15
Cyanobacterial Exopolysaccharides: Their Nature and Potential Biotechnological Applications

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Introduction

Cyanobacteria (blue-green algae) are photosynthetic prokaryotic organisms which are unicells or filaments. Of great significance biologically is the fact that certain cyanobacteria can fix elemental nitrogen (Carr and Whitton, 1982). Some cyanobacteria are capable of movement by gliding when in contact with the substrate (Bold and Wynne, 1985). Some cyanobacteria have the ability to survive desiccation and extremes of temperature, and can grow at high pH and salinity (Flaibani *et al.*, 1989). Cyanobacteria occur in most environments on earth. Their distribution in freshwater and marine environment is cosmopolitan. Cyanobacteria are also commonly found in the soil and in rocks from the tropics to polar regions, and from temperate climates to extreme arid deserts, where they sometimes participate in the formation of microbial crusts or mats (Bold and Wynne, 1985; Mazor *et al.*, 1996). A number of diazotrophic cyanobacteria grow easily in association or symbiosis with certain green algae, liverworts, water ferns, and angiosperms (Bold and Wynne, 1985).

Cyanobacteria have been known, for a long time, to produce large amounts of exopolysaccharide (Drews and Weckesser, 1982). Recently, this massive production has received increasing attention due to the potential applications of these substances as industrial gums, bioflocculants, soil conditioners and biosorbants, and to their participation in symbiotic processes in plants, in the gliding movement, and in the general interactions between microorganisms and their habitats (Bertocchi *et al.*, 1990; Painter, 1993; Morvan *et al.*, 1997; De Philippis and Vincenzini, 1998).

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Abbreviations: CPS, capsular or slime polysaccharide; RPS, released polysaccharide; LDOF, light-diffusing optical fibres; CSR, completely stirred reactor.

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Many cyanobacteria are surrounded by mucilaginous external layers which have been called capsule, sheath, mucilage, glycocalyx or slime. The exocellular mucilaginous material is mainly polysaccharidic in nature. There is, however, no uniform terminology for the external layers. There are three types of exopolysaccharide: (1) A sheath, a thin uniform structured external layer immediately next to the outer membrane, containing either concentric or radial fibres, according to the strains. (2) A capsule or slime (capsular or slime polysaccharide, CPS), more outer unstructured zones. Differentiation between these two forms may be difficult. The capsular polysaccharide is intimately associated with the cell surface and may be covalently bound with well-defined limits. In contrast, slime polysaccharide is only loosely associated with the cell surface without sharply defined limits. (3) Soluble polysaccharides (released polysaccharide, RPS), released by many cyanobacteria into the media (Bold and Wynne, 1985; Bertocchi *et al.*, 1990).

Extraction of the sheath is usually achieved by differential sucrose gradient centrifugation of the homogenized cells (Bertocchi *et al.*, 1990). Solubilization of the CPS is achieved by warm water treatment of the cell pellet (Bertocchi *et al.*, 1990), by washing with deionized water (Nakagawa *et al.*, 1987; Plude *et al.*, 1991), by resuspending of the cell pellet in low ionic strength buffer at 100°C (Filali Mouhim *et al.*, 1993), or by a sodium chloride (1.5%) solution extraction of the cell pellet at 60°C (Vincenzini *et al.*, 1990; De Philippis *et al.*, 1993). RPSs are usually precipitated by alcohol from cell-free supernatants.

The aim of this review is to describe and discuss some important studies on both classes of these potentially highly useful substances – CPS and RPS – in literature to date. These studies have indicated possible biotechnological applications of cyanobacterial exopolysaccharides apart from highlighting some fundamental aspects of their physiology and ecology.

Chemical properties of cyanobacterial exopolysaccharides

We would like to stress that an exopolysaccharide preparation from a cyanobacterium may not be homogeneous in terms of its chemical and physical properties. For example, two types of polysaccharide have been separated from the RPS produced by *Anabaena flos-aquae* A-37 by ion exchange chromatography (Wang and Tischer, 1973). Gel permeation chromatography analysis demonstrated that *Cyanospira capsulata* RPS coming from the cultures run in open ponds or in a completely stirred reactor was not homogeneous in size (Vincenzini *et al.*, 1993). The RPSs of both *Chroococcus minutus* and *Nostoc insulare* were fractionated by ion exchange chromatography into neutral and acidic fractions (Fischer *et al.*, 1997). The RPS from *Aphanothece halophytica* GR02 can also be fractionated into two major fractions by ion exchange chromatography (Li *et al.*, 2001).

The neutral sugars xylose, arabinose, fucose, rhamnose, galactose, glucose, mannose and uronic acids are the major components of both the RPSs (*Tables 15.1–15.3*) and CPSs (*Tables 15.4–15.6*) in the cyanobacteria investigated. In general, there is no noteworthy difference in the major monosaccharide compositions between cyanobacterial RPSs and CPSs. Some cyanobacterial strains were analysed for sugar compositions of both RPSs and CPSs (Moore and Tischer, 1964; Vincenzini *et al.*, 1990; Gloaguen *et al.*, 1995a; Forni *et al.*, 1997; Nicolaus *et al.*, 1999). RPSs and

CPSs in *A. flos-aquae*, *Nostoc* sp., *Palmella mucosa* and *C. capsulata* show the same monosaccharide composition (qualitatively and quantitatively) (Moore and Tischer, 1964; Vincenzini *et al.*, 1990). RPSs and CPSs in *Oscillatoria* sp. and *Phormidium cf. foveolarum* MEU exhibit the same sugar composition in different molar ratios (Gloaguen *et al.*, 1995a). By contrast, the monosaccharide compositions of RPSs in most cyanobacteria are different from those of CPSs.

RPSs in those strains that have been examined thus far contain from 0 to 9 different neutral monosaccharides, depending on the particular strain (*Tables 15.1–15.3*). Glucuronic and/or galacturonic acids are present in most cyanobacterial RPSs. Hexose and pentose are absent in the RPS of *Microcystis wesenbergii* which consists of only uronic acid (Forni *et al.*, 1997). Usually, glucose is the dominant monosaccharide of the RPS although uronic acid, xylose, arabinose, fucose, rhamnose and mannose are the dominant monosaccharides in some cyanobacterial RPSs. Galactose is the dominant sugar only in *Anabaena sphaerica* RPS. Ribose, osamine, methylsugar and unidentified residues are also present in several cyanobacterial RPSs. There is no obvious relationship between the monosaccharide compositions of RPSs and cyanobacteria belonging to different orders or genera.

The cyanobacterial CPSs consist of various neutral monosaccharides ranging from two to as many as nine units depending on the examined strains (*Tables 15.4–15.6*). Most cyanobacterial CPSs also contain glucuronic and/or galacturonic acids. Glucose is dominant in all the strains except for three. The presence of ribose, osamine and an unidentified residue in CPSs has been found in several strains. Just as with the RPS, there is no obvious correlation between the monosaccharide compositions of CPSs and cyanobacteria belonging to different orders or genera. It is noteworthy that the composition of the slime polysaccharide of *Microcystis flos-aquae* resembles that of the plant polysaccharide pectin (Plude *et al.*, 1991).

The RPSs in several cyanobacteria contain protein. The CPSs of two cyanobacteria also contain protein. The RPSs from several cyanobacteria are also characterized by the presence of pyruvate and acetate groups. A most interesting feature is the presence of sulphate groups in the RPSs and CPSs of many strains, since sulphate is thought to be limited to polysaccharides produced by eukaryotic cells (Sutherland, 1994).

The study of the structures of cyanobacterial exopolysaccharides is necessary in order to explain their physico-chemical properties. However, there have been only a few studies on the structure of cyanobacterial exopolysaccharide. It has been proposed that the RPS produced by *C. capsulata* has a branched decasaccharide or octasaccharide repeating unit (Marra *et al.*, 1990; Garozzo *et al.*, 1998). The desiccation-tolerant cyanobacterium *Nostoc commune* DRH-1 RPS possesses a 1-4-linked xylogalactoglucan backbone with D-ribofuranose and 3-O-[(R)-1-carboxyethyl]-D-glucuronic acid pendant groups (Helm *et al.*, 2000). A possible backbone of the major fraction of RPS from *A. halophytica* GR02 could contain glucose, arabinose, fucose, mannose and glucuronic acid with branch points at mannose, and the remaining glucose and glucuronic acid are at terminal positions (Li *et al.*, 2001). The CPS produced by the thermophilic cyanobacterium *Mastigocladus laminosus* was deduced to have a branched pentadecasaccharide repeating unit (Gloaguen *et al.*, 1995b, 1997, 1999). The presence of uronic acid in the side-chains was found in all four exopolysaccharides. Taken as a whole, all these results clearly demonstrate the structural complexity of cyanobacterial RPS and CPS.

Table 15.1. Chemical composition of RPSs from cyanobacteria belonging to Chroococcales (X, xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid; +, present; -, absent; d, dominant; t, trace; a, acetate; ms, methyl-sugar; o, osamine; p, pyruvate; pr, protein; r, ribose; s, sulphate)

Species	X	A	F	R	Ga	G	M	UA	Others	References
Anacystis nidulans	–	–	–	–	+	d	+	–		Sangar and Dugan, 1972
Aphanocapsa halophytia MN-11	+	–	d	+	+	+	+	–	pr, s	Sudo *et al.*, 1995
Aphanothece halophytica GR02	–	+	+	+	+	d	+	+		Li *et al.*, 2001
Chroococcocus minutus	+	+	+	+	+	d	+	+	ms, o	Fischer *et al.*, 1997
Cyanothece sp. 16Som2	+	–	+	+	+	d	+	d	s	De Philippis *et al.*, 1998
Cyanothece sp. 16Som2	+	–	+	–	+	d	+	+		De Philippis *et al.*, 1993
Cyanothece sp. CA 3	–	d	+	+	–	+	+	d	a, p, s	De Philippis *et al.*, 1998
Cyanothece sp. CE 4	+	+	+	+	+	+	–	d	a, p, s	De Philippis *et al.*, 1998
Cyanothece sp. CE 9	–	–	+	+	+	d	+	d	p, s	De Philippis *et al.*, 1998
Cyanothece sp. CH 1	+	–	d	+	+	–	+	d	a, p, s	De Philippis *et al.*, 1998
Cyanothece sp. ET 2	–	+	+	+	+	+	+	d	a, p	De Philippis *et al.*, 1998
Cyanothece sp. ET 5	d	–	+	+	+	d	+	+	a, p	De Philippis *et al.*, 1998
Cyanothece sp. IR 20	–	–	+	d	+	+	+	+	a, p, s, r	De Philippis *et al.*, 1998
Cyanothece sp. PE 13	+	–	+	+	+	d	+	+	p, s, r	De Philippis *et al.*, 1998
Cyanothece sp. PE 14	–	d	+	+	–	+	+	+	a, p, s	De Philippis *et al.*, 1998
Cyanothece sp. TI 4	+	–	+	+	+	+	+	d	a, p, s	De Philippis *et al.*, 1998
Cyanothece sp. TP 10	–	d	+	+	–	+	+	d	p, s	De Philippis *et al.*, 1998
Cyanothece sp. TP 5	+	+	+	+	–	+	–	d	p, s	De Philippis *et al.*, 1998
Cyanothece sp. VI 13	+	–	+	+	+	d	+	d	p, s	De Philippis *et al.*, 1998
Cyanothece sp. VI 22	+	–	+	+	+	+	+	d	a, p, s	De Philippis *et al.*, 1998
Microcystis aeruginosa f. *aeruginosa*	–	–	–	d	–	+	+	+		Forni *et al.*, 1997
Microcystis aeruginosa f. *flos-aquae*	–	–	–	–	–	d	+	+		Forni *et al.*, 1997
Microcystis PCC 7005	–	–	+	–	–	d	+	+		Forni *et al.*, 1997
Microcystis PCC 7941	–	–	t	–	–	d	+	+		Forni *et al.*, 1997
Microcystis viridis	+	–	–	+	–	d	+	+		Forni *et al.*, 1997
Microcystis wesenbergii	–	–	–	–	–	–	–	+		Forni *et al.*, 1997
Synechocystis PCC 6714	+	+	+	+	+	d	+	+	pr, s, o, ms	Panoff *et al.*, 1988
Synechocystis PCC 6803	+	–	+	+	+	d	+	+	pr, s, o, ms	Panoff *et al.*, 1988

Table 15.2. Chemical composition of RPSs from cyanobacteria belonging to Nostocales (X, xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid; +, present; –, absent; d, dominant; t, trace; o, osamine; p, pyruvate; pr, protein; r, ribose; s, sulphate; u, unidentified residues)

Species	X	A	F	R	Ga	G	M	UA	Others	References
Anabaena ATCC 33047	d	–	–	–	+	+	+	+	pr	Moreno *et al.*, 2000
Anabaena C5	–	–	+	–	+	+	+	d	pr	Gantar *et al.*, 1995
Anabaena cylindrica 10C	d	–	+	+	+	d	+	+	s	Lama *et al.*, 1996
Anabaena flos-aquae A-37	+	–	–	–	–	d	–	+	r	Wang and Tischer, 1973
Anabaena flos-aquae A-37	+	–	–	–	–	d	–	+	r	Moore and Tischer, 1965
Anabaena fos-aquae	+	–	–	–	–	+	–	+	r	Moore and Tischer, 1964
Anabaena sphaerica	–	+	–	–	d	+	+	–		Nicolaus *et al.*, 1999
Anabaena torulosa	d	+	+	+	+	+	–	–	o	Nicolaus *et al.*, 1999
Cyanospira capsulata	–	+	+	–	–	+	+	d	p, pr, o	Garozzo *et al.*, 1995
Nostoc 2S9B	–	–	+	–	–	d	+	+	pr	Gantar *et al.*, 1995
Nostoc calcicola 79WA01	+	+	+	+	+	d	+	+	pr	Flaibani *et al.*, 1989
Nostoc commune DRH-1	+	–	–	–	+	d	–	+	r	Helm *et al.*, 2000
Nostoc D	+	d	–	–	–	+	–	–	u, r	Cupac and Gantar, 1992
Nostoc insulare	+	+	+	+	+	d	+	+		Fischer *et al.*, 1997
Nostoc sp.	–	+	+	–	–	+	–	+		Moore and Tischer, 1964
Nostoc sp. PCC 7423	+	–	+	+	+	d	+	+		De Philippis *et al.*, 1998
Nostoc sp. PCC 7936	–	–	+	–	+	+	+	d		De Philippis *et al.*, 1998
Palmella mucosa	–	+	+	–	–	+	–	+		Moore and Tischer, 1964
Scytonema hofmanni	–	–	–	–	+	+	d	–		Nicolaus *et al.*, 1999
Tolypothrix tenuis	–	+	+	+	+	d	+	–		Nicolaus *et al.*, 1999

Table 15.3. Chemical composition of RPSs from cyanobacteria belonging to Oscillatoriales and Stygonematales (X, xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid; +, present; –, absent; d, dominant; t, trace; f, fatty acid; o, osamine; pr, protein; r, ribose; s, sulphate; u, unidentified residues)

Species	X	A	F	R	Ga	G	M	UA	Others	References
Antarctic 1	d	+	–	+	+	+	–	+		Nicolaus *et al.*, 1999
Antarctic 2	–	+	–	–	–	d	+	+		Nicolaus *et al.*, 1999
Lyngbya confervoides S9g	t	t	t	t	+	d	+	+	u	Gloaguen *et al.*, 1995a
Oscillatoria amphibia PCC 7105	+	t	t	+	+	d	+	+	o	Gloaguen *et al.*, 1995a
Oscillatoria corallinae CJ 1	+	+	+	+	+	d	+	+	u	Gloaguen *et al.*, 1995a
Oscillatoria sp.	+	–	+	+*	+	d	+	+	u, o, r*	Bender *et al.*, 1994
Oscillatoria sp.	+	+	+	–	+	d	–	+		Nicolaus *et al.*, 1999
Phormidium cf. foveolarum C 52	+	+	+	+	+	d	+	+	o, u	Gloaguen *et al.*, 1995a
Phormidium cf. foveolarum MEU	+	+	+	+	+	d	+	+	o, u	Gloaguen *et al.*, 1995a
Phormidium ectocarpi C 86	+	–	–	t	+	d	+	+	u	Gloaguen *et al.*, 1995a
Phormidium ectocarpi K 5	+	t	t	+	+	d	+	+	o, u	Gloaguen *et al.*, 1995a
Phormidium ectocarpi ME 3	+	t	t	+	+	d	+	+		Gloaguen *et al.*, 1995a
Phormidium ectocarpi N 182	t	–	t	–	+	d	+	+		Gloaguen *et al.*, 1995a
Phormidium ectocarpi PCC 7375	+	t	+	+	+	d	+	+	u	Gloaguen *et al.*, 1995a
Phormidium minutum D 5	+	–	+	t	d	d	+	+	o, u	Gloaguen *et al.*, 1995a
Phormidium minutum NB 5	+	+	–	t	+	d	+	+	o, u	Gloaguen *et al.*, 1995a
Phormidium minutum RT 6	+	d	+	+	+	d	+	+	o	Gloaguen *et al.*, 1995a
Phormidium sp.	d	+	+	+	+	d	–	–		Nicolaus *et al.*, 1999
Phormidium sp. 90-14/1	+	t	+	t	+	d	+	+	o, u	Gloaguen *et al.*, 1995a
Phormidium sp. CCAP 1463/4	+	+	t	+	+	d	+	+	u	Gloaguen *et al.*, 1995a
Phormidium sp. CCAP 1464/3	+	t	–	t	+	d	+	+	o	Gloaguen *et al.*, 1995a
Phormidium sp. PNG 91	+	+	+	+	+	d	+	t	o, u, r	Gloaguen *et al.*, 1995a
Phormidium sp. strain J-1	–	–	–	+	+	–	d	+	pr, s, f	Bar-Or and Shilo, 1987
Stygonematales										
Chlorogloeopsis sp. 6912	–	+	+	+	+	d	+	+		Nicolaus *et al.*, 1999
Fischerella muscicola	+	–	+	–	+	d	+	–		Nicolaus *et al.*, 1999

* uncertain definition

Cyanobacterial RPSs and CPSs often contain one to three pentoses, which are absent in most of polysaccharides from other prokaryotic sources (Sutherland, 1994). Cyanobacterial RPSs and CPSs are heteropolysaccharides, most of which contain five or more monosaccharides. This is strikingly different from other microbial exopolysaccharides, which are either homopolysaccharides, or heteropolysacharides composed of several different monosaccharides, commonly two to four in number (Sutherland, 1994).

Other microbial exopolysaccharides studied until recently are usually linear molecules, although some of them have side-chains (Sutherland, 1994). The above-mentioned cyanobacterial exopolysaccharides are all branched polysaccharides. The structures of cyanobacterial exopolysaccharides are more complex than those of other microbial exopolysaccharides.

Physico-chemical properties

Fattom and Shilo (1984a) demonstrated that all examined benthic cyanobacteria were hydrophobic, whereas all planktonic cyanobacteria tested were hydrophilic. The capsule or slime layers of some other cyanobacterial strains have also been reported to possess hydrophobic properties (Fattom and Shilo, 1985; Bar-Or and Shilo, 1988; Katznelson, 1989; Mazor *et al.*, 1996; Kidron *et al.*, 1999). The hydrophobicity is confined to the outer surface layers. The presence of cations is necessary for the expression of hydrophobicity. Divalent cations are more efficient than monovalent cations in affecting the expression of hydrophobicity (Fattom and Shilo, 1984a).

Multivalent metal cations, such as Ca^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Mg^{2+} and Co^{2+}, cause coagulation of *Microcystis aeruginosa* K-3A CPS to form gel (Nakagawa *et al.*, 1987). The RPS from *Cyanothece* strain 16Som2 was also found to possess gelling properties. The addition of drops of the RPS aqueous solution (1% w/v) to 0.05 M $FeCl_3$ solution leads to the formation of stable gel beads. Unstable gel beads are formed after the addition of drops of the RPS aqueous solution (1% w/v) to 0.05 M $CuCl_2$ solution (De Philippis *et al.*, 1993).

It has been proposed that metal biosorption can occur by the complexation of metal ions with carbonyl, carboxyl, hydroxyl and sulphate groups in cyanobacterial exopolysaccharides (Tease and Walker, 1987). Since oxygen present in carboxylate ions increases the anionic tendency, carboxyl group can attract more metal cations. There is at least one uronic acid in the exopolysaccharide of most cyanobacteria. Thus, carboxyl groups are the main metal sequestering sites of cyanobacterial exopolysaccharides. The predominance of galacturonic acid in *M. flos-aquae* CPS suggests that charge attraction to carboxyl groups contributes to Fe cation binding (Plude *et al.*, 1991). The metal-binding mechanism occurring in *Phormidium laminosum* is fast (Sampedro *et al.*, 1995). The different Ni adsorption values for *Aphanothece* sp. and *Rivularia* sp. present a species specific property (Asthana *et al.*, 1995). The different compositions of cyanobacterial exopolysaccharides seem to be decisive in the metal-binding role played by these external envelopes (Sampedro *et al.*, 1995).

The RPSs of cyanobacteria *Anabaena* sp. N1444, *Anabaena* sp. PC-1, *Phormidium* sp. strain J-1 and *Anabaenopsis circularis* PCC 6720 show flocculating activity (Bar-Or and Shilo, 1987; Choi *et al.*, 1998). This activity is, at least in part, due to the

presence of acidic carboxyl groups (Bar-Or and Shilo, 1987). Temperature, pH value and cation concentration can affect the flocculating activity (Fattom and Shilo, 1984b; Choi *et al.*, 1998). The flocculating activities of the CPSs in *Phormidium* sp. strain J-1 and *A. circularis* PCC 6720 were also found (Bar-Or and Shilo, 1988).

By studying the role of cyanobacterial inoculation in maintaining soil structure, the beginning of a primary clay aggregation has been found as a consequence of interaction between the exopolysaccharides produced by the two *Nostoc* strains AfS49 and KaS35 and the morphological units of the fine soil fraction. This is probably due to the interaction between the positively charged edges of the clay particles and the negatively charged cyanobacterial exopolysaccharides (Falchini *et al.*, 1996).

The aqueous solution of *C. capsulata* RPS has demonstrated some rather interesting rheological properties. The shear dependent properties are just about equivalent to those of xanthan, and the time dependent properties are more similar to those of plant gum guar (Cesàro *et al.*, 1990; Navarini *et al.*, 1990, 1992). *Anabaena* sp. ATCC 33047 RPS and Alkemir 110 dispersions display quite similar viscosity and/or shear thinning properties (Moreno *et al.*, 2000). The *Spirulina platensis* CPS exhibits a non-Newtonian behaviour and a strong pseudoplastic property (Filali Mouhim *et al.*, 1993). The *A. halophytica* GR02 RPS is xanthan-like in its shear thinning properties (Morris *et al.*, 2001). The data show a biphasic effect of metal ion concentration on *M. flos-aquae* CPS viscosity at pH7. The polysaccharide viscosity increases with increasing metal ion concentration until a maximal viscosity occurs at a specific concentration, and then the viscosity decreases with further addition of that ion. The relative abilities of various metal salts to increase capsule viscosity are as follows: $CdCl_2$, $Pb(NO_3)_2$, $FeCl_3 > MnCl_2 > CuCl_2$, $CaCl_2 > NaCl$ (Parker *et al.*, 1996).

The data suggest that the solution conformation of *C. capsulata* RPS is a random coil with a chain flexibility comparable to that of alginate (Cesàro *et al.*, 1990). *Anabaena* sp. ATCC 33047 RPS forms an intermediate structure between a random-coil polysaccharide and a weak gel in solution (Moreno *et al.*, 2000). The solution conformation of the RPS produced by *A. halophytica* GR02 is a rigid/extra-rigid rod type polysaccharide (Morris *et al.*, 2001). The average molecular mass of the RPS from *Anabaena* sp. ATCC 33047 is 1.35 MDa (Moreno *et al.*, 2000). The molecular weight of *A. halophytica* GR02 RPS is 2.1 MDa (Morris *et al.*, 2001). De Philippis and Vincenzini (1998) have listed molecular masses of some other cyanobacterial exopolysaccharides. These data indicate cyanobacterial exopolysaccharides are macromolecules.

Stability of molecular and rheological properties

C. capsulata, cultivated under both continuous light and light–dark cycles in two culture devices, an open pond and a completely stirred reactor (CSR), possesses the capacity to release a xanthan-like RPS with quite stable molecular and rheological properties. All RPS samples obtained from these cultures show the same monosaccharide composition and relative proportions among sugar units. Several RPS samples from *C. capsulata* taken from cultures at different stages of growth also show no significant variations in sugar composition and the relative proportions of the monosaccharides. The sugar composition of the RPS thus appears to be fairly constant

with respect to culture age and conditions (Vincenzini *et al.*, 1990, 1993). Although gel permeation chromatography demonstrated that RPS samples produced by cultures run in open ponds were more homogeneous in size than those obtained from cultures grown in CSRs, no significant change in the flow properties was observed among the aqueous solutions of the different RPS samples (Vincenzini *et al.*, 1993).

It should be stressed that the sugar compositions of cyanobacterial exopolysaccharides may slightly vary, both qualitatively and quantitatively, with the age of the culture and growth conditions. The proportion of galactose in CPSs extracted from *S. platensis* cultures of different ages is significantly different. This may be due to the variation of the composition of the repetitive unit and/or of the proportion of different individual CPS (Filali Mouhim *et al.*, 1993). After addition of acetate, valerate, glucose, propionate or citrate to the growth medium, the monosaccharide composition of the RPS produced by *Anabaena cylinfrica* 10C varies slightly (Lama *et al.*, 1996). The sugar composition of the RPS produced by *Synechocystis* PCC 6714 under different culture ages is strikingly different (Panoff *et al.*, 1988).

Ecological roles of cyanobacterial exopolysaccharides

It has been suggested that the ability of a microorganism to produce exopolysaccharide is a direct and logical response to selective pressures in the natural environment (Dudman, 1977). Functions attributed to bacterial exopolysaccharides include their participation in the anchorage of the bacterial cell to its substrate, protection against desiccation, protection against phagocytic predation, the masking of antibody recognition, and prevention of lysis by other bacteria and viruses (Tease and Walker, 1987). Additionally, it has been suggested that the exopolysaccharide may bind and affect the penetration to the cell surface of both useful and toxic metal ions (Dudman, 1977), create a microenvironment that is suitable for the growth and survival of the organism (Sutherland, 1988), and constitute a possible barrier against toxins, or antibiotics (Costerton *et al.*, 1987). There are some reports dealing with the ecological role of cyanobacterial exopolysaccharide. The precise role played by cyanobacterial exopolysaccharide is dependent on the natural environment of the cyanobacterium.

TOLERANCE TO DESICCATION

The *N. commune* CPS functions as a physical barrier to the environment and an infrastructure that protects cells during desiccation and subsequent rehydration (Hill *et al.*, 1994). The exopolysaccharide tends to be hygroscopic and to represent well-mixed gels. It may decrease the rate of water loss from the cells and provide a repository for water (Potts, 1994). The presence of side-chains which contain charged component (uronic acid) renders cyanobacterial exopolysaccharides soluble in water and improves the ability of cyanobacterial exopolysaccharides to bind water molecules (Sutherland, 1994). The water content of the CPS of *Gloeothece* sp. ATCC 27152 was found to be more than that of the bulk environment (Tease and Walker, 1987). An increased envelope thickness, observed in desiccated cultures of *Chroococcidiopsis*, is probably useful in the prevention of water loss (Caiola *et al.*, 1996). The *N. commune* RPS undergoes striking changes in rheological properties in response to water availability (Potts, 1994, 1997). *N. commune* secretes copious

amounts of exopolysaccharide. The *N. commune* RPS, at low concentrations, prevents the fusion of phosphatidylcholine membrane vesicles at 0% relative humidity (–400 MPa) in the presence of trehalose and sucrose. The capacity of the RPS to prevent membrane fusion, and the changes in rheological properties of the RPS in response to water availability, constitute what are likely important mechanisms for desiccation tolerance in the cyanobacterium (Hill *et al.*, 1997).

ANTI-UV

The yield of a large-scale CPS isolated from UV-B irradiated *N. commune* cultures is about three times higher than that from control cultures. The increased CPS accumulation provides much longer effective path lengths for the absorption of radiation (Ehling-Schulz *et al.*, 1997). Also, the increased CPS production provides a matrix for the UV-absorbing mycosporine, a water soluble UV-AB-absorbing pigment and the lipid-soluble UV-protective pigment scytonemin, which are found within the glycan matrix of *N. commune* (Hill *et al.*, 1994; Böhm *et al.*, 1995; Ehling-Schulz *et al.*, 1997). However, it is noteworthy that the CPS of *N. commune* generates significant quantities of superoxide radicals upon UV irradiation, a feature which indicates that the CPS is a significant source of damaging free radicals upon exposure to UV irradiation (Shirkey *et al.*, 2000).

CHELATING CATIONS

The cyanobacterial exopolysaccharides are known to interact strongly with cations. *Anacystis nidulans* exhibits significant Ni adsorption (75%) (Asthana *et al.*, 1995). The accumulation of heavy metals in CPS is also exhibited by *P. laminosum*. Its affinity sequence is Pb > Fe > Cd > Cu > Zn > Ni (Sampedro *et al.*, 1995). Approximately 30% of the iron added to the medium is accumulated in the *M. aeruginosa* K-3A CPS (Nakagawa *et al.*, 1987). Since the ratio of Fe to Na in the dialysed *M. flos-aquae* C3-40 CPS is 10^4 times that in the growth medium, the Fe adsorption is preferential (Plude *et al.*, 1991). Morvan *et al.* (1997) has listed many other cyanobacteria which can accumulate cations and heavy metals. It has been suggested that these polysaccharides could concentrate essential metal elements and provide a microenvironment rich in available metal cations around the cell (Lange, 1976). On the other hand, these exopolysaccharides may scavenge metals to use as toxins to repel predators (Tease and Walker, 1987).

GLIDING MOTILITY

The mechanism of gliding movement is thus far not fully understood. It has been suggested that the necessary propulsion for locomotion is caused by the steady secretion of slime (Bold and Wynne, 1985). The study on the cell walls of four gliding filamentous Oscillatoriaceae species, as well as the combination of structural data and light microscopic observations of slime secretion process of *Phormidium uncinatum* and *Anabaena variabilis*, strongly suggests that the necessary propulsive force for locomotion is directly generated by shear forces between the surface fibrils and the continuing flow of secreted extracellular slime. A sort of pore complex was found to

be the actual extrusion site of the slime (Hoiczyk and Baumeister, 1995, 1998). The ability to secrete slime in *Phormidium* sp. has invariably been found so long as the filaments display gliding motility. Conversely, the sheath, which is only produced in old cultures, impairs the gliding motility of the filaments. Various nutritional and environmental factors may control which type of exopolysaccharide is formed by the *Phormidium* filaments (Hoiczyk and Baumeister, 1995; Hoiczyk, 1998). It has been speculated that the sheath polysaccharide is secreted by the junctional pore complexes which are involved in the process of slime secretion: in this way, the cells may be able to switch their polysaccharide production in response to different environmental stimuli (Hoiczyk, 1998).

FORMING MICROBIAL CRUST, BIOFILM AND MAT

A key feature of microbial crusts in arid zones is the abundance of filamentous sheath-forming and polysaccharide-excreting cyanobacteria, such as *Microcoleus* sp., *Phormidium* sp., and *Nostoc* sp. (Zhou *et al.*, 1995; Mazor *et al.*, 1996; Kidron *et al.*, 1999). The presence of a crust cover leads to run-off of water on the dune, whereas no run-off is generated on a crustless dune. The cyanobacterial exopolysaccharides play a key role in the formation of run-off as well as in protecting the crust's microbial community (Mazor *et al.*, 1996). It has been suggested that run-off is formed because of hydrophobicity of the exopolysaccharide, or pore clogging caused by swelling and expansion of the exopolysaccharide (Kidron *et al.*, 1999). The microbial biofilm or mat is formed by microorganisms living on a solid surface exposed to air or water. The microbial exopolysaccharides present in adherent biofilm or mat play a major role in microbial adhesion and in maintaining the structure of the biofilm or mat (Sutherland, 1983). Cyanobacteria have been found to be included in the biofilm on areas of rough hill pasture in Southern Scotland (Sutherland, 1996). Cyanobacterial biofilms, consisting of *Phormidium*, *Lyngbya*, *Oscillatoria*, *Microcoleus*, *Aphanothece* and *Scytonema*, were also collected in the subtropical part of Taiwan from wet and irrigated rocks, stone and concrete walls, and drains (Juttner and Wu, 2000). Cyanobacterial mat, developing on the surface of groundwater recharge basins in Israel, tends to reduce the rate of deposition of effluent into the ground. *Phormidium autumnale* is dominant in the mat. The remarkable clogging capacity of *P. autumnale* is thought to be related to its production of exopolysacchride (Katznelson, 1989). The cyanobacterium *Microcoleus chthonoplastes* has also beem found to be prevelant in the microbial mat in the Guangrao Saltworks of China. The remarkable permeation-protecting capacity of *M. chthonoplastes* is related to its production of exopoly-saccharide (Wu and Liu, 1995).

FLOCCULATING THE SUSPENDED CLAY PARTICLES

Phormidium sp. strain J-1, a benthic filamentous cyanobacterium, has been found to produce significant amounts of extracellular flocculants. This flocculant is a sulphated heteropolysaccharide to which fatty acids and protein are bound (Fattom and Shilo, 1984b; Bar-Or and Shilo, 1987). Extracellular flocculants were also found to be produced by the benthic cyanobacteria *A. circularis* PCC 6720 (Bar-Or and Shilo, 1987) and *Oscillatoria* sp. (Bender *et al.*, 1994). Two planktonic cyanobacteria,

Anabaena sp. N1444 and *Anabaena* sp. PC-1 can also produce extracellular flocculants (Choi *et al.*, 1998).

In the case of benthic cyanobacteria, the excretion of extracellular flocculants may be of great importance in clarification of turbid water bodies to allow light to reach the soil–water interface. For example, the release of the flocculant by *Phormidium* sp. strain J-1 was found to bring about the flocculation and subsequent sedimentation of the suspended clay particles, which hamper light penetration through the water column to the cells (Fattom and Shilo, 1984b). It was also found that the production of extracellular cell-bound flocculants was related to the co-flocculation with suspended clay particles by the benthic cyanobacteria *Phormidium* sp. strain J-1 and *A. circularis* 6720. It is suggested that the ability of co-flocculation is conducive to the attachment of cyanobacteria to the substrate (Bar-Or and Shilo, 1988).

SYMBIOSIS AND ASSOCIATION WITH OTHER ORGANISMS

It has been found that glycoconjugates play an important role in cell recognition in the *Azolla–Anabaena* symbiosis (Ladha and Watanabe, 1984). Also, recent data indicate that the significant changes in the composition of *Nostoc punctiforme* slime polysaccharide, occurring at the developmental stage involved in the establishment of the *Geosiphon* symbiosis, could play a role in the specific recognition between the symbiosis partners (Schübler *et al.*, 1997). The mucilaginous layer of *Nostoc* 2S9B is of great importance in the formation of a firm association with the roots of wheat plants. There is tight attachment of the isolated exopolysaccharide from *Nostoc* 2S9B to the root surface (Gantar *et al.*, 1995).

ADHERENCE TO THE SUBSTRATE

Cyanobacterial exoplysaccharide may function as bioadhesives. It is proposed that the adherence of the benthic filamentous cyanobacteria *Phormidium* sp. strain J-1 and *A. circularis* 6720 to the substrate is facilitated by the hydrophobicity of the exopolysaccharides. In old cultures the decrease in hydrophobicity of the exopolysaccharides enables the cells to detach and colonize new surfaces (Bar-Or and Shilo, 1988). The same adhesion mechanism has also been demonstrated in some other benthic cyanobacteria (Fattom and Shilo, 1984a). *Phormidium* sp. strain J-1 produces a polymeric extracellular emulsifying agent (emulcyan) at the stationary phase of growth. The emulcyan contains sugar moieties, proteins and fatty acids. It has been suggested that emulcyan masks the hydrophobicity of the exopolysaccharide, thus causing detachment of the cells. Production of emulcyan by *Phormidium* cells may serve as a dispersal strategy by this cyanobacterium (Fattom and Shilo, 1985).

SOIL AGGREGATION

The effects of CPS isolated from *Nostoc muscorum* or cyanobacterial mass inoculation on a saline-sodic soil, a poorly structured silt loam soil and a clay–silt–loam mixture have been evaluated. Inoculation with living cyanobacterial mass increases oxidizable C, soluble C, microbial activity and soil aggregate stability. These increases are mainly due to the exopolysaccharide produced by *N. muscorum* and increased soil

microbial population (Rogers and Burns, 1994; Caire *et al.*, 1997; Cano *et al.*, 1997). It has also been found that *Oscillatoria prolifica* and *N. commune* can increase water stability of aggregates when they are grown separately on Peoria loess soil (Bailey *et al.*, 1973). Addition of *N. muscorum* CPS increases the amount of water-stable aggregates either by direct glueing of the particles or by increasing activity of the heterotrophic microflora, which produces more exopolysaccharide. Addition of isolated CPS results in a faster and higher increase in soil aggregate stability than cyanobacterial mass inoculation. The improvement of soil aggregate stability produced by both treatments results from the increase of soil polysaccharide concentration and soil microbial activity (Caire *et al.*, 1997).

OTHER ROLES

A Ca/Si rich external (pellicular) layer of the glycan is suggested to act as a physical barrier to epiphytic bacteria on the surface of *N. commune* colonies (Hill *et al.*, 1994). The cyanobacterial exopolysaccharide may function to flocculate cells during stress. Balkwill and Stevens (1980) have demonstrated that exposure of *Agmenellum quadruplicatum* to higher than optimal temperature or light intensity, to blue light, or to a pH below 5.5 or above 9 results in flocculation of a culture. Rohrlack *et al.* (1999) have demonstrated that the exopolysaccharide of the colony-forming *Microcystis* strains plays a decisive role in their ability to influence the ingestion rate of daphnids.

Biosynthesis and production of cyanobacterial exopolysaccharides

Bacterial exopolysaccharides are synthesized under a variety of growth conditions and in different growth phases, depending on the bacteria studied (Sutherland, 1985). The synthesis of bacterial exopolysaccharides is complex. Repeating unit exopolysaccharide from Gram-negative bacteria studied to date appears to involve essentially similar synthetic processes (Whitfield, 1988). The presence of several acidic or neutral monosaccharides in cyanobacterial exopolysaccharide indicates there may be a more complex biosynthetic pathway in cyanobacteria. Early studies of the biosynthesis of cyanobacterial exopolysaccharides indicate that they are derived from intracellular polysaccharides of the same composition in *A. flos-aquae*, *P. mucosa* and *Nostoc* (Moore and Tischer, 1964, 1965). In *A. flos-aquae*, the intracellularly synthesized polysaccharide may diffuse through the cell wall, and be released into the surrounding medium subsequently (Moore and Tischer, 1965). In contrast, the qualitative differences in composition of extracellular and intracellular polysaccharides in *Nostoc* suggest there is a selective mechanism of excretion of polysaccharide (Mehta and Vaidya, 1978). The exopolysaccharide of *A. flos-aquae* is thought to be the major end product of photosynthesis (Moore and Tischer, 1965). The results indicate that the rates of CO_2 fixation, polysaccharide synthesis, and excretion are surprisingly rapid in *A. flos-aquae* and *Nostoc* (Moore and Tischer, 1965; Mehta and Vaidya, 1978). From *Tables 15.1–15.6*, we find the sugar compositions of RPSs in most cyanobacteria are remarkably different from those of the CPSs. The biosynthesis mechanism of RPSs in most cyanobacteria could be different from that of CPSs. Different environmental, nutritional, chemical and physical parameters affect biosynthesis and production of the cyanobacterial exopolysaccharide. All these effects on exopolysaccharide production are strain-dependent.

Table 15.4. Chemical composition of CPSs from cyanobacteria belonging to Chroococcales (X, xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid; +, present; –, absent; d, dominant; t, trace; pr, protein)

Species	X	A	F	R	Ga	G	M	UA	Others	References
Microcystis aeruginosa f. aeruginosa	–	–	–	–	+	d	–	+		Forni *et al.*, 1997
Microcystis aeruginosa f. flos- aquae	–	–	–	–	+	d	–	+		Forni *et al.*, 1997
Microcystis aeruginosa K-3A	+	+	+	+	+	+	+	d	pr	Nakagawa *et al.*, 1987
Microcystis flos-aquae C3-40	+	–	–	+	+	+	+	d		Plude *et al.*, 1991
Microcystis PCC 7005	–	+	–	–	–	d	+	+		Forni *et al.*, 1997
Microcystis PCC 7941	–	–	–	–	–	d	+	+		Forni *et al.*, 1997
Microcystis viridis	–	–	–	–	–	d	–	+		Forni *et al.*, 1997
Microcystis wesenbergii	+	–	–	+	+	d	–	+		Forni *et al.*, 1997

Table 15.5. Chemical composition of CPSs from cyanobacteria belonging to Oscillatoriales (X, xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid; +, present; –, absent; d, dominant; t, trace; o, osamine; r, ribose; s, sulphate; u, unidentified residues)

Species	X	A	F	R	Ga	G	M	UA	Others	References
Lyngbya confervoides S9g	+	+	+	+	+	d	+	+	o, u	Gloaguen *et al.*, 1995a
Oscillatoria amphibia PCC 7105	+	–	+	+	+	d	+	+	s	Gloaguen *et al.*, 1995a
Oscillatoria corallinae CJ 1	+	–	+	+	+	d	+	+	s	Gloaguen *et al.*, 1995a
Oscillatoria sp.	+	+	+	–	+	d	–	+		Nicolaus *et al.*, 1999
Phormidium cf. foveolarum C52	+	–	+	+	+	d	+	+	u, s, r	Gloaguen *et al.*, 1995a
Phormidium cf. foveolarum MEU	+	t	+	+	+	d	+	+	o, u, s	Gloaguen *et al.*, 1995a
Phormidium ectocarpi C 86	+	–	+	+	+	d	+	+	u, s	Gloaguen *et al.*, 1995a
Phormidium ectocarpi K5	+	–	+	+	+	d	+	+	o, s	Gloaguen *et al.*, 1995a
Phormidium ectocarpi ME 3	+	–	t	+	+	d	+	+	s	Gloaguen *et al.*, 1995a
Phormidium ectocarpi N 182	+	–	+	+	+	d	+	+	s	Gloaguen *et al.*, 1995a
Phormidium ectocarpi PCC 7375	+	–	+	+	+	d	+	+	u	Gloaguen *et al.*, 1995a
Phormidium minutum D 5	+	–	+	+	+	d	+	+	o, s	Gloaguen *et al.*, 1995a
Phormidium minutum NB 5	+	t	t	+	+	d	+	+		Gloaguen *et al.*, 1995a
Phormidium minutum RT 6	+	+	+	–	+	d	+	+	u, s	Gloaguen *et al.*, 1995a
Phormidium sp. 90-14/1	+	+	+	+	+	d	+	+	s	Gloaguen *et al.*, 1995a
Phormidium sp. CCAP 1463/4	+	–	–	+	+	d	+	+	s	Gloaguen *et al.*, 1995a
Phormidium sp. CCAP1464/3	+	–	–	+	+	d	+	+	s	Gloaguen *et al.*, 1995a
Phormidium sp. PNG 91	+	+	+	+	+	d	+	+	u, s, r	Gloaguen *et al.*, 1995a
Phormidium uncinatum	+	+	+	+	+	d	–	–		Hoiczyk, 1998
Spirulina platensis	+	–	+	+	d	d	t	+	u, s	Filali Mouhim *et al.*, 1993
Spirulina sp.	–	–	–	–	+	d	+	–		Nicolaus *et al.*, 1999

Table 15.6. Chemical composition of CPSs from cyanobacteria belonging to Nostocales and Stigonematales (X, xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid; +, present; –, absent; d, dominant; t, trace; a, acetate; o, osamine; p, pyruvate; pr, protein; s, sulphate; su, succinate)

Species	X	A	F	R	Ga	G	M	UA	Others	References
Nostocales										
Anabaena cylindrica	+	–	t	–	+	d	+	–		Dunn and Wolk, 1970
Anabaena flos-aquae	+	–	–	–	–	+	–	+	r	Moore and Tischer, 1964
Anabaena sphaerica	–	–	–	d	d	d	+	–		Nicolaus *et al.*, 1999
Anabaena WSAF	d	+	+	+	+	+	–	+	o	Nicolaus *et al.*, 1999
Cyanospira capsulata	–	+	+	–	–	+	+	d		Vincenzini *et al.*, 1990
Nostoc sp.	–	+	+	–	–	+	–	+		Moore and Tischer, 1964
Palmella mucosa	–	+	+	–	–	+	–	+		Moore and Tischer, 1964
Scytonema hofmanni	–	–	t	–	t	d	–	–		Nicolaus *et al.*, 1999
Stigonematales										
Fischerella muscicola	+	+	+	–	+	d	+	–		Nicolaus *et al.*, 1999
Mastigocladus laminosus	+	t	+	+	+	d	+	+	pr, s, a, p, su	Gloaguen *et al.*, 1995b

NITROGEN SOURCE

Nitrogen starvation has been found to stimulate RPS release in *Cyanothece* strain 16Som2 (De Philippis *et al.*, 1993). Also, RPS production by *Anabaena* sp. ATCC 33047 appears to be particularly enhanced under conditions of nitrogen starvation, induced by low nitrogen fixation capacity of the cells (Moreno *et al.*, 1998). Nitrogen starvation causes the increase of total exopolysaccharide production of *Spirulina* (Nicolaus *et al.*, 1999). N-starvation stimulates the synthesis of CPS in *Cyanothece* strain 16Som2 and *P. laminosum* (Agardh) Gomont (strain OH-1-pCl_1) (Fresnedo and Serra, 1992; De Philippis *et al.*, 1993). High RPS production of *Anabaena* sp. ATCC 33047 was observed under diazotrophic conditions. The presence of combined nitrogen source leads to decreased RPS production, without affecting cell growth (Moreno *et al.*, 1998). The increase in nitrogen content in the medium and the absence of combined nitrogen cause the decrease of RPS production in *Anabaena cylindrica* 10C (Lama *et al.*, 1996). The same effect on the total exopolysaccharide production was observed in *Phormidium*, *Anabaena torulosa* and *Anabaena* sp. WSAF (Nicolaus *et al.*, 1999). Maximum RPS production in *Aphanocapsa halophytia* MN-11 occurs with nitrogen concentrations in the medium exceeding 100 mg/l (Sudo *et al.*, 1995). Maximum RPS production in *Cyanothece* sp. ATCC 51142 is obtained in the presence of $NaNO_3$ as the nitrogen source (Shah *et al.*, 1999). When a nitrogen source is utilized adequately, the RPS production in *A. flos-aquae* A-37 is not affected by the nature of the nitrogen source (Tischer and Davis, 1971).

PHOSPHATE SOURCE

The absence of phosphate and the increase of phosphate content give rise to the decrease of total exopolysaccharide production in *Phormidium* (Nicolaus *et al.*, 1999). The increase in phosphate content in the growth medium has little influence on total exopolysaccharide production in *A. torulosa* and *Anabaena* sp. WSAF and *Spirulina* (Nicolaus *et al.*, 1999). The absence of phosphate induces a strong increase in the total exopolysaccharide production of *Spirulina* (Nicolaus *et al.*, 1999). Phosphate limitation causes a significant enhancement of total carbohydrate synthesis in *Cyanothece* strain 16Som2 (De Philippis *et al.*, 1993). In contrast, the absence of phosphate induces a strong decrease in total exopolysaccharide production of *Anabaena* WSAF and *A. torulosa* (Nicolaus *et al.*, 1999). The same effect on the RPS production was reported in *A. cylindrica* 10C (Lama *et al.*, 1996). Phosphate deficiency causes no influence on the RPS production in *C. capsulata* (De Philippis *et al.*, 1991). Maximum exopolysaccharide production in *A. halophytia* MN-11 occurs when phosphorus concentrations in the medium exceed 40 mg/l (Sudo *et al.*, 1995).

SALINITY

The absence of NaCl causes a small decrease in total exopolysaccharide production in *Spirulina* (Nicolaus *et al.*, 1999). No effect on the amount of RPSs released by *C. capsulata* and *Cyanothece* strain 16Som2 is caused by increasing salinity (De Philippis *et al.*, 1991, 1993). The optimum NaCl concentration for RPS production in *A. halophytia* MN-11 and *Cyanothece* sp. ATCC 51142 are 3% and 4.5%, respec-

tively (Sudo *et al.*, 1995; Shah *et al.*, 1999). High NaCl concentration causes decreased RPS production in *Anabaena* sp. ATCC 33047 (Moreno *et al.*, 1998).

METAL IONS

Magnesium shortage induces a significant enhancement of the RPS production in *C. capsulata* (De Philippis *et al.*, 1991). The synthesis of carbohydrates in *Cyanothece* strain 16Som2 is not enhanced by Mg^{2+} deficiency (De Philippis *et al.*, 1993). Calcium deficiency causes no effect on the amount of RPS released by *C. capsulata* (De Philippis *et al.*, 1991), nor on the synthesis of carbohydrates in *Cyanothece* strain 16Som2 (De Philippis *et al.*, 1993). RPS production in *Phormidium* sp. strain J-1 is enhanced by the decrease of the calcium content in the growth medium (Fattom and Shilo, 1984b). The synthesis of carbohydrates in *Cyanothece* strain 16Som2 is not enhanced by K^+ deficiency (De Philippis *et al.*, 1993). Exopolysaccharide synthesis was found to be metal-specific. The effectiveness of cations on RPS synthesis in *Nostoc spongialforme* follows the order Cu > Hg > Ni (Singh *et al.*, 1999).

OTHER CHEMICAL PARAMETERS

RPS production in *A. cylindrica* and *C. capsulata* is stimulated markedly by addition of glyoxylate (Bergman, 1986; De Philippis *et al.*, 1996). This is of great interest for possible application because addition of glyoxylate causes no effect on growth. On the contrary, some nutritional deficiencies can enhance exoplysaccharide production on a per cell basis, but there is no actual enhancement of exopolysaccharide yield because these deficiencies also hamper cell multiplication (De Philippis *et al.*, 1996). Addition of acetate, valerate, glucose or citrate to the growth medium causes a decrease in *A. cylindrica* 10C RPS production (Lama *et al.*, 1996). RPS production in *Phormidium* sp. strain J-1 is enhanced by increasing its EDTA content (Fattom and Shilo, 1984b). No effect on the RPS production in *C. capsulata* is caused by pH values higher than optimum (De Philippis *et al.*, 1991). Maximum RPS production in *Cyanothece* sp. ATCC 51142 was found to occur at pH 7.0 (Shah *et al.*, 1999).

PHYSICAL PARAMETERS

The RPS production in *Anabaena* sp. ATCC 33047 is markedly enhanced by an increase in temperature (Moreno *et al.*, 1998). Conversely, the increase in temperature causes a small decrease in total exopolysaccharide production in *Spirulina* (Nicolaus *et al.*, 1999). The RPS production in *Anabaena* sp. ATCC 33047 is markedly enhanced by an increase in illumination (Moreno *et al.*, 1998). Compared with continuous illumination, the light/dark cycles cause a dramatic decrease in total exopolysaccharide yield in *Phormidium*, *A. torulosa* and *Anabaena* sp. WSAF (Nicolaus *et al.*, 1999). The CPS yield in *N. commune* isolated from UV-B irradiated cultures is about three times higher than that from control cultures (Ehling-Schulz *et al.*, 1997).

PHASE OF GROWTH

Optimal conditions for exopolysaccharide production by some cyanobacteria do not coincide with those for cell growth. Some cyanobacteria, such as *Cyanothece* sp. BK68K (Reddy *et al.*, 1996), *Nostoc calcicola* (Flaibani *et al.*, 1989), *Phormidium* J-1 (Fattom and Shilo, 1984b, 1985) and *Anabaena* sp. ATCC 33047 (Moreno *et al.*, 1998, 2000), accumulate RPSs mainly during the stationary phase, in which growth is limited by different factors. Conversely, in *C. capsulata* (Vincenzini *et al.*, 1990) and *A. halophytia* (Sudo *et al.*, 1995), RPS production starts early during growth, increasing with increasing cell density and reaching a maximum at the stationary phase of growth.

Production of exopolysaccharide on a large scale

To evaluate the technological feasibility on production of cyanobacterial exopolysaccharide on a large scale, two investigations have been carried out. *A. halophytia* MN-11 is immobilized by two methods to produce RPS. Cells immobilized on light-diffusing optical fibres (LDOF) produce about ten times more RPS than those immobilized in calcium alginate beads only. There is a problem with the conventional calcium bead method of limitation of light penetration into the gel because of the self-shading of cells. Using LDOF in the immobilization system, this problem can be overcome (Matsunaga *et al.*, 1996). In another investigation, RPS production of cyanobacteria *C. minutus* and *Nostoc nisulare* were studied in various cultivation systems. Batch cultures in 8-L flasks were found to be suitable for the laboratory scale. Batch cultures in photobioreactors with internal illumination, easy to handle, are suitable for very large scale production. In order to produce RPS continuously from cell-free media, another immobilization culture system has also been tested. Cyanobacterial cells are immobilized on white cotton towelling and grown in 470-ml and 17-L flat upright transparent chambers made of polycarbonate. However, it does not appear feasible to construct larger immobilization chambers of the type with volumes comparable to the larger photobioreactors. It is difficult to further scale-up the immobilization chambers (Fischer *et al.*, 1997).

Potential biotechnological applications

BIOFLOCCULANT

It has been found that *Phormidium* sp. strain J-1 and *A. circularis* PCC 6720 can release extracellular macromolecular flocculants into medium. This substance can flocculate bentonite particles from suspensions (Fattom and Shilo, 1984b; Bar-Or and Shilo, 1987). *Anabaena* sp. can also produce extracellular flocculant. This flocculant has a broad substrate specificity, rapid flocculating activity and thermal stability (Choi *et al.*, 1998). There are many potential uses for cyanobacterial extracellular flocculants, such as clarification of tap water, reduction of suspended solid matter in water reservoirs, wastewater treatment and clarification of solar ponds. *Anabaena* sp., *Phormidium* sp. strain J-1 and *A. circularis* 6720 also excrete extracellular cell-bound flocculants (Avnimelech *et al.*, 1982; Fattom and Shilo, 1984b; Bar-Or and Shilo,

1988). This may be used to manage lakes and other bodies of water. Water rich in cyanobacteria may be clarified through the addition of clay to the water, and turbid water may be clarified through the enhancement of cyanobacterial growth (Avnimelech *et al.*, 1982).

BIOPOLYMER

Microbial exopolysaccharides are of significant commercial value, since they represent interesting alternatives to the plant and macroalgae exopolysaccharides traditionally used in the food, textile, painting, cosmetic, paper, pharmaceutical and oil industries. Contemporary commercial production of microbial polysaccharides is built on fermentation of heterotrophic bacteria. This process requires expensive organic substrates. Photoautophic cyanobacteria can grow on inorganic media. Cyanobacteria could be a cheaper new source of these biopolymers.

Aqueous solutions of the RPSs produced by *C. capsulata* and *A. halophytica* GR02 show xanthan-like physical properties (Cesàro *et al.*, 1990; Navarini *et al.*, 1990, 1992; Morris *et al.*, 2001). *Anabaena* sp. ATCC 33047 RPS and Alkemir 110 dispersions display quite similar viscosity and/or shear thinning properties. Alkemir 110 is widely used in the food industry as a stabilizer (Moreno *et al.*, 2000). From these studies, it emerges that cyanobacterial exopolysaccharides possess promising properties and potential for industrial exploitation as emulsifiers, stabilizers or thickening agents (De Philippis and Vincenzini, 1998). Additionally, the carboxylate groups present in most cyanobacterial exopolysaccharides might be used for linking to natural or synthetic polymers to generate new polysaccharides with special physical properties.

It should be mentioned the monosaccharide composition of CPS from *M. flos-aquae* C3-40 resembles that of the plant polysaccharide pectin. Pectin, a commonly used gelling agent, requires chemical modification for certain applications. The pectin-like polysaccharide can be harvested from *M. flos-aquae* cells by washing with deionized water, a simpler procedure than that used to extract pectin from plant tissue. This pectin-like polysaccharide may be more suitable for certain applications than pectin (Plude *et al.*, 1991). The potential use of these biopolymers has also been reported by De Philippis and Vincinzini (1998).

BIOACTIVE SUBSTANCE

Sulphated polysaccharides can interfere with the absorption and penetration of viruses into host cells and inhibit various retroviral reverse transcriptases (Nakanishi *et al.*, 1987; Bagasa and Lischner, 1988). Calcium spirulan, a sulphated polysaccharide from *S. platensis*, is known to inhibit tumour invasion and metastasis (Mishima *et al.*, 1998). The presence of sulphated groups has indicated that many cyanobacterial exopolysaccharides possess promising potential applications in the pharmaceutical industry.

SOIL CONDITIONER

Soil microbial polysaccharides are the most important substances influencing soil

aggregate formation (Metting, 1988). The direct effect of polysaccharides on soil aggregation is to bind soil particles into microaggregates (Cheshire *et al.*, 1983, 1984). The long-term effect of polysaccharides on soil aggregation may be a result of the microbial mineralization of exopolysaccharides and the formation of their microbial degradation products (Piccolo and Mbagwu, 1989; Haynes *et al.*, 1991; Rogers and Burns, 1994).

Cyanobacteria, a common component of all terrestrial habitats, have an influence on soil structure and fertility, due to their exopolysaccharide-excreting capacity as well as dinitrogen-fixing activity. It was demonstrated that inoculation with N_2-fixing cyanobacterium *N. muscorum* not only had a pronounced effect on soil physical, chemical, and biological properties, but also improved seedling emergence. Inoculation with *N. muscorum* leads to a drastic increase in soil polysaccharide content and a subsequent improvement in soil aggregate, mainly due to exopolysaccharide secreted by *N. muscorum* (Rao and Burns, 1990; Rogers and Burns, 1994; Caire *et al.*, 1997; Cano *et al.*, 1997). Compared with synthetic polyacrylamide soil conditioners (Wallace and Wallace, 1986), much lower *N. muscorum* application rates are necessary to achieve the same increase in lettuce emergence (Rogers and Burns, 1994). Rice cultivation alters the soil structure and reduces the amount of soil organic matter. Inoculation with *Tolypothrix tenuis* and *N. muscorum* in post-harvest soil generates the increase of soil organic matter content and an improved stability of soil aggregates (Mulé *et al.*, 1999).

Cyanobacteria are ubiquitous in soil, and may form a microbial crust or mat on the surface of bare soil as primary colonizers. Their contribution is significant in desert and semi-arid soils. Exopolysaccharides excreted by these cyanobacteria are important in the improvement of soil water, the provision of organic carbon, the stabilization of soil, and the *de novo* formation of soil (Flaibani *et al.*, 1989; Mazor *et al.*, 1996). Exopolysaccharide-forming N_2-fixing cyanobacteria and microalgae have been developed for use as a soil supplement to improve soil characteristics and crop productivity (Bar and Visnovsky, 1997). With respect to the potential of cyanobacterial exopolysaccharides in desert reclamation, one could refer to another review (Painter, 1993). These studies indicate that the N_2-fixing cyanobacteria which produce exopolysaccharides are a promising source of soil bioconditioner.

REMOVAL AND RECOVERY OF DISSOLVED HEAVY METALS

Increasing contamination of aquatic resources with heavy metals has become a major global concern. A number of techniques, such as adsorption, chemical precipitation, chemical oxidation and reduction, ion-exchange and evaporative recovery, have been used for removal and recovery of valuable or toxic metals from wastewater (Singh *et al.*, 1998). Since these physico-chemical methods are very expensive, the application of biological methods is emerging as a viable alternative (Muraleedharan *et al.*, 1991). Algae, bacteria, fungi, mosses, macrophytes and several higher plants have been employed for metal recovery from water systems (Shannon and Unterman, 1993; Singh *et al.*, 1998). In an attempt to find more economically viable biological methods, much interest has been focused on finding new organisms able to bind large amounts of metal quickly. The metal binding capacity of algal and bacterial exopolysaccharides is of major concern in metal removal (Lester *et al.*, 1984). Some

cyanobacteria, which produce large amounts of exopolysaccharides, could well be important in the removal and recovery of dissolved heavy metals.

Biosorption consists of two phases: (1) passive adsorption, which is generally a rapid cell surface adsorption, and (2) active uptake, which is an energy-dependent slow process (Rai *et al.*, 1998). There is no significant difference in metal adsorption between living and dead cyanobacterial cells, indicating that the adsorption process does not necessarily involve cell metabolism (Singh *et al.*, 1989; Asthana *et al.*, 1995). The biosorption of metal ions by capsulated cyanobacteria could be due mainly to the presence of the carboxyl groups and hydroxyl groups in the cyanobacterial CPSs. The CPS of *Chroococcus paris* is known to have the ability to remove Cu, Cd and Zn (Les and Walker, 1984). Pretreatment of biomass of the cyanobacterium *P. laminosum* with alkali leads to increased Cr, Pb and Cu metal ion adsorption. The effect of calcium ions on the adsorption of Cu, Cr and Zn metal ions by *P. laminosum* can be considered negligible. The relatively easy desorption of Fe, Cd, Zn and Cu metal ions from *P. laminosum* biomass should allow re-use of the biomass. These characteristics indicate that *P. laminosum* seems to be suitable for use in practical metal depollution systems, even in the presence of high concentrations of Ca^{2+} (Sampedro *et al.*, 1995). Some immobilized cyanobacteria have proved effective in metal removal from solution (Singh *et al.*, 1989; Flemming *et al.*, 1990; Mallick and Rai, 1994). This provides the feasibility of high biomass production and repeated use, important requirements for practical systems.

There have been some reports on the metal biosorption by capsulated *Microcystis*. *Microcystis* is an abundantly occurring water-bloom cyanobacterium in many eutrophic lakes, ponds and reservoirs worldwide. Large amounts of slime are produced during the frequent and extensive *Microcystis* blooms (Gorham and Carmichael, 1988). The available data indicate that *Microcystis* slime can interact strongly with cations due to the presence of galacturonic acid (Nakagawa *et al.*, 1987; Doers and Parker, 1988; Plude *et al.*, 1991). The naturally occurring cyanobacterium *Microcystis* not only has the excellent capacity for metal removal but also has greater affinity for Cd^{2+} than Ni^{2+} (Rai *et al.*, 1998). The affinity of *Microcystis* for Fe^{3+} is also greater than for Cu^{2+} (Singh *et al.*, 1998). Copper sorption by *M. aeruginosa* is largely independent of its metabolic state. Furthermore, heat-killed, HCHO-treated, and air-dried *M. aeruginosa* exhibits substantial sorption of copper, cadmium, and nickel (Parker *et al.*, 1998). Sorption of metals by *M. aeruginosa* is pH dependent. *M. aeruginosa* has greater affinity for copper, followed by nickel and zinc (Subhashree *et al.*, 1998). These data indicate the nuisance cyanobacterium *Microcystis* may be used as metal biosorbant.

The metal sorption by cyanobacteria appears to be both species- and metal-specific. Some exopolysaccharide-forming cyanobacteria may be suitable candidates for removal and recovery of metal from contaminated water.

POTENTIAL APPLICATION RELATED TO ADHESION

Cyanobacteria may be immobilized in columns for industrial processes due to their adhesion to a solid surface (Fattom and Shilo, 1984a). The bioadhesive property of cyanobacterial exopolysaccharides is also of importance in creating novel associations between agronomically important plants and N_2-fixing cyanobacteria (Gantar *et*

al., 1995). In contrast, cyanobacteria are known to be pioneering fouling organisms due to their adhesive capacity (Scott *et al.*, 1996). Serious economic losses have been caused by the significant marine biofouling effects on ships and various equipment placed in the marine environment (Evans, 1981). Cyanobacterial exopolysaccharides may result in the detoxification of antifouling paint, enhanced corrosion of steel surfaces and the conditioning of surfaces for subsequent colonizing organisms (Scott *et al.*, 1996). Prevention of the formation of cyanobacterial slime or capsular layer could be of great importance for antifouling technology.

Concluding remarks

Fundamental studies on cyanobacterial exopolysaccharides are relatively few in number compared to other polysaccharides. There are indeed many gaps in our knowledge on fundamental questions such as the structure, physico-chemical properties and biosynthesis of cyanobacterial exopolysaccharides. Thus, research is urgently needed on these aspects. Answers to these questions will not only be of fundamental interest, but also of critical importance in developing appropriate biotechnology.

Although the monosaccharide compositions of exopolysaccharides present in a number of species of cyanobacteria have been determined, only the structures of four of them have thus far been presented. There is an urgent need to know more about the structure of these polysaccharides. We know little about the biosynthesis of cyanobacterial exopolysaccharides. Hence, research is also urgently needed on the biochemical steps of exopolysaccharide biosynthesis in cyanobacteria. A particularly important question is whether the synthetic mechanisms are similar to those of other bacterial exopolysaccharides. Another question is if the synthetic mechanism of cyanobacterial RPS is different from that of CPS because the monosaccharide compositions of RPSs in most cyanobacteria investigated are different from those of CPSs. The ecological roles of some cyanobacterial exopolysaccharides have been proposed, but the precise role of many exopolysaccharides is still unclear, and more than one role may be possible.

The data currently available indicate cyanobacterial exopolysaccharides have considerable biotechnological importance, but further studies are necessary to evaluate the feasibility of their practical application. The marked differences and the complexity in monosaccharide composition in cyanobacterial exopolysaccharides suggest some of them may possess special chemical or rheological properties and offer certain advantages over other commercially available gums. However, extensive studies on the structure, the rheological properties, the molecular and rheological stability of cyanobacterial exopolysaccharides to temperature, pH, and salt concentration are all necessary for establishing the potential of cyanobacteria as a new source of natural biopolymers. In terms of the utilization of cyanobacterial exopolysaccahrides as biosorbant, the natural system is usually much more complex than ideal experimental systems. This may limit the practical application of cyanobacterial exopolysaccharides as a metal-chelating agent. Thus, further work is needed to determine whether cyanobacterial exopolysaccharides have high and selective affinities for metal ions in a given natural system. It is proposed that the use of microscopic soil (edaphic) algae as biofertilizers seems to offer the only realistic hope of halting and reversing desert encroachment in the semi-arid regions. The exopolysaccharides produced by edaphic

cyanobacteria are of central significance in soil neogenesis (Painter, 1993). Further field tests of their water-retaining and particle-aggregating properties are essential for the development of soil conditioner biotechnology.

Exopolysaccharide mutants of cyanobacteria *Synechocyctis* strain PCC 6803 and *Cyanothece* sp. BK68K have been successfully isolated (Panoff and Joset, 1989; Reddy *et al.*, 1996). In order to make cyanobacterial exopolysaccharide more suitable for certain applications, the production, structure and physico-chemical property of cyanobacterial exopolysaccharide could be modified through genetic method.

Cyanobacteria are known to be present in every ecosystem, including extreme environments (Carr and Whitton, 1982). This capacity of cyanobacteria is of particular interest, giving the possibility of utilizing under-exploited resources for the production of valuable chemicals. The production of exopolysaccharides by some halophilic or halotolerant cyanobacteria suggests a possible approach to obtain new under-explored resources from hypersaline environments (Rachel and Elisha, 1985; Liu and Lin, 1993; De Philippis *et al.*, 1993, 1998; Sudo *et al.*, 1995).

Some cyanobacteria, such as *N. commune* in China, *Aphanothece sacrum*, *Nostoc verrucosum*, *N. commune* and *Brachutrichia* in Japan, have been utilized as a human food (Lee, 1989). *Spirulina* has become a most nutritious food source because it contains much good-quality protein as well as carotenoids, vitamins, fatty acids and minerals (Hayashi and Hayashi, 1996). The success in the commercial production of *Spirulina* indicates that production and utilization of cyanobacteria are reasonable and practical. As a final comment, *Spirulina* is a promising source for the commercial production of high-value phycobilin pigments for colouring foodstuffs. Thus, it appears to be reasonable to carry out a multi-product strategy when we study the use of cyanobacterial exopolysaccharides.

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