REVIEW ARTICLE

Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly

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Abstract

Cyanobacterial extracellular polymeric substances (EPS) are mainly composed of high-molecular-mass heteropolysaccharides, with variable composition and roles according to the microorganism and the environmental conditions. The number of constituents – both saccharidic and nonsaccharidic – and the complexity of structures give rise to speculations on how intricate their biosynthetic pathways could be, and how many genes may be involved in their production. However, little is known regarding the cyanobacterial EPS biosynthetic pathways and regulating factors. This review organizes available information on cyanobacterial EPS, including their composition, function and factors affecting their synthesis, and from the *in silico* analysis of available cyanobacterial genome sequences, proposes a putative mechanism for their biosynthesis.

Introduction

A wide range of bacteria are able to synthesize and secrete extracellular polymeric substances (EPS) (Neu & Marshall, 1990), mainly of polysaccharidic nature, which can remain covalently linked or loosely attached to the cell surface, or be released into the surrounding environment (De Philippis & Vincenzini, 2003). These exopolysaccharides can be divided into two groups: homopolysaccharides and heteropolysaccharides (Sutherland, 2001). The homopolysaccharides are composed of only one type of monosaccharide, and are synthesized from sucrose by the action of a sucrase. The heteropolysaccharides consist of high-molecular-mass hydrated molecules made up of different sugar residues, and are synthesized by the combined action of different types of glycosyltransferases (De Vuyst & Degeest, 1999; De Vuyst *et al*., 2001; Van Hijum *et al*., 2006; Arskold *et al*., 2007). These complex polymers can also contain acetylated amino sugar moieties, as well as noncarbohydrate constituents such as phosphate, lactate, acetate and glycerol (De Vuyst & Degeest, 1999; De Vuyst *et al*., 2001; Ruas-Madiedo *et al*., 2002; Girard & Schaffer-Lequart, 2007). The composition of the bacterial EPS varies with the type of microorganism (Vaningelgem *et al*., 2004; Panhota *et al*., 2007), nutrient availability (De Vuyst & Degeest, 1999; Ricciardi *et al*., 2002), growth phase and environmental conditions (Fischer *et al*., 2003; Bahat-Samet *et al*., 2004). However, the mechanisms involved in the synthesis of EPS seem to be relatively conserved for Gram-negative and Gram-positive bacteria (De Vuyst *et al*., 2001; Jolly & Stingele, 2001; Laws *et al*., 2001; Sutherland, 2001; Welman & Maddox, 2003; Whitfield, 2006). In terms of biotechnological applications, bacterial EPS are a good alternative to the polysaccharides of plant and algal origin due to the higher growth rates of the producing bacteria, the reproducible physicochemical properties of their EPS, the easier manipulation of these

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properties by genetically engineering the producing microorganisms, the presence of novel functionalities and the overall economical costs of producing them (Selbmann *et al.*, 2002; Parikh & Madamwar, 2006). This versatility could be useful in a wide range of applications in many industrial sectors such as textiles, detergents, adhesives, oil recovery, wastewater treatment, dredging, brewing, downstream processing, cosmetology, pharmacology and food additives.

Cyanobacteria are a large and widespread group of photoautotrophic microorganisms that combine the ability to perform oxygenic photosynthesis (similar to that of the chloroplasts) with typical prokaryotic features (Whitton & Potts, 2000). Certain strains also have the ability to fix atmospheric dinitrogen, thus displaying the simplest nutritional requirements (Fay, 1992; Bergman *et al.*, 1997). They possess a unique cell wall that combines the presence of an outer membrane and lipopolysaccharides, as in Gram-negative bacteria, with a thick and highly cross-linked peptidoglycan layer similar to Gram-positive bacteria (Hoiczyck & Hansel, 2000; Stewart *et al.*, 2006). Moreover, many cyanobacterial strains have polysaccharidic structures surrounding their cells (De Philippis & Vincenzini, 2003). However, a lack of information regarding both the genes encoding the proteins involved in the EPS biosynthetic pathways, and the factors controlling these processes strongly limits their potential for biotechnological applications (Morvan *et al.*, 1997; Otero & Vincenzini, 2003; Potts, 2004).

The aim of this review is to summarize the current knowledge on the composition and the macromolecular characteristics of the cyanobacterial exopolysaccharides. We consider their ecological role and the factors that may affect their synthesis, as well as analyze the available genome sequences to gather information on the genes encoding products putatively involved in the production of EPS in cyanobacteria.

Composition and macromolecular characteristics of cyanobacterial exopolysaccharides

The cyanobacterial EPS can be divided in two main groups: the ones associated with the cell surface and the polysaccharides released into the surrounding environment (released polysaccharides, RPS). The EPS associated with the cell surface can be referred to as sheaths, capsules and slimes, according to their thickness, consistency and appearance (De Philippis & Vincenzini, 1998, 2003). The sheath is defined as a thin, dense layer loosely surrounding cells or cell groups usually visible in light microscopy without staining. The capsule generally consists of a thick and slimy layer intimately associated with the cell surface with sharp outlines, which is structurally coherent, excluding particles (e.g. India ink). The slime refers to the mucilaginous material dispersed around the organism but not reflecting the shape of the cells. The RPS are soluble aliquots of polysaccharidic material released into the medium, either from the external layer(s) or derived from a biosynthetic process not directly related to the synthesis of EPS. Despite some evidence, this last point is still controversial. Differences in the sulphur content and in the monosaccharidic composition reported for the sheath and RPS of some cyanobacteria strongly support the hypothesis of different biosynthetic pathways (Tease *et al.*, 1991; Ortega-Calvo & Stal, 1994; Li *et al.*, 2002; Micheletti *et al.*, 2008b). However, further studies are needed to fully elucidate these pathways in cyanobacteria.

The RPS can be easily recovered from liquid cultures and, due to their physicochemical properties, are suitable for a variety of industrial applications, making cyanobacteria one of the most attractive sources of new polymers (De Philippis & Vincenzini, 1998, 2003). The available data on the monosaccharidic composition of cyanobacterial EPS (Table 1) reveal some peculiar features of these polymers when compared with those produced by other microorganisms, such as the presence of one or two uronic acids, constituents rarely found in the EPS produced by other microbial groups. Cyanobacterial EPS also contain sulphate groups, a feature unique among bacteria, but shared by the EPS produced by archaea and eukaryotes. Both the sulphate groups and the uronic acids contribute to the anionic nature of the EPS, conferring a negative charge and a 'sticky' behaviour to the overall macromolecule (Decho, 1990; Sutherland, 1994; Leppard *et al.*, 1996; Arias *et al.*, 2003; De Philippis & Vincenzini, 2003; Mancuso Nichols *et al.*, 2005). The anionic charge is an important characteristic for the affinity of these EPS towards cations, notably metal ions. However, the ability to chelate metal ions is related not only to the amount of charged groups but also to their distribution on the macromolecules and their accessibility (Brown & Lester, 1982; De Philippis *et al.*, 2000; Mancuso Nichols *et al.*, 2005; Micheletti *et al.*, 2008b). On the other hand, many cyanobacterial EPS are also characterized by a significant level of hydrophobicity, which is due to the presence of ester-linked acetyl groups (up to 12% of EPS dry weight), peptidic moieties and deoxysugars such as fucose and rhamnose (Table 1). The presence of these hydrophobic groups contributes significantly to the emulsifying properties of the polysaccharides, which would otherwise be highly hydrophilic, and it is also essential for determining their rheological properties (Neu *et al.*, 1992; Shepherd *et al.*, 1995).

Cyanobacterial EPS are complex heteropolysaccharides, with *c.* 75% of the polymers described so far composed of six or more different kinds of monosaccharides. This feature contrasts with the polymers synthesized by other bacteria or macroalgae, which contain a lower number of different monomers, usually less than four (De Philippis & Vincenzini, 1998). To date, up to 12 different monosaccharides have

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Table 1a. Main constituents of EPS produced by unicellular cyanobacteria

			Wt/EPS dry weight (%)						
Unicellular organisms	Ecological origin	No. of monosaccharides	Uronic acids	Deoxysugars	Sulphate	Pyruvate	Acetate	Peptides	References
Aphanocapsa halophytia MN-11	Saline lake, Japan	6	0	52.4	11.9			10.3	Sudo *et al*. (1995)
Aphanothece halophytica GR02	Saltworks, China	7	14.1	27.8	0			0	Li *et al*. (2001)
Aphanothece sacrum (Sur.) *Okada**	Freshwater spring, Japan	4		p					Kabata *et al*. (2005)
Chroococcus minutus B 41.79	Water reservoir, India	14	4.2	17.1				3.2	Fischer *et al*. (1997)
Chroococcus submarinus†	Brackish ponds, French Polynesia	10	10	24.4	14			14	Richert *et al*. (2005)
Cyanothece 16Som2	Saltworks, Somalia	7	20.6	8.2	p	0	0		De Philippis & Vincenzini (1998)
Cyanothece CA3	Hypersaline ponds, Italy	7	66.8	23.2	p	2.7	0.6		De Philippis & Vincenzini (1998)
Cyanothece CE4	Saltworks, Italy	7	80.1	36.6	p	0.4	0.7		De Philippis & Vincenzini (1998)
Cyanothece CE9	Saltworks, Italy	6	35.7	33.5	p	1.2	0.0		De Philippis & Vincenzini (1998)
Cyanothece CH1	Saltworks, Greece	6	27.4	39.4	p	1	0.5		De Philippis & Vincenzini (1998)
Cyanothece ET2	Alkaline lake, Ethiopia	8	63.1	21.5	0	2.3	4.2		De Philippis & Vincenzini (1998)
Cyanothece ET5	Alkaline lake, Ethiopia	8	29.4	22.3	0	0.4	2.5		De Philippis & Vincenzini (1998)
Cyanothece IR20	Hypersaline lake, Israel	7	9.8	80.2	p	2.1	0.8		De Philippis & Vincenzini (1998)
Cyanothece PE13	Tidal pool, Greece	8	20.9	8.9	p	2.1	0		De Philippis & Vincenzini (1998)
Cyanothece PE14	Tidal pool, Greece	7	21.7	4.4	p	0.2	0.3		De Philippis & Vincenzini (1998)
Cyanothece sp. ATCC 51142	Intertidal area, Texas	3	0	0	p			18.3	Parikh & Madamwar (2006)
Cyanothece sp. 113	Sea, China	1	0	0				0	Chi *et al*. (2007)
Cyanothece sp. PCC 8801	Rice fields, Taiwan	8	35	33.3		0.5	1.4		De Philippis & Vincenzini (1998)
Cyanothece TI4	Hypersaline pond, Italy	7	58.2	39.9	p	1.4	1		De Philippis & Vincenzini (1998)
Cyanothece TP10	Saltworks, Italy	7	31.3	42.7	p	3.9			De Philippis & Vincenzini (1998)
Cyanothece TP5	Saltworks, Italy	6	40.4	57.5	p	1.1	0		De Philippis & Vincenzini (1998)
Cyanothece VI13	Tidal pool, Italy	8	32.1	37.2	p	0.3	0		De Philippis & Vincenzini (1998)
Cyanothece VI22	Tidal pool, Italy	7	40.8	31.7	p	0.2	0.6		De Philippis & Vincenzini (1998)
Gloeocapsa gelatinosa	Unknown	10	31.4	14.2				28.2	Raungsomboon *et al*. (2006)
Gloeothece sp. PCC 6909	Unknown	6	5	3	13.8			6.2	Tease *et al*. (1991)
Johannesbaptistia pellucida†	Brackish ponds, French Polynesia	9	5	7.6	19			10	Richert *et al*. (2005)
Microcystis aeruginosa†	Water reservoir, Brazil	8	8.9	28.3					Gouvea *et al*. (2005)
Microcystis aeruginosa f. *aeruginosa*	Unknown	4	5.2 $\mu g\,mg^{-1}$	64.6					Forni *et al*. (1997)
Microcystis aeruginosa f. *flos-aquae*	Unknown	3	2.6 $\mu g\,mg^{-1}$	0					Forni *et al*. (1997)
Microcystis flos-aquae C3-40	Unknown	6	83	5.5				< 1	Plude *et al*. (1991)
Microcystis PCC 7005	Lake, Wisconsin	4	0.05 $\mu g\,mg^{-1}$	15.7					Forni *et al*. (1997)
Microcystis PCC 7941	Lake, Canada	4	0.04 $\mu g\,mg^{-1}$	tr					Forni *et al*. (1997)
Microcystis sp.	Water reservoir, Taiwan	5	P	p					Huang *et al*. (2007)
Microcystis viridis	Unknown	6	5.8 $\mu g\,mg^{-1}$	7.3					Forni *et al*. (1997)
Microcystis wesenbergii	Unknown	1	100	0					Forni *et al*. (1997)
Rhabdoderma rubrum†	Brackish ponds, French Polynesia	8	2	8.7	13			7	Richert *et al*. (2005)
Synechococcus elongatus f. *A. nidulans*†	Lake, Ohio	3	0	0					Sangar & Dugan (1972)
Synechocystis sp. PCC 6714	Freshwater, California	11	16.7	31.4	1.2	0		20	Panoff *et al*. (1988)
Synechocystis sp. PCC 6803	Freshwater, California	12	16.4	6.7	1.0	0		40	Panoff *et al*. (1988)

The cyanobacteria are sourced from a culture collection unless specified otherwise.

*Field sample.

†Monocyanobacterial culture.

p, present, but not quantified; tr, trace.

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Table 1b. Main constituents of EPS produced by filamentous cyanobacteria

Filamentous organisms	Ecological origin	No. of mono-saccharides	Wt/EPS dry weight (%)						References
			Uronic acids	Deoxysugars	Sulphate	Pyruvate	Acetate	Peptides	
Geitlerinema sp.*	Brackish ponds, French Polynesia	4	6	tr	0			8	Richert *et al.* (2005)
Leptolyngbya sp. VRUC 135	Domus Aurea, Italy	5	0	3					Piro *et al.* (2005)
Lyngbya confervoides S9g	Surface of *Lithophyllum lichenoides*, France	9	38.6	tr					Gloaguen *et al.* (1995)
Microcoleus sp.*	Sand dunes, Israel	5	0	p				6.0	Mazor *et al.* (1996)
*Microcoleus vaginatus**	Desert algal crusts, China	10	8	9.9				50.3	Hu *et al.* (2003a)
Oscillatoria amphibia PCC 7105	Unknown marine habitat	8	6.7	4.5	9.1				Gloaguen *et al.* (1995)
Oscillatoria corallinae CJ1	Leaves of *Posidonia Oceanica*, France	9	24.2	5.1	19.3				Gloaguen *et al.* (1995)
Oscillatoria sp.†	Microbial mats, Florida	10	4.6	15.9					Bender *et al.* (1994)
Oscillatoria sp.	Pantelleria Island, Italy	5	p	1					Nicolaus *et al.* (1999)
Oscillatoria sp.	Contaminated soil, India	3	0	0	0			34.4	Parikh & Madamwar (2006)
Oscillatoria sp.	Water reservoir, Taiwan	3	0	p					Huang *et al.* (2007)
Phormidium ectocarpi ME3	Marine habitat	9	18.9	2.7	15.2				Gloaguen *et al.* (1995)
Phormidium ectocarpi C86	Marine habitat	7	29.9	tr	12.2				Gloaguen *et al.* (1995)
Phormidium ectocarpi K5	Marine habitat	9	28.7	1.8	12.4				Gloaguen *et al.* (1995)
Phormidium ectocarpi N182	Marine habitat	6	28.7	tr	13.6				Gloaguen *et al.* (1995)
Phormidium ectocarpi PCC7375	Marine plankton, Massachusetts	8	41.5	10.4					Gloaguen *et al.* (1995)
Phormidium foveolarum	unknown	9	p	17.4					Matulewicz *et al.* (1984)
Phormidium foveolarum C52	Marine habitat	9	29.4	2	11.2				Gloaguen *et al.* (1995)
Phormidium foveolarum MEU	Freshwater	8	0.5	9.8	0.5				Gloaguen *et al.* (1995)
Phormidium minutum D5	Marine habitat	7	17.1	0.9	4.5				Gloaguen *et al.* (1995)
Phormidium minutum NB5	Marine habitat	7	24.4	tr					Gloaguen *et al.* (1995)
Phormidium minutum RT6	Marine habitat	9	20.1	7.1	7.7				Gloaguen *et al.* (1995)
Phormidium sp.	Lake, Antarctica	9	9	24.5				13	Matulewicz *et al.* (1984)
Phormidium sp.	Unknown	6		5.8					Nicolaus *et al.* (1999)
Phormidium sp. 90-14/1	Intertidal habitat, New Guinea	8	3.0	5.8	3				Gloaguen *et al.* (1995)
Phormidium sp. CCAP1463/4	Phosphorescent bay, Massachusetts	9	13.0	6.7	3.5				Gloaguen *et al.* (1995)
Phormidium sp. CCAP1464/3	Marine habitat	8	26.1	tr	4.4				Gloaguen *et al.* (1995)
Phormidium sp. J-1	Benthic, drainage canal, Israel		34.0		1.65			4.4	Bar-Or & Shilo (1987)
Phormidium sp. PNG91	Intertidal habitat, New Guinea	9	tr	5.6	3.6				Gloaguen *et al.* (1995)
*Phormidium tenue**	Desert algal crusts, China	8	tr	12.7				21.9	Hu *et al.* (2003a)
Phormidium uncinatum	Unknown	5	0	< 5	0				Hoiczyk (1998)
*Plectonema battersii**	Brackish ponds, French Polynesia	8	7	11.1	16			11	Richert et al. (2005)
*Plectonema golenkinianum**	Brackish ponds, French Polynesia	8	11	20.4	0			19	Richert *et al.* (2005)
Spirulina maxima	China	8	p	p					Nie *et al.* (2002)
Spirulina platensis	China	5	20.0	p					Tseng & Zhao (1994)
Spirulina platensis PCC 8005	Unknown	10	40	13.6	5				Filali Mouhim *et al.* (1993)

The cyanobacteria are sourced from a culture collection unless specified otherwise.

*Monocyanobacterial culture.

†Field sample.

p, present, but not quantified; tr, trace.

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Table 1c. Main constituents of EPS produced by filamentous heterocystous cyanobacteria

			Wt/EPS dry weight (%)						
Filamentous heterocystous organisms	Ecological origin	No. of monosaccharides	Uronic acids	Deoxy-sugars	Sulphate	Pyruvate	Acetate	Peptides	References
Anabaena cylindrica	Unknown	5	0	0					Dunn & Wolk (1970)
Anabaena cylindrica 10 C	Unknown	7	1.7	10.1	p*				Lama *et al*. (1996)
Anabaena cylindrica CCAP1403/2	Freshwater pond, UK	6	28	6					Bishop *et al*. (1954)
Anabaena flos-aquae	Freshwater	4	p	0					Moore & Tischer (1964)
Anabaena flos-aquae A-37	Unknown	4	58.4	0					Wang & Tischer (1973)
Anabaena flos-aquae A-37	Unknown	4	0.9	0					Moore & Tischer (1965)
Anabaena sp.	Water reservoir, Taiwan	3	0	p					Huang *et al*. (2007)
Anabaena sp. ATCC 33047	Algal mat, Texas	5	19.4	0	0			7	Moreno *et al*. (2000)
Anabaena sp. C5*	Soil, Yugoslavia	5	p					51.5	Gantar *et al*. (1995)
Anabaena sp. PC-1	Unknown		5		p			12	Choi *et al*. (1998)
Anabaena sphaerica	Unknown	4	p	0					Nicolaus *et al*. (1999)
*Anabaena spiroides**	Water reservoir, Brazil	8	9	33.9					Gouvea *et al*. (2005)
Anabaena torulosa	Soil, France	7	0	8.7					Nicolaus *et al*. (1999)
Anabaenopsis circularis PCC 6720	Drainage canal, Israel		0		0			0	Bar-Or & Shilo (1987)
Chlorogloeopsis sp. PCC 6912	Soil, India	6	p	10.2					Nicolaus *et al*. (1999)
Cyanospira capsulata	Alkaline lake, Kenya	7	37.5	12.5		p		p	Garozzo *et al*. (1995)
Cyanospira capsulata ATCC 43193	Alkaline lake, Kenya	5	36.5	15.4	0	1.5	0	2.0	Vincenzini *et al*. (1990)
Fischerella maior Nav 10 bis	Catacombs, Italy	7	12.8	21.4	p				Bellezza *et al*. (2006)
Fischerella muscicola	Unknown	5		17.6					Nicolaus *et al*. (1999)
Mastigocladus laminosus†	Thermal spring, France	11	p	p					Gloaguen *et al*. (1999)
Nostoc flagelliforme†	Desert China	3	0	0					Huang *et al*. (1998)
Nostoc calcicola 79WA01	Soils, Washington	9	21.8	15.5				30	Flaibani *et al*. (1989)
Nostoc carneum	Contaminated soil, India	2	0	0	0			27.2	Parikh & Madamwar (2006)
Nostoc commune†	Mountain area, China	7	13.3	5.8					Huang *et al*. (1998)
Nostoc commune	Freshwater, China	6	18.4	0					Brüll *et al*. (2000)
Nostoc commune DRH-1	Desert, Mongolia	7	22.7	0	0				Helm *et al*. (2000)
Nostoc commune UTEX584	Unknown	9	42	9					Flaibani *et al*. (1989)
Nostoc insulare 54.79	Soil	4	26.4	0	0	tr	tr	0.7	Volk *et al*. (2007)
Nostoc insulare 54.79	Soil	8	25.3	8.9				3.5	Fischer *et al*. (1997)
Nostoc linckia f. muscorum	Unknown	6	p	p					Mehta & Vaidya (1978)
Nostoc sp.	Contaminated soil, India	2	0	0	0			40.1	Parikh & Madamwar (2006)
Nostoc sp.	Unknown	4	p	p					Moore & Tischer (1964)
Nostoc sp.	Unknown	6	p	p					Mehta & Vaidya (1978)
Nostoc sp.*	Desert algal crusts, China	6	0	3.5				7.5	Hu *et al*. (2003a)
Nostoc sp. 221	Unknown	3	41.3	0					Mehta & Vaidya (1978)
Nostoc sp. 2S9B*	Soil, Yugoslavia	4	p					2.8	Gantar *et al*. (1995)
Nostoc sp. D	Unknown	6	0	0					Cupac & Gantar (1992)
Nostoc sp. PCC 6302	Unknown	8	69.4	3.9	0	0	0.5	6.9	De Philippis *et al*. (2000)
Nostoc sp. PCC 6310	Pond, Israel	9	4.3	1.6	p	1.4	3.3	69.5	De Philippis *et al*. (2000)
Nostoc sp. PCC 6705	Botanical garden, California	9	26.8	2.1	p	3.2	1.7	6.9	De Philippis *et al*. (2000)
Nostoc sp. PCC 6719	Soil water culture, California	9	9.9	0.7	p	5.0	2.4	0.8	De Philippis *et al*. (2000)
Nostoc sp. PCC 6720	Soil, Indonesia	8	13.5	4.1	p	2.1	1.1	3.1	De Philippis *et al*. (2000)
Nostoc sp. PCC 7107	Pond, California	9	26.7	6.7	p	0.7	2.7	3.4	De Philippis *et al*. (2000)
Nostoc sp. PCC 7119	Unknown	6	5.2	0.5	p	5.0	3.4	0.9	De Philippis *et al*. (2000)
Nostoc sp. PCC 7413	Soil, UK	9	19.3	2.0	p	6.2	3.3	0.6	De Philippis *et al*. (2000)
Nostoc sp. PCC 7416	Shallow pool, California	8	23.6	4.6	p	0	4.0	9.7	De Philippis *et al*. (2000)
Nostoc sp. PCC 7422	Cycas roots	8	26.8	5.4	p	2.4	2.0	4.7	De Philippis *et al*. (2000)
Nostoc sp. PCC 7423	Dried soil, Senegal	7	18.8	0.8	p	3.2	6.8	18.4	De Philippis *et al*. (2000)

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Table 1c. Continued.

Filamentous heterocystous organisms	Ecological origin	No. of mono-saccharides	Wt/EPS dry weight (%) Uronic acids	Deoxy-sugars	Sulphate	Pyruvate	Acetate	Peptides	References
Nostoc sp. PCC 7706	Water below calcareous stone, France	7	25.2	1.2	0	0	0.8	10.3	De Philippis *et al.* (2000)
Nostoc sp. PCC 7803	Sand dunes, UK	8	22.0	0.8	0	0.2	1.1	5.5	De Philippis *et al.* (2000)
Nostoc sp. PCC 7807	Soil, France	7	28.7	0.6	p	6.1	2.0	10.2	De Philippis *et al.* (2000)
Nostoc sp. PCC 7906	Freshwater,	8	37.7	3.3	0	0	1.9	15.6	De Philippis *et al.* (2000)
Nostoc sp. PCC 7933	Mud, Finland	8	33.7	4.8	p	0	1.4	5.9	De Philippis *et al.* (2000)
Nostoc sp. PCC 7936	Rice field, India	5	51.1	0.6	p	5.8	1.8	2.1	De Philippis *et al.* (2000)
Nostoc sp. PCC 7937	Freshwater, Mississippi	7	4.9	0.9	p	3.5	3.0	3.2	De Philippis *et al.* (2000)
Nostoc sp. PCC 8009/1	Coralloid roots of *Macrozamia lucida*	8	12.7	1.2	p	0.4	3.7	3.3	De Philippis *et al.* (2000)
Nostoc sp. PCC 8109	Unknown	7	62.4	1.1	0	0.6	0.7	24.6	De Philippis *et al.* (2000)
Nostoc sp. PCC 8112	Laundromat discharge pond, Michigan	9	42.2	5.0	p	0.4	0.9	11.7	De Philippis *et al.* (2000)
Nostoc sp. PCC 8113	Unknown	8	6.6	5.1	p	5.8	0	3.8	De Philippis *et al.* (2000)
Nostoc sp. PCC 8306	Soil, West Africa	7	56.7	3.2	0	0.7	0	21.7	De Philippis *et al.* (2000)
Nostoc sp. PCC 9202	Rice field, Spain	9	37.7	2.0	0	0	0	5.0	De Philippis *et al.* (2000)
Nostoc sp. PCC 9305	Anthoceros, California	8	13.2	4.4	0	2.3	0	4.5	De Philippis *et al.* (2000)
Nostoc sp. WV2	Unknown	6	p	p					De Philippis & Vincenzini (1998)
Scytonema hofmanni PCC 7110	Cave (limestone), Bermuda	3		0					Nicolaus *et al.* (1999)
*Scytonema javanicum**	Desert algal crusts, China	10	tr	7.4				50.2	Hu *et al.* (2003a)
Scytonema ocellatum CP8-2	Catacombs, Italy	7	16.6	12.5	p				Bellezza *et al.* (2006)
Scytonema sp.	Thallus of *D. glabratum*, Brazil	10		11					Sassaki *et al.* (2005)
Tolypothrix tenuis PCC 7101	Soil, Borneo	6		14.3					Nicolaus *et al.* (1999)

The cyanobacteria are sourced from a culture collection unless specified otherwise.
*Monocyanobacterial culture.
†Field sample.
p, present, but not quantified; tr, trace.

been identified in cyanobacterial EPS (Table 1): the hexoses, glucose, galactose, mannose and fructose, the pentoses, ribose, xylose and arabinose, the deoxyhexoses, fucose, rhamnose and methyl rhamnose, and the acidic hexoses, glucuronic and galacturonic acid (De Philippis & Vincenzini, 1998, 2003; De Philippis *et al.*, 2001). In a few cases, the presence of additional types of monosaccharides (i.e. methyl sugars and/or amino sugars) such as *N*-acetyl glucosamine, 2,3-*O*-methyl rhamnose, 3-*O*-methyl rhamnose, 4-*O*-methyl rhamnose and 3-*O*-methyl glucose have been reported (Hu *et al.*, 2003a). The monosaccharide most frequently found at the highest concentration in cyanobacterial EPS is glucose, although there are polymers where other sugars, such as xylose, arabinose, galactose or fucose, are present at higher concentrations than glucose (Tease *et al.*, 1991; Bender *et al.*, 1994; Gloaguen *et al.*, 1995; Fischer *et al.*, 1997; De Philippis & Vincenzini, 1998, 2003; Parikh & Madamwar, 2006).

The high number of different monosaccharides found in cyanobacterial EPS and the consequential variety of linkage types is usually considered a reason for the presence of complex repeating units, as well as for a broad range of possible structures and architectures of these macromolecules. As one consequence of this complexity, the cyanobacterial EPS are less well characterized than those of other microorganisms and only a few structures have been proposed (Table 2). The polysaccharides produced by *Nostoc commune* DRH-1, *Nostoc insulare* and *Cyanothece* sp. ATCC 51142 are composed of repeating units of six, four and three monosaccharides, respectively (Helm *et al.*, 2000; Huang *et al.*, 2000; Shah *et al.*, 2000; Volk *et al.*, 2007). On the other hand, the structures proposed for the EPS produced by *Mastigocladus laminosus* and *Cyanospira capsulata* are far more complex, with repeating units of 15 and eight monosaccharides, respectively (Garozzo *et al.*, 1995, 1998; Gloaguen *et al.*, 1995, 1999). For *Spirulina platensis*, no structure was proposed, but it was demonstrated that its EPS repeating unit contains at least 15 sugar residues (Filali Mouhim *et al.*, 1993).

The knowledge of the structure of a polysaccharide is generally considered necessary to infer its physicochemical properties (De Philippis & Vincenzini, 1998). Indeed, the

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Table 2. Overview of the published structures of the heteropolysaccharides produced by cyanobacteria

Organisms (ecological origin)	Repeating units	References
Unicellular		
Cyanothece sp. ATCC 51142 (intertidal area, Texas)	[4) α-D-Ido*p*-2-*C*-carboxylic acid(1 → 4) 2-Deoxy-2-C-methyl-α-D-Glc*p* (1]$_n$ 3 ↓ 6 3-Deoxy-α-D-Ido*p*-3-*C*-sulfonic acid	Shah *et al.* (2000)
Filamentous heterocystous		
Cyanospira capsulate (unknown)	[3) α-D-Glc*p*NAc (1 → 3) α-D-Gal*p*A (1 → 3) α-L-Fuc*p* (1]$_n$ α-D-Glc*p*NAc 4 ← 1 α-L-Ara*p* α-D-Gal*p*A 2 ← 1 α-D-Gal*p*A α-L-Fuc*p* 4 ← 1 β-D-Glc*p* 3 ← 1 β-D-man*p* 4 ← 1 (S)-CH_3CH(–CO_2H)-4-β-D-man*p*	Garozzo *et al.* (1995, 1998)

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Table 2. Continued.

Organisms (ecological origin)	Repeating units	References
*Mastigocladus laminosus** (thermal spring, France)	α-D-Glc*p*A (1 → 3) on β-D-Gal*p*; β-D-Glc*p* (1 → 3) α-L-Fuc*p* (1 → 6) β-D-Gal*p* (1 → 4) on β-D-man*p*; [4) α-D-Glc*p*A (1 → 2) α-D-Gal*p*A (1 → 2) β-D-man*p* (1 → 4) β-D-Gal*p* (1 → 2) α-L-Rha*p* (1]$_n$; 3 of α-D-Glc*p*A ← 1 of β-D-Glc*p*; β-D-Xyl*p* (1 → 4) α-L-Fuc*p* (1 → 3) β-D-Glc*p*; α-D-Glc*p*A (1 → 3) on α-L-Fuc*p*; β-D-Xyl*p* (1 → 4) on β-D-Glc*p*; β-D-Glc*p* (1 → 3) on α-L-Rha*p*	Gloaguen *et al.* (1995)
Nostoc commune DRH-1 (desert, Mongolia)	β-NosA (1 → 6) on β-D-Glc*p*; [4) β-D-Glc*p* (1 → 4) α-D-Gal*p* (1 → 4) β-D-Glc*p* (1 → 4) β-D-Xyl*p* (1]$_n$; α-L-Rib*f* (1 → 3) on β-D-Xyl*p*	Helm *et al.* (2000), Huang *et al.* (2000)
Nostoc insulare 54.79 (soil)	[1) Glc*p* (3 → 1) Glc*p* (4 → 1) Glc*p*A (4]$_n$; 3-*O*-methyl-Ara*f* (1 → 3) on the second Glc*p*	Volk *et al.* (2007)

The cyanobacteria are sourced from a culture collection unless specified otherwise.
GlcA, glucuronic acid; GalA, galacturonic acid; NosA, nosturonic acid; Ido, idose; Glc, glucose; Gal, galactose; Fuc, fucose; Ara, arabinose; Man, mannose; Rha, rhamnose; Xyl, xylose; Rib, ribose. *p*, pyranose form; *f*, furanose form; α, anomer α; β, anomer β.
*Field sample.

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Table 3. Molecular masses of the EPS released by cyanobacteria

Organism	Ecological origin	Apparent molecular mass (kDa)	References
Unicellular			
Chroococcus minutus B 41.79	Water reservoir, India	1200–1600	Fischer *et al*. (1997)
Filamentous			
*Microcoleus vaginatus**	Desert algal crusts, China	380	Hu *et al*. (2003a)
Oscillatoria sp.†	Microbial mats, Florida	≥200	Bender *et al*. (1994)
Phormidium 94a*	Arid soil, Mexico	2000	Vicente-Garcia *et al*. (2004)
Phormidium J-1	Drainage canal, Israel	1200	Bar-Or & Shilo (1987)
*Phormidum tenue**	Desert algal crusts, China	380	Hu *et al*. (2003a)
Spirulina platensis	China	81–98	Tseng & Zhao (1994)
Filamentous heterocystous			
Anabaena circularis PCC 6720	Drainage canal, Israel	>1200	Bar-Or & Shilo (1987)
*Anabaena spiroides**	Water reservoir, Brazil	2000	Colombo *et al*. (2004)
Anabaena sp. ATCC 33047	Algal mat, Texas	1350	Moreno *et al*. (2000)
Cyanospira capsulata ATCC 43193	Alkaline lake, Kenya	1400–1900	Vincenzini *et al*. (1993), Cesàro *et al*. (1990)
Nostoc insulare 54.79	Soil	540–1300	Fischer *et al*. (1997)
Nostoc sp.*	Desert algal crusts, China	460	Hu *et al*. (2003a)
Schizothrix sp.*	Intertidal marine stromatolites, Bahamas	300	Kawaguchi & Decho (2002)
*Scytonema javanicum**	Desert algal crusts, China	110–380	Hu *et al*. (2003a)

The cyanobacteria are sourced from a culture collection unless specified otherwise.
*Monocyanobacterial culture.
†Field sample.

interest in cyanobacteria as producers of high-molecular-weight polysaccharides is related to the capability of these biopolymers to modify the rheological properties of water, acting as thickening agents (Sutherland, 1996), and to stabilize the flow properties of aqueous solutions. Thus, one of the key features of a polysaccharide, which determines most of the properties generally considered to be useful for industrial applications, is its high molecular mass (Shepherd *et al*., 1995), as this characteristic has a direct influence on the rheological properties of solutions of the polymer (Kamal *et al*., 2003). The molecular masses reported thus far for the exopolysaccharides released by cyanobacteria are listed in Table 3; the highest molecular masses were found for the polysaccharides produced by *C. capsulata*, *Anabaena spiroides* and *Phormidium* 94, which are about 2 MDa. These values, significantly higher than that of xanthan gum, which has a molecular mass of about 1 MDa (Kamal *et al*., 2003), point to the potential of these polymers for biotechnological exploitation as viscosifying or suspending agents. In this context, it is worth mentioning that the viscosity of some of the cyanobacterial exopolysaccharides is comparable to, or even higher than, that of aqueous solutions of xanthan gum at similar concentrations (Sutherland, 1996; De Philippis *et al*., 2000). However, even if only a very limited number of cyanobacterial EPS have been fully described regarding their flow properties (Cesàro *et al*., 1990; Navarini *et al*., 1990; Lapasin *et al*., 1992; Moreno *et al*., 2000; Morris *et al*., 2001), there are a few reports emphasizing the dependence of viscosity on the shear rate of water solutions of cyanobacterial EPS and of commercial xanthan gum. Comparing the viscosity data (Table 4), it is possible to conclude that some of the RPS produced by cyanobacteria (e.g. the RPS synthesized by *Cyanothece* strains CE4 and CA3) possess very high viscosities, up to four times higher than that of xanthan gum. However, it should be stressed that, to make a reliable comparison of the flow properties of two polysaccharides, it is necessary to evaluate additional rheological properties, as well as to assess the dependence of these properties on factors such as pH, temperature and the ionic strength of the solution.

It has been reported that cyanobacterial EPS are not only composed of carbohydrates but also of other macromolecules such as polypeptides (Kawaguchi & Decho, 2000). Polypeptides enriched with glycine, alanine, valine, leucine, isoleucine and phenylalanine have been reported in the EPS of *C. capsulata* and *Nucula calcicola* (Flaibani *et al*., 1989; Marra *et al*., 1990), and in *Schizothrix* sp., small proteins specifically enriched with aspartic and glutamic acid have been observed (Kawaguchi & Decho, 2002). In general, the chemical composition, the type and the amount of the exopolysaccharides produced by a given cyanobacterial strain are stable features, mostly depending on the species and the cultivation conditions (Nicolaus *et al*., 1999). However, the sugar composition of the EPS produced by a certain strain may, qualitatively and quantitatively, vary slightly, especially with the age of the culture (Gloaguen *et al*., 1995; De Philippis & Vincenzini, 1998).

According to the chemical and physicochemical features of the cyanobacterial exopolysaccharides summarized

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Table 4. Viscosity (expressed as mPa s and measured at 10.1 s^{-1} shear rate) of 0.1% (w/v) water solutions of pure cyanobacterial polysaccharides (RPS) or of xanthan gum (Kelco Keltrol, commercial grade)

Organism	Viscosity (mPa s)	References
Anabaena ATCC 33047	100.0*	Moreno *et al.* (2000)
Aphanothece halophytia GR02	9.5†	Morris *et al.* (2001)
Cyanospira capsulata	158.5	De Philippis & Vincenzini (1998)
Cyanothece CA3	398.1	De Philippis & Vincenzini (1998)
Cyanothece CE4	400.0	De Philippis *et al.* (2001)
Cyanothece ET2	5.6	De Philippis & Vincenzini (1998)
Cyanothece IR20	80.0	De Philippis & Vincenzini (1998)
Cyanothece PE14	158.5	De Philippis & Vincenzini (1998)
Nostoc cameum	6.9‡	Parikh & Madamwar (2006)
Nostoc PCC 6705	40.0	De Philippis *et al.* (2001)
Nostoc PCC 6705	19.5	De Philippis *et al.* (2000)
Nostoc PCC 7119	125.9	De Philippis *et al.* (2000)
Nostoc PCC 7422	7.9	De Philippis *et al.* (2000)
Nostoc PCC 7423	158.5	De Philippis *et al.* (2000)
Nostoc PCC 7937	12.3	De Philippis *et al.* (2000)
Nostoc sp.	11.7‡	Parikh & Madamwar (2006)
Oscillatoria sp.	12.1‡	De Philippis *et al.* (2000)
Xanthan gum	78.9	De Philippis & Vincenzini (1998)

*Exopolysaccharides in a 0.4% (w/w) solution.
†Exopolysaccharides in a 0.6% (w/v) solution.
‡Intrinsic viscosity (μ) in deionized water.

above, there are at least three possible fields of application for these polymers: (1) in the food, cosmetic, textile or painting industries, for the modification of the flow properties of water, i.e. as thickening, suspending or emulsifying agents (De Philippis & Vincenzini, 1998, 2003; Li *et al.*, 2002; Parikh & Madamwar, 2006); (2) in the pharmaceutical industry, because of their antiviral or immuno-stimulating properties or the capability of slowly releasing drugs (Schaeffer & Krylov, 2000; Jensen *et al.*, 2001; Pugh *et al.*, 2001; Ghosh *et al.*, 2009); (3) in waste water treatment plants or the goldsmith industry, for the chelation of toxic or valuable metal ions from water solutions, i.e. as biosorbents (De Philippis & Micheletti, 2009).

Considering the extensive literature claiming the potential for industrial exploitation of these biopolymers, one would expect that at least for some of them the technology transfer had already occurred. However, in spite of a significant number of patents available, covering the use of cyanobacterial polysaccharides in various industrial fields (see, for instance, the review published in 2006 by Sekar & Paulraj on the patents filed at the US Patent and Trademark Office), no industrial product derived from these biopolymers is available in the market. In our opinion, the main reason for this discrepancy is the presence in the market of well-established industrial processes for heterotrophic microorganisms that in the short term would be expensive to convert for cyanobacteria. In the case of thickening agents in foods, it has to be stressed that there are already other microbial polysaccharides in the market, the most important being xanthan gum, gellan and pullulan, respectively, produced by *Xanthomonas campestris*, *Pseudomonas elodea* and *Aureobasidium pullulans*, and dextran, produced by several lactic acid bacteria belonging to the genera *Leuconostoc*, *Lactobacillus* and *Streptococcus*. These biopolymers have already undergone the complex, expensive and time-consuming procedures for their approval as food additives. Thus, even if some of the cyanobacterial exopolysaccharides, such as the one produced by *C. capsulata* (Navarini *et al.*, 1990), show better rheological properties, the differences would not be significant enough to risk a new technology transfer in competition with well-established commercial products. Similarly, the exploitation of cyanobacteria producing polysaccharides with good antiviral activity has not been considered worth developing new drugs. This is due to the long and very expensive procedures needed for the commercialization of new pharmaceutical products.

The possible use of exopolysaccharide-producing cyanobacteria for the recovery of valuable metals from industrial wash waters seems to be more promising than most of the above-mentioned applications. Indeed, the high economical value of the metal, which can be easily recovered from the biosorbent, might justify the investment necessary for the production of the biomass. However, this field of application is still in its infancy and needs more research to establish a simple and cheap technology for the production and utilization of the cyanobacterial biomass as biosorbent, as well as for the recovery of the metal.

Putative roles of EPS in cyanobacteria

The capability of cyanobacteria to survive in severe habitats (e.g. at the surface of a sand dune in a desert or exposed to high UV radiation on the lithic surfaces of monuments) has been related to the protective mechanisms that they have developed. Among these mechanisms, one of the most diffused within the cyanobacteria is the ability to synthesize external polysaccharidic layers that protect the cells from unfavourable environmental conditions. Many studies, in fact, have shown that a coating of extracellular polysaccharidic material can protect bacteria against dehydration, phagocytosis, antibody recognition and even lysis by viruses (Dudman, 1977; Tease & Walker, 1987; Hill *et al.*, 1994; Scott *et al.*, 1996; Hoiczyk, 1998; De Vuyst & Degeest, 1999; Sutherland, 1999; Ruas-Madiedo *et al.*, 2002; De Philippis

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& Vincenzini, 2003; Tsuneda *et al.*, 2003; Welman & Maddox, 2003), or confer to the cells the ability to adhere to a solid substrate, preventing them from being washed away from their natural habitat by water flow (Dudman, 1977; Scott *et al.*, 1996; De Philippis *et al.*, 2005).

A number of cyanobacteria are capable of surviving nearly without water, producing both internal and external polysaccharides, which help to stabilize the macromolecular constituents of the cell, as well as the cell structure. It has been suggested that these polysaccharides can form hydrogen bonds with proteins, lipids and DNA, thus replacing the water shell that usually surrounds these macromolecules (Potts, 1994). EPS, owing to their hydrophilic/hydrophobic characteristics (see previous section), are able to trap and accumulate water, creating a gelatinous layer around the cells that regulates water uptake and loss, and stabilizes the cell membrane during periods of desiccation (Grilli Caiola *et al.*, 1993, 1996; Tamaru *et al.*, 2005). Upon rehydration, cyanobacteria can rapidly recover metabolic activities and repair cellular components (Scherer *et al.*, 1984, 1986; Satoh *et al.*, 2002; Fleming & Castenholz, 2007). A good example of this is the filamentous EPS-producing cyanobacterium *N. commune*, which is ubiquitously distributed from the tropics to the polar regions of the Earth. These cyanobacteria form macroscopic colonies in which the entangled filaments are embedded in massive polysaccharidic structures. In their natural environment, these colonies are subjected to frequent desiccation and rewetting cycles, during which they release large quantities of protective proteins and compounds such as mycosporine-like amino acids, UV-screen pigments and active Fe-containing superoxide dismutase (Hill *et al.*, 1994; Böhm *et al.*, 1995; Shirkey *et al.*, 2000).

Another important consequence of the above-described hydrophobicity of the cyanobacterial EPS becomes evident in desert microbial crusts, where the polysaccharides contribute to the hydrological properties of the soil by clogging sand particles and by causing the run-off of water on the dune, protecting the microbial community of the crusts from being washed away by the water flow (Mazor *et al.*, 1996; Kidron *et al.*, 1999).

Recently, it was demonstrated that the cyanobacterial sheath can protect the cells from the detrimental process of biomineralization (Phoenix *et al.*, 2000; Benning & Mountain, 2004). In fact, permeability studies demonstrated that the sheath of *Calothrix* sp. was impermeable to particles of at least 11 nm diameter, thus preventing the colloids from biomineralizing the sensitive components of the cell wall (Phoenix *et al.*, 2000; Benning *et al.*, 2004).

Furthermore, the presence of negatively charged polysaccharidic layers surrounding cyanobacterial cells may play an important role in the sequestration of metal cations, and in creating a microenvironment enriched in those metals that are essential for cell growth but are present at very low concentrations in some environments (Parker *et al.*, 1996; Sutherland, 1999). On the other hand, the presence of a polysaccharidic layer surrounding the cells can also prevent direct contact between the cells and toxic heavy metals that may be present in the environment. Actually, it was recently suggested that the high viscosity of the cultures of *C. capsulata*, due to the solubilization in the culture medium of large amounts of a high molecular mass RPS, hindered the free diffusion of copper ions into the culture (De Philippis *et al.*, 2007).

The UV-absorbing pigment scytonemin was found in the sheath of a number of cyanobacteria living in environments characterized by a high level of solar irradiation (Garcia-Pichel & Castenholz, 1991; Ehling-Schulz *et al.*, 1997; Ehling-Schulz & Scherer, 1999). Moreover, in the sheath of some cyanobacterial strains, mycosporine-like amino acid compounds (MMAs) were also found (Adhikary & Sahu, 1998), confirming the role of the sheath in harbouring UV-absorbing substances, and thus protecting the cyanobacterial cells from the deleterious effects of UV radiation.

EPS may also play an important role in the locomotion of gliding cyanobacteria. Indeed, the secretion of slime can provide the necessary propulsive force for movement (Li *et al.*, 2002). Cyanobacterial exopolysaccharides may also protect nitrogenase (the complex responsible for nitrogen fixation) from the deleterious effects of oxygen (Kallas *et al.*, 1983).

Only a few of the ecological roles attributed to the cyanobacterial exopolysaccharides are fully supported by experimental data or detailed ecological observations. For instance, their role in protecting the cells against desiccation was experimentally demonstrated by a number of authors, in particular by Malcom Potts' group (Potts, 1994, 1999, 2004; Shaw *et al.*, 2003; Wright *et al.*, 2005) and Tamaru *et al.* (2005). These publications described in detail some of the chemical and molecular mechanisms by which the exocellular polysaccharidic layers prevent possible cell damage caused by desiccation and rewetting processes. Moreover, there is evidence that most of the cyanobacteria isolated from very dry environments (desert soils, lithic surfaces of monuments located in arid environments, etc.) are characterized by the capacity to synthesize large amounts of exocellular polysaccharidic material (Danin *et al.*, 1998; Brüll *et al.*, 2000; Belnap & Lange, 2001; Hu *et al.*, 2003b; Crispim & Gaylarde, 2005; Rivera-Aguilar *et al.*, 2006; Zhang *et al.*, 2006), thus supporting the role of these macromolecules in the survival of cyanobacteria in arid habitats. Another possible role that has been thoroughly investigated is the capacity of the sheath, capsules and slime to protect the cyanobacterial cells from the harmful effects of UV radiations. It was demonstrated that UV irradiation induces the synthesis of the extracellular polysaccharidic matrix in *N. commune* (Ehling-Schulz *et al.*, 1997; Wright *et al.*, 2005) and also that the UV-screen pigments are

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accumulated in the sheath and in the extracellular matrix, constituting a barrier against the penetration of the harmful UV radiations (Böhm *et al.*, 1995; Ehling-Schulz & Scherer, 1999; Dillon *et al.*, 2002; Fleming & Castenholz, 2007, 2008).

On the other hand, the observed capacity of the cyanobacterial exopolysaccharides to chelate metal ions has been reported to enable cells to accumulate the metals necessary for their growth and/or to prevent cells from direct contact with metals with toxic effects. Indeed, this assumption arises from experiments demonstrating that most cyanobacterial EPS are anionic in nature due to the presence of charged constituents, such as uronic acids, sulphate and ketal-linked pyruvate groups (Table 1). Additionally, many studies demonstrated the affinity of exopolysaccharides for metals (Micheletti *et al.*, 2008a; De Philippis & Micheletti, 2009). However, direct experimental evidence demonstrating the ecological role of this metal-uptake capacity is not yet available.

The role of the cyanobacterial polysaccharidic investments seems to differ from strain to strain, and to be dependent on the physical and chemical characteristics of the natural habitat or culture medium in which the organism grows. A more accurate perception of the ecological roles of these polymers will be possible when the information on the genetic machinery related to their production is available. This will enable the conditions under which the genes are transcribed/expressed to be investigated.

Factors affecting biosynthesis of cyanobacterial EPS

The use of cyanobacterial EPS for biotechnological applications depends on the identification of culture parameters that influence the synthesis and/or the characteristics of the EPS, and, subsequently, the establishment and control of the conditions that optimize the productivity and the suitable characteristics of the polymer. During the last three decades, several main factors controlling the production of the cyanobacterial EPS have been identified. These include energy availability and the C : N ratio (De Philippis & Vincenzini, 1998; Li *et al.*, 2002). However, other important factors such as the amounts of other nutrients as well as growth parameters such as light intensity, salinity and temperature have been largely disregarded, and very few exhaustive studies on factors influencing the production of cyanobacterial EPS are available in the literature. Moreover, the responses of cyanobacteria to changes in culture conditions appear to be frequently strain-dependent, making the optimization of EPS production even more difficult. The known key factors affecting EPS production are summarized in Table 5.

Nitrogen

Nitrogen is one of the most important elements for the synthesis of cell material, and cyanobacteria are either dependent on a combined nitrogen source or, in a restricted number of strains, can fix atmospheric nitrogen. Correlation between the source/amount of nitrogen and the production of EPS has been evaluated for several cyanobacteria and different results were observed depending on the strain tested. Usually, as can be observed in Table 5, the presence of a combined nitrogen source in the culture medium resulted in an increase in EPS synthesis, probably due to the lower energy requirement necessary for the assimilation of combined nitrogen compared with the energy needed for nitrogen fixation (Otero & Vincenzini, 2003; Kumar *et al.*, 2007). In some cyanobacteria, the amount of polymer produced varied according to the nitrogen source used (De Philippis & Vincenzini, 1998), whereas *Anabaena flos-aquae* A37 showed similar EPS production when supplied with different nitrogen sources such as $Mg(NO_3)_2$, KNO_3, $NaNO_3$, NH_4NO_3 and NH_4Cl (Tischer & Davis, 1971). Moreover, it was also demonstrated that the composition of the polymer released by *Anabaena cylindrica* 10C was slightly modified when the strain was cultivated with different nitrogen sources (De Philippis & Vincenzini, 1998). Nitrogen starvation has often been described as a condition that enhances EPS synthesis (De Philippis *et al.*, 1993; Otero & Vincenzini, 2003), probably because this contributes to the increase in the C : N ratio, thus promoting the incorporation of carbon into polymers (Otero & Vincenzini, 2003; Kumar *et al.*, 2007). Nevertheless, it is difficult to detect a direct correlation between diazotrophic and nitrogen-limiting conditions because other factors, such as differences in the carbon fixation efficiency and in the control of the equilibrium between internal and extracellular carbon pools, may explain the variations observed in the production of EPS under different culture conditions (De Philippis & Vincenzini, 1998; Otero & Vincenzini, 2003). Indeed, it was observed that in the nitrogen-fixing cyanobacterium *C. capsulata*, the mere diversion of carbon flux from protein synthesis, caused by the addition of various inhibitors of nitrogen assimilation, induced the accumulation of intracellular carbohydrate reserves (i.e. glycogen), whereas an effective enhancement of the amount of carbon available to the cells, induced by the addition of glyoxylate, which is known to stimulate the CO_2 fixation rate, caused an increase in the amount of EPS synthesized and released by the cells (De Philippis *et al.*, 1996).

Phosphate

The importance of phosphate supply in regulating the growth of cyanobacteria is widely recognized, especially in aquatic environments. Increased phosphate levels together with favourable weather conditions, for example water surface temperatures over 20 °C, often result in the development of widespread cyanobacterial blooms. The relationship

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Table 5. Effects of culture conditions on the EPS production in cyanobacteria

Organisms	Effects							References
	Presence of combined nitrogen	Phosphate starvation	Increase in NaCl concentration	Continuous air flow	Increase in temperature	Continuous light	Increase in light intensity	
Unicellular								
Aphanocapsa halophyta MN11	+	–	+				+	Sudo *et al*. (1995), Matsunaga *et al*. (1996)
Cyanothece sp. 113	+		+	+		+	+	Su *et al*. (2007)
Cyanothece sp. 16Som2	–	+	=					De Philippis *et al*. (1993), De Philippis & Vincenzini (1998)
Cyanothece sp. ATCC 51142	+		+					Nicolaus *et al*. (1999), Shah *et al*. (1999)
Gloeocapsa gelatinosa	+					+	+	Raungsomboon *et al*. (2006)
Gloeothece sp. ATCC 27152	+							Tease & Walker (1987)
Synechococcus elongatus f. *A. nidulans*	+							Sangar & Dugan (1972)
Synechococcus sp.	+	+	=					Roux (1996)
Synechococcus sp. BG0011						+		De Philippis & Vincenzini (1998)
Filamentous								
Microcoleus vaginatus	+		+					Hu *et al*. (2003a), Chen *et al*. (2006)
Phormidium laminosum (OH-1-pCl$_1$)	–							Fresnedo & Serra (1992)
Phormidium sp.	+	–		+		+		Nicolaus *et al*. (1999)
Phormidium tenue	+							Hu *et al*. (2003a)
Spirulina sp.	–	+	+		–			Nicolaus *et al*. (1999)
Filamentous heterocystous								
Anabaena cylindrica 10 C	+	–						Lama *et al.* (1996)
Anabaena flos-aquae A37	=							Tischer & Davis (1971)
Anabaena sp. ATCC 33047	–		–	+	+		+	Moreno *et al*. (1998)
Anabaena sp. PC-1	+		+					Choi *et al*. (1998)
Anabaena sp. WSAF	+	–		+		+		Nicolaus *et al*. (1999)
Anabaena sp.	+	+						Huang *et al*. (2007)
Anabaena torulosa	+	–		+		+		Nicolaus *et al*. (1999)
Cyanospira capsulata		=	=			+		De Philippis *et al*. (1991)
Nostoc commune	+							Huang *et al*. (1998)
Nostoc sp.	–							Hu *et al*. (2003a)
Nostoc sp. PCC 7413	+						+	Otero & Vincenzini (2003)
Nostoc sp. PCC 7936	–				=		+	Otero & Vincenzini (2003, 2004)
Nostoc sp. PCC 8113	–						+	Otero & Vincenzini (2003, 2004)
Scytonema javanicum	–							Hu *et al*. (2003a)
Westiellopsis prolifica ARM 366			+					Jha *et al*. (1987)

+, positive effect (increased production); –, negative effect (decreased production); =, no changes.

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between the available amounts of phosphate and the production of EPS is not straightforward, as the overall effect might be dependent on a set of interlinked variables such as the amount of phosphate, nitrate and sulphate (Grillo & Gibson, 1979). In most cases, phosphate starvation or low levels of phosphate induced an increase in EPS production (De Philippis *et al.*, 1993; Roux, 1996; Nicolaus *et al.*, 1999; Huang *et al.*, 2007); however, in *C. capsulata*, the absence of phosphate had no significant effect (De Philippis *et al.*, 1991), and in *Anabaena* spp. and *Phormidium* sp., it significantly decreased EPS production (Nicolaus *et al.*, 1999). Generally, an increase in phosphate concentration in the growth medium has little effect on the amount of exopolymers.

Sulphate

Cyanobacterial EPS contain sulphate groups, a unique feature among bacteria and shared by the EPS produced by archaea and eukaryotes (Sutherland, 1994; De Philippis *et al.*, 1998; De Philippis & Vincenzini, 2003; Micheletti *et al.*, 2008b). It has been reported that sulphur limitation has a dramatic impact on the cells, resulting in morphological and physiological changes similar to those due to nitrogen limitation (Wanner *et al.*, 1986). In *Gloeothece* sp. PCC 6909, sulphur starvation caused significant morphological alterations in the cells, such as the synthesis of a structureless sheath, the accumulation of cyanophycin, polyhydroxybutyrate and glycogen granules and the disintegration of thylakoid membranes. Most of these changes were reversed by the addition of sulphate to the culture (Ortega-Calvo & Stal, 1994; Ariño *et al.*, 1995).

Salt (NaCl)

The acquisition of salt tolerance in some cyanobacteria living in extreme environments induces various structural and metabolic changes, including a decrease in respiration and an increase in the production of some carbohydrates, notably sucrose, which functions as an osmotic solute protecting membranes from desiccation (Chen *et al.*, 2006). Generally, under salt stress (about 0.5 M), cyanobacteria also produce larger amounts of EPS (Table 5). It has been postulated that the increased export of EPS can have a function equivalent to that of sucrose, i.e. improving salt tolerance and carbohydrate metabolism (Chen *et al.*, 2003). However, some exceptions are reported, in which an increase in NaCl concentration did not affect or even lowered the EPS productivity. In *C. capsulata* (De Philippis *et al.*, 1991) and *Cyanothece* sp. 16Som2 (De Philippis & Vincenzini, 1998), the presence of a thick and firmly attached capsule probably provided enough protection against osmotic shocks, and in *Synechococcus* sp., EPS production increased only in the stationary phase, possibly because a nutrient limitation is necessary for the activation of EPS production (Roux, 1996). In *Anabaena* sp. ATCC 33047 growing under diazotrophic conditions, EPS production was enhanced only under conditions in which the nitrogenase activity and phycobiliprotein content were low, and production decreased in the presence of higher NaCl concentrations. However, the authors did not provide any explanation for this behaviour (Moreno *et al.*, 1998). In the halophilic cyanobacterium *Aphanothece halophytica* GR02 grown in the presence of various NaCl concentrations, a variation in the relative amounts of rhamnose and galactose, two of the seven monosaccharides constituting the RPS, was observed (Li *et al.*, 2001).

Aeration

Aeration seems to be vital for increasing the production of EPS by cyanobacteria, with the few studies available reporting that EPS production reached a maximum with continuous aeration (Moreno *et al.*, 1998; Nicolaus *et al.*, 1999; Su *et al.*, 2007). A possible explanation is that the increase in culture turbulence may facilitate the release of the polysaccharides from the cell surface, thus stimulating the synthesis of new exopolysaccharides. However, it is also possible that the higher turbulence due to the aeration provides a better stirring of the viscous cultures, which may increase the light penetration and consequently may induce a higher biosynthetic activity of the cells.

Temperature

The majority of the studies dealing with the production of EPS in cyanobacteria use the optimal growth temperature for the organism under investigation and, again, the limited data available indicate that the effect of the temperature variation is strain dependent. For *Anabaena* sp. ATCC 33047, an increase in the temperature (from 30/35 to 40/45 °C) led to a noticeable increase in the production of the EPS (Moreno *et al.*, 1998), probably because at higher temperatures, the time required to reach the onset of stationary phase was shorter than that required at 30/35 °C. In contrast, the temperature increase (from 30 to 35 °C) did not affect the EPS productivity in *Nostoc* sp. PCC 7936 (Otero & Vincenzini, 2004), and temperatures > 30 °C even caused a small decrease in EPS production in *Spirulina* sp. (Nicolaus *et al.*, 1999).

Light

The synthesis and release of EPS are particularly light dependent, even though different light regimens (continuous light and light–dark cycles) do not seem to have a significant effect on the quality of the polymer, i.e. monosaccharidic composition and relative proportions of the sugar units (Vincenzini *et al.*, 1993; De Philippis &

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Vincenzini, 1998). However, generally, EPS production is enhanced by continuous light and high light intensities (up to 400 μmol photons m^{-2} s^{-1}), but it is important to consider both the culture volume and the geometry of the culture flasks/bioreactors when adjusting the light intensity (see, e.g. Fischer *et al.*, 1997). Moreover, it was demonstrated that certain wavelengths influence EPS production; notably, in the heterocystous *N. commune*, UV-B irradiation stimulates extracellular glycan production as well as induces the synthesis of photoprotective pigments (Ehling-Schulz *et al.*, 1997).

Other factors

Many other factors that can influence EPS production, notably pH, dilution rate, growth phase, presence/absence of magnesium, calcium, potassium and heavy metals, as well as the addition of glycoxylate, acetate, valerate, glucose, citrate and EDTA have been sporadically studied (Li *et al.*, 2002), but not consistently evaluated.

In summary, although the key factors controlling the production of the cyanobacterial EPS have been identified, comprehensive strain-specific studies taking into account the interaction between the variables to understand the system response to changes, are still missing. This requires a better knowledge of the genes and metabolic pathways involved in the production of EPS in cyanobacteria.

Genes and biosynthetic pathways related to the production of EPS

Over the past decade, several studies have been initiated to try to understand the genetics and biochemistry of EPS biosynthesis in bacteria (Van Kranenburg *et al.*, 1999; De Vuyst *et al.*, 2001; Jolly & Stingele, 2001; Laws *et al.*, 2001; Sutherland, 2001; Welman & Maddox, 2003; Whitfield, 2006). However, cyanobacteria have not been thoroughly examined and, consequently, the information available is extremely limited (Yoshimura *et al.*, 2007). Studies performed in both Gram-negative and Gram-positive bacteria revealed that the EPS biosynthetic pathways are very complex, including, besides the enzymes directly involved in the EPS synthesis, enzymes engaged in the formation of the cell wall polysaccharides and lipopolysaccharides (Mozzi *et al.*, 2003). However, the mechanisms involved in the synthesis of EPS are relatively conserved throughout bacteria. Typically, this process comprises four distinct steps occurring in different cellular compartments: (1) the activation of the monosaccharides and conversion into sugar nucleotides in the cytoplasm, (2) the assembly of the repeating units by sequential addition of sugars onto a lipid carrier by glycosyltransferases at the plasma membrane, (3) the polymerization of the repeating units at the periplasmic face of the plasma membrane and (4) the export of the polymer to the cell surface (Yamazaki *et al.*, 1996; De Vuyst & Degeest, 1999; Kleerebezem *et al.*, 1999; Whitfield & Roberts, 1999; De Vuyst *et al.*, 2001; Jolly & Stingele, 2001; Sutherland, 2001). A schematic representation is depicted in Fig. 1. The sugar activation/modification enzymes and the glycosyltransferases are strain dependent, whereas the proteins involved in the polymerization, chain length control and export are conserved among bacteria. Some of these conserved proteins, as well as their interactions, are highlighted in Fig. 1, and their putative roles are discussed below.

The genes related to the production of surface polysaccharides can be divided into three classes: (1) those encoding the enzymes involved in the biosynthetic pathways of nucleotide sugars, or other components, needed for polysaccharide synthesis and not otherwise available in the cells; (2) those coding for the glycosyltransferases; and (3) those

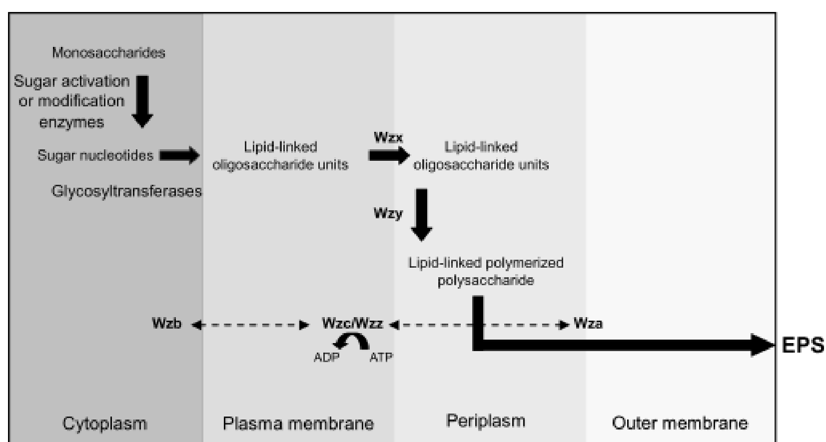

Fig. 1. Sequence and compartmentalization of the putative biosynthetic pathway, polymerization and export of EPS in cyanobacteria, based on the information gathered for other bacteria as well as the genes present in the available cyanobacterial genomes. Some of the proteins involved in the polymerization, chain length control and export are highlighted along with the interactions among them (dashed line arrows).

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required for the oligosaccharide or polysaccharide processing (Reeves *et al.*, 1996).

The first class is a vast and diverse group of genes not specific for EPS biosynthesis, given that sugar nucleotides are needed for the synthesis of a range of polysaccharides, and this group, therefore, will not be extensively discussed in this work. Among these genes are *rfbABCD*, also frequently called *rml* genes (Reeves *et al.*, 1996), which encode proteins involved in the biosynthesis of L-rhamnose. L-Rhamnose is a 6-deoxyhexose commonly present in bacteria, but only as a component of surface polysaccharides (Li & Reeves, 2000). Indeed, dTDP-rhamnose is commonly found in the EPS of Gram-negative and Gram-positive bacteria (Li & Reeves, 2000) and is a key constituent of the O-antigens of lipopolysaccharides of Gram-negative bacteria (Reeves, 1993). Furthermore, rhamnose is one of the sugars frequently found in the cyanobacterial EPS, and the proteins encoded by the *rfb* genes are listed in CyanoBase (http://bacteria.kazusa.or.jp/cyanobase/) as involved in the assembly of cyanobacterial surface polysaccharides. However, the participation of these proteins in the biosynthesis of both lipopolysaccharides and EPS makes it very difficult to determine their specific role. Moreover, the presence of several acidic or neutral monosaccharides in cyanobacterial EPS indicates that their biosynthetic pathway may be even more complex (Sutherland, 2001; Li *et al.*, 2002).

Glycosyltransferases are key enzymes for the biosynthesis of the EPS repeating unit, catalyzing the transfer of the sugar nucleotides from activated donor molecules to specific acceptor molecules – most probably a lipid carrier – in the plasma membrane. A large number of genes encoding glycosyltransferases have been identified, given the structural diversity of the bacterial extracellular polysaccharides, and consequently the number of possible linkages. The diverse function of the transferases, which in addition are strain specific, is reflected in the heterogeneity of their DNA sequences (Reeves *et al.*, 1996; De Vuyst *et al.*, 2001; Jolly & Stingele, 2001; Samuel & Reeves, 2003). *In silico* analysis of the cyanobacterial genomes revealed the presence of numerous genes putatively encoding glycosyltransferases; however, the enzymes have not been biochemically characterized, which makes it impossible to assign their function to the synthesis of EPS.

In bacteria, the genes encoding the proteins responsible for polymer extension and processing are usually clustered and organized in a similar way (De Vuyst & Degeest, 1999), often constituting long operons. Within these clusters, three different regions can be discerned: a central region constituted by the genes encoding the glycosyltransferases, flanked by two regions comprising the genes encoding enzymes involved in the polysaccharide polymerization, chain length control and export (De Vuyst & Degeest, 1999). The nomenclature of the latter genes is very diverse, for example being named *eps* and *cps* for lactic acid bacteria, *wz_* and *kps* for *Escherichia coli*, *exo* for *Sinorhizobium meliloti* and *gum* for *X. campestris* (De Vuyst & Degeest, 1999; De Vuyst *et al.*, 2001; Jolly & Stingele, 2001; Sutherland, 2001; Welman & Maddox, 2003; Whitfield & Paiment, 2003; Whitfield, 2006). Recently, a region containing 18 ORFs putatively involved in polysaccharide biosynthesis was identified for the cyanobacterium *Anabaena* sp. PCC 7120 (Yoshimura *et al.*, 2007).

Despite the variety of bacterial exopolysaccharides, bacteria use a limited repertoire of assembly and secretion strategies, which are represented in *E. coli* (Whitfield & Roberts, 1999; Whitfield & Paiment, 2003; Whitfield, 2006). For this organism, two models have been proposed for the biosynthesis and assembly of the different types of capsules based on genetic and biochemical criteria, with the one proposed for the capsules of groups 1 and 4 being the most common among Gram-negative bacteria (Rahn *et al.*, 1999; Whitfield, 2006; Whitfield & Larue, 2008). This mechanism is Wzy-dependent, in contrast to the mechanism for groups 2 and 3 capsules, which are assembled via ABC-transporter-dependent pathways (Whitfield, 2006). Using the *E. coli* Wzy-dependent model, together with the information derived from *Anabaena* sp. PCC 7120 (Yoshimura *et al.*, 2007) and the cyanobacterial genome sequences, a putative mechanism was put forward for cyanobacteria (Fig. 2). This is a hypothetical working model, on which further studies aiming to elucidate the mechanisms involved in the production of cyanobacterial EPS can be based. Assuming that a lipid carrier (in most of the Gram-negative bacteria an undecaprenol diphosphate – see Skorupska *et al.*, 2006) is also present in cyanobacteria, the glycosyltransferases will pass the sugar nucleotides to this acceptor, where repeating units are assembled. This step takes place at the interface between the cytoplasm and the plasma membrane. The newly synthesized lipid-linked repeating units are then flipped across the membrane in a process requiring Wzx, an integral plasma membrane protein. This provides the substrate for the blockwise polymerization of the repeating units that takes place at the periplasmic face of the membrane, a step carried out by the Wzy protein. The polymerization also requires the auxiliary protein Wzc to act at the interface between the membrane and the periplasmic space (Skorupska *et al.*, 2006), probably for the control of the chain length of the growing polymer. Transphosphorylation of Wzc and its dephosphorylation by Wzb is required to regulate the polysaccharide polymerization and export. The translocation process is mediated by the outer-membrane auxiliary protein Wza, which forms a channel, allowing the externalization of the growing polysaccharide to the cell surface. The translocation may require the physical association of proteins located in both membranes, notably Wzc and Wza (Whitfield & Paiment, 2003; Skorupska *et al.*, 2006; Whitfield, 2006; Collins & Derrick, 2007).

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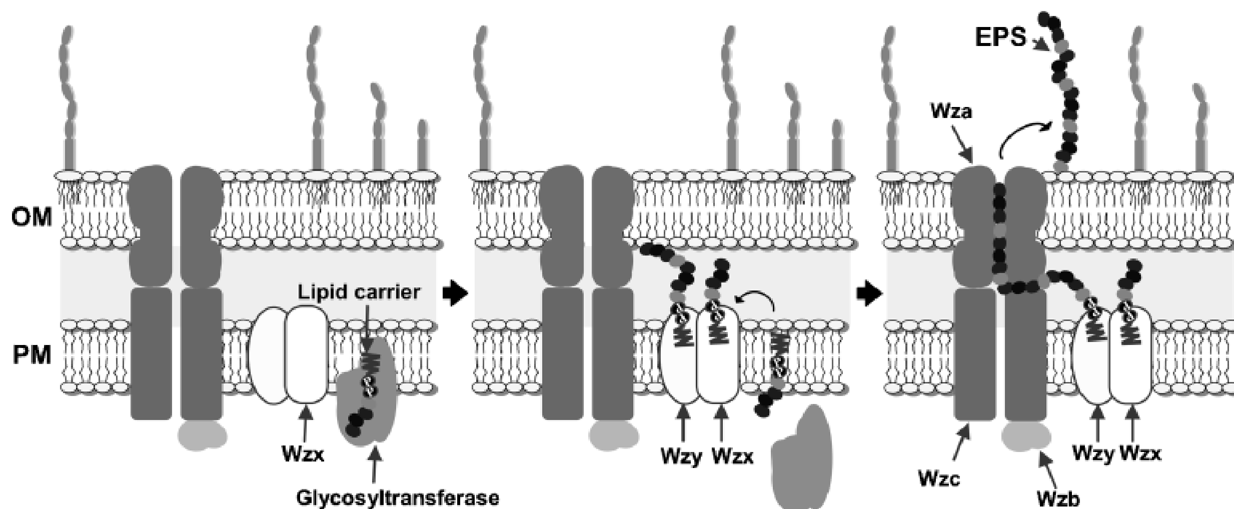

Fig. 2. Proposed model for the assembly and export of cyanobacterial EPS based on the information gathered both for other bacteria and genes found in cyanobacterial genomes. (1) Glycosyltransferases transfer the nucleotide sugars onto a putative lipid carrier, and the lipid-linked repeated units are assembled at the interface between the cytoplasm and the plasma membrane. (2) Newly synthesized units are 'flipped' across the membrane in a process requiring the integral membrane protein Wzx. Subsequently, Wzy assembles the polysaccharide by addition of new repeating units to the growing polysaccharide chain. (3) The polymer is translocated in a process requiring the Wzc and Wzb proteins. In the final stage, the carbohydrate polymer is translocated across the outer membrane through the outer-membrane lipoprotein Wza (adapted from Whitfield, 2006). OM, outer membrane; PM, plasma membrane.

An *in silico* analysis of the cyanobacterial genomes revealed that the genes putatively involved in the production of exopolysaccharides are sometimes clustered, present in different regions of the genome, and often occur in multiple copies. This last feature is not common in *E. coli*, *Klebsiella pneumoniae* and lactic acid bacteria, where the genes are frequently clustered and transcribed as one or two operons (Roberts, 1996; De Vuyst & Degeest, 1999; De Vuyst *et al.*, 2001; Jolly & Stingele, 2001; Whitfield & Paiment, 2003; Whitfield, 2006). Examples of the physical organization of *wz_* genes in three morphologically distinct types of cyanobacteria are depicted in Fig. 3. For the unicellular *Cyanothece* sp. only two copies of each gene were found, with the exception of *wzx*, which appears to be single (its sequence may not be complete). It was not possible to determine the relative position of these genes for this organism as the genome annotation process is still in progress. In general, as the complexity of the organism/size of the genome increases, more copies of the genes putatively involved in the production of the EPS are found, as can be observed for *Lyngbya* sp. and *Nostoc punctiforme*. In the last case, one needs to consider the presence of heterocysts that also have a polysaccharidic layer in their envelope. However, in the filamentous strains, a genome region containing all *wz_*, except *wzb*, could be identified, but it remains to be shown whether these genes are indeed specifically related to EPS production, and whether they constitute a transcriptional unit. Only by construction of deletion mutants will it be possible to understand the function of each of the proteins encoded by these ORFs and to start to unveil the biosynthetic pathways of cyanobacterial EPS.

The synthesis and secretion of EPS in cyanobacteria probably follow the pathways previously described for other bacteria. However, as a consequence of the cyanobacteria's ability to perform oxygenic photosynthesis and the unique characteristics of their EPS, some differences are expected. The production of exopolysaccharides is intimately dependent on the balance between the catabolic pathways of sugar degradation and the anabolic pathways of sugar nucleotide synthesis. This balance is certainly different in cyanobacteria compared with heterotrophic bacteria. Moreover, the presence of a higher number of different sugars in cyanobacterial EPS suggests that the synthesis of the sugar nucleotides is more complex, involving a higher number of different enzymatic reactions. In addition, although several genes encoding proteins putatively involved in the Wzy-dependent mechanism of EPS polymerization and export were identified in cyanobacterial genomes, their physical organization differs from what is observed in other microorganisms, suggesting that in cyanobacteria, this mechanism may be under a different type of regulation.

Concluding remarks

As discussed previously by De Philippis & Vincenzini (1998), the data on the chemical composition and on the

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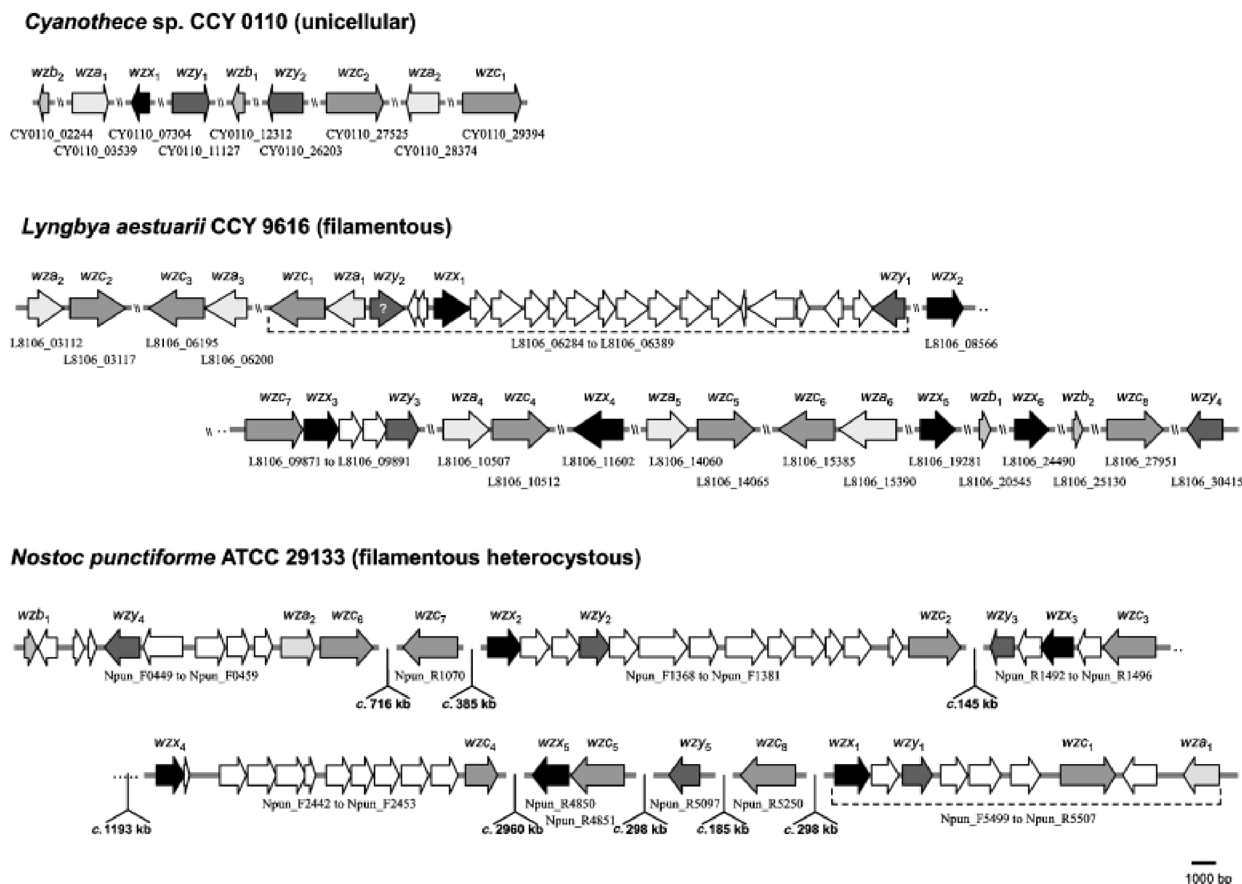

Fig. 3. Physical map of the putative genes involved in the polymerization, chain length control and export of EPS in the three morphologically distinct types of cyanobacteria. The deduced protein sequences encoded by these genes were submitted to an *in silico* analysis to identify the conserved motifs of interest. This analysis was performed using the following bioinformatic tools: BLASTP, CDART (at NCBI – http://www.ncbi.nlm.nih.gov/), and SMART (at EMBL – http://smart.embl-heidelberg.de/). In general, several copies of a specific gene could be identified in a single cyanobacterial strain. In a given organism, the copy that has the highest probability to be related to EPS production is designated by subscript 1 (taking into account both the percentage of identity with the corresponding sequences in other organisms and the position of the gene in relation to others involved in the same process); the other copies are numbered subsequently. In *Lyngbya* sp. and *Nostoc punctiforme*, the genome region containing all *wz_*, except *wzb*, is underlined with a dashed line. ? indicates that the gene *wzy2* from *Lyngbya* is the one with the lowest homology to the available *wzy* sequences. Genbank accession numbers: AAXW00000000 (*Cyanothece* sp. CCY 0110), AAVU00000000 (*Lyngbya* sp. PCC 8106) and CP001037 (*N. punctiforme* ATCC 29133).

rheological properties of the cyanobacterial EPS are not always comparable due to the different hydrolytic procedures and analytical methods used. Therefore, some of the results reported in the literature were not included in this review as they were not consistent with the majority of the data available.

The information gathered strongly underlines the complexity of both the chemical features of the cyanobacterial EPS and their putative biosynthetic pathways. As a result, it is not surprising that the data available on the structures of these macromolecules are still scarce and little is known about the genes encoding the proteins involved in their synthesis. Consequently, it is important to generate knowledge to unveil the pathways utilized by cyanobacteria for the synthesis of these biopolymers, which not only play a decisive ecological role, allowing these organisms to survive in adverse environmental conditions, but also have a high potential for biotechnological applications. The identification of the genes involved in the biosynthesis of EPS would also offer the possibility to investigate (1) the factors regulating the expression of these genes and (2) the possible genetic modification that could be introduced. This will make it possible to maximize the production of the polymer, as well as to introduce specific alterations in the composition/structure, producing polymers more suitable for specific applications. The construction of deletion mutants will help to define the role of each gene product and to clarify the function of EPS in natural habitats.

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