Exopolysaccharides from cyanobacteria and microalgae and their commercial application

Dhanesh Kumar[1,2], Petr Kaštánek[2] and Siba P. Adhikary[1,3,*]

[1]Department of Biotechnology, Visva-Bharati, Santiniketan 731 235, India
[2]Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Prague-6, Czech Republic
[3]Fakir Mohan University, Balasore 756 020, India

Cyanobacteria and green algae are phototrophic microorganisms showing high diversity in their cellular structure in response to the environmental conditions of the niche where they occur. Production of exopolysaccharides (EPS) in response to adverse conditions is one of the important features of these organisms. EPS are present mainly around their cells/filaments as an enveloped layer and released outside. EPS play protective functions and are important for their survival in stressed habitats exposed to radiation, desiccation and elevated temperatures. This review discusses the production, extraction and possible commercial applications of EPS produced by cyanobacteria and green algae.

Keywords: Cyanobacteria, carbohydrate, exopolysaccharides, green algae.

CYANOBACTERIA are prokaryotic, oxygen evolving, unicellular or filamentous microorganisms among which some can fix atmospheric nitrogen. They are phylogenetically related to eubacteria and algae because cyanobacteria share the characteristics of both. Green algae are eukaryotic lower plants. Both cyanobacteria and green algae show similarity in autotrophic mode of nutrition through photosynthesis. Many cyanobacteria and green algae are surrounded by a special mucilaginous covering around their cells or filaments. Chemical analysis revealed that these are mainly composed of carbohydrates produced and secreted outside the cell and are termed as exopolysaccharides (EPS). These EPS form different types of layers around the cells depending upon the species and are termed as slimes, sheaths and/or capsules[1–4]; however these terminologies are not strictly followed. Generally slimes are layers which can be removed from cells by simple mechanical processes like vortex and centrifugation while sheaths/capsules are rigid in structure and can be removed using harsh mechanical procedures such as cell mill or French press followed by density gradient centrifugation[5].

These sheaths, slimes, and/or capsules that remain associated with the cell surface, are termed as capsular polysaccharides (CPS) while EPS released by cells to their surrounding environment are termed as released polysaccharides (RPS). The amount of RPS and CPS depends on the producing microorganism and its culturing conditions[6]. It is generally accepted that synthesis of exocellular polysaccharides represents a metabolic strategy for survival and growth of cyanobacteria, especially under unfavourable environmental conditions[7,8]. Production of exocellular polysaccharides is reported to respond to changes in several external factors, such as temperature, nitrogen concentration or light irradiance[9–12].

With the development of biotechnological tools and technology, a wide range of algae and cyanobacteria have attracted the attention of researchers due to their capacity to produce EPS in large quantities. These polysaccharides from biological sources can be commercially exploited in many ways such as for use as gums, food additives, food thickener, soil stabilizer and in phytoremediation of contaminated waste. This review focuses on general aspects of EPS produced by cyanobacteria and green algae including their composition, role of EPS for survival of the organism under extreme environments, and their possible commercial applications.

Arrangement of EPS on cells/filaments and their chemical composition

The EPS attached to cell surface can be grouped into sheaths, capsules and slimes based on their thickness, consistency and appearance[4]. The sheath is defined as a thin, dense layer loosely surrounding cells or cell groups usually visible in light microscopy without staining. Figure 1 shows a thick layer of EPS around the cell/filaments of cyanobacteria and green algae (Figure 2). Capsule generally consists of a thick and slimy layer intimately associated with cell surface with sharp outlines. Slime refers to the mucilaginous material dispersed around the organism but not reflecting the shape of the cells.

Many studies have analysed the chemical composition of EPS using various chromatographic and mass spectrometric techniques. Glucose, galactose, rhamnose, fucose, arbinose, xylose, mannose, orthomethyl sugar and acidic residues of glucuronic acid and galacturonic acid are the principal sugars present in the EPS of cyanobacteria (Table 1)[5,13] and green algae (Table 2). Glucose is the most frequently occurring sugar and shares a major

*For correspondence. (e-mail: adhikarysp@gmail.com)

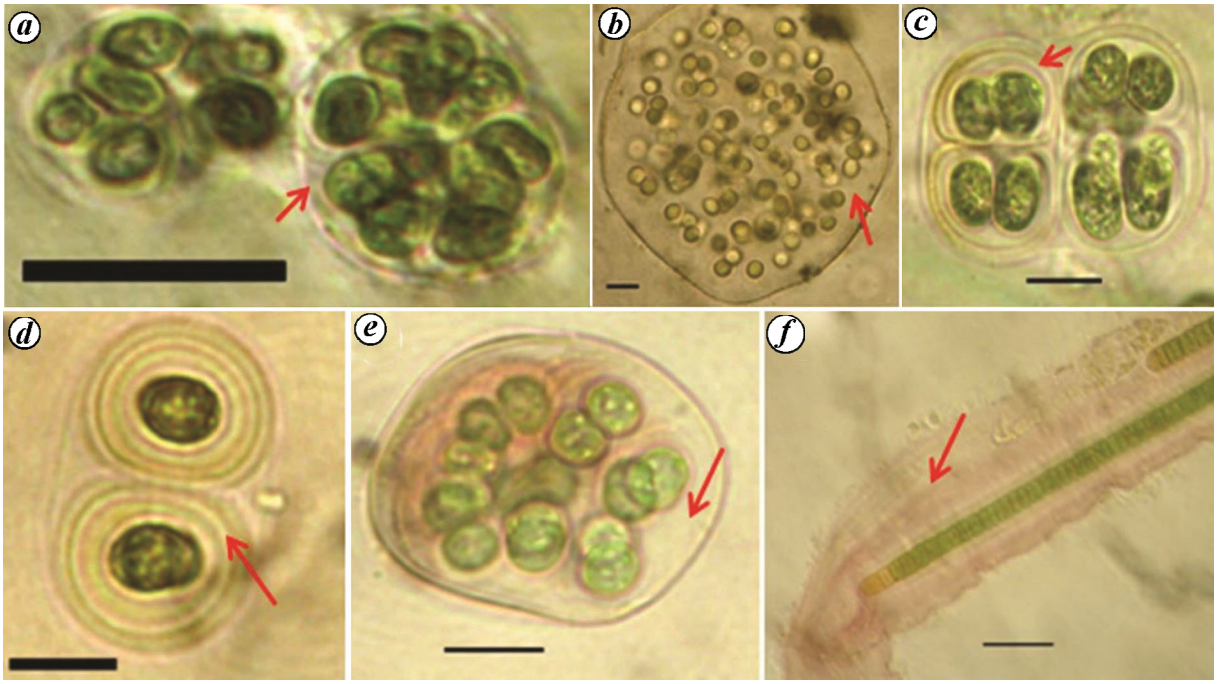

Figure 1. Cyanobacteria enclosed EPS sheath: ***a***, *Aphnanothece saxicola*; ***b***, *Aphanocapsa gravillei*; ***c***, *Gloeocapsa caldariorum*; ***d***, *Gloeothece rupestris*; ***e***, *Asterocapsa submerse*; ***f***, *Porphyrosiphon notarisii*. Scale bar = 10 µm.

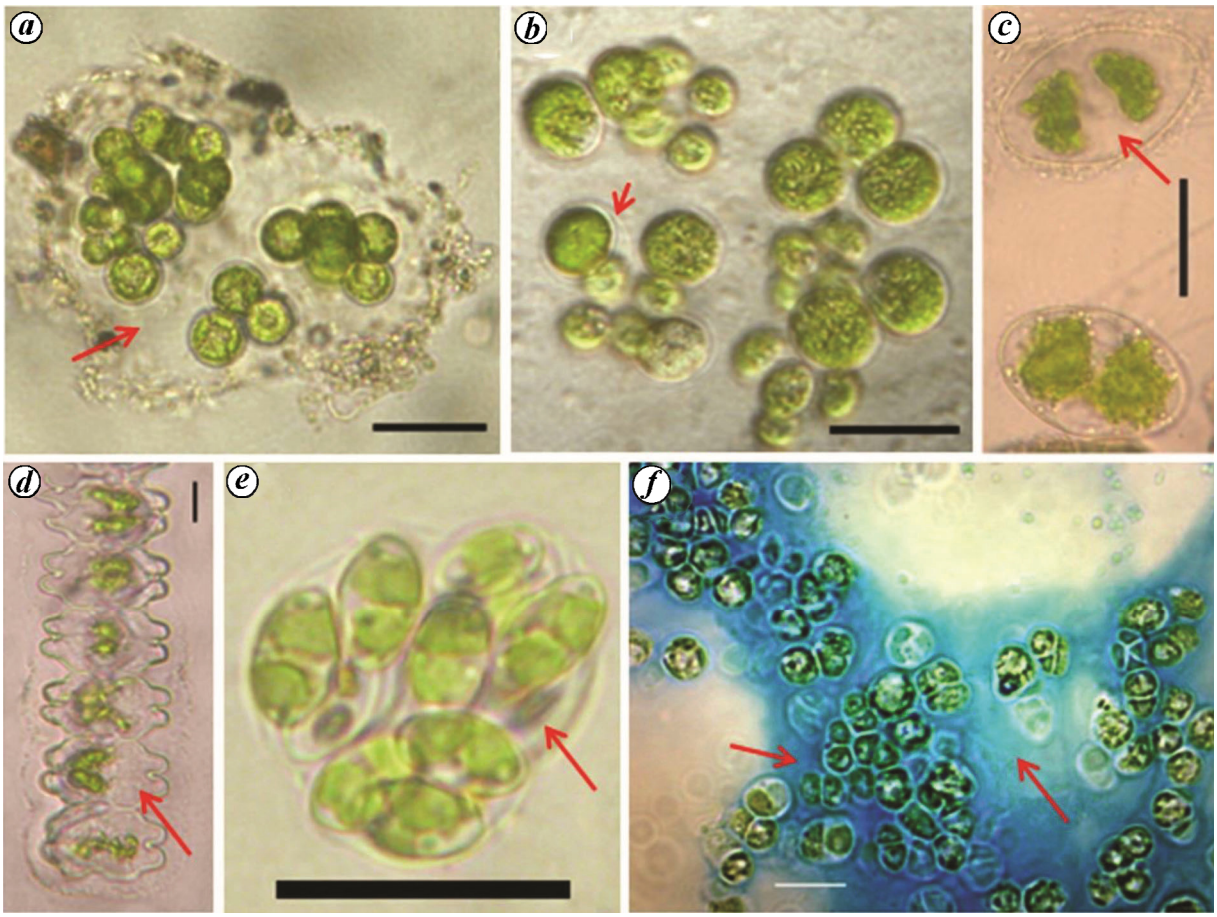

Figure 2. Green microalgae enclosed EPS sheath (arrows): ***a***, *Bracteacoccus* sp.; ***b***, *Chlorococcum humicola*; ***c***, *Cylindrocystis obesa*; ***d***, *Desmidium* sp.; ***e***, *Oocystis lacustris*; ***f***, *Dictyosphaerium chlorelloides*. Scale bar = 10 µm.

Table 1. Chemical composition of EPS sheath of cyanobacteria

Organism	Polysaccharides (sugar composition)	Other	Reference
Chroococcus minutus SAG B 41.79	Glc (+), Man, Xyl, Ara, 4MS	AA	2
Gloeothece PCC 6501	Gal (+), Glc, Man, Rha, Xyl, 1MS	UA, AA	52
Calothrix parietina	Gal, Glc (+), Man, Rha, Ara, Xyl, Fuc, 2 MS	UA, AA, As	53
Fischerella PCC 7414	Gal, Glc (+), Man, Xyl, Fuc, 1MS	UA, AA, As, sulphate	54
Anabaena cylindrical	Gal, Glc (+), Man, Xyl, Fuc		55
Nostoc IARI 221	Gal, Glc (+), Man, Rha, Xyl, Ara	UA	56
Microcystis flos-aquae C3-40	Gal, Glc, Man, Rha, Xyl	UA (+)	57
Phormidium foveolarum MEU, C52	Gal, Glc (+), Man, Ara±, Fuc, Rha, Xyl, Rib±	UA, AS±, sulphate	58
Phormidium ectocarpi N 182, K5, ME3, C86 PCC 7375	Gal, Glc (+), Man, Rha, Xyl, Fuc	UA, AS±, sulphate	58
Phormidium minutum D5, NB5, RT6	Gal, Glc (+), Man, Rha, Xyl, Ara, Fuc	UA, AS±, sulphate	58
Phormidium sp. PNG 91, 90-14/1,CCAP 1464/3, CCAP1464/4	Gal, Glc (+), Man, Rha, Xyl, Ara±, Fuc±, Rib±	UA, sulphate	58
Oscillatoria amphibian PCC 7105	Gal, Glc (+), Man, Rha, Xyl, Fuc	UA, sulphate	58
Oscillatoria corallinae CJ 1	Gal, Glc (+), Man, Rha, Xyl, Fuc	UA, sulphate	58
Lyngbacon fervoides S 9 g	Gal, Glc (+), Man, Rha, Xyl, Ara, Fuc	UA, AS	58
Cyanothece 16Som2	Gal, Glc (+), Man, Xyl, Fuc, Rha	UA	3
Cyanothece CA3	Glc, Man, Ara (+), Fuc, Rha	UA	3
Cyanothece CE9	Gal, Glc (+) Man, Fuc, Rha	UA	3
Cyanothece ET2	Gal, Glc, Man Ara (+), Fuc, Rha	UA	3
Cyanothece IR20	Gal, Glc, Man, Rib, Fuc, Rha (+)	UA	3
Mastigocladus laminosus	Rha, Fuc, Xyl, Man, Gal, Glc		59
Microcoleus vaginatus	Ara, Rha, Fuc, Xyl, Man (+), Gal, Glc	UA, MS	60
Nostoc sp.	Rha, Xyl, Man, Gal, Glc (+)	MS, AA	60
Phormidium tenue	Ara (+), Rha, Man, Gal, Glc	UA	60
Scytonema javanicum	Ara, Rha, Xyl, Man, Gal, Glc (+)		60
Phormidium tenue	Ara (+), Rha, Fuc, Xyl, Man, Gal, Glc	AA	21
Plectonema battersii strain GF	Ara, Fuc Xyl Glc (+) Gal Man	UA, sulphate	61
Chroococcus submarines strain BM	Rha, Fuc, Xyl (+), Glc, Gal, Man	UA, sulphate	61
Rhabdoderma rubrum strain CH	Fuc, Xyl (+), Glc, Gal, Man	UA, sulphate	61
Johannesbaptistia pellucida strain GC	Ara, Rha, Fuc, Xyl, Glc, Gal, Man	UA, sulphate	61
Cyanothece sp. ATCC 51142	Rib, Xyl, Glc (+)	AA	40
Oscillaria sp.	Rib, Xyl, Glc (+)	AA	40
Nostoc carneum	Xyl, Man (+)	AA	40
Nostoc insulare 54.79	Glc	UA, MS, AA	62
Arthrospira platensis	Ara, Rha, Fuc, Xyl, Man, Gal, Glc	UA, AA	63
Nostoc commune	Xyl, Man, Gal, Glc (+)	AA	64
Nostoc verrucosum	Xyl, Man (+), Glc (+)	UA	64
Arthrospira platensis strain MMG-9	Fruc, Fucose, Gal, Glc (+), Man, Rha, Rib, Xyl	UA	65
Microcoleus vaginatus	Rha, Xyl, Man, Gal, Glc (+)	AA	66
Nostoc carneum	Xyl (+), Glc	UA, sulphate	67

portion in EPS composition with few exceptions. Presence of sulphate groups in cyanobacterial EPS is a rare feature among eubacteria, but common in EPS produced by archaea and eukaryotes. Sulphate groups, glucuronic acid along with galacturonic acid contribute in the anionic nature of the EPS. The negative charge gives a sticky character to the overall macromolecule[4]. It has been shown that due to anionic and sticky nature, EPS have the affinity towards positively charged ions, mainly metal ions[4]. This feature is useful for bioremediation[14]. Some EPS are of hydrophobic nature due to presence of ester-linked acetyl groups, peptidic moieties and deoxysugars such as fucose and rhamnose. These hydrophobic groups give emulsifying properties to EPS, a property important for industrial uses[15].

Chemical compositions of EPS produced by various cyanobacteria are different. Even for the same strain, composition changes with age and condition of culture. Temperature, intensity of light, concentration of sulphur, phosphate, potassium and other metal ions also affect the composition of EPS. Changes in these physiological conditions have an effect on cellular morphology too. According to Otero and Vincenzini[16], capsulated strain of *Nostoc* (PCC 7936 and PCC 8113) became uncapsulated and stopped releasing the slime in the media in the presence of nitrate affecting the viscosity and rheological properties of culture, but these strains revert to their capsular form once they are transferred to nitrate free media. The amount of total carbohydrate was also large in culture grown in high light with nitrate media than low light and nitrate free media. Based on chemical composition, these EPS can be divided into two groups: homopolysaccharides and heteropolysaccharides[17]. Homopolysaccharides are composed of only one type of monosaccharide, and are synthesized from sucrose by the action of a sucrase[18]. Heteropolysaccharides are made up of different

Table 2. Chemical composition of EPS sheath of microalgae

Organism	Polysaccharides (sugar composition)	Other	Reference
Chlamydomonas mexicana	Ara, Rha, Fuc, Rib, Xyl, Man, Gal, Glc (+)	UA	68
Chlamydomonas sajao	Ara, Rha, Fuc, Rib, Xyl, Man, Gal (+), Glc	UA	68
Botryococcus braunii	Ara, Rha, Fuc, Gal (+), Fuc, Rha	UA, MS	69
Chlorella sp.	Ara, Glc, fuc	UA	70
Desmococcus olivaceus	Ara, Rha, Xyl, Man, Gal, Glc (+)	UA	60
Desmococcus olivaceus	Ara, Rha, Xyl, Man, Gal (+), Glc	MS	21
Dunaliella salina	Gal, Glc (+), Xyl, Fru		71
Nannochloropsis sp.	Glc (+), Man, Fuc, Man, Xyl	UA	72
Dunaliella tertiolecta	Gal, Xyl, Glc, Rib	UA	73
Chlamydomonas reinhardtii	Ara, Rha, Rib, Xyl, Gal, Glc	UA	74
Graesiella sp.	Fuc (+), Gal, Ara, Glc, Man, Xyl, Rib, Rha	UA, AA, sulphate	63

Fru, Fructose; Gal, Galactose; Glc, Glucose; Man, Mannose; Rha, Rhamnose; Ara, Arabinose; Xyl, Xylose; Fuc, Fucose; Rib, Ribose; MS, O-methyl sugars; UA, Uronic acids; AS, Amino sugars; AA, Amino acids. $^{+}$Major component; $^{\pm}$Presence or absence in different strains of the same species.

sugars of high molecular mass[1] and synthesized by the combined action of different types of glycosyltransferases[19,20].

Cyanobacteria produce complex EPS composed of, on the whole, by different monosaccharides including pentoses, which are usually absent in other polysaccharides of prokaryotic origin that contain a lower number of different monomers, usually less than four[3]. To date, up to 12 different monosaccharides have been identified in cyanobacterial EPS. These are 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[3,4,19]. Hu *et al.*[21] also reported the presence of 2, 3-O-methyl rhamnose, 3-O-methyl rhamnose, 4-O-methyl rhamnose and 3-O-methyl glucose in trace amount.

Role of EPS

The EPS produced by cyanobacteria and green algae are of diverse types and play different roles in the life cycle of these microorganisms ranging from biofilm formation, symbiosis and protection from predation to resistance from various types of stresses such as ultraviolet (UV) radiation, oxidation, temperature and desiccation stress[20,22,23]. Several cyanobacterial species reported from extreme habitats such as exterior of stone monuments in a tropical country like India are subjected to high solar irradiation[12–24]. Many studies have reported the presence of scytonemin, a UV-absorbing pigment in the sheath of these cyanobacteria living in such habitats[12,22,25]. Other types of UV-absorbing pigments, the mycosporine-like amino acid compounds were also found in the sheath of some cyanobacterial strain[26], which confirms the protective role of capsular EPS from the deleterious effects of UV radiation.

EPS also help in the gliding movement of cyanobacteria. Hoiczyk and Baumeister[27] studied the envelope of the filamentous cyanobacteria *Oscillatoria princeps*, two strains of *Phormidium uncinatum* and *Lyngbya aeruginosa* and suggested that necessary propulsive force for the gliding of cyanobacteria is generated by shear forces between the specially arranged fibrils on the cell envelope and the continuing flow of secreted extracellular slime. Prosperi[28] reported that mucilaginous heterocysts of *Nostoc cordubensis* were able to fix nitrogen at high oxygen concentrations, whereas heterocysts without mucilage were unable to fix nitrogen at oxygen concentrations higher than 20% suggesting the role of EPS to protect nitrogenase (the enzyme complex responsible for nitrogen fixation) from the harmful effects of oxygen. It has been experimentally established that algal and cyanobacterial polysaccharides protect the cells against desiccation stress[29,30]. A number of cyanobacteria are reported to survive without water by producing both internal and external polysaccharides. Due to their hydrophilic and hydrophobic properties, EPS can absorb and retain water forming a gelatinous layer around the cell and regulate the water uptake[22,31] and water loss. Some sugars help in the stabilization of proteins, membranes, and whole cells under water deficient conditions but in case of extreme water loss only the disaccharides trehalose and sucrose were able to provide protection[29]. These trehalose or sucrose enable the microorganism to cope the desiccation stress by protecting from drying of liposomes, cellular enzymes, membrane components and dried cells as well[29,32].

Soon after rehydration, the cyanobacteria cells started showing metabolic activities such as photosynthesis and respiration and repair of their cellular components[33,34]. Apart from protection against desiccation, EPS of cyanobacteria and green algae also accumulate heavy metals[14]. Due to the presence of various functional groups (carbonyl, carboxyl, hydroxyl and sulphate), EPS are able

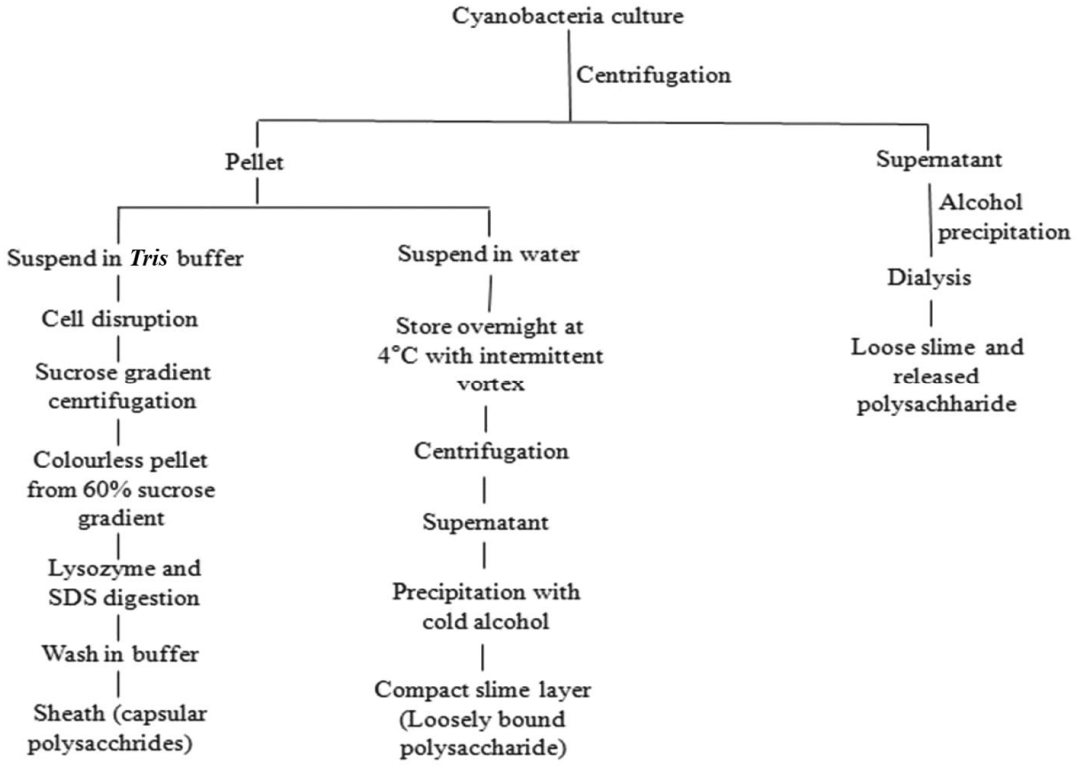

Figure 3. Schematic representation of extraction of EPS from cyanobacteria and green algae.

to bind various positively charged metal ions which can be used in an organism's own physiological processes such as nitrogen fixation and growth or as toxin against other predators[29,35]. Thus EPS covering the cell avoid direct contact with heavy metals with toxic effects. Difference in chemical composition of the sheath of *Gloeothece* ATCC 27152 in various concentrations showed the variation in their metal binding capacity[35]. EPS also help microorganisms for anchorage with other substratum and an masking the antibody recognition[27].

Extraction methods

Extraction of EPS from cyanobacteria and green algae is important for determining their quantity, chemical compositions, purification, product formation and commercialization. Various studies have applied different techniques for extraction of EPS from cyanobacteria and micro-algae. The overall extraction procedure of cyanobacterial EPS is described[5,13,36] in Figure 3.

According to De Philippis *et al.*[36], after removing the cells by centrifugation (14,000 *g*, 20 min at 10°C), crude EPS were obtained by addition of 2-propanol to the medium. For purification of crude polymers, RPS samples were dispensed again into distilled water, dialyzed for 24 h against distilled water and lyophilised. Total carbohydrates, uronic acid and hexos-amine contents of purified RPS samples were extracted using phenol ± sulphuric acid[37], carbazole[38] and Ehrlich's reagent[39] respectively. Elemental composition of the RPS was determined colorimetrically using automated elemental analyser.

In the method followed by Parikh and Madamwar[40], cells were separated from the growth medium by centrifugation at 15,000 *g* at 15°C for 40 min. The supernatant was concentrated to one fourth volume on magnetic stirrer at 60°C for 10 h. The polysaccharide from this concentrated supernatant was precipitated by gradually adding an equal volume of cold acetone to the supernatant and kept at 4°C overnight. The precipitated EPS was redissolved in Milli Q water. This process of precipitation and redissolution was repeated. Crude EPS was further purified by dialysis against Milli Q water for 20 h at 4°C and freeze dried.

Application of EPS

In recent times, EPS produced by cyanobacteria and algae are drawing the attention of many researchers due to their advantage over EPS produced by other sources such as algae produced continuously throughout the year in less space in open ponds, raceway ponds and photobioreactors[41,42]. EPS are mainly used in industries as gelling and thickening agents suspending or stabilizing the aqueous phase. EPS produced by different organisms have different characteristics; hence they have specific

industrial applications[43]. The EPS from microbial sources are used in food (jelly, cakes, ice-creams, candy, dressing, sauce, gravy, beverages), pharmaceuticals (capsule covering, anti-tumour activity of drugs), textiles (printing, dye and pigment suspensions), cosmetics (body lotion, emulsifier), detergents, oil recovery etc.[5]. Many cyanobacteria produced sulphated polysaccharides. These polysaccharides interfere in the absorption and penetration of virus into host cells and inhibit the transcriptase activities[44]. According to Moreno[45], EPS extracted from *Anabaena* sp. showed pseudoplasticity which is an advantageous flow property and useful in commercial applications specially when mixed with other materials. The viscosity of *Anabaena* EPS dispersions was similar to other commercially available food grade polysaccharides and very near xanthangum[45]. *Anabaena* EPS also have certain advantageous properties over other commercially available gums in terms of their mitigation capacity to temperature, pH and salt concentration on EPS[45].

Algal EPS are also used in bioremediation and as bioflocculant. The biofilms mainly formed by cyanobacteria synthesize the negatively charged EPS that are released in water and bind a number of positively charged heavy metals causing them to settle down at the bottom of water bodies[46]. EPS of the cyanobacterium *Anabaena spiroides* were efficient in binding the heavy metals Mn(II), Cu(II), Pb(II) and Hg(II)[47]. Algal EPS can also be used in agriculture for prevention of soil erosion. EPS produced by cyanobacteria and algae entrap and bind soil particles together, increasing the size of soil aggregates. As soil aggregates enlarge, they become heavier, have a greater surface area and are more difficult for wind or water to remove[48]. This association of cyanobacteria, algae and soil on earth surface is also known as biological soil crusts or microbial mat. This mat encourages the growth of other beneficial soil microorganisms, improves soil water-holding capacity, increases soil organic matter and makes phosphates more soluble[49]. Falchini *et al*.[50] studied the role of cyanobacterial inoculation in maintaining soil structure and reported that EPS produced by the two *Nostoc* strains AfS49 and KaS35 were responsible for primary clay aggregation and maintenance of soil structure[50]. It is also possible to increase the production of EPS and to introduce some specific alteration in the composition or in the structure of EPS making it more suitable for specific applications[51]. However, lack of concrete information regarding the genes encoding the proteins involved in the EPS biosynthetic pathways, and the factors controlling these processes limits their potential for biotechnological applications[16]. Hence further research is needed for proper understanding of the pathways for the formation of EPS at molecular level.

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