Carbohydrate Polymers **12** (1990) 127–153

Review Paper

Polysaccharides from Cyanobacteria†

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(Received 14 November 1988; revised version received 15 February 1989; accepted 17 February 1989)

ABSTRACT

This review deals with the different glycans produced by cyanobacteria such as reserve polymers, polysaccharides from the cell envelope and exocellular polysaccharides. For each group a description of previous work is reported, quoting all different strains tested, methods of polysaccharide extraction and chemical and physical characterization. In particular, chemical composition and sequence (when known) are reported although inhomogeneity of results reveals that sometimes methods used are outdated. Emphasis is put on exocellular polysaccharides, the most interesting type of polymers from the technological point of view.

INTRODUCTION

Cyanobacteria are the most widely distributed photosynthetic prokaryotes in nature. These microorganisms live in seawater, freshwater, on rocks and also in environments where climatic conditions may

†This paper is Part 1 in a series on: Polysaccharides from Cyanobacteria. Part 2, pp. 169–87.

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Carbohydrate Polymers 0144-8617/90/$03.50 — © 1990 Elsevier Science Publishers Ltd, England. Printed in Great Britain

hamper the development of superior plants. They are constituted of a cell typical of Gram-negative bacteria with a photosynthetic apparatus similar in functional and structural respects to that contained in the eucaryotic chloroplast (Stanier & Cohen-Bazire, 1977). This peculiar property causes much interpretative controversy between microbiologists and botanists; hence in many works these microorganisms are sometimes named cyanobacteria and sometimes blue-green algae.

The discovery of fossils dating back to 3500 million years ago suggests that the beginning of cyanobacteria evolution may be established approximately at that date (Carr & Whitton (eds.), 1982). Some cyanobacteria are morphologically and structurally comparable to photosynthetic microorganisms of 2800 million years ago, thus indicating scarce evolution if compared, for example, to green-algae which have undergone greater evolution although they appeared more recently.

Many cyanobacteria have been used as a food source for a long time (Lem & Glick, 1985). More recently, they have been proposed as a valuable food supplement. *Spirulina* for example, in addition to being a rich source of vitamin B_{12}, also contains significant amounts of vitamins B_1, B_2 and, in some species, of vitamin A. The proteins of some species of *Spirulina* are easily digestible and the amino acid content compares favourably with that contained in traditional foods (egg, soybean). Moreover, cyanobacteria produce a series of useful chemical compounds like phycobiliproteins, phosphorescent dyes, drugs and vaccines (Gudin & Thepenier, 1986; Ramus & Jones (eds.), 1988). Finally, the fact that cyanobacteria are able to produce O_2 and, in some cases, exocellular compounds, that specifically bind metal cations, has proved useful in waste-water management (Lem & Glick, 1985). The use of cyanobacteria in biotechnological applications as biofertilizers and in desert reclamation (Lem & Glick, 1985) is due to the adaptability of these microorganisms to wide ranging habitats and their ability to fix atmospheric nitrogen and to stimulate the synthesis of plant growth promoters.

Like many microorganisms, cyanobacteria produce polysaccharides (Harada, 1985; Slodki, 1987; Sutherland, 1987; Zevenhuizen, 1987). Cyanobacterial polysaccharides are divided into three groups (Painter, 1983*a*): storage, cell envelope and exocellular polysaccharides. The cell envelope is properly divided into the cell wall and the external layers, which comprise sheath, capsule and slime. The definition of these different layers and the extraction of the polysaccharides are given in detail in the section on Cell Envelope Polysaccharides.

While some other microbial polysaccharides have been extensively studied (Sutherland, 1985; Brant & Marchessault, 1987; Sutherland,

1987; Whitfield, 1988), cyanobacterial polysaccharides are reported in literature mainly for their monosaccharidic composition. Their structure and properties are described only in some rare cases.

The purpose of this review is to report and to discuss the most important results achieved in literature to date. In fact, to our knowledge, an exhaustive review on cyanobacterial polysaccharides complete with composition, structure and property data and/or applications has not been published yet. It was deemed advisable to collect and to comment on works available in literature for the sake of forwarding further studies.

CELLULAR MORPHOLOGY

The vegetative cyanobacterial cell, schematically shown in Fig. 1, is surrounded by a multilayer cell wall typical of the Gram-negative type. The photosynthetic apparatus is located in thylacoids, which are the site of pigments accounting for the photochemical reaction. Attached to the thylacoids there are organelles known as phycobilisomes containing phycobiliproteins which are light-harvesting pigments. In some cyanobacteria thylacoids and phycobilisomes are not present. In this case phycobiliproteins are located on the inner surface of the cellular membrane. Granules containing reserve compounds (for example, cyanophycin, polyphosphate, glycogen and in some cases poly-β-hydroxybutyrate granules) are found within the cytoplasm. Carboxysomes, prokaryotic organelles responsible for CO_2 fixation, are also present.

The vegetative cells of some cyanobacteria can differentiate into two types of cells (heterocysts and akinetes), with distinguishable morphologies and structures. Heterocysts are responsible for nitrogen fixation. Akinetes are structures allowing the survival of cyanobacteria in harsh environmental conditions; their germination occurs whenever the circumstances favour growth.

Cyanobacteria have been classified by Rippka *et al.* (1979) in five sections on the basis of their structure and reproduction. According to their structure, cyanobacteria are divided into unicellular and filamentous microorganisms. The former are either single or aggregate cells; the latter are made up of a cell chain known as a trichome. Unicellular cyanobacteria are further subdivided into two subgroups: in the first group, reproduction takes place by binary fission or gemmation (section I); in the second, reproduction takes place by multiple fission giving rise to smaller daughter cells known as baeocytes (section II). Filamentous

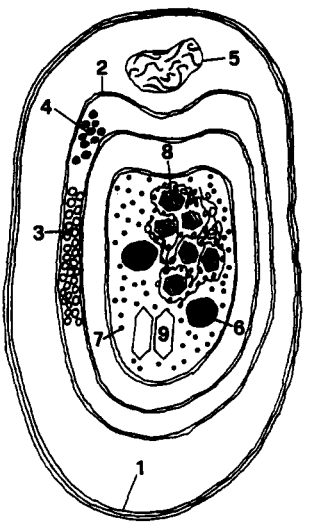

Fig. 1. Schematic representation of the vegetative cell. 1, Cell membrane; 2, thylacoid; 3, phycobilisomes; 4, glycogen granules; 5, cyanophycin granules; 6, polyphosphate granules; 7, ribosomes; 8, carboxysome surrounded by nucleoplasm; 9, gas vescicle.

cyanobacteria reproduce by random trichome breakage thereby forming hormogonia, which are small chains of cells (sections III, IV, V). The cyanobacteria of sections IV and V may also at times reproduce by akinete germination. Table 1 shows the genera according to the classification by Rippka *et al.* (1979).

STORAGE POLYSACCHARIDES

Microorganisms accumulate polysaccharides as carbon and energy reserve for cells. It has been quoted (Painter, 1983*a*) that the first indication of the presence of glucydic type reserve material in cyanobacteria dates back to 1938. In 1952 an amylopectin-like glucan was detected in a species of *Oscillatoria* (Hough *et al.*, 1952). Its average chain length between branch-points was 23–26 glucose units, according to the method used. More recently (Chao & Bowen, 1971) electron microscopy investigations revealed the presence of α-granules containing glycogen-like polysaccharide in the filamentous cyanobacteria *Nostoc muscorum*. The α-granules, isolated by means of sucrose density gradient centrifugation, are uniform in size and shape (on average 31 nm in diameter and 65 nm in length). Acid hydrolysis of these granules, which still contained small amounts of proteins (3%) notwithstanding purification, revealed the presence of glucose in amounts greater than 95% of granules dry weight. The digestion of granules with amylase

TABLE 1
Classification of Cyanobacteria According to Rippka *et al.* (1979)

Section I	*Gloeobacter*
	Gloeothece
	Cyanothece[a]
	Synechococcus
	Cyanobium[a]
	Cyanobacterium[a]
	Synechocystis
	Chamaesiphon
	Gloeocapsa
Section II	*Dermocarpa*
	Xenococcus
	Dermocarpella
	Myxosarcina
	Chroococcidiopsis
	Pleurocapsa
Section III	*Spirulina*
	Oscillatoria
	Gruppo LPP-A[b]
	Gruppo LPP-B[b]
	Pseudanabaena
Section IV	*Anabaena*
	Nodularia
	Cylindrospermum
	Nostoc
	Scytonema
	Calothrix
	Cyanospira[c]
Section V	*Fischerella*
	Chlorogloeopsis

[a]Quoted by Florenzano (1984).
[b]Lyngbya — Phormidium — Plectonema.
[c]New genus according to Florenzano *et al.* (1984).

yields maltose and α-macrodextrin in amounts ranging from 11 to 14% of the polysaccharide weight. Since α-amylase cannot break $\alpha(1-4)$ linkages neighbouring $\alpha(1-6)$ linkages, a high yield in α-macrodextrin reflects the high complexity in the branching of the polysaccharide contained in the α-granules. The staining of granules with iodine gives a UV absorption maximum at λ max 410 nm, comparable with that of the

iodine-glycogen complex obtained from other sources. Periodate oxidation revealed that the average length of these glycogen-like chains is about 13 glucose units, in agreement with the 10–14 unit range reported by Merrick (1979) for other bacterial glycogen.

The glycogen produced by the unicellular cyanobacteria *Anacystis nidulans* (genus *Synechococcus*) (Weber & Wöber, 1975) has been isolated and studied. This glycogen has been debranched by bacterial isoamylase; the oligosaccharides thereby obtained have been separated by means of gel filtration chromatography. The glycogen isolated from *Anacystis nidulans* has an average chain length of 9 glucose residues and a chain distribution, after the debranching treatment, which more closely resembles that of amylopectin fractions obtained from sweet corn than that of glycogen obtained from bacterial sources (*Arthrobacter, Escherichia coli*).

Recently (Casu *et al.*, 1980), the storage polysaccharide of *Spirulina platensis* was studied. The polysaccharide was obtained by extraction with water, after the removal of lipids, carbohydrates and low-molecular weight polyalcohols. The acidic hydrolysate of the purified polysaccharide only contained glucose. The structural characterization of glucan was carried out with classic chemical methods (periodate oxidation, optical rotation and iodine absorption) and with ^{1}H, ^{13}C-NMR and IR spectra. The results show that the polymer is constituted of chains made of $\alpha(1-4)$ glucose (85% of glucose) and $\alpha(1-6)$ branches. The polysaccharide was degraded by β-amylase, yielding maltose and a limit-dextrin as major products of enzymolysis.

CELL ENVELOPE POLYSACCHARIDES

Like the cells of many Gram-negative bacteria, cyanobacterial ones are surrounded by many layers playing a fundamental role in the interaction of cells with their environment. These layers constitute the cell envelope comprising the cytoplasmic membrane, the cell wall, and the external layers. Figure 2 shows a schematic diagram of the cell envelope of a cyanobacterium.

The cytoplasmic membrane and the cell wall are typical structures existing in the cell of every bacterium. Their function is to allow the flow of substances from the outside to the inside of the cell and vice versa.

The cell wall, constituted of peptidoglycan, proteins and lipopolysaccharides, determines and maintains the size and shape of the cell. These lipopolysaccharides have an overall structure similar to that of many other lipopolysaccharides of Gram-negative bacteria, and are

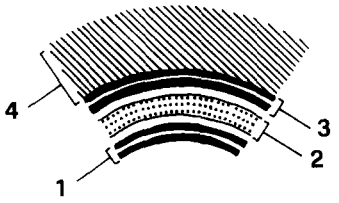

Fig. 2. Schematic diagram of the cell envelope. 1, Cytoplasmic membrane; 2, cell wall; 3, sheath; 4, capsule and slime.

formed by different types of sugars with amide-linked β-hydroxy-fatty acids (Stanier & Cohen-Bazire, 1977).

The external layers comprise sheath, capsule and slime (Martin & Wyatt, 1974). The sheath is a thin uniform layer with an homogeneous fibrillar structure; it surrounds the cell wall reflecting the shape of the microorganism and is visible even in the absence of staining. Extraction of the sheath is usually carried out by differential and sucrose gradient centrifugation of the homogenized cells.

The capsule is a non-uniform thick layer surrounding the sheath. Solubilization of the polysaccharide contained in the capsule is achieved by warm water treatment of the cell pellet. The slime is the most external layer which lacks definite margins although it is physically detectable as mucillagine.

Figure 3 shows schematic extraction procedures of the different cyanobacterial polysaccharides.

Many authors did not properly define the external layers into sheath, capsule and slime; therefore the structural and functional differences among these layers are not always evident in the literature. Concerning the nomenclature of the external layers, the assignment sometimes appears to be arbitrary and the generic name, cell investment has also been used by some authors to indicate the wall external layers. When these layers are present (Rippka *et al.*, 1979), they are thought to protect the cell from unfavourable environmental conditions. They are at times involved in the formation of pluricellular organisms and in the motility of microorganisms.

Among the macromolecular constituents of the cyanobacterial cell envelope, studies have concentrated on polysaccharides contained in the sheath and in the cell wall. However, these studies have been limited to the composition in monosaccharides. Furthermore, in most cases, these studies have not always been carried out on an isolated polysaccharide; often enough the reported composition in monosaccharides refers to the whole cell wall or the external layers. With a few exceptions, information on the structure of polysaccharides in the cell envelope is very scarce and very little is known about the physico-chemical properties of these polysaccharides.

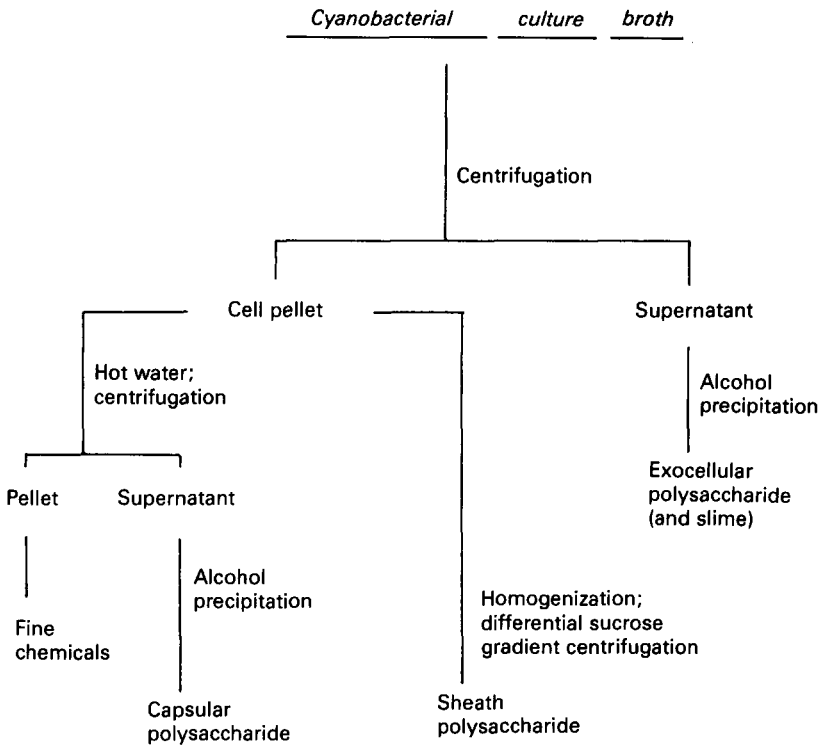

Fig. 3. Schematic extraction procedures of the cyanobacterial polysaccharides.

Notwithstanding the forementioned confusion, it seems proper to report separately on the polysaccharide of the cell wall and from each external layer (sheath, capsule and slime). This choice allows one to note, whenever the data is available, differences between the polysaccharides of the various layers.

Cell wall

The cell envelope polysaccharides which received more attention, especially from a structural point of view, are the ones produced by *Anabaena cylindrica*. Dunn and Wolk (1970) isolated and studied the polysaccharides contained in the walls of vegetative cells, of the heterocysts and of the akinetes of this filamentous cyanobacteria. Paper chromatography yielded the sugar composition of these polysaccharides. Glucose is the major sugar found in the glycans of the cell wall of heterocysts and of akinetes, whereas in the walls of vegetative cells mannose is

the major component. Fucose is also detected in the latter whereas it is not to be found at all in other cases. Galactose and xylose were also found in all these polysaccharides. The composition of the polysaccharide of the heterocyst cell wall was found to be similar to that of the akinete cell wall. Cardemil and Wolk (1976; 1979) confirmed these results, obtained for the polysaccharides of heterocysts and akinetes, showing the chemical and structural identity of these two polymers. Figure 4 shows the structure proposed by the authors, by means of enzymatic hydrolysis and classical methods of structure determination. The backbone consists of mannose and glucose linked by $\beta(1-3)$ glycosidic bonds in a 1:3 molar ratio. Mannose, glucose, galactose and xylose are at terminal positions in the side chains.

Polysaccharides in the heterocyst and akinete cell walls of *Cylindrospermum licheniforme* and *Anabaena variabilis* have also been studied (Cardemil & Wolk, 1981). With the exception of the polysaccharide from the akinetes of *Cylindrospermum licheniforme*, which is a galactan with terminal glucose residues in its side branches, the composition in the monosaccharides of the backbone is always identical to that of the *Anabaena cylindrica* polysaccharides. Terminal residues in their side branches are to a certain extent similar to those identified for *Anabaena cylindrica*, but *Anabaena variabilis* has a terminal arabinosyl residue instead of a mannosyl residue and in *Cylindrospermum licheniforme* the two branches linked to a glucose of the backbone are not present. The authors conclude that with the exception of the polysaccharide from the

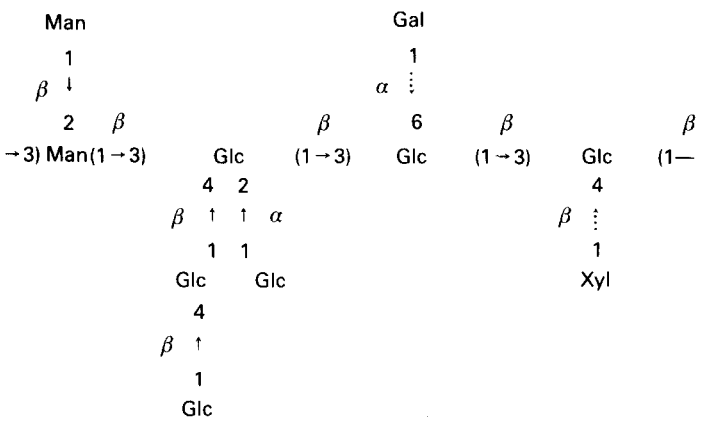

Fig. 4. Approximate repeating unit structure of the polysaccharide from the cell envelopes of heterocysts and akinetes of *Anabaena cylindrica* (Cardemil & Wolk, 1976 and 1979). Dashed lines indicate an incomplete substitution.

akinete cell wall of *Cylindrospermum licheniforme*, the structure of all other polysaccharides is similar to the one proposed for *Anabaena cylindrica*, as shown in Fig. 5.

A glucose and mannose rich polysaccharide has been isolated from the cell wall of a strain of *Nostoc* (strain 221, IARI, India) (Mehta & Vaidya, 1978). Paper chromatographic separation of the polysaccharide yielded glucose, glucuronic acid, mannose, arabinose, galactose, rhamnose, xylose in amounts of 5·7, 3·2, 2·7, 1·2, 0·3, 0·3, 0·1% respectively, accounting for 13% of cell dry weight. Traces of ribose and galacturonic acid were also detected. The composition of this cell wall polysaccharide differs from that of the polysaccharide isolated from the cell wall of *Nostoc muscorum* strains in the 1950s (Biswas, 1957). Such a polysaccharide is made up of glucose, galactose, xylose, arabinose, ribose, rhamnose and two non-identified sugars. These composition discrepancies have been ascribed to different extraction techniques and different analysis methods (Mehta & Vaidya, 1978).

The presence of mannose in the cell wall of some cyanobacteria has also previously been observed by Höcht *et al.* (1965) in the cell walls of *Anacystis nidulans, Phormidium faveolarum* and *Tolypothrix tenuis* (genus *Calothrix*). The cell wall of these species are constituted of murein (50%) and of polysaccharides ($\approx$ 50%) containing respectively: mannose; mannose and glucose; mannose, glucose and xylose.

A polysaccharidic fraction of cell wall of a strain of *Anacystis nidulans* was extracted with the same procedure as above, with the only difference that, after the phenol/water extraction, the polymer was recovered by ethanol precipitation but no further purification by enzymatic treatment was carried out (Drews & Gollwitzer, 1965). The presence of mannose was evidentiated by paper chromatography together with glucose and two other non-defined neutral sugars.

Another mannose-rich polysaccharide, presumably belonging to the cell wall of *Chlorogloeopsis* PCC 6912 (Schrader *et al.*, 1982*a*), was extracted by phenol/water with a 0·8% biomass yield. A chromatographic and mass-spectrometric analysis revealed a high mannose content together with 6-*O*-methyl-D-mannose, uronic acids and other monosaccharides.

An unusual homopolysaccharide has been isolated and characterized from the cell wall of the cyanobacterium *Spirulina platensis* (Van Eykelenburg, 1978), already mentioned as a food supplement and fine chemical source. The homopolysaccharide was obtained with a low yield of less than 1% of its cell dry weight. It was proposed to contain only $\beta(1-2)$ glucose on the basis of comparison with a glucan isolated from *Agrobacterium tumefaciens*, which, indeed, produces also in larger

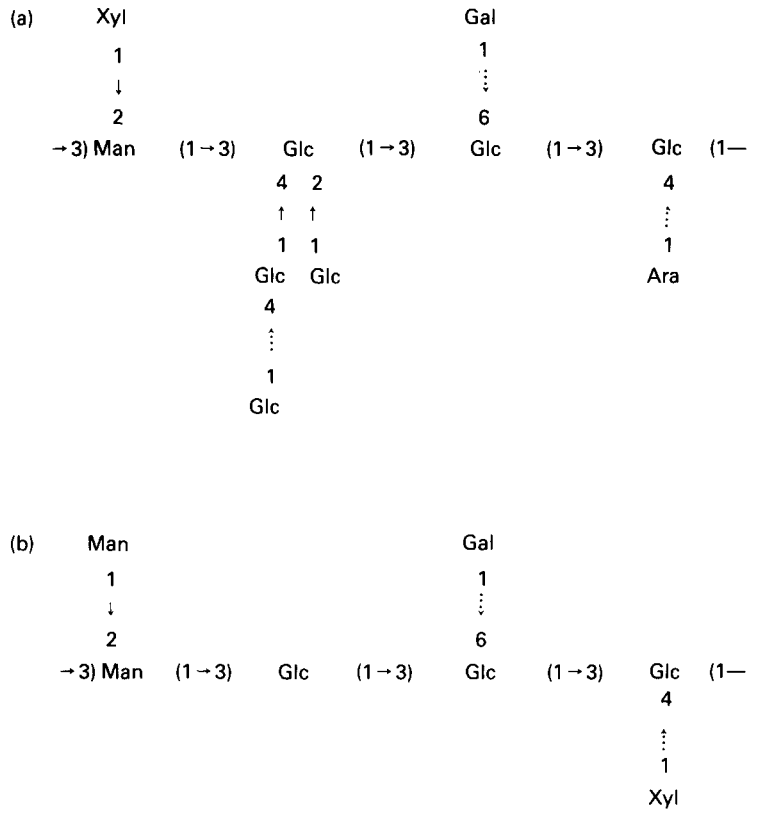

Fig. 5. Possible structures of the polysaccharides from (a) the cell envelope of heterocysts and akinetes of *Anabaena variabilis* and (b) the cell envelopes of heterocysts of *Cylindrospermum licheniforme* (Cardemil & Wolk, 1981). Dashed lines indicate an incomplete substitution.

amounts high molecular weight glucans (Hisamatsu *et al.*, 1977). It must also be quoted that $\beta(1-2)$glucans are produced by soil microorganisms, such as *Rhizobium*, and have later been demonstrated to have cyclic oligomeric structure (Himasatsu *et al.*, 1982).

Phosphodiester linkages were detected between a peptidoglycan and a polysaccharide isolated from the non-ensheathed unicellular cyanobacterium *Synechocystis* PCC 6714 (Jürgens & Weckesser, 1986). The molecular weight of the free polysaccharide is about 3×10^4 and the sugar composition determined by gas chromatography gives mannose, glucose, and three aminosugars (glucosamine, galactosamine, mannosamine). In addition to mannose and glucose, aminosugars (glucosamine, mannosamine) were also found in another peptidoglycan-linked polysaccharide from *Gloeobacter* PCC 7421 (Schneider *et al.*, 1988).

Sheath

A large number of polysaccharides from the external layers studied, are those from the sheath. Studies of the composition of the sheath of *Anabaena cylindrica* (Dunn & Wolk, 1970) have evidenced a substantial amount of carbohydrates accounting for almost the total weight of the sheath. The monosaccharidic composition is distinctly different from that of the cell wall, although the type of sugar is the same (i.e. glucose, mannose, galactose, xylose).

In *Chlorogloeopsis* PCC 6912 (Schrader *et al.*, 1982*b*) a sheath fraction (20–30% of total biomass) has been isolated in order to analyse its carbohydrate content and composition by chromatography. According to the authors, the sheath carbohydrates, accounting for 38% of its dry matter, belong to two different polysaccharides. The major one was not separated from the protein moiety of the sheath by hot phenol/water. It contained glucose as its main constituent in addition to other monosaccharides. The other polysaccharide contained galactose as a major constituent in addition to other sugars.

The presence of methyl-glycosides is not to be considered as an exception in the constituents of cell envelope polysaccharides. For example, studies have been carried out on sugars of the polysaccharidic fraction of the sheath of the unicellular cyanobacteria *Chroococcus minutus* SAG B.41.79 (genus *Gloeocapsa*) (Adhikary *et al.*, 1986) and *Gloeothece* PCC 6501 (Weckesser *et al.*, 1987).

In the case of *Chroococcus minutus* the presence of glucose and of four *O*-methyl substituted sugars was identified. The sheath obtained by centrifugation, appears as a thin gelatinous layer in the sediment; it yielded a 28% cell dry weight and was purified by removing cell wall components by lysozyme and detergent treatment.

The sheath isolated from *Gloeothece* PCC 6501 was obtained in a 6% amount of its cell dry weight. It contains a carbohydrate fraction whose thin-layer chromatographic and mass-spectrometric analysis showed the presence of uronic acids and various neutral sugars, in particular 2-*O*-methyl-D-xylose. After further purification in order to remove any cell wall contaminants, the sugars revealed themselves to be the same as those previously identified with a slight increase in glucose and galactose.

Concurrently, 2-*O*-methyl-D-xylose was also found in the composition of the polysaccharide isolated from the sheath of cells of *Gloeothece* ATCC 27152 (Tease & Walker, 1987; Tease *et al.*, 1988). The total amount of carbohydrates, containing neutral sugars and uronic acids, accounts for 41 or 55% in sheath dry weight, respectively, when the sheath is extracted from cells cultivated with or without nitrate. The

neutral sugars composition remains the same for materials extracted from the two cultures. The presence of pyruvic acid, *O*-methyl and *O*-acetyl groups and sulphate shows a high level of complexity of the sheath polysaccharidic material. It was observed that the sheath polysaccharide is able to bind cadmium ions. This property depends on culture conditions: the polysaccharide of the sheath extracted from cells grown in the presence of nitrate is able to bind greater amounts of cadmium. According to the authors this property may be linked to the presence of functional groups which might represent the binding sites for the cations.

Polysaccharides of the sheath of *Fischerella* PCC 7414 (Pritzer *et al.*, 1988) and *Gloeobacter* PCC 7421 (Schneider *et al.*, 1988) have recently been studied. Both glycans are composed of various neutral sugars, uronic acids and in the case of *Fischerella*, glucosamine.

Wang & Hill (1977) carried out paper and gas-chromatography of the phenol/water extract from cells of *Anabaena flos-aquae* A3 and found a heteropolysaccharide composed of mannose and glucose (5:1 molar ratio) in the organic phase, and a glucan in the aqueous phase, both without further characterization. Whether these polysaccharides belong to the sheath or to the cell wall is not clear.

Capsule and slime

Little data is available in literature regarding the polysaccharides from the capsule and slime of cyanobacteria.

Different species of the genus *Nostoc* have been proved to produce mucillagineous capsule and slime.

Nostoc 221, IARI, India (Mehta & Vaidya, 1978), already mentioned, has been observed to produce a capsular polysaccharide in addition to cellular and exocellular polysaccharides. The capsule was solubilized by treating the cell pellet with hot water. The extract was very viscous and upon concentration set to a gel below 20°C.

The hot-water treatment was also used for the extraction of a polysaccharidic fraction from *Nostoc calcicola* Gietler, strain 79WA01 (Flaibani *et al.*, 1989). This filamentous cyanobacterium produces a mucilage in an amount of $\approx$ 70% of its biomass. The hot water treatment of the cells isolates a fraction of the exocellular products, which is composed of a glycuronoglycan moiety in addition to an arginine-rich polypeptide moiety. This material, previously purified, was divided into acid-soluble (30%) and acid-insoluble (70%) fractions by carefully adjusting the pH value of an aqueous solution to two. The behaviour of the hot-water extract in acidic media in addition to other findings leads to the conclusion that glycuronoglycan and polypeptide moieties are

bonded, although the precise nature of the carbohydrate-peptide bonding has yet to be established. The sugar analysis of the whole hot-water extract by using gas-chromatography reveals glucose, galactose, xylose, glucuronic acid, fucose, mannose, arabinose, galacturonic acid and rhamnose. This composition varies quantitatively in the different fractions. Glucose is the major component in both acid-soluble and acid-insoluble fractions. Xylose is the most abundant pentose and the content of glucuronic acid is twice the galacturonic acid in all fractions.

Martin and Wyatt (1974) have classified a large number of *Nostoc* strains by means of the amount of slime; some of these strains do not produce any slime at all. The amount of slime was correlated with the motility of the microorganisms; the composition of the slime constituents has not been reported.

Two species of the genus *Cyanospira* have been isolated and characterized (Florenzano *et al.*, 1985); the cells of *Cyanospira rippkae* lack the capsule, whereas *Cyanospira capsulata* (ex *Anabaena spiroides*) have been found to produce an external capsular layer of considerable thickness. The capsular polysaccharide produced by *Cyanospira capsulata* has recently been studied (Sili *et al.*, 1984). The extracted polysaccharide, accounting for more than 90% of the total biomass carbohydrates, has a monosaccharidic composition which is the same as that found for the exocellular product (see next paragraph). A preliminary rheological characterization of this polymer reveals interesting properties comparable with those of the exocellular polysaccharide (Cesàro *et al.*, unpublished).

In some cases (Hough *et al.*, 1952; Tischer & Moore, 1964) products designated as mucilage or capsular polysaccharide have been extracted by using methods not commonly used; these extractions can therefore lead to products that can be slime or even exocellular polysaccharide as well as capsular polysaccharide.

EXOCELLULAR POLYSACCHARIDES

Like other microorganisms (Sandford & Laskin (eds.), 1977), many cyanobacterial species secrete various amounts of organic compounds. The polysaccharides can be soluble in the media and/or form a gelatinous layer around the cells, bestowing particular rheological properties to the culture broth. Thanks to the industrial success of some microbial polysaccharides, there is a continuous interest in new polymers from other bacterial species, including cyanobacteria. However, the properties of some cyanobacterial exocellular polysaccharides were only recently

and partially investigated. Usually, exopolysaccharides are extracted in high yields by alcohol precipitation from cell-free supernatants, with a simpler laboratory process than the one used for the isolation of cell envelope polysaccharides.

As in the case of cell wall and external layers polysaccharides, it is possible to find much literature about the composition in monosaccharides of exocellular polysaccharides, whereas to our knowledge, no definite structural study has been published.

One of the first exopolysaccharides which had been studied, extracted from *Anabaena cylindrica* in the 1950s (Bishop *et al.*, 1954), contained glucose, galactose, arabinose, xylose, rhamnose and uronic acids. Such polysaccharide had been extracted with a procedure which could have isolated slime and/or capsule material too.

Paper chromatography of the exocellular polysaccharide extracted from a culture of *Palmella mucosa* (Tischer & Moore, 1964) in exponential growth phase yielded glucose, fucose, arabinose, and glucuronic acid in molar ratios of 11:6:3:1. However this composition can vary in quantity and quality by changing the carbon and nitrogen sources. This polysaccharide is produced in amounts of 400 mg/litre of growth medium after 14 days.

Mehta and Vaidya (1978) report the data obtained on the polysaccharide secreted exocellularly by a strain of *Nostoc*. This polysaccharide is produced in amounts depending on both the culture conditions and fermentation time; it accounts for 14–18% of its cell dry weight, and it is the largest polysaccharidic fraction produced by this cyanobacterium. The exopolysaccharide, containing glucuronic acid, glucose and xylose, differs greatly from the cell wall polysaccharide isolated from the same organism (see above), a fact which led the authors to raise the question of the existence of a selective mechanism involved in the excretion of exopolysaccharides.

The exocellular polymers produced by *Nostoc calcicola* Gietler, strain 79WA01, already mentioned, have been extensively studied by Flaibani *et al.* (1989). Addition of cetyltrimethylammonium bromide to the cell-free centrifugates co-precipitated a polypeptide in addition to a glycuronoglycan moiety. All attempts to separate the glycuronoglycan and polypeptide components by physical methods were unsuccessful. The reported findings provided convincing evidence of close association between these exopolymers. The sugar composition of the glycuronoglycan moiety is qualitatively identical to that obtained for the hot-water extract already mentioned; in both cases the molar ratios of the nine different kinds of sugar residue suggest considerable irregularity in the structure.

Anabaena flos-aquae A37 (Moore & Tischer, 1965; Wang & Tischer, 1973) secretes two exopolysaccharides with a total yield of 250 mg/litre of culture into the medium. They have been separated by ion exchange chromatography and identified by paper chromatography and colorimetric reactions. Glucose was the major component in both polysaccharides. One of them was neutral and contained glucose and xylose (approximated molar ratio 8:1). The other was acidic and contained glucose, xylose, ribose and a non-identified uronic acid (approximated molar ratio 6:1:1:10).

Apart from exopolysaccharides of which only the composition in monosaccharides is known, there is a small number of cases in which the physico-chemical properties have been and are still being studied.

The cyanobacterium *Phormidium* J-1 (Fattom & Shilo, 1984; Fattom & Shilo, 1985; Bar-Or & Shilo, 1987) was isolated from a drainage canal in Israel in 1984; it produced a high molecular weight ($1{\cdot}2 \times 10^6$) exocellular polysaccharide, called emulcyan, with very interesting flocculating properties. It contains rhamnose, mannose and galactose in molar ratio of 1:2:0·5 respectively, and a non-identified uronic acid which is neither galacturonic, glucuronic or mannuronic. Notwithstanding purification, proteins and fatty acids still remained linked to the polysaccharide. Moreover, sulphate groups which have never before been found in microbial exopolysaccharides were present. The fact that sulphate groups have previously been found in many polysaccharides extracted from eukaryote organisms (red macroalgae, for example) suggests the intermediate position of cyanobacteria between prokaryotes and photosynthetic eukaryotes. Sulphate groups are important for flocculant activity, indeed the desulphation of polysaccharides leads to an 80% loss of flocculant activity.

The same authors have extracted exocellular polysaccharides from *Anabaenopsis circularis* (genus Nostoc) and *Calothrix desertica* (Bar-Or & Shilo, 1987). Both polysaccharides showed flocculating properties. The first contained neutral sugars and keto acid residues, but no sulphate groups were found. The composition of the second is not known. It is worth mentioning that secretion of the polysaccharide from *Anabaenopsis circularis* does not occur in certain culture conditions. The flocculating activity of the polysaccharide of *Phormidium* J-1 is greater than that of *Anabaenopsis circularis*, since its anionic density (sulphate groups and acids) is much higher. The authors have also indicated a few possible applications for these polysaccharides: reduction of solid matter in water reservoirs, soil conditioning for the improvement of the water-holding capacity in dry soils and waste-water management.

Other exocellular polysaccharides were extracted from cultures of two different strains of *Synechocystis* PCC 6803 (Panoff & Joset, 1988) and PCC 6714 (Panoff *et al.*, 1987) with yields ranging from 160–350 mg/litre of culture according to the conditions. These anionic polysaccharides have been reported to have gelling properties; the study of their composition indicates the presence of a large number of monosaccharides including sulphate residues.

Another interesting polysaccharide was extracted from *Cyanospira capsulata* ATCC 43193 (Sili *et al.*, 1984; Florenzano *et al.*, 1985; De Philippis *et al.*, 1988), a microorganism, isolated in 1983 from an alkaline soda lake in Kenya, which produces an abundant and highly viscous slime in the culture medium. The exocellular polysaccharide is excreted in the medium independently of the growth phase with a 3 g/litre yield 9 days after inoculation. The polysaccharidic fraction was isolated and characterized by gas-chromatography yielding arabinose, fucose, mannose, glucose and galacturonic acid; the exopolysaccharide also contains pyruvil residues. A rheological analysis of the purified polymer characterized its viscoelastic properties (Bertocchi *et al.*, 1988; Navarini *et al.*, 1990), which are comparable with other microbial polysaccharides already on the market, and preliminary technological tests of the exocellular product proved its good suspending and emulsifying properties.

CONCLUSIONS

This review is intended to cover the little attention which has been paid till now to polysaccharides from Cyanobacteria. Indeed, some preliminary physico-chemical properties, especially in exopolysaccharides, suggest that it is worthwhile to carry out further studies in this new field.

The collection of the published papers presently reported reveals interesting features when a critical comparison is made among the polysaccharides belonging to the same group (storage, cell envelope, exocellular). Among the cell envelope polysaccharides, with separate analyses of cell wall, sheath, capsule and slime polymers, some correlation is found if strains are classified according to the sections proposed by Rippka *et al.* (1979).

Concerning the monosaccharidic composition of the polysaccharides from the cell wall (Table 2) the presence of glucose, which occurs in all microorganisms mentioned, is remarkable. Mannose is present in most cases whereas galactose and xylose are very frequent only in the section IV. Furthermore most of these polysaccharides do not contain uronic acids. Only one case of homopolysaccharides was found in the cell wall.

TABLE 2
Carbohydrates Found in the Cell Wall Polysaccharides

	Glc	*Gal*	*Man*	*Ara*	*Xyl*	*Rib*	*Rha*	*Fuc*	*Ur.A*	*M-G*[a]	*N-G*[b]	*Reference*
Section I												
Gloeobacter PCC 7421	*		*								*	Schneider *et al.*, 1988
Synechocistis PCC 6714	*		*								*	Jürgens & Weckesser, 1986
Anacystis nidulans	(*)[c]		*								*	Höcht *et al.*, 1965; Drews & Gollwitzer, 1965
Section III												
Spirulina platensis	*											Van Eykelenburg, 1978
Phormidium faveoloarum	*		*									Höcht *et al.*, 1965
Section IV												
Anabaena cylindrica												Dunn & Wolk, 1970; Cardemil & Wolk, 1979
— heterocysts & akinetes	*	*	*		*							
— vegetative cell	*	*	*		*			*				
Anabaena variabilis												Cardemil & Wolk, 1981
— heterocysts & akinetes	*	*	*	*	*							

Cylindrospermum licheniforme										Cardemil & Wolk, 1981
— heterocysts	*	*	*		*					
— akinetes	*	*								
Nostoc 221	*	*	*	*	*		*	*[d]		Mehta & Vaidya, 1978
Nostoc muscorum	*	*		*	*	*	*			Biswas, 1957
Tolyphotrix tenuis	*		*		*					Höcht *et al.*, 1965
Section V										
Chlorogloeopsis PCC 6912	*	'[f]	*	'	'		*	*[d,e]	*	Schrader *et al.*, 1982

[a]M-G: Methyl-sugar.
[b]N-G: Amino-sugar.
[c](*): additional sugar found.
[d]*: Glca.
[e]*: GalA.
[f]': traces.

The sheath polysaccharides (Table 3) reveal the presence of glucose, galactose, mannose in all cases with the exception of *Chroococcus minutus* (Adhikary *et al.*, 1986) where only glucose and methyl-sugars were found. Methyl-sugars have also been detected in many cases, in contrast to cell wall polysaccharides. The only time the sheath polysaccharides of two different microorganisms of the same genus (*Gloeothece* (Tease & Walker, 1987; Weckesser *et al.*, 1987)) were studied, revealed a result very similar in composition for the two polymers. Further data would permit to find a correlation between a genus of ensheathed cyanobacteria and the corresponding sheath polysaccharides composition.

The large number of sugars present in the polysaccharides of the cell envelope could be correlated to the scarce evolution of cyanobacteria, according to the proposal of Painter (1983*b*) that evolution has resulted in a reduction in the number of different sugar residues. It must however also be borne in mind that the low yield in cell envelope polysaccharides might have hindered their isolation and further purification and thereby the analysis of a product free of other glucydic contaminants. In fact, for example, Van Eykelenburg (1978), in his study on the glucan present in the cell wall of *Spirulina platensis*, reports the chromatographic results obtained on the isolated and hydrolysed polysaccharide as well as on the hydrolysate of the total cell mass. While the isolated polysaccharide contained only glucose the cell mass contained glucose, galactose, xylose, rhamnose and mannose.

Exopolysaccharides are composed of different monosaccharides in numbers ranging from 2–9 units according to the species (Table 4). The molar ratio varies from species to species and at times within the same species, if the growth conditions are changed. Glucose is again the most frequent sugar among the exoses whereas xylose is the most common among the pentoses. The presence of uronic acids has frequently been observed, although the type of acid has not been always identified. The high variability of sugar types — especially pentoses — in the polysaccharides from cyanobacteria, which is not so common in polysaccharides from other bacterial sources has to be observed and kept in mind. The presence of non-saccharidic substituent groups (e.g. acetyl, pyruvic and sulphated groups) has not always been verified.

Many microbial polysaccharides reveal substituents, the content of which can be controlled by changing the microbial growing conditions. These substituents play a crucial role in the definition of some physicochemical properties.

Microbial polysaccharides offer greater advantages than polysaccharides derived from other sources because they are obtained from

TABLE 3
Carbohydrates Found in the Sheath Polysaccharides

	Glc	*Gal*	*Man*	*Ara*	*Xyl*	*Rha*	*Fuc*	*Ur.A*	*M-G*[a]	*N-G*[b]	*Reference*
Section I											
Chroococcus minutus	*								*		Adhikari *et al.*, 1986
Gloeothece PCC 6501	*	*	*		*	*		*[c,d]	*		Weckesser *et al.*, 1987
Gloeothece ATCC 27152	*	*	*	*	*	*		[c,d,e]	*		Tease & Walker, 1987
Gloeobacter PCC 7421	*	*	*	*		*		*			Schneider *et al.*, 1988
Section IV											
Anabaena cylindrica	*	*	*		*		[f]				Dunn & Wolk, 1970
Section V											
Chlorogloeopsis PCC 6912	*	*	*	*	*			*[c]			Schrader *et al.*, 1982
Fischerella PCC 7414	*	*	*		*		*	[f]		*	Schneider *et al.*, 1988

[a]M-G: Methyl-sugar.
[b]N-G: Amino-sugar.
[c]*: GlcA.
[d]*: GalA.
[e]*: ManA.
[f]: traces.

TABLE 4
Carbohydrates Found in Exocellular Polysaccharides

	Glc	*Gal*	*Man*	*Ara*	*Xyl*	*Rib*	*Rha*	*Fuc*	*Ur.A*	*Reference*
Section III										
Phormidium J-1		*	*				*		*	Bar-Or & Shilo, 1987
Section IV										
Anabaena cylindrica	*	*		*	*		*		*	Bishop *et al.*, 1954
Nostoc 221	*				*				*[a]	Mehta & Vaidya, 1978
Nostoc calcicola	*	*	*	*	*		*	*	*[a,b]	Flaibani *et al.*, 1988
Palmella mucosa	*			*				*	*[a]	Tischer & Moore, 1964
Anabaena flos-aquae A37										Wang & Tischer, 1973
— neutral fraction	*				*					
— acidic fraction	*				*	*			*	
Cyanospira capsulata	*		*	*				*	*[b]	Bertocchi *et al.*, 1988

[a]*: GlcA.
[b]*: GalA.

cultures of selected strains with high constant yields in reproducible conditions. Moreover, they have a high molecular weight and regular sequence.

Polysaccharides produced by various natural sources (vegetal, animal, algal, bacterial and fungal) highlighted interesting properties for many industrial purposes (Sandford *et al.*, 1984). These properties comprise for example, the ability to form stable gels; stabilize suspensions and emulsions; increase the viscosity of aqueous solutions; form fibres, films, liquid crystals and the ability to be employed as flocculants. All the above-mentioned properties favour their applications in many fields as for example, foodstuffs (gelatines, puddings, icecreams); pharmaceuticals (controlled release systems, anti-tumoral activity drugs); textiles (printing pastes); oil (enhanced oil recovery); cosmetics (suspendants and emulsifiers).

As regards cyanobacteria, some strains produce polysaccharides whose aqueous solutions have interesting rheological and flocculant properties if compared to those of the microbial polysaccharides already on the market. It must also be acknowledged that the growing conditions of photosynthetic microorganisms are most cost-effective compared to those of other microorganisms. In fact, the medium for the growing of cyanobacteria does not require carbon sources. Furthermore growth of microalgae for polysaccharide production has also been exploited in open-air ponds or in tubular reactors (Sili *et al.*, 1984, Vonshak, 1988) thereby avoiding the steps of medium and fermenter sterilization. In the case of a polysaccharide being released in the medium, its extraction permits that other fine chemicals accumulated intracellularly may be recovered. Further studies of cyanobacterial polysaccharides not only provide further information on the understanding of cyanobacterial metabolic processes but are a prerequisite in order to find new industrially profitable products from renewable sources.

ACKNOWLEDGMENT

This work has been carried out within a project supported by EniChem S.p.A., Research & Development (Milano, Italy) to POLY-biòs (Trieste, Italy). C.B. and L.N. are recipients of fellowships under this programme. The authors are grateful to Professor R. Materassi and M. Vincenzini (Firenze, Italy), and to Professor L. P. T. M. Zevenhuizen (Wageningen, The Netherlands) for helpful discussions and suggestions.

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