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Exocellular polysaccharides from cyanobacteria and their possible applications

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Abstract

Cyanobacteria are photoautotrophic prokaryotes which include a large variety of species of widespread occurrence and with diverse morphological, physiological and biochemical properties. Many cyanobacteria are known to be able to synthesise outermost slimy investments and to release polysaccharidic material into the culture medium during cell growth. These released polysaccharides (RPSs), being easily recoverable from the culture medium, are attracting much interest in view of their possible uses in several industrial applications. In this paper, an overview of the current knowledge on both RPS-producing cyanobacterial strains (including the possible roles of the exopolysaccharides) and chemical characteristics of the cyanobacterial RPSs is given, with particular emphasis on RPS properties and possible industrial applications. On the whole, cyanobacterial RPSs are characterised by a great variety in both number (from two to 10) and type of constitutive monosaccharides (various arrangements of acidic and neutral sugars). Most polymers show an anionic nature due to the presence of uronic acids and/or other charged groups such as pyruvyl or sulfate. Polypeptide moieties as well as acetyl substituents have also sometimes been found, causing additional structural complexity. All the cyanobacterial RPSs so far tested showed a pseudoplastic behaviour, but with marked differences in both viscosity values and shear thinning. In terms of RPS production, the responses of cyanobacteria to changes of culture conditions appear strain-dependent. RPS productivities shown by some cyanobacteria are well comparable with those reported for other photosynthetic microorganisms proposed for polysaccharide production, but very low in comparison with those of heterotrophic microorganisms. Nevertheless, cyanobacteria may be regarded as a very abundant source of structurally diverse polysaccharides, some of which may possess unique properties for special applications, not fulfilled by the polymers currently available. However, much work has still to be done to bridge the wide gap existing between data on the biology of the RPS-producer strains and information concerning technological and other useful properties of the cyanobacterial RPS.

Keywords: Cyanobacterium; Cyanobacterial outermost cell investment; Exopolysaccharide release; Polysaccharide production; Potential application of cyanobacterial polysaccharide

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Contents

1. Introduction

Cyanobacteria are a major and phylogenetically coherent group of Gram-negative prokaryotes possessing the unifying property of performing oxygenic plant-like photosynthesis with autotrophy as their dominant mode of nutrition [1]. However, in spite of their typically aerobic photosynthetic nature, some of the cyanobacterial species can grow in the dark on organic substrates [2,3] and others under anaerobic conditions with sulfide as electron donor for photosynthesis [4]. Certain strains have the ability to fix atmospheric dinitrogen into organic nitrogen-containing compounds, so displaying the simplest nutritional requirements of all microorganisms [5,6]. Cyanobacteria are also characterised by a great morphological diversity, unicellular as well as filamentous species being included with a cell volume ranging over more than five orders of magnitude [7]. Representatives of the group have been found, frequently in abundance, in most of the natural illuminated environments examined so far, both aquatic and terrestrial, including several types of extreme environments [7]. This widespread distribution reflects a large variety of species, covering a broad spectrum of physiological properties and tolerance to environmental stress [8].

Owing to their ecological and biochemical diversity, cyanobacteria, as well as several species of microalgae, have been regarded as good candidates for various biotechnological applications and their potential in the conversion of light energy into renewable forms of useful chemicals for food, feed, pharmaceutical and other industries has often been claimed and assessed. Indeed, since the first major volume edited by Burlew in 1953 [9], extensive studies on the practical exploitation of these microorganisms have been carried out and reviewed [10–14].

A relatively new field of possible exploitation of cyanobacteria has arisen in the last decade by the growing industrial interest towards polysaccharides of microbial origin, that often show advantages over the polysaccharides extracted from plants or marine macroalgae. As a result, a wide search for bacterial strains able to produce good yields of new polysaccharides with potentially useful properties has been undertaken, also involving cyanobacteria because of the well-known capability of some strains to excrete mucilaginous material [15–18]. Indeed, several cyanobacterial strains possess, outside their outer membrane, additional surface structures, mainly of a polysaccharidic nature, that comprise a wide variety of outermost investments differing in thickness, consistency and appearance after staining. These structures, in spite of the rather arbitrary terminology sometimes used, can be referred to as three distinct types, namely sheaths, capsules and slimes. The sheath (Fig. 1) is defined as a thin, electron-dense layer loosely surrounding cells or cell groups and is usually visible by light microscopy without staining; the capsule (Fig. 2) generally consists of a thick and slimy layer intimately associated with the cell surface with sharp outlines and structurally coherent to exclude particles (e.g. India ink); the slime (Fig. 3) refers to the mucilaginous material dispersed around the organism but not reflecting the shape of the cells. During cell growth in batch cultures, aliquots of the polysaccharidic material of both capsules and slimes may be released as water-soluble material into the surrounding medium, causing a progressive increase of its viscosity. These water-soluble released polysaccharides (RPSs), being easily

Fig. 1. Nomarski differential interference contrast photomicrographs of sheathed cyanobacterial strains. Top: *Chroococcus* sp. (1000×); bottom: *Phormidium* sp. (1000×).

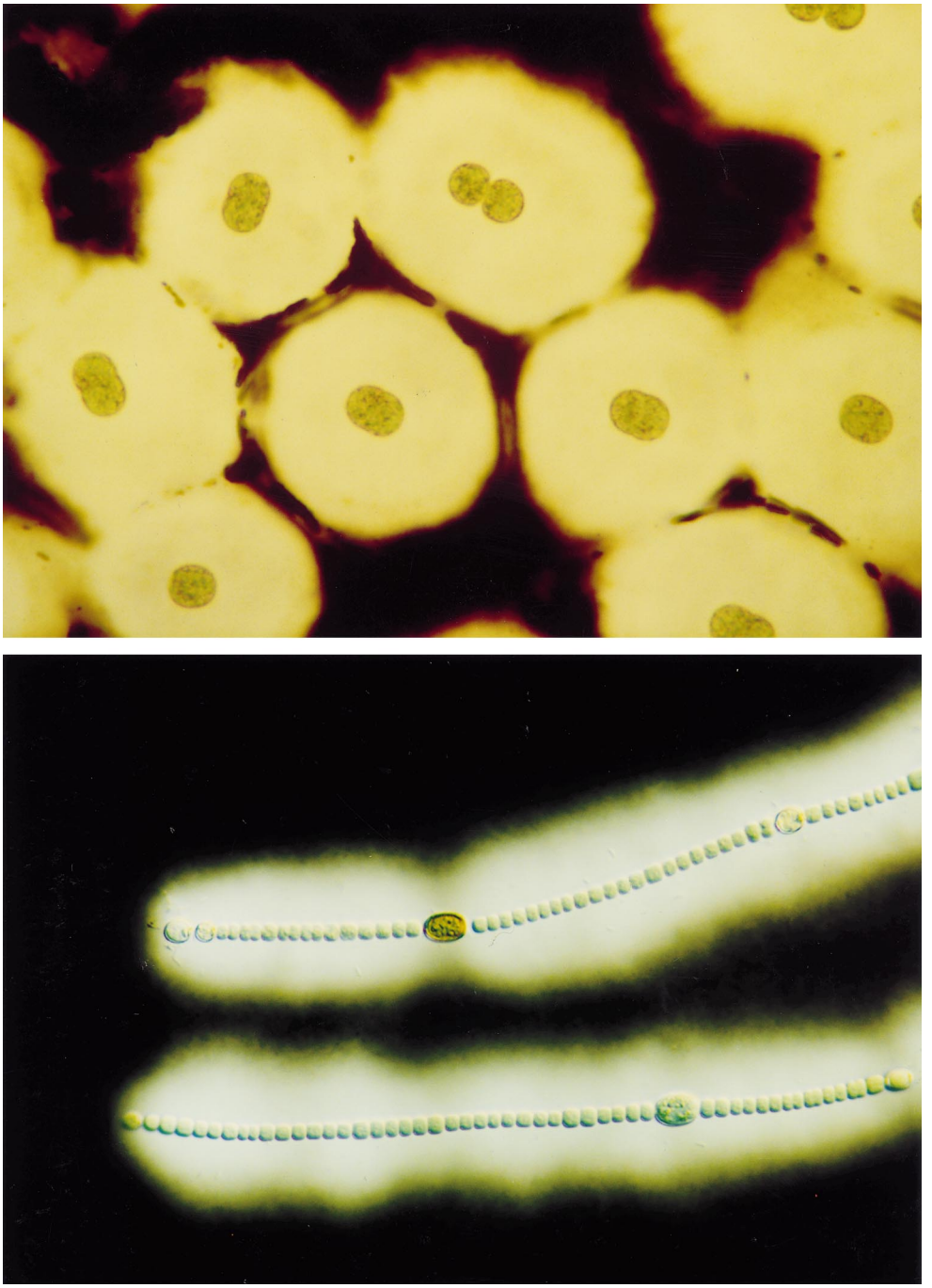

Fig. 2. Photomicrographs of negatively stained cyanobacterial strains with capsule. Top: *Cyanothece* CE 4 (775×; bright field); bottom: *Nostoc* sp. (480×; Nomarski differential interference contrast).

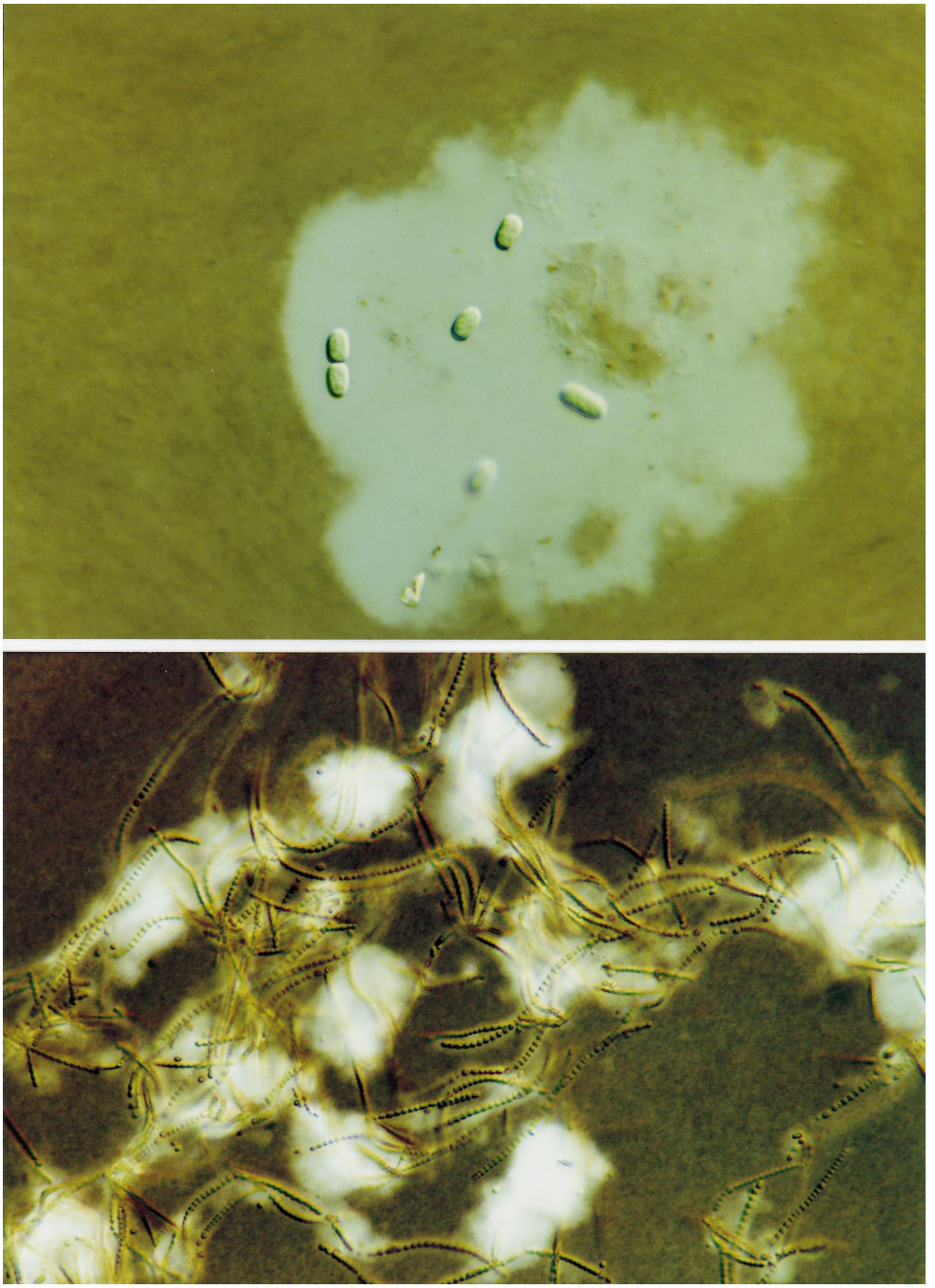

Fig. 3. Nomarski differential interference contrast photomicrographs of cyanobacterial strains with slime. Top: *Cyanothece* PCC9224 (775×); bottom: *Nostoc* PCC7906 (194×).

recoverable from liquid cultures, are currently attracting much interest because of their suitability for a variety of industrial purposes and make cyanobacteria one of the most attractive sources of new polymers.

The aim of this paper is to give an overview of the current knowledge on exocellular polysaccharides released by cyanobacteria, with particular emphasis on RPS properties and possible industrial applications.

Throughout the text, cyanobacterial strains will be designated as they were cited in the original literature; to get a useful correspondence between strain names as they will appear in the text and current cyanobacterial taxonomy as well as between strain numbers in the different culture collections, the reader is referred to the strain catalogue of the Pasteur Culture Collection [19].

2. Possible roles of exopolysaccharides in cyanobacteria

The synthesis of exocellular investments of a polysaccharidic nature by bacterial cells is generally considered to be directly related to environmental constraints on the producing microorganism [20]. Therefore, the main function attributed to capsules or other polysaccharidic investments is to serve as a boundary between the bacterial cell and its immediate environment. More specifically, it could fulfil a protective role against desiccation, antibacterial agents (e.g. antibiotics, antibodies, bacteriocins, phages, phagocytic cells, surfactants) or predation by protozoans [21–23]. Moreover, exocellular polysaccharides may furnish microorganisms with the capability to form biofilms on solid surfaces [21–23].

It has been hypothesised that the synthesis of exocellular polysaccharides in microorganisms, including cyanobacteria, plays a major role in protecting cells from stress in extreme habitats and from other harmful conditions. Many studies have focused on the capability of some polysaccharide-producing cyanobacteria to overcome stress due to desiccation or to low water activity in desert or saline environments. For a desiccation-tolerant *Nostoc commune* strain, Hill et al. [24] proposed that the secreted glycan provides a repository for water, thereby acting as a buffer between cells and the atmosphere and representing the key component of the mechanism used by this cyanobacterium to tolerate desiccation. In a following study [25], performed with the polymer released by *N. commune* strain CHEN, it was found that the addition in vitro of the polysaccharide to artificial membrane vesicles prevented membrane fusion, which is the main damage process occurring when cells desiccate and subsequently rehydrate. Thus, the authors concluded that the polysaccharide, together with the synthesis of a mixture of trehalose and sucrose, may represent the key mechanism in the stabilisation in vivo of *N. commune* cells when they are dried in air. Mazor et al. [26], studying the release of polysaccharides by some cyanobacterial strains isolated from sand dunes in the Negev Desert (Israel), concluded that these polymers play an important role in maintaining the moisture in desert microbial crusts where, for many months, the only source of water was, occasionally, the morning dew. A role as buffer compound for the accumulation and the slow release of water has also been suggested for the polysaccharide released by a *Chroococcidiopsis* strain [27].

Recently, in a review on the ecology of the genus *Nostoc*, Dodds et al. [28] pointed out that the dense mucilage surrounding the trichomes of many strains could make them less preferred food in comparison with other microalgae that are devoid of capsules.

In benthic cyanobacteria, it has been suggested that the attachment of cells to the sediment is modulated by cell hydrophobicity [29], which is usually determined by extracellular polymeric substances (EPS). These are polysaccharides bearing non-sugar components. For instance, *Phormidium* J-1 synthesises a sulfated heteropolysaccharide, named emulcyan, which contains fatty acids and proteins that confer variable degrees of hydrophobicity on the macromolecule [30]. The attachment of *Phormidium* J-1 as well as of several other benthic cyanobacteria is also enhanced by the co-flocculation of polysaccharide-producing cells with sedimentary clay particles [31,32].

For cyanobacterial exopolysaccharides, other specific roles have been proposed as well. Since the energetic metabolism of cyanobacteria depends on the availability of light, it has been suggested that the release of emulcyan by *Phormidium* J-1 also plays a role in flocculation of suspended clay particles, there-

Table 1
Monosaccharide composition of RPSs produced by cyanobacterial strains belonging to subsection I (Chroococcales) [1]

Species	Hydrolytic procedure	Monosaccharides (molar ratios)												Ref.
		Ara	Fuc	Gal	Glc	Man	Rha	Rib	Xyl	GalA	GlcA	UrA[a]	Others	
Aphanocapsa halophytica MN11	A,B	–	26.5	1.5	12.5	7.5	1.0	–	1.5	–	–	–	–	[44]
Anacystis nidulans	C	–	–	1.0	4.7	1.4	–	–	–	–	–	–	–	[45]
Chroococcus minutus B 41.79	ns	5.1	4.6	9.5	19.1	10.6	10.0	–	10.7	1.0	2.8		b	[46]
Cyanothece sp. CA 3	D	9.2	2.0	–	1.4	tr	1.0	–	–	+	+	66.8	–	[47]
Cyanothece sp. CE 4	D	0.4	0.3	tr	1.3	–	1.0	–	0.5	+	–	80.1	–	[47]
Cyanothece sp. CE 9	D	–	1.1	0.3	2.8	0.7	1.0	–	–	+	–	35.7	–	[47]
Cyanothece sp. CH 1	D	–	3.1	1.4	–	0.6	1.0	–	0.9	+	–	27.4	–	[47]
Cyanothece sp. ET 2	D	5.8	1.5	1.4	1.3	0.8	1.0	–	–	+	+	63.1	–	[47]
Cyanothece sp. ET 5	D	–	2.1	2.2	3.1	1.8	1.0	–	3.3	+	+	29.4	–	[47]
Cyanothece sp. IR 20	D	–	1.5	0.1	0.1	2.3	10.0	0.1	–	–	+	9.8	–	[47]
Cyanothece sp. PCC 8801	D	–	0.2	0.6	1.0	0.2	1.2	–	0.9	+	+	35.0	–	c
Cyanothece sp. PE 13	D	–	3.8	11.6	22.0	5.9	1.0	0.3	5.9	–	+	20.9	–	[47]
Cyanothece sp. PE 14	D	6.1	0.2	–	0.5	0.3	0.1	–	–	+	+	21.7	–	[47]
Cyanothece sp. TI 4	D	–	2.7	1.2	2.9	0.4	1.0	–	0.7	–	+	58.2	–	[47]
Cyanothece sp. TP 5	D	0.4	1.2	–	0.9	–	1.0	–	0.3	+	–	40.4	–	[47]
Cyanothece sp. TP 10	D	1.8	0.8	–	0.6	0.1	1.0	–	–	+	+	31.3	–	[47]
Cyanothece sp. VI 13	D	–	1.5	0.1	2.0	0.5	1.0	–	1.5	+	+	32.1	–	[47]
Cyanothece sp. VI 22	D	–	1.8	0.2	2.8	1.0	1.0	–	1.8	–	+	40.8	–	[47]
Cyanothece sp. 16Som2	E	–	1.6	2.4	6.8	4.8	–	–	2.9	2.0	1.0		–	[48]
Cyanothece sp. 16Som2	D	–	1.0	0.1	1.8	0.4	1.0	–	1.2	+	+	20.6	–	[47]
Gloeothece sp. PCC 6909	F	–	–	4.2	4.9	2.6	1.6	–	1.0			2.4[d]	e	[49]
Microcystis aeruginosa K 3A	ns	+	+	+	+	+	+	–	+	+	–		–	f
Microcystis flos-aquae C3-40	G,H	–	–	1.0	1.0	3.0	3.0	–	2.0	43.0	–		–	[50]
Synechocystis sp. PCC 6714[g]	I	5.5	2.1	6.0	34.8	3.8	2.8	–	2.8			16.7	h	[51]
Synechocystis sp. PCC 6803[g]	I	–	6.0	1.0	6.7	3.9	3.6	–	3.5			16.4	i	[51]

Abbreviations: Ara = arabinose; Fuc = fucose; Gal = galactose; Glc = glucose; Man = mannose; Rha = rhamnose; Rib = ribose; Xyl = xylose; GalA = galacturonic acid; GlcA = glucuronic acid; UrA = uronic acid (not identified); + = present (not quantified); – = absent; ns = not specified; tr = traces.

Notes: A = 2 N TFA (trifluoroacetic acid) at 100°C for 6–12 h; B = 4 N HCl at 100°C for 6–12 h; C = 2 N TFA at 121°C for 2 h; D = 2 N TFA at 120°C for 45 min; E = 2 N TFA at 120°C for 10 and for 60 min; F = 1 N HCl at 100°C for 10 h; G = 0.5, 1, 2 N H_2SO_4 at 100°C (time not stated); H = 2 N H_2SO_4 at 121°C (time not stated); I = 2 N HCl at 100°C for 2 h. a: Expressed as percent of total carbohydrates. b: Deoxyhexose, 6-deoxy-2-O-methylhexose, 2-*O*-methylhexose; 3-*O*-methylhexose, glucosamine. c: Our unpublished data. d: Expressed as molar ratio. e: 2-*O*-Methylhexose. f: Nagakawa et al. (1987) in [52]. g: Composition of RPSs from young cultures (15 days of batch cultivation); in older cultures (2 months of batch cultivation) the quantitative composition changed. h: 3-*O*-Methylpentose, glucosamine, galactosamine. i: 3-*O*-Methyldeoxyhexose, 4-*O*-methylhexose, methylhexose, glucosamine, galactosamine.

by clearing the water column and increasing the amount of light available for the cyanobacterium growing as a mat on the bottom sediments [33]. In *Nostoc* sp., the slimy shrouds surrounding trichomes have been suggested to facilitate the homogeneous dispersion of trichomes into the liquid medium, so improving light utilisation and nutrient uptake [15].

Another possible role of cyanobacterial polysaccharides could be to protect nitrogenase from harmful effects of oxygen. Indeed, the thick mucilaginous envelope surrounding the heterocysts of a strain of *Nostoc cordubensis* appeared to be essential for the protection of nitrogenase activity from the inactivation due to the atmospheric oxygen [34]. However, it

is worth mentioning that the nitrogenase activity of a sheathless mutant of the unicellular cyanobacterium *Gloeothece* proved to be as sensitive to oxygen as that of the sheathed strain [35]. More recently, Reddy et al. [36], discussing the positive effect on aerobic nitrogen fixation by the mucilaginous investments that surround the colonies of *Cyanothece* BH68 growing on agar plates, suggested that the polysaccharide, in addition to acting as a physical barrier to the atmospheric oxygen, could also serve as a chelator for iron and calcium, which are both essential for nitrogen fixation. In several other cases, it has been suggested that cyanobacterial polysaccharides, which are mostly characterised by their anionic nature, play an important role in the sequestering or immobilisation of metal ions, which are respectively essential or harmful to bacterial life [37–40]. In particular for cyanobacteria like *Microcystis flos-aquae* C3-40, living in alkaline habitats, the polysaccharide capsule that surrounds the cells seems to play a useful function in nutrition, allowing the accumulation of both iron and manganese, metals essential for cyanobacterial growth but relatively insoluble in aerobic alkaline waters [41].

Finally, for some cyanobacteria that live in association or symbiosis with higher plants, it has been suggested that the EPS may act as an adhesive for cyanobacterial cells. For instance, the release of an exopolysaccharide by the cyanobiont *Anabaena azollae* has been considered essential for the attachment of this cyanobacterium to the surfaces and the cavities of the fronds of the host plant *Azolla filiculoides* [42]. More recently, Gantar et al. [43] showed that the firm association of the filaments of a *Nostoc* strain to the roots of wheat are due to the binding of the polysaccharide matrix that surrounds the trichomes. In the same study, it was noted that the polysaccharide surrounding the cells of an *Anabaena* strain was not able to bind to the root surface, so that only a loose association was established between the cyanobacterium and the roots.

The only conclusion that can be drawn from the above reported studies is that cyanobacterial exopolysaccharides may fulfil a variety of different roles, depending on the strain and on the physico-chemical characteristics of the natural habitat in which the organisms thrive.

3. Cyanobacterial exopolysaccharides

Since the beginning of the 1950s, many cyanobacteria have been reported to be capable of synthesising exocellular polysaccharides and, in some cases, of releasing them into the surroundings. To date, about 70 strains have been studied with regard to their production of RPSs, most of the studies being devoted to the determination of the sugar composition of the polymers. All these studies deal with RPS-producer strains that belong to subsections I, III or IV of the cyanobacterial classification (Tables 1–3), with the single exception of a recent paper, reporting the composition of a polysaccharide released by a strain of *Mastigocladus laminosus* [52]. No data are available on the production of RPSs by strains included in subsection II.

On the whole, only 10 different monosaccharides have been found in the cyanobacterial RPSs: the hexoses glucose, galactose and mannose, the pentoses ribose, xylose and arabinose, the deoxyhexoses fucose and rhamnose and the acidic hexoses glucuronic and galacturonic acid. In a few cases, the presence of additional types of monosaccharides (i.e. methyl sugars and/or amino sugars) has been reported [40,46,49,51,53,55]. However, a precise identification of these sugars concerns only the RPSs produced by one *Chroococcus* [46], one *Gloeothece* [49] and two *Synechococcus* [51] strains. The monosaccharide most frequently found in the RPSs is glucose (in more than 90% of the polymers), followed by galactose, mannose and rhamnose (80–85% of the polymers). In a large number of RPSs, glucose is also the most abundant monosaccharide, but there are also polymers where other sugars, like arabinose, galactose or fucose, are present at higher concentrations than glucose; ribose has only been found in a small number of polymers (about 9% of the RPSs analysed). The presence of acidic sugars in the macromolecules accounts for the anionic nature observed for almost all the polysaccharides studied so far. When identification of uronic acids was carried out, only glucuronic and/or galacturonic acid was found, the simultaneous presence of both of them having been observed in about half of the polymers analysed.

Information from papers giving only a partial description of the monosaccharide composition of cy-

Table 2
Monosaccharide composition of RPSs produced by cyanobacterial strains belonging to subsection III (Oscillatoriales) [1]

Species	Hydrolytic technique	Monosaccharides (molar ratios)												Ref.
		Ara	Fuc	Gal	Glc	Man	Rha	Rib	Xyl	GalA	GlcA	UrA	Others	
Lyngbya confervoides S9g	A	tr	tr	17.8	40.2	3.5	tr	–	tr			38.6	a	[53]
Microcoleus sp. (2 strains)	B	–	+	+	+	+	+	–	–			–	–	[26]
Oscillatoria amphibia PCC 7105	A	tr	tr	16.0	33.0	21.7	4.5	–	12.3			6.7	b	[53]
Oscillatoria corallinae CJ1	A	5.1	1.7	15.0	29.6	15.6	3.4	–	4.0			24.2	a	[53]
Oscillatoria sp.[c]	C	–	5.4	9.9	18.3	8.0	–[d]	4.7[d]	5.5	1.0	1.0		e	[40]
Phormidium ectocarpi C86	A	–	–	3.1	34.7	23.2	tr	–	8.0			29.9	a	[53]
Phormidium ectocarpi K5	A	tr	tr	12.6	36.6	10.9	1.8	–	7.3			28.7	f	[53]
Phormidium ectocarpi ME3	A	tr	tr	4.1	59.1	8.7	2.7	–	6.5			18.9	–	[53]
Phormidium ectocarpi N182	A	–	tr	4.1	52.1	15.2	–	–	tr			28.7	–	[53]
Phormidium ectocarpi PCC 7375	A	tr	7.8	8.3	25.8	8.1	2.6	–	3.2			41.5	a	[53]
Phormidium foveolarum[g]	D,E,F	1.0	2.0	4.0	8.0	2.0	2.3	–	1.5	+	+		–	[54]
Phormidium foveolarum C52	A	0.6	0.5	3.4	43.0	15.3	1.5	–	5.5			29.4	f	[53]
Phormidium foveolarum MEU	A	1.7	7.0	28.2	37.7	12.0	2.8	–	3.8			0.5	f	[53]
Phormidium minutum D5	A	–	0.9	34.8	33.6	11.2	tr	–	1.1			17.1	f	[53]
Phormidium minutum NB5	A	5.0	–	7.2	32.7	18.0	tr	–	9.5			24.4	f	[53]
Phormidium minutum RT6	A	18.9	6.0	7.4	19.1	12.7	1.1	–	12.0			20.1	b	[53]
Phormidium sp. CCAP1463/4	A	2.1	tr	13.4	49.2	7.1	6.7	–	7.4			13.0	a	[53]
Phormidium sp. CCAP1464/3	A	tr	–	10.5	57.4	4.1	tr	–	1.6			26.1	b	[53]
Phormidium sp.[g]	D,E,F	1.0	2.0	2.0	4.0	2.0	2.5	–	1.5	+	+	9.0[h]	–	[54]
Phormidium sp. J-1	G	–	–	0.5	–	2.0	1.0	–	–			34.0[h]	–	[30]
Phormidium sp. PNG91	A	2.5	2.1	12.9	30.3	22.0	3.5	tr	13.6			tr	f	[53]
Phormidium sp. 90-14/1	A	tr	5.8	9.4	39.9	29.2	tr	–	9.4			3.0	f	[53]
Spirulina platensis[i]	H	–	0.7	2.7	2.0	tr	0.3	–	1.3	+	+	40.0[h]	l	[55]
Spirulina platensis[m]	I	–	–	+	–	–	+	–	+	–	20.0[h]		n	[56]

Abbreviations: Ara = arabinose; Fuc = fucose; Gal = galactose; Glc = glucose; Man = mannose; Rha = rhamnose; Rib = ribose; Xyl = xylose; GalA = galacturonic acid; GlcA = glucuronic acid; UrA = uronic acid (not identified); + = present (not quantified); – = absent; tr = traces.

Notes: A = 2 N HCl at 100°C for 2 h; B = 3 N HCl at 100°C for 4 h; C = 2 N TFA at 121°C for 2 h; D = 90% acetic acid; E = 72% H_2SO_4; F=2 N TFA at 95°C for 16 h; G = 2 N TFA at 120°C for 2 h; H = 4 N TFA at 100°C for 4 h; I = 4 N H_2SO_4 at 100°C for 6 h. a: Unknown sugar, probably methyl sugar. b: Hexosamines. c: Not axenic culture. d: Uncertain identification; authors give the two possibilities, Rib or Rha. e: Unknown sugar and unknown amino sugar. f: Hexosamines and unknown sugar, probably methyl sugar. g: Capsular polysaccharide (CPS) extracted with hot water at 100°C for 3 h. h: Expressed as percent of total carbohydrates. i: CPS extracted with buffer at 100°C for 20 min. l: An unknown methyl sugar and an unknown uronic acid. m: CPS extracted with hot water; the polymer is composed of three fractions with the same composition, with the exception of one lacking uronic acids. n: Unknown sugar.

Table 3
Monosaccharide composition of RPSs produced by cyanobacterial strains belonging to subsections IV (Nostocales) and V (Stigonematales) [1]

Species	Hydrolytic technique	Monosaccharides (molar ratios)												Ref.
		Ara	Fuc	Gal	Glc	Man	Rha	Rib	Xyl	GalA	GlcA	UrA	Others	
Subsection IV														
Anabaena cylindrica CCCAP1403/2	A	1.0	–	1.0	5.0	–	1.0	–	4.0	–	4.0		–	[57]
Anabaena cylindrica	B	–	tr	1.0	8.7	4.7	–	–	4.7			–	–	[58]
Anabaena flos-aquae A37	C	–	–	–	88.0	–	–	3.0	39.0	–	1.0		–	[59,60]
Anabaena flos-aquae A37[a]	D	–	–	–	8.0	–	–	–	1.0			–	–	[61]
Anabaena flos-aquae A37[b]	D	–	–	–	6.0	–	–	1.0	1.0			10.0	–	[61]
Anabaena sp. C5	E	–	+	+	+	+	–	–	–			51.5[c]	–	[43]
Anabaena sp. 10C	F	–	1.0	1.0	2.5	1.5	1.0	–	3.1	–	1.7[c]		d	[62]
Cyanospira capsulata ATCC 43193	G	1.0	1.0	–	1.0	1.0	–	–	–	2.0	–		–	[63]
Nostoc calcicola 79WA01	H	1.0	2.8	3.8	5.9	1.7	1.0	–	6.1	3.0	2.8		–	[64]
Nostoc commune UTEX584[e]	H	1.6	1.7	6.5	2.0	1.3	1.0	–	2.8	4.0	6.7		–	[64]
Nostoc insulare 54.79	ns	22.9	11.1	0.2	53.2	2.9	1.0	–	0.2	3.6	25.3		–	[46]
Nostoc linckia f. *muscorum*	ns	+	–	+	+	–	+	–	+			+	–	f
Nostoc sp. PCC 7423	I	–	0.5	4.2	10.0	1.7	0.1	–	4.0	–	+	18.8[c]	–	g
Nostoc sp. PCC 7936	I	–	0.6	11.0	10.0	8.0	–	–	–	–	+	51.1[c]	–	g
Nostoc sp. WV2	G	–	–	+	+	–	+	–	+	+	+		–	[65]
Nostoc sp. 221[h]	L	–	–	–	1.0	–	–	–	1.0	–	1.2		–	[66]
Nostoc sp. 2S9B	E	–	1.0	–	2.0	1.0	–	–	–	–	1.0		–	[43]
Nostoc sp.	ns	–	–	+	+	–	+	–	+	+	+		i	l
Nostoc sp.	C	+	+	–	+	–	–	–	–	–	+		–	[60]
Subsection V														
Mastigocladus laminosus	M	+	+	+	+	+	+	–	+			+	–	[52]

Abbreviations: Ara = arabinose; Fuc = fucose; Gal = galactose; Glc=glucose; Man = mannose; Rha = rhamnose; Rib = ribose; Xyl = xylose; GalA = galacturonic acid; GlcA = glucuronic acid; UrA = uronic acid (not identified); + = present (not quantified); – = absent; ns = not specified; tr = traces.

Notes: A = 2.5% H_2SO_4 at 97°C for 24 h; B = 2 N H_2SO_4 at 110°C for 1 h; C = 1 N H_2SO_4 at 100°C for 6 h; D = 1 N H_2SO_4 at 100°C for 2 h; E = 1 N H_2SO_4 at 100°C for 18 h; F = 0.5 or 2 N TFA at 80°C for 16 h; G = 2 N TFA at 120°C for 45 min and 3 h; H = 1 N H_2SO_4 at 98°C for 12 h; I = 2 N TFA at 120°C for 45 min; L = 2.5% H_2SO_4 at 100°C for 24 h; M = methanol/1 N HCl at 80°C for 24 h. a: Neutral fraction. b: Acidic fraction. c: Expressed as percent of total carbohydrates. d: Unknown UrA. e: Capsular polysaccharide extracted with 0.1 M EDTA at 22°C overnight. f: Kokyrsta and Chekoi (1972) in [66]. g: Our unpublished results. h: Extracted with hot water. i: Unknown sugar. l: Hough et al. (1952) in [66].

anobacterial RPSs has not been included in Tables 1–3. In this connection, Reddy et al. [36] reported the presence of glucuronic acid in addition to six unidentified neutral sugars in the polymer produced by *Cyanothece* sp. ATCC 51142 and Bar-Or and Shilo [30] described the bioflocculant released by *Anabaena circularis* PCC 6720 as a polymer not containing uronic acids. Polymers obtained from nature have also not been included in the tables, owing to the difficulty of establishing which is the cyanobacterium that produced the polysaccharide. In any case, the monosaccharide composition of these polymers appears to be consistent with those reported in Tables 1–3. Indeed, Gloaguen et al. [67] described the composition of the capsular polymers extracted by hot water treatment from floating and benthic mats of a thermal spring, respectively dominated by *Mastigocladus laminosus* and *Phormidium* sp. They found rhamnose, glucose, xylose, mannose, galactose and fucose as the major components, ara-

Table 4
Polysaccharide daily production by cyanobacterial strains

Species	Culture device and duration (days)	Crude RPS production (mg RPS l^{-1} day^{-1})	Reference
Subsection I			
A. halophytica MN11	4-l flask, 20 days	32.0	[44]
A. nidulans	3-l flask, 21 days	20.0	[45]
C. minutus B 41.79	8-l flask, 39 days	12.7	[46]
C. minutus B 41.79	250-l photobioreactor, 50 days	8.6	[46]
Cyanothece BH68K	2-l flask, 16 days	8.0	[36]
Cyanothece sp. CA 3	0.5-l flask, 8 days	19.0[a]	[47]
Cyanothece sp. CE 4	0.5-l flask, 8 days	43.0[a]	[47]
Cyanothece sp. CE 9	0.5-l flask, 8 days	67.0[a]	[47]
Cyanothece sp. CH 1	0.5-l flask, 8 days	60.0[a]	[47]
Cyanothece sp. ET 2	0.5-l flask, 8 days	38.0[a]	[47]
Cyanothece sp. ET 5	0.5-l flask, 8 days	19.0[a]	[47]
Cyanothece sp. IR 20	0.5-l flask, 8 days	80.0[a]	[47]
Cyanothece sp. PE 13	0.5-l flask, 8 days	62.0[a]	[47]
Cyanothece sp. PE 14	0.5-l flask, 8 days	47.0[a]	[47]
Cyanothece sp. TI 4	0.5-l flask, 8 days	33.0[a]	[47]
Cyanothece sp. TP 5	0.5-l flask, 8 days	17.0[a]	[47]
Cyanothece sp. TP 10	0.5-l flask, 8 days	27.0[a]	[47]
Cyanothece sp. VI 13	0.5-l flask, 8 days	21.0[a]	[47]
Cyanothece sp. VI 22	0.5-l flask, 8 days	20.0[a]	[47]
Cyanothece sp. 16Som2	0.5-l flask, 8 days	25.0[a]	[47]
Cyanothece sp. 16Som2	0.5-l flask, 21 days	29.0	[48]
Synechocystis sp. PCC 6803	ns, 90 days	3.0	[51]
Synechococcus sp. BG0011	4-l flask, 30 days	33.3	[88]
Subsection IV			
A. cylindrica 10C	1-l fermenter, 21 days	15.0	[62]
A. flos-aquae A37	1-l flask, 7 days	36.0[b]	[61]
A. flos-aquae A37	1-l flask, 12 days	20.0	[87]
A. flos-aquae A37	2-l Pyrex column, 12 days	46.4	[60]
Anabaena sp. C5	10-l flask, 30 days	4.7	[43]
C. capsulata ATCC 43193	6-l open pond, 28 days	116.0	[39]
C. capsulata ATCC 43193	3-l fermenter, 21 days	144.0	[65]
N. insulare 54.79	8-l flask, 52 days	47.0	[46]
N. insulare 54.79	12-l photobioreactor, 70 days	18.4	[46]
Nostoc sp.	2-l Pyrex column, 12 days	34.6	[60]
Nostoc sp. 221	0.25-l flask, 20 days	45.4	[66]
Nostoc sp. 2S9B	10-l flask, 30 days	1.4	[43]

Abbreviations: ns=not specified.
Notes: a: Expressed as soluble carbohydrates. b: Mixotrophic growth with glucose.

binose at very low concentrations and variable amounts of uronic acids that were identified, in a later paper [68], as glucuronic and galacturonic acids.

From the data on the monosaccharide composition of cyanobacterial RPSs, it is possible to draw some peculiar features of these polymers in comparison with those released by other microbial sources. (i) Most cyanobacterial polysaccharides are characterised by an anionic nature, many of them containing two different uronic acids, a feature rarely found in the polymers released by strains belonging to other microbial groups [69]. (ii) Cyanobacterial RPSs often show the presence of one or two pentoses, sugars that are usually absent in other polysaccharides of prokaryotic origin [69]. (iii) Most RPSs synthesised by cyanobacteria (about 80% of the polymers) are quite complex, being composed of six or more monosaccharides. This is a striking difference from the polymers synthesised by other bacteria or by macroalgae, in which the number of monomers is usually less than four [70], and it has been suggested that it is due to the low position of cyanobacteria in the evolutionary scale [17]. The large number of different monosaccharides present in a polymer and the variety of linkage types usually produce quite complex repeating units and a broad range of possible structures and architectures of the macromolecules [71]. The few studies available confirm this picture also for cyanobacterial RPSs: for the polysaccharides produced by *Spirulina platensis* [55] and *Mastigocladus laminosus* [52], repeating units of 15 sugars have been suggested, while for the RPS from *Cyanospira capsulata* a decasaccharide [72] or octasaccharide repeating unit [73] has been proposed.

Very few studies have been devoted to verifying if RPS-releasing cyanobacterial strains produce polymers with a stable chemical composition, not changing from batch to batch and unaffected by growth conditions, a very important feature for microbial strains proposed for industrial applications. In some cases, it has been reported that the composition changes during cell growth: two *Synechocystis* strains modified the molar ratio among the RPS constitutive monosaccharides with culture age [51], one of them also producing an additional polymer with a different qualitative and quantitative composition from that produced by the younger culture; *Spirulina platensis* PCC8005 [55] showed only a slight modification of the RPS quantitative composition, decreasing the relative amount of galactose with culture ageing, but this change was attributed to a different ratio among the individual polymers that were simultaneously synthesised by the cyanobacterium during the growth period. On the other hand, the RPS produced by *Cyanospira capsulata* showed a stable monosaccharide composition throughout growth phases and under different growth conditions. Indeed, it was shown that RPS samples taken from different phases of cell growth possessed the same monosaccharide composition [63]. Moreover, the utilisation of different light regimens (continuous light and light-dark cycles) to grow the cyanobacterial strain did not affect the monosaccharide composition or the relative proportions among sugar units [74]. It is noteworthy that the composition of the RPS synthesised by *C. capsulata* is still the same after more than 10 years of cultivation (our unpublished observations). These findings also demonstrate that this cyanobacterium proved to be stable as a RPS producer, since its capability to synthesise the capsule and to release the RPS has been maintained through the years. This feature is of great technological significance, considering that many other microorganisms, accumulating significant amounts of exopolysaccharides in natural habitats, lose this property when cultivated under laboratory conditions, where the environmental pressure has been removed [21], and thus need to be tested with regard to their stability of RPS production [75].

The composition of the polymer released by *Anabaena cylindrica* 10C was slightly modified by cultivating the strain with different nitrogen sources or under phosphorus starvation [62], whereas the RPS synthesised by *C. capsulata* showed the same composition also when various nutritional deficiencies (Mg, Ca, P) or salinities were tested (our unpublished data). In some cases, different chemical compositions have been reported for RPSs produced by the same strain. One example of this, from both a qualitative and a quantitative point of view, is the polymer produced by *Cyanothece* 16Som2 (Table 1): the polysaccharide was first described as composed of five neutral sugars and galacturonic and glucuronic acids [48], whereas a different batch, carried out 5 years later, showed the additional presence of rhamnose

and rather different molar ratios among the monosaccharides [47]. Even if the hydrolytic conditions were not the same, the observed discrepancy could be most likely ascribed to changes in the synthetic capability of the strain during the long period of cultivation and conservation under laboratory conditions.

As a concluding remark to this section, it must be stressed that qualitative and quantitative analyses of the monosaccharides can be strongly affected by the conditions utilised for the hydrolysis of the polymers. In particular, the determination of uronic acids seems to be very critical: the presence of a carboxyl group stabilises the glycosidic linkage between monosaccharides and thus an incomplete acid hydrolysis may occur [76,77]; otherwise, the uronic acids can undergo some degradation [54,78] or lactonisation [76], once a complete hydrolysis has been achieved. Consequently, the presence of uronic acids should be excluded only after a careful check made by means of specific colorimetric analysis (e.g. the methods described by Galambos [79], Blumenkranz and Asboe-Hansen [80] or Taylor and Buchanan-Smith [81]). In this connection, the absence of uronic acids in the RPSs produced by *Aphanocapsa halophytica* MN11 [44] and by *Anabaena cylindrica* [58] should be regarded with a certain caution. Furthermore, since other sugars, particularly pentoses, can be degraded in the presence of strong acids [57], the hydrolytic conditions should be carefully selected for each polymer in order to give sound results.

4. Exopolysaccharide release and factors affecting polymer production

Usually, polysaccharide-releasing cyanobacteria are characterised by the presence of capsules or slimes enclosing cells or cell groups. Consequently, cyanobacterial RPSs are generally believed to be originated from the mere solubilisation of these external layers of the mucilaginous outermost investments. The few available data seem to support this hypothesis: indeed, at least for *A. flos-aquae* [59,66], some *Phormidium* strains [53] and *C. capsulata* [63], the capsular and the released polysaccharides showed the same monosaccharide composition; in the case of *C. capsulata*, the two polymers also showed quite similar molecular masses (our unpublished results). With regard to the morphological changes that may occur during polysaccharide release, it was observed that, in *C. capsulata* [63] and *Cyanothece* 16Som2 [48], the thickness of the capsule surrounding the cells remained almost constant throughout growth phases and under all the culture conditions tested, in spite of the large amounts of polysaccharide released into the culture medium. Thus, the processes of synthesis and release of the polymer very likely occurred at the same rate. On the contrary, the RPS-producing red microalgae *Porphyridium* and *Rhodella* showed a quite different behaviour, the capsule thickness increasing with culture ageing [82,83]. Gantar et al. [43], on the other hand, reported that the mucilaginous sheath of *Nostoc* 2S9B was mainly synthesised in the aseriate stage of the developmental cycle of the cyanobacterium and then released, as empty shells, when hormogonia were liberated. In *Anabaena* C5, the same authors found a quite different behaviour, the sheath being continuously synthesised and released so that cell filaments appeared devoid of any polysaccharide envelope [43].

In any case, apart from the origin of the RPS, it seems that there is not a common behaviour of polymer release among the RPS-producing cyanobacterial strains described so far. In *A. halophytica* [44], *S. platensis* [55] and *C. capsulata* [63], polysaccharide production parallels biomass production so that the polymer may be considered a primary metabolite, as defined by Filali Mouhim et al. [55] in the case of *S. platensis*. Among these strains, *C. capsulata*, a heterocystous, akinete-forming cyanobacterium isolated from the alkaline soda lake Magadi (Kenya) [84], is the most extensively studied with regard to the process of polymer release. In batch cultures, this cyanobacterium showed an almost constant specific rate of polysaccharide release (expressed as mg of RPS per mg of protein per day) and maintained the same capsule thickness throughout growth phases, mature akinetes being the only cells completely devoid of capsule [63,85]. This kinetics of polysaccharide release has been considered by Vincenzini et al. [85] to be the result of a complex dynamic equilibrium among different processes: trichome elongation and akinete germination, in which the polysaccharide synthesis is mainly directed towards the formation

of the capsule, and, as opposed processes, trichome fragmentation and akinete differentiation, which cause the release of the polymer into the culture medium. Other cyanobacteria produce the polysaccharide as a typical secondary metabolite: *Cyanothece* BH68K showed a significant release of polysaccharide only starting from the late exponential growth phase [36], like *N. calcicola* [64] and *Phormidium* J-1 [33,86]. On the other hand, in *A. flos-aquae* A37 [60,87] and *A. cylindrica* 10C [62], RPS production occurred during all growth phases, but the highest production rates were observed starting from the late exponential or from the stationary phase. Conversely, in the case of a *Nostoc* strain, the highest rates of polysaccharide synthesis and release were achieved by young cultures [66].

All these findings point out that each strain should be carefully tested in order to envisage the right culture strategies aimed at optimising RPS production. In this connection, many data are now available on RPS daily production by cyanobacterial strains; these are reported in Table 4. Although most strains have only been tested in small culture devices and in studies not oriented to maximising RPS production, it appears that almost all strains attained very low RPS productivities, apart from *C. capsulata* and some *Cyanothece* strains. In particular, the most promising productivities have been shown by *C. capsulata*, with daily RPS productions of 116 mg l^{-1} by cultures run in open ponds and 144 mg l^{-1} by cultures grown in fermenters. These data are quite comparable with those reported for other photosynthetic microorganisms proposed for polysaccharide production. Indeed, strains of the red microalga *Porphiridium* have been reported to release a sulfated polysaccharide with a daily production of 55–75 mg l^{-1} in 2.5-m^2 open ponds [89] or 133 mg l^{-1} in 1-m^2 open ponds [90], while a *Botryococcus braunii* strain produced a polysaccharide with interesting rheological properties at a daily productivity of 130–145 mg l^{-1} in 1-l columns [91]. On the other hand, it has to be stressed that the photosynthetic organisms tested so far are characterised by polysaccharide productivities very low in comparison with the heterotrophic microorganisms industrially utilised for biopolymer production: for instance, in a typical batch for xanthan gum production, *Xanthomonas campestris* can reach productivities of 7–10 g (PS) l^{-1} day^{-1} [75]. This serious drawback of photosynthetic microorganisms compared to the RPS-producing heterotrophic bacteria could be partially disregarded in view of some advantages, of both environmental and economic impact, that cyanobacteria could achieve: (i) they are capable of utilising renewable and cheap substrates, being photoautotrophs and, many of them, nitrogen fixers; (ii) many strains can grow in brackish or in waste waters; (iii) it is possible to utilise as carbon source the CO_2 emitted by industrial plants; (iv) the economy of the process could be enhanced by recovering more than one useful compound, with a multiproduct strategy as already proposed for microalgae [92].

The possibility of stimulating polysaccharide release by means of an optimisation of the culture conditions has been poorly considered. Most of the available studies were mainly devoted to assaying the effects of nitrogen deficiency, which has been shown to stimulate polysaccharide synthesis in some RPS-producing microalgae [93–95]. However, the response of cyanobacteria to nitrogen starvation is not univocal. Some strains, like *A. nidulans* [45] and several *Cyanothece* [47,48], released larger amounts of polysaccharides under conditions of nitrogen limitation and others, like *A. cylindrica* [62] and *A. flos-aquae* [87], varied the amount of polymer produced depending on the nitrogen source used. On the other hand, in strains like *Synechocystis* [51], some *Cyanothece* [47], *C. capsulata* [96] and *Phormidium* [33], nitrogen starvation did not affect the exocellular production of polysaccharides. In the nitrogen-fixing cyanobacterium *C. capsulata*, when the metabolic carbon flux was affected by cultivating the organism under conditions of nitrogen deficiency due to the presence of an argon atmosphere or to the use of inhibitors of nitrogen assimilation (like *O*-diazoacetyl-L-serine, D,L-7-azatryptophan or methionine-D,L-sulfoximine) [96], an accumulation of intracellular carbon reserves instead of an increase in the production of RPS was observed. In contrast, when the metabolic carbon flux was directly stimulated by the addition of glyoxylate, a stimulator of both CO_2 and nitrogen fixation rates in *Anabaena cylindrica* [97], the cyanobacterium released larger amounts of polysaccharide, roughly corresponding, in terms of carbon balance, to the amount of the organic compound added. It is worth stressing that the possibility

Table 5
Molecular masses of the exopolysaccharides released by cyanobacteria

Species	Apparent molecular mass (kDa)	Technique for MW determination	Reference
C. minutus B 41.79	1200–1600	GPC	[46]
Oscillatoria sp.	200	GPC	[40]
Phormidium J-1	1200	GPC	[30]
S. platensis	81–98	GPC	[56]
A. circularis PCC 6720	>1200	GPC	[30]
C. capsulata ATCC 43193	1400–1900	LALLS; GPC	[74,78]
N. insulare 54.79	540–1300	GPC	[46]

Abbreviations: GPC = gel permeation chromatography; LALLS = low angle laser light scattering.

of increasing the amount of polysaccharide released without affecting growth, as it occurs in *C. capsulata* cultures carried out with the addition of glyoxylate, is very promising owing to the actual enhancement of the final yield of the polymer achieved by this way.

In some cases, the effects of other nutrient deficiencies or variations of growth parameters (e.g. light intensity, salinity, pH, etc.) have also been tested. In two *Synechocystis* strains, the release of polysaccharide was not affected by the addition of 0.5 M NaCl and glyoxylate nor by decreasing light intensity [51]. On the other hand, *Cyanothece* 16Som2 increased the exopolysaccharide release under conditions of phosphorus starvation, being unaffected by magnesium, calcium or potassium deficiencies as well as by salinities up to 2 M [48]. The release of the polysaccharidic bioflocculant by *Phormidium* J-1 was stimulated by calcium starvation but not by phosphate or sulfate deficiencies [33]. In *C. capsulata*, magnesium starvation, but not calcium or phosphate deficiencies, stimulated polysaccharide release, the amount of RPS being about 17% higher than in cultures grown on the standard medium [39]. In *A. cylindrica* 10C phosphorus shortage as well as the addition of acetate, propionate, valerate, citrate or glucose to the culture medium reduced RPS production [62].

In spite of the phototrophic nature of cyanobacteria, almost no studies are available on the influence of light on RPS production, apart from two studies carried out with *C. capsulata* [65] and *Synechococcus* BG0011 [88] that were grown under light-dark cycles. These two strains produced smaller amounts of exocellular polysaccharide in comparison with the control cultures carried out under continuous light, this reduction roughly corresponding to the shorter light period experienced by the cells. These findings point out that the processes of polymer synthesis and release are tightly light-dependent.

The above data demonstrate that, in terms of RPS production, the responses of cyanobacteria to alterations of culture conditions are strain-dependent. This behaviour possibly reflects different physiological roles played by the exocellular polysaccharides in different strains. For instance, when significant enhancements of RPS release are achieved by the shortage of some metallic ion, a role of the polymer as chelating agent for cations essential for cell life, as suggested for some algal and cyanobacterial anionic polysaccharides [23,37,98], may be envisaged. On the other hand, when polymer release is stimulated by nitrogen starvation, the polymer could act as a product of overflow metabolism, so that it is excreted to allow cells to get rid of the carbon excess [99].

5. Properties and possible applications of cyanobacterial RPSs

Most cyanobacterial polysaccharides described in the previous sections have not yet been studied with regard to their physical, structural or rheological properties, so that the assessment of their potential use for various industrial purposes or specific applications is a quite arduous task. In such a situation, in order to have useful guidelines to envisage the possible applications of these polymers, one should refer to some recent overviews on properties and uses of polysaccharides from other microbial sources [69,70,100–103]. In any case, the main features of possible industrial interest of cyanobacterial RPSs will be briefly considered.

Table 6
Substituent groups and protein content of the exopolysaccharides released by cyanobacteria

Species	Substituent groups (% on RPS dw)			Protein content (% on RPS dw)	Reference
	Acetate	Pyruvate	Sulfate		
Subsection I					
A. halophytica MN11	nd	nd	11.9	10.3	[44]
C. minutus B 41.79	nd	nd	nd	3.2	[46]
Cyanothece sp. CA 3	0.62	2.72	tr	nd	[47]
Cyanothece sp. CE 4	0.66	0.36	tr	nd	[47]
Cyanothece sp. CE 9	−	1.17	tr	nd	[47]
Cyanothece sp. CH 1	0.52	1.04	tr	nd	[47]
Cyanothece sp. ET 2	4.2	2.28	−	nd	[47]
Cyanothece sp. ET 5	2.5	0.39	−	nd	[47]
Cyanothece sp. IR 20	0.75	2.11	+	nd	[47]
Cyanothece sp. PCC 8801	1.40	0.50	nd	nd	a
Cyanothece sp. PE 13	−	2.08	tr	nd	[47]
Cyanothece sp. PE 14	0.32	0.17	tr	nd	[47]
Cyanothece sp. TI 4	0.98	1.37	tr	nd	[47]
Cyanothece sp. TP 5	−	1.10	+	nd	[47]
Cyanothece sp. TP 10	nd	3.86	+	nd	[47]
Cyanothece sp. VI 13	−	0.34	+	nd	[47]
Cyanothece sp. VI 22	0.55	0.23	+	nd	[47]
Cyanothece sp. 16Som2	−	−	+	1.4	[47,48]
Gloeothece sp. PCC 6909	nd	nd	13.8	6.2	[49]
M. aeruginosa K3A	nd	nd	nd	+	b
M. flos-aquae C3-40	nd	nd	nd	−	[50]
Synechocystis sp. PCC 6714[c]	nd	−	1.2	20.0	[51,106]
Synechocystis sp. PCC 6803[c]	nd	−	1.0	40.0	[51,106]
Subsection III					
Microcoleus sp. (2 strains)	nd	nd	nd	6.0	[26]
Phormidium sp.	nd	nd	−	13.0	[54]
Phormidium sp. J-1	nd	nd	1.6	4.4	[30]
S. platensis[d]	nd	nd	5.0	nd	[55]
Subsection IV					
A. circularis PCC 6720	nd	nd	−	−	[30]
A. cylindrica	nd	nd	nd	5.0[e]	[58]
A. cylindrica 10C	nd	nd	+	nd	[62]
Anabaena sp. C5	−	−	−	0.6	[43]
C. capsulata ATCC 43193	−	1.5	−	2.0	[63,72]
N. calcicola 79WA01	nd	nd	nd	7.9	[64]
N. commune UTEX584	nd	nd	nd	16.7	[64]
N. insulare 54.79	nd	nd	nd	3.5	[46]
Nostoc sp. PCC 7423	12.9	3.2	nd	nd	a
Nostoc sp. PCC 7936	3.1	5.9	nd	nd	a
Nostoc sp. 2S9B	−	−	−	2.8	[43]

Abbreviations: nd = not determined; dw = dry weight; + = present (not quantified); − = absent; tr = traces.
Notes: a: Our unpublished results. b: Nakagawa et al. (1987) in [50]. c: Composition of RPSs from young cultures (15 days of batch cultivation); in older cultures (2 months of batch cultivation) the quantitative composition changed. d: CPS extracted with buffer at 100°C for 20 min. e: Expressed as amino components.

One of the most important prerequisites of a polysaccharide, which determines many of the properties generally considered useful for its industrial utilisation (i.e. high viscosity of its aqueous solutions, ca-

pability of forming gels with good tensile strength, stabilising emulsions, etc.), is that it must possess, together with an adequate composition and structure [104], a high molecular mass (MW) [105]. In this respect, only seven cyanobacterial polysaccharides have been tested, five of them showing MWs higher than 1000 kDa (Table 5). The highest MW values, in the range of 1400–2800 kDa, depending on the analytical techniques utilised for MW determination, have been reported for the RPS produced by *C. capsulata* [74,78].

Another important feature that contributes to the chemical and physico-chemical properties of the polysaccharides is the presence of a polypeptide moiety or other non-saccharidic components such as organic (e.g. acetyl, pyruvyl, succinyl groups) or inorganic (e.g. sulfate or phosphate groups) substituents [69]. However, systematic investigations of the presence of these non-saccharidic components in cyanobacterial RPSs have been undertaken only recently, so that data are available only for almost half of the polysaccharides described (Table 6). A proteinaceous moiety has been found in almost all the polymers investigated, but only for *C. capsulata* [72] and *N. calcicola* [64] its amino acid composition is available; in the other cases, only the protein content has been determined, by colorimetric or Kjeldahl methods. For *C. capsulata*, an amino acid composition rich in glycine, alanine, valine, leucine, isoleucine and phenylalanine has been reported. Large amounts of the same amino acids have also been found in the polypeptide moiety of the RPS produced by *N. calcicola*, where they are reported to contribute significantly to the hydrophobicity of the macromolecule [64].

It must be stressed that, in those cyanobacterial RPSs reported to have a very high protein content, contamination due to cell lysis could have been occurred. However, most authors agree in considering amino acidic nitrogen not reducible below a certain value (usually in the range of 1–3% of RPS dry weight), even after repeated purifications, thus pointing out that it is a true component of the polymers. On the other hand, since the removal of the proteinaceous moiety drastically reduced the viscosity of aqueous solutions of the polysaccharides produced by some microalgae, a structural role of this constituent in or among the macromolecules has been suggested [107,108]. In the case of the polysaccharide released by *Nostoc* 2S9B, it was observed that the protein removal from the polymer significantly reduced the adhesive capacity of the polysaccharide to the roots of *Triticum vulgare* L., so underlining the importance of the polypeptide moiety in the process of root colonisation by the nitrogen-fixing cyanobacterium [43].

For many years in the past, only eukaryotic cells were believed to be capable of producing sulfated polysaccharides, the sulfation of the polymer occurring in the Golgi apparatus [109–112]. In the last 10 years, this opinion has been falsified because of the increasing number of cyanobacterial RPSs produced by strains isolated from both fresh water and saline or hypersaline environments that showed the presence of sulfate groups (Table 6). The current opinion is that sulfate groups may be present also in some prokaryotic polysaccharides, but only in those produced by strains belonging to cyanobacteria or archea [69]. The occurrence of phosphate residues, frequently found in many polysaccharides synthesised by other bacterial groups, has never been investigated in cyanobacterial RPSs, although phosphate-containing polysaccharides are attracting much interest because of their possible immunological significance [70]. In this connection, a certain biological activity could be also displayed by cyanobacterial RPSs bearing sulfate groups, since it is known that sulfated polysaccharides possess inhibitory properties against various types of viruses [113,114] and tumours [115,116]. With regard to the organic substituents, pyruvyl and acetyl groups, as shown in Table 6, have been frequently found, whereas succinyl residues have been reported only for the capsular polysaccharides obtained from cyanobacterial mats of a thermal spring [67]. The frequent presence of these inorganic and organic constituents introduces a further cause of structural complexity in cyanobacterial RPSs, but, on the other hand, makes these polymers excellent markers of biodiversity. However, a recent investigation [117], carried out on the RPSs produced by several *Cyanothece* strains isolated from saline environments, demonstrated that the chemical composition of the RPSs cannot be utilised for clustering the strains into more homogeneous subgroups and thus it is not a reliable chemotaxonomic marker.

Table 7
Anionic charge density (determined according to [123] as modified by [30]; our unpublished results), uronic acid, pyruvate and sulfate content of the exopolysaccharides released by some cyanobacterial strains

Strain	Uronic acid (% on crude RPS dry weight)	Pyruvate (% on crude RPS dry weight)	Sulphate	Anion density[a]
Cyanothece CA 3	18.8	2.72	tr	1.1
Cyanothece CE 4	24.8	0.36	tr	1.4
Cyanothece ET 2	7.3	2.28	−	0.1
Cyanothece ET 5	12.6	0.39	−	< 0.1
Cyanothece IR 20	4.6	2.11	+	1.3
Cyanothece PE 14	9.4	0.17	tr	1.6
Cyanothece TI 4	24.7	1.37	tr	1.5
Cyanothece VI 22	17.5	0.23	+	2.2
Cyanothece 16Som2	11.2	−	+	1.8
C. capsulata ATCC 43193	13.6	1.5	−	1.2

Abbreviations: tr = traces; + = present; − = absent.
Notes: a: mg of Alcian blue linked per mg of crude polysaccharide.

As already noted, any correlation between chemical composition and physical properties of the polysaccharides is generally hazardous in the absence of information about secondary and tertiary structures of the macromolecules, but, in spite of this, some behaviours can be expected. A significant presence of ester-linked acetyl groups (see Table 6) as well as of deoxy sugars like fucose and rhamnose (see Tables 1–3) has been suggested to give an appreciable contribution to the emulsifying properties of the polysaccharides, owing to a certain lipophilic character introduced by these small molecules in the macromolecules that otherwise would be highly hydrophilic [105,118]. However, some polysaccharides

Table 8
Effect of changes in pH, temperature and NaCl concentration on viscosity of 0.1% (w/v) aqueous solutions of RPS from *C. capsulata* and of commercial xanthan gum (Kelco Keltrol, commercial grade) at two shear rates

	Viscosity (log mPa s)			
	Xanthan gum		*C. capsulata* RPS	
	shear rate = 0.1 s^{-1}	shear rate = 10.2 s^{-1}	shear rate = 0.1 s^{-1}	shear rate = 10.2 s^{-1}
pH				
2.2	nd	1.40	nd	1.50
3.7	2.30	1.56	2.78	1.78
5.6	2.70	1.80	3.05	1.95
7.5	2.95	1.90	3.32	2.08
8.4	2.30	1.65	nd	2.05
10.0	nd	nd	2.95	1.90
11.8	nd	1.48	nd	1.60
Temperature				
25°C	2.95	1.92	3.40	2.08
40°C	2.78	1.83	3.23	2.05
60°C	2.10	1.61	3.08	1.99
25°C[a]	2.48	1.80	3.30	2.08
[NaCl]				
0.1 M	1.82[b]	1.40	1.60[b]	1.40
0.5 M	1.80[b]	1.42	1.65[b]	1.40
1.0 M	1.83[b]	1.42	1.70[b]	1.48

Abbreviations: nd = not determined.
Notes: a: Determined at the end of a thermal cycle (from 25°C to 60°C and back to 25°C). b: Measured at shear rate = 1.0 s^{-1}.

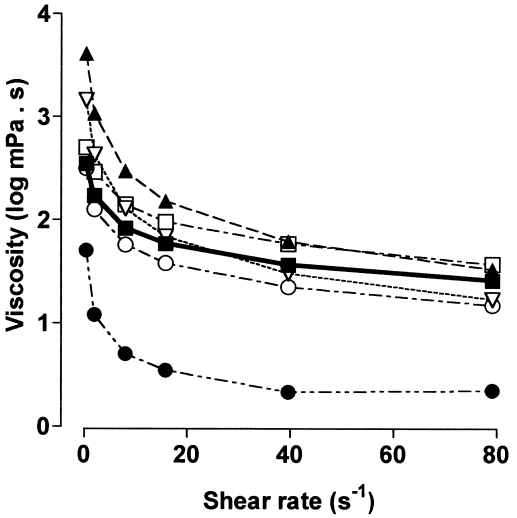

Fig. 4. Viscosity dependence of aqueous solutions of polysaccharides (0.1% w/v) on shear rate: xanthan gum (■), RPS produced by *Cyanospira capsulata* (□) and RPSs produced by *Cyanothece* strains CA 3 (▲); ET 2 (●); IR 20 (○) and PE 14 (▽).

containing high levels of rhamnose did not show any particular emulsifying activity [119]. It has also been reported that the presence of acetyl groups on the macromolecules may hinder cation binding, as occurs in bacterial alginates [120], or gel formation, as was observed in gellan [121], but may facilitate gel swelling, as in the case of alginate gel beads [102]. Moreover, acetyl groups may contribute to the stabilisation of the ordered form of the polymers, as has been reported for the xanthan structure [69].

Polysaccharides characterised by high concentrations of charged components (like uronic acids, sulfate or phosphate groups, pyruvate ketals) usually form stable gels in the presence of metallic ions [69] and are the most promising for the removal of toxic metals from polluted waters [40,68,122]. However, the mere determination of the quantity of charged groups is not enough for anticipating the actual metal binding capability of a polymer, because, depending on the conformational structure of the macromolecules, some of the charged groups could be hardly accessible for the ions. Indeed, the ion uptake depends on both charge density and charge distribution on the polymers [69]. Useful information about the actual metal binding capability of a polysaccharide can be obtained by the determination of the anion density through simple methods, like that based on the ability of the negatively charged polymers to link the Alcian blue pigment [123]. Using this method as modified by Bar-Or and Shilo [30], for instance, no evident correlations were found between the quantity of charged groups in some cyanobacterial RPSs and their anionic density (Table 7). In any case, the number of charged groups in the macromolecules, and particularly of sulfate substituents [18], has been reported to play a key role in flocculating suspended clay particles [30]. Charged groups also significantly contribute to the solubility in water of the polysaccharides, which otherwise would be insoluble [69], and improve the ability of the macromolecules to bind water molecules. Consequently, the viscosity of their aqueous solutions, tightly related to the effective volume occupied by the macromolecules, increases [124]. On the other hand, the protective role against desiccation, suggested for microbial exopolysaccharides [37,85,98], is particularly effective as the presence of acidic components in the macromolecules increases their water-retaining capability [125].

If the above reported considerations are useful to give an idea of the expected behaviour of a polysaccharide, on the other hand they underline the lack of experimental evidence on the properties of cyanobacterial RPSs. Indeed, a very small number of these polysaccharides has been tested with regard to their physico-chemical and rheological properties.

Two cyanobacterial strains, studied with regard to the viscosity of their cultures, underlined the importance of cell morphology in determining culture viscosity. In the case of the coccoid strain *Synechococcus* BG0011, the increase of culture viscosity, observed during its photoautotrophic growth, was mainly due to the released polysaccharide, the difference in viscosity between whole culture and cell-free medium being less than 5% [88]. In contrast, in *C. capsulata* the capsulated trichomes contributed significantly to the overall viscosity of the culture, particularly in the first period of growth [39]. In a further study [126], it was shown that whole cultures of *C. capsulata* are characterised by a pseudoplastic behaviour, the viscosity decreasing with the increase in shear rate, the more marked the older the culture.

A larger, but still limited, number of studies is available on the rheological properties of aqueous solutions of cyanobacterial RPSs, most of them

only considering the viscosity dependence on shear rate. All the RPSs tested showed the pseudoplastic behaviour typical of this kind of biopolymer, but, if their viscosity dependence on shear rate is compared with that of solutions of xanthan gum at the same concentrations, many differences become evident. The RPS produced by *Synechococcus* BG0011 showed a more marked shear thinning behaviour than solutions of xanthan gum but, at the same concentrations, lower values of viscosity [88]. The polymer produced by *S. platensis* PCC8005 showed a pseudoplastic behaviour at very low concentrations and was also characterised by a significant decrease of viscosity with increasing ionic strength of the solution [55]. On the other hand, a rather wide range of rheological behaviours has been observed in aqueous solutions of the RPSs produced by several *Cyanothece* strains or by *C. capsulata* (Fig. 4). Indeed, the flow properties highly differentiated these polymers: some of them showed a behaviour quite comparable to or even better than xanthan gum, while some of the others showed a more marked shear thinning behaviour, a property that could be of particular interest for some applications, e.g. for the formulation of oil drilling muds [102]. Finally, the remaining RPSs showed very low viscosities.

Deeper investigations on the flow properties of the aqueous solutions of RPS are available only for the polymer produced by *C. capsulata*. In this case, it was demonstrated that the pseudoplastic behaviour is the more marked the higher the polymer concentration and that the performances, at least with regard to the viscosity dependence on shear rate, are comparable with systems possessing a high suspending capability [127]. Moreover, it was suggested that the polymer has a random-coil behaviour, but with relevant interactions among the macromolecules [127]; the chain flexibility was considered comparable to that of alginate [78]. Other comparative data between xanthan gum and the RPS from *C. capsulata*, concerning the dependence of viscosity on pH, NaCl concentration and temperature, demonstrated a very promising behaviour of the cyanobacterial polymer (Table 8).

As a concluding remark, it has to be kept in mind that all the studies available on cyanobacterial RPSs involve laboratory experiments and there is an absolute lack of information on technological, economic and applicative aspects of these polymers. Hence, the very small number of patents issued during the last 10 years on cyanobacterial exopolysaccharides (Table 9) should not surprise.

6. Future prospects

The present knowledge of cyanobacterial RPSs, described in the previous sections, suggests that these polymers may cover a broad range of complex chemical structures and consequently different properties. Moreover, it is reasonable to anticipate that further studies on RPS-producing cyanobacteria will lead to new polymers possessing properties different from those of the polymers already available. This great diversity per se does not imply that cyanobacterial RPSs will find applications as integrative or alterna-

Table 9
Patents on cyanobacterial RPSs issued in the last 10 years (information drawn from J. Appl. Phycol., section 'Patents' compiled bimonthly by M.A. Borowitzka)

Year	Patent no.	Country	Title	RPS producer strain
1989	4,826,624	USA	Bioemulsifyer for dispersing liquid hydrocarbon(s) in a second liquid – is a polymeric product produced by cyanobacteria.	*Phormidium* spp.
1990	4,894,161	USA	Clarification of particulate containing liquid with bioflocculant produced by *Phormidium* genus cyanobacterium.	*Phormidium* spp.
1992	4,370,098	Japan	Continuous production of polysaccharide used as agar-agar substitutes etc.	*Spirulina platensis*
1993	5,049,491	Japan	High yield polysaccharide production.	*Aphanocapsa halophytica*
1993	5,250,201	USA	Secondary recovery of petroleum from subterranean formations.	*Phormidium* spp.
1994	6,040,880	Japan	Cosmetic material with sufficient skin-whitening effect and high stability and safety.	*Aphanocapsa* spp.

tive options to biopolymers currently in use, so having actual industrial perspectives for uses with a large market or for profitable market niches. The polymers already on the market (of microbial or plant origin) appear quite adequate for most applications and requirements of the industries; furthermore, they can rely on a mature industrial technology for their production and, some of them, on the sanitary authorisations necessary for their use in foods. Notwithstanding, it has been envisaged that the use of 'greener' technologies will most probably increase in the immediate future [102], so that the possibility of utilising photoautotrophic microorganisms to produce natural biopolymers from renewable resources will very likely attract more and more attention. However, as emphasised at the international symposium recently held in Israel ('Polysaccharide Biotechnology: an interdisciplinary approach'; Beer Sheva, 18–19 May 1998), one should bear in mind that the development of any microbial polysaccharide into biotechnological products requires co-ordinated efforts of both basic and applied research, involving all the necessary expertise to move from the laboratory culture of a polysaccharide-producing strain to the biopolymer market. Unfortunately, in the case of cyanobacterial polysaccharides, a wide gap exists between the current knowledge and what is needed to carry out a complete biotechnology programme. In this context, any research activity aimed at assessing and/or promoting the effectiveness of a cyanobacterial RPS will be greatly appreciated as well as any practical suggestion on the properties that a new polymer should possess in view of its specific applications.

Cyanobacterial RPSs destined to special applications, such as those in the biomedical sector, deserve separate consideration, owing to their particularly high added value. Indeed, cyanobacterial RPSs could have a great biomedical significance, as demonstrated in the case of the sulfated polysaccharide isolated from *S. platensis* that showed anti-herpes simplex virus and anti-human immunodeficiency virus activities [128,129]. In this perspective, cyanobacterial strains of saline or hypersaline origin, among which the ability to synthesise and to release sulfated exocellular polysaccharides seems to be frequent [47], may provide a great resource for the production of new bioactive polymers.

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