings and other food emulsions (Raymundo et al. 2005).

4.4 Other Applications

Another little known field of application is as drag reducers. Only a few studies were conducted in order to determine whether polysaccharides had the potential of drag-reducing ability (Ramus et al. 1989; Gasljevic et al. 2008), in order to extend their functionalities to engineering applications (Table 4), namely, naval engineering. It is known for some years that the efficiency of **PSs** drag-reducing properties is improved by the high molecular and linear structure of the polymers, associated with a strong resistance to mechanical degradation (Gasljevic et al. 2008). In fact, these researchers have already studied the potential of several marine microalgal **PSs** as drag reducers. *P. cruentum* and *R. maculata* were the ones whose polysaccharides showed the higher drag-reducing power at lower concentrations, followed by *Schizochlamydella* (former *Chlorella*) *capsulata*. However, the **PSs** of some of these microalgae proved to be more powerful than others. As a matter of fact, to have the same level of drag-reduction effectiveness, 25 % more **PS** of *R. maculata* is required in relation to *P. cruentum* and almost three times more than the polysaccharide of *S. capsulata* (Gasljevic et al. 2008). Thus, if applied to the hulls of the vessels, these high-molecular **EPSs** could reduce friction losses by reducing flow turbulence due to the elasticity of the polymers. Therefore, there could be a reduction in the fuel consumption and in the propelling power for a ship to achieve a certain velocity (Gasljevic et al. 2008).

Another promising and emerging application of microalgae might be associated to the production of nanofibers from the biomass of *A. platensis* to be used as extracellular matrices for the culture of stem cells in order to treat spinal cord injuries (Raposo et al. 2010).

Their gluing and adhesive capacities and also their strong cohesive and binding strength, allied to their nontoxic and nonirritating properties, make these bioadhesive **PSs** produced by marine microalgae good candidates as mucobioadhesives or glues for bone gluing and soft tissue closure after surgery, promising to be, in the near future, the substituents of metallic screws and traditional wound closure methods, respectively (Laurienzo 2010).

Other areas of application of the marine microalgal **sPSs** could be as diverse as cosmetics or as ion exchangers, due to their chemical composition, rheological characteristics, and ion affinity.

Finally, besides all these applications, the adhesion properties of the **sPSs** produced by microalgae seem to play an important role in either the locomotion of some algae (Wetherbee et al. 1998) or in the aggregation of soil and sand particles (Paterson 1989; Sutherland et al. 1998), influencing stability and cohesiveness of sediments.

5 Mechanisms of Action

5.1 Antibacterial Activity

Some researchers have found that some **PSs** have an inhibitory effect on some bacteria: the extract from *Chaetomorpha aerea* inhibited the growth of *S. aureus* (Pierre et al. 2011); **EPS** from *P. cruentum* inhibited the growth of *S. enteritidis* (Raposo et al. 2013); fucoidan from the brown seaweed *Laminaria japonica* inhibited *E. coli* (Li et al. 2010). This inhibitory effect might be explained by the anti-adhesive properties of sulfated exopolysaccharides of some microalgae against

the adherence of microorganisms. Several **sPSs** inhibited the adherence of both *Helicobacter pylori* to HeLa S3 cell line and three fish pathogens to spotted sand bass gills, gut, and skin cultured cells (Guzman-Murillo and Ascensio 2000). **PSs** may compete with carbohydrates as recognition sites to which microorganisms can attach to, this mechanism having already been evidenced for other carbohydrates in cell surfaces (Ofek et al. 1978). Heparan sulfate glycosaminoglycan was identified as a receptor in host cells, this interaction having been associated with the net charge and molecular stereochemistry of the polymer (Ascencio et al. 1993).

However, the same **PS** may also not show activity against other bacteria (*C. aerea* extract against *S. enteritidis*, Pierre et al. 2011; *P. cruentum* extract against *S. aureus*, Raposo et al. 2014). The different reactions of various bacteria to the biological extracts of **PSs** could be due to the composition of the bacterial cell wall, to the absence of a specific structure in the bacteria, or also to the ability of the bacteria to change the chemical structure of the extract (Michael et al. 2002). The antibacterial activity may also be related to the antibiofilm formation ability of the **EPS** (Bernal and Llamas 2012) and, therefore, with the anti-adhesive properties. Most evidence suggests that these molecules act by modifying the physical properties of biotic surfaces (Rendueles and Ghigo 2012). Gram-negative *E. coli* and Gram-negative *S. enteritidis* present different cell surfaces that might explain the differences found between the correspondent inhibitions by the **EPS** (Raposo et al. 2014).

Rendueles and Ghigo (2012) suggest that **PSs**, as surfactant molecules, may modify the physical properties of the bacterial cell surfaces. **PSs** from microalgae might act in a similar way as *E. coli* exopolysaccharides, which can inhibit the autoaggregation via adhesins of bacterial cells (Valle et al. 2006; Rendueles et al. 2011). Polysaccharides, as sugar polymers, have also the capacity to act as inhibitors of lectin, which, being mainly located on the surface of bacteria cells, facilitate the attachment or adherence of bacteria to host cells by binding to the glycan substrates present on the surface of those host cells (Esko and Sharon 2009). **PSs** compete with the sugar-binding domain of lectins and inhibit the lectin-dependent adhesion of pathogens and biofilm formation, therefore reducing the occurrence of infection.

5.2 Antiviral Activity

Several mechanisms have already been proposed to explain the antiviral activity of **EPSs**, either involving the inhibition of the virus penetration into the host cells (Hayashi et al. 1996b), by competing with the glycoprotein attachment sites of the membrane/envelope of the viruses (Damonte et al. 2004; Radonic et al. 2010; Rashid et al. 2009), or relating to the replication during the early phases of the virus cycle (Martinez et al. 2005; Kim et al. 2012).

The general mechanism of the antiviral activity of most **PS** against enveloped viruses could be based on shielding off the positively charged sites in the viral envelope glycoprotein through ionic interactions between the anionic (mainly sulfate) groups in the polysaccharide and the basic amino acids of the glycoprotein (Damonte et al. 2004; Radonic et al. 2010; Rashid et al. 2009), in a similar way to what happens when a virus attaches to a cell through the cell surface heparan sulfate receptor (Witvrouw and De Clercq 1997). Therefore, the **EPS** could compete with the amino acids of the virus glycoprotein, blocking the viral adsorption process, in an identical way to what happens in relation to bacteria, already mentioned. There is evidence that carrageenan, a sulfated polysaccharide from a macroalgae, could directly bind to human papillomavirus capsid (Buck et al. 2006). The **EPS** from *P. cruentum* is a good candidate to protect against viruses (Raposo et al. 2013), since uronic acids, along with the half-ester sulfate groups and the carboxyl groups of the **EPS**, contribute to its anionic properties. Raposo et al. (2014) found that the **EPS** from a Spanish strain of *P. cruentum* revealed, in general, greater antiviral activity than the **EPS** from an Israeli strain, and

DOI 10.1007/978-3-319-03751-6_47-1

Table 5 Some food and feed applications of microalgae and safety aspects

Microalgae/ cyanobacteria	Species	Safety aspect	Microalgae/ cyanobacteria	Species	Safety aspect
Cyanophytes	*Arthrospira/spirulina*	GRAS	Diatoms	*Navicula*	NT
	Synechococcus	NT		*Nitzschia dissipata*	NT
Prasinophyte	*Tetraselmis*	NT		*P. tricornutum*	NT
Chlorophytes	*Chlamydomonas reinhardtii*	NT		*Thalassiosira pseudonana*	NT
	H. pluvialis	NT		*Odontella aurita*	NT
	Dunaliella	NT		*Skeletonema*	NT
	Chlorococcum	NT	Eustigmatophytes	*Monodus subterraneus*	NT
	Scenedesmus	NT		*Nannochloropsis*	NT
	Desmodesmus	NT	Haptophytes	*Isochrysis*	NT
	Parietochloris incisa	NT		*Pavlova*	NT
	Chlorella	GRAS	Dinoflagellate	*Crypthecodinium cohnii*	GRAS
Rhodophyta	*P. cruentum*	GRAS			

Adapted from Enzing et al. (2014)
NT no toxins known, *GRAS* generally recognized as safe

this fact might be related to the higher degree of sulfation of the former, although they did not discard other factors.

6 Safety and Regulatory Aspects

The safety hazards of a **PS** as food or food ingredient is directly or indirectly associated to the microalgae which it was extracted or secreted from, respectively.

Some microalgae have been widely commercialized and used, mainly as nutritional supplements for humans and as animal feed additives, and some have the GRAS status being attributed by the FDA (Food and Drug Administration) of the USA (Table 5).

In the EU, several *Chlorella* species and *A. platensis* were on the market as food or food ingredient, and consumed to a significant degree, before 15 May 1997. Thus, in general, they are not subject to the Novel Food Regulation EC No. 258/97. However, in some EU Member States, specific legislation may restrict the placing of these products in the market, and it is recommended to check this issue with the national competent authorities.

Safety hazards related to algae may include allergens and toxins, heavy metals and pesticides, and pathogens (van der Spiegel et al. 2013). Allergenicity has been reported for the cyanobacteria *Phormidium fragile* and *Nostoc muscorum* (Sharma and Rai 2008) and the green algal genus *Chlorella* (Tiberg and Einarsson 1989). No toxins have been found in *Arthrospira* and *Chlorella*, but toxic microcystines were detected in other cyanobacteria (Kerkvliet 2001; Heussner et al. 2012). Extracts from *Aphanizomenon flos-aquae*, *Spirulina*, and *Chlorella*, or mixtures thereof, were cytotoxic (Heussner et al. 2012). Pheophorbides may be formed in *Chlorella*, which give rise to photosensitization in some humans (Kerkvliet 2001). *A. platensis* remarkably reduced the incidence of liver tumors and prevented DBN-induced hepatotoxicity in rats without causing any side effects or organ toxicity (Ismael et al. 2009). Microalgae may also accumulate heavy metals, depending on

DOI 10.1007/978-3-319-03751-6_47-1

the conditions under which they are grown (Hung et al. 1996; Wong et al. 1996). The presence of pathogenic microorganisms is another crucial safety aspect that must be considered. If microalgae are cultivated in open tanks, this may result in microbiological contamination from birds, insects, or rodents (Kerkvliet 2001). These safety problems might be eliminated by growing the microalgae in closed bioreactors (Amara and Steinbuchel 2013).

PSs from microalgae might be a valuable material for a wide potential range of uses, including food, feed, and biomedical applications due, in general, to the absence of or to no known toxicity problems. Most toxicological data from the **PSs** from microalgae are driven from in vitro tests. Raposo et al. (2014) found no cytotoxic effects of the **EPS** from *P. cruentum* for the concentration (100 μg/mL) and cell lines tested. Other studies with Vero cells indicated that the cytotoxic effect only occurred for concentrations higher than 250 μg/mL, and some other in vivo assays indicated that this type of polysaccharide does not show any cytotoxic effect at 2 mg/mL (Huleihel et al. 2001).

The treatment of aortic endothelial smooth muscle cultured cells with depolymerized sodium spirulan (NaSP), an **sPS** obtained from a hot water extract of *A. platensis*, resulted in a significant inhibition of the proliferation of the arterial smooth muscle cells, therefore preventing atherosclerosis without exhibiting any toxic effects on the integrity of the vascular endothelial cell layers (Yamamoto et al. 2006). Some **sPSs** may interfere with blood coagulation and, therefore, have the potential to be used in some biomedical applications and not in another (Raposo et al. 2013).

It should be remarked that any **PS** extracted from microalgae/cyanobacteria and refined to be used as food is considered a new product and, thus, falls under the Novel Food Law of EU. For biomedical applications the novel biological products, in the USA, must comply with FDA Biologics Control Act (21 CFR 600 Biological Products General, Subpart A – General Provisions, Sec.600.3 Definitions) (Whiteside 2011). In the EU the term "biological product" was first published in the Directive 2003/63/EC, amending Annex I of the Directive 2001/83/EC (Noffz 2011). This implies that companies have to provide information on the safety of the food product (including results of animal testing) to the EFSA or FDA, before commercialization is authorized. For instance, carrageenans, which are polysaccharides from seaweeds, not from microalgae, are used as a food additive (E-407), have very low toxicity, and have been shown not to be teratogenic (Necas and Bartosikova 2013). However, the poligeenan, formerly referred to as degraded carrageenan, is not a food additive, exhibiting toxicological properties at high doses (Cohen and Ito 2002). Nilson and Wagner (1959) found no adverse effects on rats lifelong fed with kappa-/lambda-carrageenan from *C. crispus* or *G. mamillosa* at concentrations up to 25 %, while in two other unidentified strains of carrageenan, they found evidence of hepatic cirrhosis, but only at a concentration of 25 % and with no effect on mortality. Therefore, any **PS** from a novel source must be tested prior to be used in human applications.

7 Bioavailability and Metabolism

To our knowledge, there are no known bioavailability studies on the **PSs** from marine microalgae, the bioavailability of **PSs** from microalgae being yet to be studied on humans (Raposo et al. 2013). The **EPS** from *P. cruentum* is not hydrolyzed in the gastrointestinal tract; therefore, its bioavailability is null or very reduced (Arad et al. 1993). However, some knowledge may be withdrawn from studies carried out with polysaccharides from macroalgae, plants, or microorganisms of structures similar to the **PSs** from microalgae.

Sulfated **PSs** from seaweeds, such as fucoidan from brown seaweeds (Senthilkumar et al. 2013) and carrageenans, alginates, and porphyrans from red seaweeds, are known to have biological

DOI 10.1007/978-3-319-03751-6_47-1

Table 6 Preclinical trials of polysaccharides from marine microalgae (see also Table 4)

Microalgae species	Compound	Experimental model	Effects	References
Arthrospira sp.	Biomass	Several models	Hypolipidemic, antioxidant, and anti-inflammatory activities	Deng and Chow 2010
A. platensis	CaSp	Raw macrophages	Synthesis of TNF-α	Parages et al. 2012
Spirulina sp.	Spirulan-like substance	Several types of cell cultures	Antiviral activity against human cytomegalovirus (HCMV), herpes simplex virus (HSV-1), human herpes virus type 6 (HHV-6), human immunodeficiency virus type 1 (HIV-1), non-susceptibility of Epstein-Barr virus (EBV), and human influenza A virus (A/WSN/33)	Rechter et al. 2006
N. flagelliforme	Nostoflan	Vero, HEL, MDCK, and HeLa cells	Antiviral activity against HSV-1 (HF), HSV-2 (UW-268), HCMV (Towne), influenza (NWS)	Kenji et al. 2005
C. pyrenoidosa; *C. ellipsoidea*	Polysaccharide complex	Natural killer cells	Immunostimulating properties, inhibition of the proliferation of *Listeria monocytogenes* and *Candida albicans*	Barrow and Shahidi 2008
C. ellipsoidea	Chlorellan	Reticuloendothelial system in rats	Stimulate phagocytic activity of the reticuloendothelial system	Kojima et al. 1974
P. cruentum	(**EPSs**) degraded by Hermetic microwave and H2O2 under ultrasonic waves	Mouse tumor model, peritoneal macrophage activation, splenocyte proliferation assay	Antitumor and immunomodulatory activities of different molecular weight	Sun et al. 2012
Porphyridium sp.	Polysaccharide	NIH/3T3 cells	Antiviral activity against retrovirus MuSV-124 and MuSV/MuLV	Talyshinsky et al. 2002
P. aerugineum				
P. cruentum	**EPS**	Confluent cultures of human erythroleukemia cell line (HEL)	Antiviral activity against HSV type 1 (HSV-1; strain KOS), HSV type 1 (HSV-1; strain TK-KOS ACVr) and type 2 (HSV-2; strain G), vaccinia virus, and vesicular stomatitis virus	Raposo et al. 2014

effects that could be useful in the prevention or reversal of metabolic syndrome (Holdt and Kraan 2011).

As discussed previously in point 4.3, **PSs** could act as prebiotics. In fact, these **PSs** cannot be digested by human endogeneous enzymes, belonging, therefore, to the dietary fibers (Baird et al. 1977). They are able to modify gastrointestinal hormone secretion, glycemia regulation, and lipid metabolism, preventing, in this way, obesity (Parnell and Reimer 2012). One of the physico-chemical properties of **PSs** is the ability to be fermented by the human colonic microbiota, resulting in beneficial health effects (Mišurcová et al. 2012). Also, in vitro and in vivo animal studies highlighted anti-hyperlipidemic and anti-hyperglycemic activities of **PSs** from microalgae (Raposo et al. 2013).

DOI 10.1007/978-3-319-03751-6_47-1

8 Clinical Trials

Reporting to what was already mentioned in Sect. 4 on bioactivity and applications, **PSs** constitute a good source for potential development of novel food ingredients and biomaterials. However, the exploitation of the biomedical potential of these **PSs** will be a long and challenging road, as the regulatory context of medical devices and, in this case, advanced therapy medicinal products are very demanding. Also, the lack of industrial-scale extraction and purification of many of these molecules is an obstacle for their application development. In fact, any clinical application will demand for the implementation and validation of industrial manufacturing methods. Besides this issue, the natural provenience of **PSs** imposes a strict control of their purity, stability, and safety, which imply extensive and, above all, expensive studies (Silva et al. 2012).

In spite of all these considerations, a large number of preclinical essays have already been conducted with microalgae. Recently, extensive studies have been performed to evaluate the therapeutic benefits of microalgae on several disease conditions including hypercholesterolemia, hyperglycerolemia, cardiovascular diseases, inflammatory diseases, cancer, and viral infections. Some studies reported the antioxidant and/or anti-inflammatory activities of *Spirulina* or its extracts, containing **PSs**, in vitro and in vivo, suggesting that *Spirulina* may provide a beneficial effect in managing cardiovascular conditions (Deng and Chow 2010). Some of the preclinical trials of polysaccharides from marine microalgae that have been carried out are listed in Table 6.

With respect to clinical essays and applications in the market, the area of cosmetics is one of the few known. An **EPS** from a cyanobacterium was reported to be effective in skin aging (Loing et al. 2011). There is a recent US patent defending the use of **sPSs** in cosmetics. Indeed, microalgae extracts, mainly from *Arthrospira* and *Chlorella* (Stolz and Obermayer 2005; Spolaore et al. 2006), are incorporated in many face and skin care products (e.g., antiaging cream, refreshing or regenerating care products, emollient, and anti-irritant in peelers), sun protection, and hair care products (Martins et al. 2014). Alguard™ is a purified **sPS** from *P. cruentum* that is used in cosmetics worldwide as antiaging, anti-inflammatory, anti-irritant, skin maintenance, UVB damage prevention, soothing and healing creams, and lip balms. Also a mixture of **PSs** from microalgae makes part of the composition of a commercial product called Algenist™ antiaging skin care formulas in the form of alguronic acid.

There is a patent (WO 2007066340 A1, Arad and Atar 2007) defending the use of a material composed of algal polysaccharides for viscosupplementation in the treatment of degenerative joint disorders related to joint lubrication, preferably osteoarthritis, rheumatoid arthritis, gout, trauma, and age-related degeneration.

9 Conclusion

Microalgae are easy to grow organisms, and, in comparison with seaweeds, their culture conditions are easily controlled in closed systems, thus avoiding safety hazards. The chemical composition and structure and the rheological behavior of the polysaccharides they produce are relatively stable no matter the period/phase of harvest. However, the polysaccharides synthetized and/or released by marine microalgae can be so heterogeneous and structurally different that research on these compounds can be a very challenging task.

Polysaccharides may be regarded as key ingredients for the production of bio-based materials in life sciences (e.g., food, cosmetics, medical devices, pharmaceutics). The biological source and biodegradability of these biopolymers, coupled to the large variety of chemical functionalities they

encompass, make them promising compounds. Despite having showed several interesting properties, including for human nutrition and health, their use in humans and clinical trials and bioapplications are yet to be explored, one of the reasons being the high molecular weights of the polymers. It would be an interesting issue to explore its use orally and therapeutically in human subjects, considering their anti-inflammatory, hypoglycemic, and anticoagulant/antithrombotic activities. However, the toxicity and bioavailability of such compounds are yet to be studied on humans. Although there are a large number of products on the market with biomass or extracts from microalgae, there are very few commercial products from polysaccharides isolated and purified on the market, but the outlook for such products is considered of major importance. Only a few patent applications on microalgae polysaccharides exist. However, the properties of polysaccharide-based products indicate great potential in the food and biomedical sectors.

Other areas of interest, due to their biochemical characteristics and rheological behavior, could be in engineering fields, such as naval (as drag-reducing agents), in food science/engineering, or in biomedical applications, as joint biolubricants and in arthritis treatment.

The extensive use of marine microalgae polysaccharides in these fields, however, would require a reliable supply of raw materials to guarantee the affordable price, sufficient purity, and constant high quality of these bioproducts. In addition, the possibility of obtaining polysaccharides in high quantities from modern microalgae biorefineries might be a competitive advantage with other sources.

Acknowledgments

This work was supported by National Funds from FCT through project PEst-OE/EQB/LA0016/2013.

References

Allard B, Casadeval E (1990) Carbohydrate composition and characterization of sugars from the green alga *Botryococcus braunii*. Phytochemistry 29(6):1875–1878

Allard B, Guillot JP, Casadeval E (1987) The production of extracellular polysaccharides by freshwater microalgae. Investigation of the polysaccharide components. In: Grassi G, Delmon B, Molle JF, Zibetta H (eds) Biomass for energy and industry. Elsevier Applied Science, London, pp 603–607

Amara A, Steinbüchel A (2013) New medium for pharmaceutical grade *Arthrospira*. Int J Bacteriol 2013:9 p, Article ID 203432. doi:10.1155/2013/203432

Arad S(M) (1988) Production of sulphated polysaccharides from red unicellular algae. In: Stadler T, Mollion J, Verdus MC, Karamanos Y, Morvan H, Christiaen D (eds) Algal biotechnology. Elsevier Applied Science, London, pp 65–87

Arad S(M) (1999) Polysaccharides of red microalgae. In: Cohen Z (ed) Chemicals from microalgae. Taylor & Francis, London, pp 282–291

Arad S(M), Atar D (2007) Viscosupplementation with algal polysaccharides in the treatment of arthritis. Il. Patent WO/2007/066340

Arad S(M), Weinstein J (2003) Novel lubricants from red microalgae: interplay between genes and products. J Biomed (Israel) 1:32–37

Arad S(M), Adda M, Cohen E (1985) The potential of production of sulphated polysaccharides from *Porphyridium*. Plant Soil 89:117–127

Arad S(M), Lerental YB, Dubinsky O (1992) Effect of nitrate and sulfate starvation on polysaccharide formation in *Rhodella reticulata*. Bioresour Technol 42:141–148

Arad (M) S, Keristovsky G, Simon B, Barak Z, Geresh S (1993) Biodegradation of the sulphated polysaccharide of *Porphyridium* sp. by soil bacteria. Phytochemistry 32:287–290

Arad S(M), Rapoport L, Moshkovich A, van Moppes D, Karpasan M, Golan R, Golan Y (2006) Superior biolubricant from a species of red microalga. Langmuir 2:7313–7317

Archibald PJ, Fenn MD, Roy AB (1981) ^{13}C NMR studies of D-glucose and D-galactose monosulphates. Carbohydr Res 93:177–190

Ascencio F, Fransson LA, Wadström T (1993) Affinity of the gastric pathogen *Helicobacter pylori* for the *N*-sulphated glycosaminoglycan heparin sulphate. J Med Microbiol 38:240–244

Badel S, Callet F, Laroche C, Gardarin C, Petit E, El Alaoui H, Bernardi T, Michaud P (2011a) A new tool to detect high viscous exopolymers from microalgae. J Ind Microbiol Biotechnol 38:319–326

Badel S, Laroche C, Gardarin C, Petit E, Bernardi T, Michaud P (2011b) A new method to screen polysaccharide cleavage enzymes. Enzyme Microb Technol 48:248–252

Bae SY, Yim JH, Lee HK, Pyo S (2006) Activation of murine peritoneal macrophages by sulphated exopolysaccharide from marine microalga *Gyrodinium impudicum* (strain KG03): involvement of the NF-kappa B and JNK pathway. Int Immunopharmacol 6:473–484

Baird IM, Waltersa RL, Daviesa PS, Hilla MJ, Drasara BS, Southgate DAT (1977) The effects of two dietary fiber supplements on gastrointestinal transit, stool weight and frequency, and bacterial flora, and fecal bile acids in normal subjects. Metabolism 26(2):117–128

Banerjee A, Sharma R, Chisti Y, Banerjee UC (2002) *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. Crit Rev Biotechnol 22(3):245–279

Barrow C, Shahidi F (2008) Marine nutraceuticals and functional foods. CRC Press/Taylor & Francis Group, Boca Raton, USA

Basedow AM, Elber KH, Feigenbutz W (1980) Polymer-solvent interactions: dextrans in water and DMSO. Die Makromolekulare Chemie 181:1071–1080

Bergman B (1986) Glyoxylate induced changes in the carbon and nitrogen metabolism of the cyanobacterium *Anabaena cylindrica*. Plant Physiol 80:698–701

Bernal P, Llamas MA (2012) Promising biotechnological applications of antibiofilm exopolysaccharides. Microb Biotechnol 5(6):670–673

Bleicher P, Mackin W (1995) Betafectin PGG-glucan: a novel carbohydrate immunomodulator with anti-infective properties. J Biotechnol Healthc 2:207–222

Buck CB, Thompson CD, Roberts JN, Muller M, Lowy DR, Schiller JT (2006) Carrageenan is a potent inhibitor of papillomavirus infection. PLoS Pathog 2(7):671–680

Burgaleta C, Territo MC, Quan SG, Golde DW (1978) Glucan-activated macrophages: functional characteristics and surface morphology. J Reticuloendothel Soc 23:195–204

Challouf R, Trabelsi L, Dhieb RB, El Abed O, Yahia A, Ghozzi K, Ammar JB, Omran H, Ouada HB (2011) Evaluation of cytotoxicity and biological activities in extracellular polysaccharides released by cyanobacterium *Arthrospira platensis*. Braz Arch Biol Technol 54(4):831–838

Chen H, Zhang M, Qu Z, Xie B (2008) Antioxidant activities of different fractions of polysaccharide conjugates from green tea (*Camellia sinensis*). Food Chem 106:559–563

Chen B, You B, Huang J, Yu Y, Chen W (2010) Isolation and antioxidant property of the extracellular polysaccharide from *Rhodella reticulata*. World J Microbiol Biotechnol 26:833–840

Chen C-S, Anaya JM, Zhang S, Spurgin J, Chuang C-Y, Xu C, Miao A-J, Chen EY-T, Schwehr KA, Jiang Y, Quigg A, Santschi PH, Chin W-C (2011) Effects of engineered nanoparticles on the assembly of exopolymeric substances from phytoplankton. PLoS ONE 6(7):1–7 (open access e21865)

Ciferri O (1983) Spirulina, the edible microorganism (algae, single-cell protein). Microbiol Rev 47(4):551–578

Clowes AW, Clowes MM (1987) Regulation of smooth muscle proliferation by heparin in vitro and in vivo. Int Angiol 6:45–51

Cohen SM, Ito N (2002) A critical review of the toxicological effects of carrageenan and processed *Eucheuma* seaweed on the gastrointestinal tract. Crit Rev Toxicol 32(5):413–444

Collins PM, Munasinghe VRN (1987) In: Collins PM (ed) Carbohydrates. Chapman and Hall, London, p 719

Dade WB, Davis JD, Nichols PD, Nowell ARM, Thistle D, Trexler MB, White DC (1990) Effects of bacterial exopolymer adhesion on the entrainment of sand. Geomicrobiol J 8(1):1–16

Damonte EB, Matulewicz MC, Cerezo AS (2004) Sulphated seaweed polysaccharides as antiviral agents. Curr Med Chem 11(18):2399–2419

De Philippis R, Sili C, Tassinato G, Vincenzini M, Materassi R (1991) Effects of growth conditions on exopolysaccharide production by *Cyanospira capsulata*. Bioresour Technol 38:101–104

De Philippis R, Margheri MC, Pelosi E, Ventura S (1993) Exopolysaccharide production by a unicellular cyanobacterium isolated from a hypersaline habitat. J Appl Phycol 5:387–394

De Philippis R, Sili C, Vincenzini M (1996) Response of an exopolysaccharide-producing heterocystous cyanobacterium to changes in metabolic carbon flux. J Appl Phycol 8:275–281

Deng R, Chow T-J (2010) Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *spirulina*. Cardiovasc Ther 28:e33–e45

Dubinsky O, Barak Z, Geresh S, Arad S(M) (1990) Composition of the cell-wall polysaccharide of the unicellular red alga *Rhodella reticulata* at two phases of growth. In: Advances in algal biotechnology. Tiberias, p 17

Dubinsky O, Simon B, Karamanos Y, Geresh S, Barak Z, Arad S(M) (1992) Composition of the cell wall polysaccharide produced by the unicellular red alga *Rhodella reticulata*. Plant Physiol and Biochem 30(4):409–414

Dvir I, Maislos M, Arad S(M) (1995) Feeding rodents with red microalgae. In: Cherbut C, Barry JL, Lairon D, Durand M (eds) Dietary fiber, mechanisms of action in human physiology and metabolism. John Libbey Eurotext, Paris, pp 86–91

Dvir I, Chayoth R, Sod-Moriah U, Shany S, Nyska A, Stark AH, Madar Z, Arad S(M) (2000) Soluble polysaccharide of red microalga *Porphyridium* sp. alters intestinal morphology and reduces serum cholesterol in rats. Br J Nutr 84:469–476

Dvir I, Stark AH, Chayoth R, Madar Z, Arad S(M) (2009) Hypocholesterolemic effects of nutraceuticals produced from the red microalga *Porphyridium* sp. in rats. Nutrients 1:156–167

Enzing C, Ploeg M, Barbosa M, Sijtsma L (2014) Microalgae-based products for the food and feed sector: an outlook for Europe. Mauro Vigani, Claudia Parisi, Emilio Rodríguez Cerezo (eds) Joint Research Centre Scientific and Policy Reports, European Commission Brighton, UK

Esko J, Sharon N (2009) Microbial lectins: hemagglutinins, adhesins, and toxins. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR et al (eds) Essentials of glycobiology, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, Chapter 34

Eteshola E, Karpasas M, Arad S(M), Gottlieb M (1998) Red microalga exopolysaccharides: 2. Study of the rheology, morphology and thermal gelation of aqueous preparations. Acta Polym 49:549–556

Evans LV, Callow ME, Percival E, Fareed VS (1974) Studies on the synthesis and composition of extracellular mucilage in the unicellular red alga *Rhodella*. J Cell Sci 16:1–21

Fabregas J, García D, Fernandez-Alonso M, Rocha AI, Gómez-Puertas P, Escribano JM, Otero A, Coll JM (1999) In vitro inhibition of the replication of viral haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antivir Res 44:67–73

Fareed VS, Percival E (1977) The presence of rhamnose and 3-*O*-methylxylose in the extracellular mucilage from the red alga *Rhodella maculata*. Carbohydr Res 53:276–277

Fernandes HL, Tomé MM, Lupi FM, Fialho AM, Sá-Correia I, Novais JM (1989) Biosynthesis of high concentrations of na exopolysaccharide during the cultivation of the microalga *Botryococcus braunii*. Biotechnol Lett 11(6):433–436

Flaibani A, Olsen Y, Painter TJ (1989) Polysaccharides in desert reclamation: compositions of exocellular proteoglycan complexes produced by filamentous blue-green and unicellular green edaphic algae. Carbohydr Res 190(2):235–248

Ford CW, Percival E (1965a) The carbohydrates of *Phaeodactylum tricornutum*. Part I. Preliminary examination of the organism, and characterization of low molecular weight material and of a glucan. J Chem Soc 1298:7035–7041

Ford CW, Percival E (1965b) The carbohydrates of *Phaeodactylum tricornutum*. Part II. A sulphated glucuronomannan. J Chem Soc 1299:7042–7046

Franco JM, Raymundo A, Sousa I, Gallegos C (1998) Influence of processing variables on the rheological and textural properties of lupin protein-stabilized emulsions. J Agric Food Chem 46:3109–3115

Gandhi NS, Mancera RL (2008) The structure of glycosaminoglycans and their interactions with proteins. Chem Biol Drug Des 72:455–482

Garcia D, Morales E, Dominguez A, Fábregas J (1996) Productividad mixotrófica del exopolisacárido sulfatado com la microalga marina *Porphyridium cruentum.* Communicaciones del III Congreso Ibérico de Biotecnología – Biotec'96. Universidad de Valladolid (eds), pp 591–592

Gardeva E, Toshkova R, Minkova K, Gigova L (2009) Cancer protective action of polysaccharide derived from microalga *Porphyridium cruentum*-a biological background. Biotechnol Biotechnol Equip 23:783–787

Gasljevic K, Hall K, Chapman D, Matthys EF (2008) Drag-reducing polysaccharides from marine microalgae: species productivity and drag reduction effectiveness. J Appl Phycol 20:299–310

Geresh S, Arad S(M) (1991) The extracellular polysaccharides of the red microalgae: chemistry and rheology. Bioresour Technol 38:195–201

Geresh S, Dubinsky O, Arad S(M), Christian D, Glaser R (1990) Structure of 3-O-(α-D-glucopyranosyluronic acid)-L-galactopyranose, an aldobiuronic acid isolated from the polysaccharides of various unicellular red algae. Carbohydr Res 208:301–305

Geresh S, Lupescu N, Arad S(M) (1992) Fractionation and partial characterization of the sulfated polysaccharide of the red alga *Porphyridium* sp. Phytochemistry 31(12):4181–4186

Geresh S, Dawadi RP, Arad S(M) (2000) Chemical modifications of biopolymers: quaternization of the extracellular polysaccharide of the red microalga *Porphyridium* sp. Carbohydr Polym 63:75–80

Geresh S, Adin I, Yarmolinsky E, Karpasas M (2002a) Characterization of the extracellular polysaccharide of *Porphyridium* sp.: molecular weight determination and rheological properties. Carbohydr Polym 50:183–189

Geresh S, Mamontov A, Weinstein J (2002b) Sulfation of extracellular polysaccharides of red microalga: preparation, characterization, properties. J Biochem Biophys Methods 50:179–187

Ghosh T, Chattopadhyay K, Marschall M, Karmakar P, Mandal P, Ray B (2009) Focus on antivirally active sulfated polysaccharides: from structure-activity analysis to clinical evaluation. Glycobiology 19:2–15

Ginzberg A, Cohen M, Sod-Moriah UA, Shany S, Rosenshtrauch A, Arad S(M) (2000) Chickens fed with biomass of the red microalga *Porphyridium* sp. have reduced blood cholesterol levels and modified fatty acids composition in egg yolk. J Appl Phycol 12:325–330

Ginzberg A, Korin E, Arad S(M) (2008) Effect of drying on the biological activities of a red microalga polysaccharide. Biotechnol Bioeng 99(2):411–420

Gloaguen V, Ruiz G, Morvan H, Mouradi-Givernaud A, Maes E, Krausz P, Srecker G (2004) The extracellular polysaccharide of *Porphyridium* sp.: an NMR study of lithium-resistant oligosaccharidic fragments. Carbohydr Res 339:97–103

Glore SR, van Treeck D, Knehans AW, Guild M (1994) Soluble fiber and serum lipids: a literature review. J Am Dietetic Assoc 94(4):425–436

Gouveia L, Batista A P, Sousa I, Raymundo A, Bandarra M (2008) Microalgae in novel food products. In: Papadopoulos KN (ed) Food chemistry research developments. Nova Science Publishers Inc., New York, USA

Guzman S, Gato A, Lamela M, Freire-Garabal M, Calleja JM (2003) Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phytother Res 17:665–670

Guzmán-Murillo MA, Ascencio F (2000) Anti-adhesive activity of sulphated exopolysaccharides of microalgae on attachment of the red sore disease-associated bacteria and *Helicobacter pylori* to tissue culture cells. Lett Appl Microbiol 30:473–478

Hasui M, Matsuda M, Okutani K, Shigeta S (1995) In vitro antiviral activities of sulphated polysaccharides from a marine microalga (*Cochlodinium polykrikoides*) against human immunodeficiency virus and other enveloped virus. Int J Biol Macromol 17(5):293–297

Hayakawa Y, Hayashi T, Hayashi K, Osawa T, Niiya K, Sakuragawa N (1996) Heparin cofactor II-dependent antithrombin activity of calcium spirulan. Blood Coagul Fibrinolysis 7:554–560

Hayakawa Y, Hayashi T, Hayashi K, Osawa T, Niiya K, Sakuragawa N (1997) Calcium spirulan as an inducer of tissue-type plasminogen activator in human fetal lung fibroblasts. Biochim Biophys Acta 1355(3):241–247

Hayakawa Y, Hayashi T, Lee JB, Osawa T, Niiya K, Sakuragawa N (2000) Activation of heparin cofactor II by calcium spirulan. J Biol Chem 275:11379–11382

Hayashi K, Hayashi T, Kojima IA (1996a) A natural sulphated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus. AIDS Res Hum Retroviruses 12:1463–1471

Hayashi T, Hayashi K, Maeda M, Kojima I (1996b) Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J Nat Prod 59(1):83–87

Hernandez-Corona A, Nieves I, Meckes M, Chamorro G, Barron BL (2002) Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. Antiviral Res 56:279–285

Heussner AH, Mazija L, Fastner J, Dietrich DR (2012) Toxin content and cytotoxicity of algal dietary supplements. Toxicol Appl Pharmacol 265(2):263–271

Holdt S, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:543–597

Huang J, Chen B, You W (2005) Studies on separation of extracellular polysaccharide from *Porphyridium cruentum* and its anti-HBV activity in vitro. Chin J Mar Drugs (Chinese) 24:18–21

Huang J, Liu L, Yu Y, Lin W, Chen B, Li M (2006) Reduction in the blood glucose level of exopolysaccharide of *Porphyridium cruentum* in alloxan-induced diabetic mice. J Fujian Norm Univ (Chinese) 22:77–80

Huleihel M, Ishanu V, Tal J, Arad S(M) (2001) Antiviral effect of the red microalgal polysaccharides on *Herpes simplex* and *Varicella zoster* viruses. J Appl Phycol 13:127–134

Huleihel M, Ishanu V, Tal J, Arad S(M) (2002) Activity of *Porphyridium* sp. polysaccharide against *Herpes simplex* viruses in vitro and in vivo. J Biochem Biophys Methods 50:189–200

Hung KM, Chiu ST, Wong MH (1996) Sludge-grown algae for culturing aquatic organisms.1. Algal growth in sludge extracts. Environ Manag 20(3):361–374

Kaji T, Fujiwara Y, Hamada C, Yamamoto C, Shimada S, Lee JB, Hayashi T (2002) Inhibition of cultured bovine aortic endothelial cell proliferation by sodium spirulan, a new sulphated polysaccharide isolated from *Spirulina platensis*. Planta Med 68:505–509

Kaji T, Okabe M, Shimada S, Yamamoto C, Fujiwara Y, Lee J-B, Hayashi T (2004) Sodium spirulan as a potent inhibitor of arterial smooth muscle cell proliferation in vitro. Life Sci 74:2431–2439

Kaplan D, Christiaen D, Arad S(M) (1987) Chelating properties of extracellular polysaccharides from *Chlorella* spp. Appl Environ Microbiol 53(12):2953–2956

Kenji LK, Kanekiyo K, Lee JB, Hayashi K, Takenaka H, Hayakawa Y, Endo S, Hayashi T (2005) Isolation of an antiviral polysaccharide, nostoflan, from a terrestrial cyanobacterium, *Nostoc flagelliforme*. J Nat Prod 68:1037–1041

Kerkvliet JD (2001) Algen en zeewieren als levensmiddel: een overzicht. De Ware(n)chemicus 31:77–104

Kieras JH (1972) Study of the extracellular polysaccharide of *Porphyridium cruentum*. PhD thesis, University of Chicago, Department of Biology

Kieras JH, Chapman D (1976) Structural studies on the extracellular polysaccharide of the red alga *Porphyridium cruentum*. Carbohydr Res 52:169–177

Kim M, Yim JH, Kim S-Y, Kim HS, Lee WG, Kim SJ, Kang PS, Lee CK (2012) In vitro inhibition of influenza A virus infection by marine microalga-derived sulfated polysaccharide p-KG03. Antivir Res 93:253–259

Kojima M, Kasajima T, Imai Y, Kobayashi S, Dobashi M, Uemura T (1974) New *Chlorella* polysaccharide and its accelerating effect on the phagocytic activity of the reticuloendothelial system. Recent Adv Res 13:101–107

Kroen WK, Rayburn WR (1984) Influence of growth status and nutrients on extracellular polysaccharide synthesis by the soil alga *Chlamydomonas mexicana* (Chlorophyceae). J Phycol 20(2):253–257

Laroche C, Michaud P (2007) New developments and prospective applications for β-(1,3)-glucans. Recent Pat Biotechnol 1:59–73

Laurienzo P (2010) Marine polysaccharides in pharmaceutical applications: an overview. Mar Drugs 8:2435–2465

Lee J-B, Hayashi T, Hayashi K, Sankawa U, Maeda M, Nemoto T, Nakanishi H (1998) Further purification and structural analysis of calcium spirulan from *Spirulina platensis*. J Nat Prod 61:1101–1104

Lee J-B, Hayashi T, Hayashi K, Sankawa U (2000) Structural analysis of calcium spirulan (Ca-SP)-derived oligosaccharides using electrospray ionization mass spectrometry. J Nat Prod 63:136–138

Leiro JM, Castro R, Arranz JÁ, Lamas J (2007) Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh. Int Immunopharmacol 7:879–888

Li P, Liu Z, Xu R (2001) Chemical characterization of the released polysaccharides from the cyanobacterium *Aphanothece halophytica* GR02. J Appl Phycol 13:71–77

Li L-Y, Li L-Q, Guo C-H (2010) Evaluation of in vitro antioxidant and antibacterial activities of *Laminaria japonica* polysaccharides. J Med Plants Res 4(21):2194–2198

Liu Y, Wang W, Zhang M, Xing P, Yang Z (2010) PSII-efficiency, polysaccharide production, and phenotypic plasticity of *Scenedesmus obliquus* in response to changes in metabolic carbon flux. Biochem Syst Ecol 38:292–299

Loing E, Briatte S, Vayssier C, Beaulieu M, Dionne P, Richert L, Moppert X (2011) Cosmetic compositions comprising exopolysaccharides derived from microbial mats, and use thereof US 20110150795 A1

Lupescu N, Arad S(M), Geresh S, Bernstein MA, Glaser R (1991) Structure of some sulfated sugars isolated after acid hydrolysis of the extracellular polysaccharide of *Porphyridium* sp., a unicellular red alga. Carbohydr Res 210:349–352

Lupi FM, Fernandes HML, Sá-Correia I, Novais JM (1991) Temperature profiles of cellular growth and exopolysaccharide synthesis by *Botryococcus braunii* Kütz. UC 58. J Appl Phycol 3:35–42

Marceliano MB (2009) Structure and function of polysaccharide gum-based edible films and coatings. In: Embuscado ME, Huber KC (eds) Edible films and coatings for food applications. Springer, Dordrecht

Marlett J (2001) Dietary fibre and cardiovascular disease. In: Cho SS, Dreher MD (eds) Handbook of dietary fibers. Marcel Dekker, New York, pp 17–30

Martinez MJA, del Olmo LMB, Benito PB (2005) Antiviral activities of polysaccharides from natural sources. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 30. Elsevier B. V., London, pp 393–418

Martins A, Vieira H, Gaspar H, Santos S (2014) Marketed marine natural products in the pharmaceutical and cosmeceutical industries: tips for success. Mar Drugs 12(2):1066–1101

Matsui SM, Muizzudin N, Arad S(M), Marenus K (2003) Sulfated polysaccharides from red microalgae anti-inflammatory properties in vitro and in vivo. Appl Biochem Biotechnol 104:13–22

Mendiola JA, Jaime L, Santoyo S, Reglero G, Cifuentes A, Ibanez E, Senorans FJ (2007) Screening of functional compounds in supercritical fluid extracts from *Spirulina platensis*. Food Chem 102:1357–1367

Metting B, Rayburn WR (1983) The influence of a microalgal conditioner on selected Washington soils: an empirical study. Soil Sci Soc Am J 47:682–685

Michael TM, John MM, Jack P (2002) Brock microbiology of microorganisms, 10th edn. Prentice Hall, New Jersey

Mishra A, Kavita K, Jha B (2011) Characterization of extracellular polymeric substances produced by micro-algae *Dunaliella salina*. Carbohydr Polym 83:852–857

Mišurcová L, Škrovánková S, Samek D, Ambrožová J, Machu L (2012) Health benefits of algal polysaccharides in human nutrition. Adv Food Nutr Res 66:75–145

Mulloy B, Linhardt RJ (2001) Order out of complexity – protein structures that interact with heparin. Curr Opin Struct Biol 11:623–628

Namikoshi M (1996) Bioactive compounds produced by cyanobacteria. J Int Microbiol Biotechnol 17:373–384

Necas J, Bartosikova L (2013) Carrageenan: a review. Vet Med 58(4):187–205

Nilson HW, Wagner JA (1959) Feeding test with carrageenan. Food Res 24:235–239

Noffz G (2011) Novel medical products: conventional biological or ATMPs? MSc thesis, University of Bonn, Germany

Nomoto K, Yokokura T, Satoh H, Mutai M (1983) Anti-tumor effect by oral administration of *Chlorella* extract, PCM-4 by oral admission (article in Japanese). Gan To Kagaku Zasshi 10:781–785

Oakenfull D (2001) Physicochemical properties of dietary fiber: overview. In: Cho SS, Dreher MD (eds) Handbook of dietary fibers. Marcel Dekker, New York, pp 195–206

Ofek L, Beachery EH, Sharon N (1978) Surface sugars recognition in bacterial adherence. Trends Biochem Sci 3:159–160

Ogawa K, Yamaura M, Maruyama I (1997) Isolation and identification of 2-*O*-methyl-L-rhamnose and 3-*O*-methyl-L-rhamnose as constituents of an acidic polysaccharide of *Chlorella vulgaris*. Biosci Biotechnol Biochem 61(3):539–540

Ogawa K, Yamaura M, Ikeda Y, Kondo S (1998) New aldobiuronic acid, 3-*O*-α-D-glucopyranuronosyl-L-rhamnopyranose, from an acidic polysaccharide of *Chlorella vulgaris*. Biosci Biotechnol Biochem 62(10):2030–2031

Ogawa K, Ikeda Y, Kondo S (1999) A new trisaccharide, α-D-glucopyranuronosyl-(1→3)- α-L-rhamnopyranosyl-(1→2)- α-L-rhamopyranose from *Chlorella vulgaris*. Carbohydr Res 321:128–131

Otero A, Vincenzini M (2003) Extracellular polysaccharide synthesis by *Nostoc* strains as affected by N source and light intensity. J Biotechnol 102:143–152

Parages ML, Rico RM, Abdala-Díaz RT, Chabrillón M, Sotiroudis TG, Jiménez C (2012) Acidic polysaccharides of *Arthrospira* (*Spirulina*) *platensis* induce the synthesis of TNF-α in RAW macrophages. J Appl Phycol 24(6):1537–1546

Parnell JA, Reimer RA (2012) Prebiotic fiber modulation of the gut microbiota improves risk factors for obesity and the metabolic syndrome. Gut Microbes 3:29–34

Patchen ML, Lotzova E (1980) Modulation of murine hemopoiesis by glucan. Exp Hematol 8:409–422

Patel AK, Laroche C, Marcati A, Ursu AV, Jubeau S, Marchal L, Petit E, Djelveh G, Michaud P (2013) Separation and fractionation of exopolysaccharides from *Porphyridium cruentum*. Bioresour Technol 145:345–350

Paterson DM (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelic diatoms. Limnol Oceanogr 34:223–234

Penna A, Berluti S, Penna N, Magnani M (1999) Influence of nutrient ratios on the in vitro extracellular polysaccharide production by marine diatoms from Adriatic Sea. J Plankton Res 21(9):1681–1690

Percival E, Foyle RAJ (1979) The extracellular polysaccharides of *Porphyridium cruentum* and *Porphyridium aerugineum*. Carbohydr Res 72:165–176

Pereira MS, Vilela-Silva AC, Valente AP, Mourão PA (2002) A 2-sulfated,3-L-linked alpha-L-galactan is an anticoagulant polysaccharide. Carbohydr Res 337:2231–2238

Pierre G, Sopena V, Juin C, Mastouri A, Graber M, Mangard T (2011) Antibacterial activity of a sulphated galactan extracted from the marine alga *Chaetomorpha aerea* against *Staphylococcus aureus*. Biotechnol Bioproc Eng 16:937–945

Pignolet O, Jubeau S, Vaca-Garcia C, Michaud P (2013) Highly valuable microalgae: biochemical and topological aspects. J Ind Microbiol Biotechnol 40:781–796

Pletikapic G, Radic TM, Zimmermann AH, Svetlicic V, Pfannkuchen M, Maric D, Godrjan J, Zutic V (2011) AFM imaging of extracellular polymer release by marine diatom *Cylindrotheca closterium* (Ehrenberg) Reiman & JC Lewin. J Mol Recognit 24:436–445

Prajapati VD, Maheriya PM, Jani GK, Solanki HK (2014) Carrageenan: a natural seaweed polysaccharide and its applications. Carbohydr Polym 105:97–112

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65:635–648

Radonic A, Thulke S, Achenbach J, Kurth A, Vreemann A, König T, Walter C, Possinger K, Nitsche A (2010) Anionic polysaccharides from phototrophic microorganisms exhibit antiviral activities to *Vaccinia* virus. J Antivir Antiretrovir 2(4):51–55

Ramus J, Robins DM (1975) The correlation of Golgi activity and polysaccharide secretion in *Porphyridium*. J Phycol 11:70–74

Ramus J, Kenney BE, Shaughnessy EJ (1989) Drag-reducing properties of microalgal exopolymers. Biotechnol Bioeng 33:550–557

Raposo MFJ, de Morais RMSC (2011) *Chlorella vulgaris* as soil amendment: influence of encapsulation and enrichment with rhizobacteria. Int J Agric Biol 13:719–724

Raposo MFJ, Oliveira SE, Castro PM, Bandarra NM, Morais RM (2010) On the utilization of microalgae for brewery effluent treatment and possible applications of the produced biomass. J Inst Brew 116(3):285–292

Raposo MFJ, de Morais RMSC, de Morais AMMB (2013) Bioactivity and applications of sulphated polysaccharides from marine microalgae, a review. Mar Drugs 11(1):233–252

Raposo MFJ, de Morais AMMB, de Morais RMSC (2014) Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from *Porphyridium cruentum*. Life Sci 101:56–63

Rashid ZM, Lahaye E, Defer D, Douzenel P, Perrin B, Bourgougnon N, Sire O (2009) Isolation of a sulphated polysaccharide from a recently discovered sponge species (*Celtodoryx girardae*) and determination of its anti-herpetic activity. Int J Biol Macromol 44:286–293

Raymundo A, Franco J, Gallegos C, Empis J, Sousa I (1998) Effect of thermal denaturation of lupin protein on its emulsifying properties. Nahrung 42:220–224

Raymundo A, Gouveia L, Batista AP, Empis J, Sousa I (2005) Fat mimetic capacity of *Chlorella vulgaris* biomass in oil-in-water food emulsions stabilized by pea protein. Food Res Int 38:961–965

Rechter S, König T, Auerochs S, Thulke S, Walter H, Dörnenburg H, Walter C, Marschall M (2006) Antiviral activity of *Arthrospira*-derived spirulan-like substances. Antiviral Res 72(3):197–206

Rendueles O, Ghigo JM (2012) Multi-species biofilms: how to avoid unfriendly neighbors. FEMS Microbiol Rev 36(5):972–989

Rendueles O, Travier L, Latour-Lambert P, Fontaine T, Magnus J, Denamur E, Ghigo J (2011) Screening of *Escherichia coli* species biodiversity reveals new biofilm-associated antiadhesion polysaccharides. MBio 2:e00043–e00011. doi:10.1128/mBio.00043-11

Riggi SJ, DiLuzio NR (1961) Identification of a reticuloendothelial stimulating agent in zymosan. Am J Physiol 200:297–300

Rincé Y, Lebeau T, Robert JM (1999) Artificial cell-immobilization: a model simulating immobilization in natural environments? J Appl Phycol 11:263–272

Senni K, Pereira J, Gueniche F, Delbarre-Ladrat C, Sinquin C, Ratiskol J, Godeau G, Fisher AM, Helley D, Colliec-Jouault S (2011) Marine polysaccharides: a source of bioactive molecules for cell therapy and tissue engineering. Mar Drugs 9:1664–1681

Senthilkumar K, Manivasagan P, Venkatesan J, Kim SK (2013) Brown seaweed fucoidan: biological activity and apoptosis, growth signaling mechanism in cancer. Int J Biol Macromol 60C:366–374

Sharma NK, Rai AK (2008) Allergenicity of airborne cyanobacteria *Phormidium fragile* and *Nostoc muscorum*. Ecotoxicol Environ Saf 69(1):158–162

Shepherd R, Rockey J, Sutherland IW, Roller S (1995) Novel bioemulsifiers from microorganisms for use in foods. J Biotechnol 40(3):207–217

Shopen-Katz O, Ling E, Himelfarb Y, Lamprecht SA, Arad SM, Shany S (2000) The effect of *Porphyridium* sp. biomass and of its polysaccharide in prevention and inhibition of human colon cancer. Proceedings of the Int Conference in the Era of Biotechnology. Beer-Sheva, Israel, p 32

Silva TH, Alves A, Popa EG, Reys LL, Gomes ME, Sousa RA, Silva SS, Mano JF, Reis RL (2012) Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. Biomatter 2(4):1–12

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101(2):87–96

Staats N, de Winder B, Stal LJ, Mur LR (1999) Isolation and characterization of extracellular polysaccharides from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. Eur J Phycol 34:161–169

Stolz P, Obermayer B (2005) Manufacturing microalgae for skin care. Cosmet Toiletries 120:99–106

Subramanian BS, Yan S, Tyagi RD, Surampalli RY (2010) Extracellular polymeric substances (EPS) producing bacterial strains of municipal wastewater sludge: isolation, molecular identification EPS characterization and performance for sludge settling and dewatering. Water Res 44:2253–2266

Sun L (2010). Preparation of polysaccharides from *Porphyridium cruentum* and their biological activities. PhD thesis dissertation posted at Globethesis.com. http://www.globethesis.com/?t=1101360275957885 on 23 May 2010. Last assess 27 Jan

Sun HH, Mao WJ, Chen Y, Guo SD, Li HY, Qi XH, Chen YL, Xu J (2009a) Chemical characteristics and antioxidant properties of the polysaccharides from marine fungus *Penicillium* sp. F23-2. Carbohydr Polym 78:117–124

Sun L, Wang C, Shi Q, Ma C (2009b) Preparation of different molecular weight polysaccharides from *Porphyridium cruentum* and their antioxidant activities. Int J Biol Macromol 45:42–47

Sun L, Wang L, Zhou Y (2012) Immunomodulation and antitumor activities of different-molecular-weight polysaccharides from *Porphyridium cruentum*. Carbohydr Polym 87:1206–1210

Sutherland TF, Grant J, Amos CL (1998) The effect of carbohydrate production by the diatom *Nitzschia curvilineata* on the erodibility of sediment. Limnol Oceanogr 43:65–72

Talyshinsky MM, Souprun YY, Huleihel MM (2002) Anti-viral activity of red microalgal polysaccharides against retroviruses. Cancer Cell Int 2(8):1–7

Tannin-Spitz T, Bergman M, van Moppes D, Grossman S, Arad S(M) (2005) Antioxidant activity of the polysaccharide of the red microalga *Porphyridium* sp. J Appl Phycol 17:215–222

Tao Y, Zhang L, Yan F, Wu X (2007) Chain conformation of water insoluble hyperbranched polysaccharide from fungus. Biomacromolecules 8:2321–2328

Tiberg E, Einarsson R (1989) Variability of allergenicity in 8 strains of the green algal genus chlorella. Intl Arch Allergy Appl Immunol 90(3):301–306

Trabelsi L, M'sakni NH, Ouada HB, Bacha H, Roudesli S (2009) Partial characterization of extracellular polysaccharides produced by cyanobacterium *Arthrospira platensis*. Biotechnol Bioprocess Eng 14:27–31

Tzianabos AO (2000) Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clin Microbiol Rev 13(4):523–533

Valle J, Da Re S, Henry N, Fontaine T, Balestrino D, Latour-Lambert P, Ghigo J (2006) Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. Proc Natl Acad Sci U S A 103:12558–12563

van der Spiegel M, Noordam MY, van der Fels-Klerx HJ (2013) Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr Rev Food Sci Food Saf 12(6):662–678

Vieira VV, Morais RMSC (2008) Composições constituídas por polissacarídeos com actividade anti-viral e anti-adesão bacteriana, respectivas formulações, processo de elaboração das mesmas e suas utilizações. Portugal Patent 38122.08

Vischer P, Buddecke E (1991) Different action of heparin and fucoidan on arterial smooth muscle cell proliferation and thrombospondin and fibronectin metabolism. Eur J Cell Biol 56:407–414

Wetherbee R, Lind JL, Burke J, Quatrano RS (1998) The first kiss: establishment and control of initial adhesion by raphid diatoms. J Phycol 34:9–15

White RC, Barber GA (1972) An acidic polysaccharide from the cell wall of *Chlorella pyrenoidosa*. Biochim Biophys Acta 264(1):117–128

Whiteside PA (2011) Biotechnology medicinal products: back to basics. Regul Rapporteur 8:4–5

Wijesekara I, Pangestuti R, Kim S-K (2011) Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carbohydr Polym 84:14–21

Witvrouw M, De Clercq E (1997) Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. Gen Pharmacol 29:497–511

Wong MH, Hung KM, Chiu ST (1996) Sludge-grown algae for culturing aquatic organisms: part II. Sludge-grown algae as feeds for aquatic organisms. Environ Manag 20(3):375–384

www.vilastic.com. A structural view of rheology. Vilastic Scientifica. www.vilastic.com/tech4.html. Accessed 03 Apr 2012

Xing RE, Yu HH, Liu S (2005) Antioxidant activity of differently regioselective chitosan sulfates in vitro. Bioorg Med Chem 13(4):1387–1392

Yamamoto C, Nakamura A, Shimada S, Kaji T, Lee J-B, Hayashi T (2003) Differential effects of sodium spirulan on the secretion of fibrinolytic proteins from vascular endothelial cells: enhancement of plasminogen activator activity. J Health Sci 49(5):405–409

Yamamoto C, Fujiwara Y, Kaji T (2006) The biological effects of depolymerized sodium spirulan and sulfated colominic acid on vascular cells are beneficial in preventing atherosclerosis. J Health Sci 52(3):205–210

Yim JH, Kim SJ, Ahn SH, Lee CK, Rhie KT, Lee HK (2004) Antiviral effects of sulphated polysaccharide from the marine microalga *Gyrodinium impudicum* strain KG03. Mar Biotechnol 6:17–25

Yim JH, Son E, Pyo S, Lee HK (2005) Novel sulfated polysaccharide derived from red-tide microalga *Gyrodinium impudicum* strain KG03 with immunostimulating activity in vivo. Mar Biotechnol (NY) 7:331–338

Yim JH, Kim SJ, Ahn SH, Lee HK (2007) Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. Bioresources Technol 98:361–367

Zhou FG, Sun YP, Xin H, Zhang YN, Li ZE, Xu ZH (2004) In vivo antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from *Chondrus ocellatus*. Pharmacol Res 50:47–53