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A Review: Bioflocculant and Biosurfactant Activity of Exo- Polysaccharides Produced by Marine Cyanobacteria- Nostoc

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ABSTRACT: Hasty industrialization is leading to the use of large quantities and varieties of synthetic chemicals, which are considered to be xenobiotic and subsidize to environmental pollution. Two important methods to pull out the pollutants are physical and chemical method. Unlike physicochemical methods, biological degradation is a natural process in which microorganisms make use of organic pollutants for its growth and other cellular processes; is termed as bioremediation. Bioremediation has been recognized as a prudent and effective treatment for these contaminations. Exo-polysaccharides from *Nostoc species* can act as a good flocculant compared with the commercial flocculants. This review would give the reader a better perceptiveness and familiarity about the EPS and the usage as an emulsifier in pharmaceutical industry.

KEY WORDS: Xenobiotic, Exo-polysaccharide (EPS), Bioremediation, Bioremediators, Nostoc species.

I. INTRODUCTION

Cyanobacteria also known as "Cyanophyta", is a phylum of bacteria that obtain their energy through photosynthesis [1]. The name "cyanobacteria" comes from the colour of the bacteria. They are often called as blue-green algae. Cyanobacteria are a major and phylogenetically coherent group of gram-negative prokaryotes possessing a unique property of performing oxygenic plant-like photosynthesis with autotrophy as their dominant mode of nutrition. Representatives of this group has been found frequently in abundance, in most of the natural illuminated environments examined so far, i.e., both aquatic and terrestrial, including several types of extreme environments. They can occur as planktonic cells or form phototrophic biofilms. They are found in almost every endolithic ecosystem. A relatively new possible exploitation of cyanobacteria has arisen in the last decade by the growing industrial interest towards polysaccharides of microbial origin, that often show advantages over the polysaccharides extracted from plants or marine macro algae. As a result, a wide search for bacterial strains able to produce good yields of new polysaccharides with potentially useful properties has been undertaken; also involving cyanobacteria because of the well-known capability of some strains to excrete mucilaginous material [4]. On the other hand, the EPS that is produced by these cyanobacteria are essentially used in various industrial applications.

II. CHARACTERISTICS OF CYNOBACTERIA

Cyanobacterial growth depends on the availability of light, it has been suggested that the release of emulcyan by *Phormidium J-1* also plays a role in flocculation of suspended clay particles, thereby clearing the water column and increasing the amount of light available for the cyanobacterium growing as a mat on the bottom sediments [4]. Benthic cyanobacteria are those that are present in the benthic region of the ocean. They have some special characteristics that are very exclusive to these cyanobacteria as they are able to survive in those harsh conditions. In benthic cyanobacteria, it has been suggested that the attachment of cells to the sediment is modulated by cell

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hydrophobicity, which is usually determined by extracellular polymeric substances (EPS). These are polysaccharides bearing non-sugar components. Some cyanobacteria that live in association or symbiosis with higher plants, it has been suggested that the EPS may act as an adhesive for cyanobacterial cells. More recently, a research showed that the arm associations of the elements of a *Nostoc* strain to the roots of wheat are due to the binding of the polysaccharide matrix that surrounds the trichomes. In the same study, it was noted that the polysaccharide surrounding the cells of an *Anabaena* strain was not able to bind to the root surface, so that only a loose association was established between the cyanobacterium and the roots [4]. Most cyanobacterial polysaccharides are characterized by an anionic nature, many of them containing two different uronic acids, a feature rarely found in the polymers released by strains belonging to other microbial groups. Cyanobacterial RPSs (released polysaccharides) often show the presence of one or two pentoses, sugars that are usually absent in other polysaccharides of prokaryotic origin. Most RPSs synthesized by cyanobacteria (about 80% of the polymers) are quite complex, being composed of six or more monosaccharides [4].

TABLE I.
TOTAL CARBOHYDRATE, PROTEIN AND MONOSACCHARIDE COMPOSITION OF
CYANOBACTERIAL EPS.

Organism	Total Protein (mg/g)	Total Carbohydrate (mg/g)	Comparative sugar composition of purified EPS				Reference
			Ribose (%)	Xylose (%)	Glucose (%)	Mannose (%)	
<i>Nostoc sp.</i>	40.1 ± 0.1	685 ± 10	-	31.7 ± 0.7	-	68.3 ± 0.5	[6]
<i>Nostoc carneum</i>	27.2 ± 0.1	560 ± 10	-	33.2 ± 1.8	-	66.8 ± 0.4	

Thirty days old cultures were used for EPS extraction and purification. Hydrolyzed EPSs were used for the identification of monosaccharides.

III. EXTRACELLULAR POLYMERIC SUBSTANCES (EPS)

Extracellular polymeric substances (EPS) are high-molecular weight (polymers) compounds secreted by cyanobacteria into their environment [2]. EPS are mostly composed of polysaccharides (exopolysaccharides) and proteins, but include other macromolecules such as DNA, lipids and humic substances. EPS are the construction material of bacterial settlements and either remains attached to the cell's outer surface, or are secreted into its growth medium. Exo-polysaccharides generally consist of monosaccharide and some non-carbohydrate substituent (such as acetate, pyruvate, succinate, sulphate, uronic acid and phosphate).

TABLE II.
THE TABLE BELOW GIVES THE PROFILE OF NOSTOC STRAINS THAT ARE INVOLVED IN
THE EPS PRODUCTION AND ITS DIVERSIFIED COMPOSITION

WEIGHT / EPS DRY WEIGHT (%)									
Filamentous heterocystous organisms	Ecological origin	No. of monosaccharides	Uronic acids	Deoxysugars	Sulphate	Pyruvate	Acetate	Peptides	References
<i>Nostoc flagelliforme</i>	Desert China	3	0	0	-	-	-	-	[10]
<i>Nostoc carneum</i>	Contaminated soil, India	2	0	0	0	-	-	27.2	[6]
<i>Nostoc commune</i>	Mountain area, China	7	13.3	5.8	-	-	-	-	[10]

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<i>Nostoc commune</i>	Freshwater, China	6	18.4	0	-	-	-	-	[12]
<i>Nostoc commune DRH-1</i>	Desert, Mongolia	7	22.7	0	0	-	-	-	[13]
<i>Nostoc insulare</i> 54.79	Soil	4	26.4	0	0	tr	tr	0.7	[14]
<i>Nostoc sp.</i>	Contaminated soil, India	2	0	0	0	-	-	40.1	[6]
<i>Nostoc sp.</i> PCC 6310	Pond, Israel	9	4.3	1.6	p	1.4	3.3	69.5	[16]
<i>Nostoc sp.</i> PCC 6705	Botanical garden, California	9	26.8	2.1	p	3.2	1.7	6.9	[16]
<i>Nostoc sp.</i> PCC 6719	Soil water culture, California	9	9.9	0.7	p	5.0	2.4	0.8	[16]
<i>Nostoc sp.</i> PCC 6720	Soil, Indonesia	8	13.5	4.1	p	2.1	1.1	3.1	[16]
<i>Nostoc sp.</i> PCC 7107	Pond, California	9	26.7	6.7	p	0.7	2.7	3.4	[16]
<i>Nostoc sp.</i> PCC 7413	Soil, UK	9	19.3	2.0	p	6.2	3.3	0.6	[16]
<i>Nostoc sp.</i> PCC 7416	Shallow pool, California	8	23.6	4.6	p	0	4.0	9.7	[16]
<i>Nostoc sp.</i> PCC 7422	Cycas roots	8	26.8	5.4	p	2.4	2.0	4.7	[16]
<i>Nostoc sp.</i> PCC 7423	Dried soil, Senegal	7	18.8	0.8	p	3.2	6.8	18.4	[16]
<i>Nostoc sp.</i> PCC 7706	Water below calcareous stone, France	7	25.2	1.2	0	0	0.8	10.3	[16]

tr = trace (<1%) , p= present

IV. CHARACTERISTIC OF THE EPS

The cyanobacterial EPS can be divided in two main groups: the one 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 [17, 18].

SHEATH:

The sheath is defined as a thin, dense layer loosely surrounding cells or cell groups usually visible in light microscopy without staining.

CAPSULE:

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).

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SLIME:

The slime refers to the mucilaginous material dispersed around the organism but not reflecting the shape of the cells.

RPS (RELEASED POLYSACCHARIDES):

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.

V. CYANOBACTERIAL EPS COMPOSITION

Cyanobacterial EPS are complex heteropolysaccharides, with 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 [18].

The different monosaccharide present in the EPS up to date are: Hexoses, Glucose, Galactose, Mannose, Fructose, Pentose, Ribose, Xylose, Arabinose, Deoxy hexose, Fucose, Rhamnose, Methyl Rhamnose, Acidic hexoses, Glucuronic acid, Galacturonic Acid. 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 [15]. 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 fructose, are present at higher concentrations than glucose [3, 11, 17, 18, 19, 20].

VI. CHEMICAL STRUCTURE OF EPS

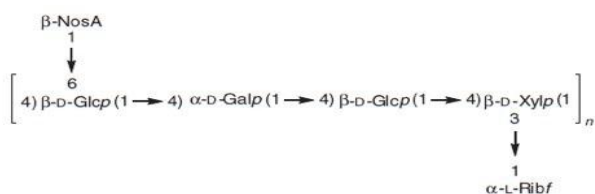


Fig. 1. *Nostoc commune* DRH-1

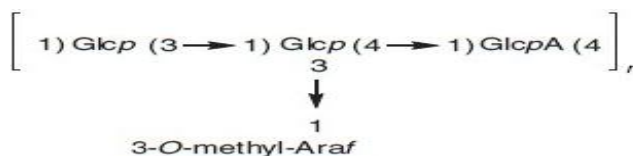


Fig. 2. *Nostoc insulare* 54.79 (Soil)

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 [3, 16, 21]. Cyanothecae strains CE4 and CA3 possess very high viscosities, up to four times higher than that of xanthan gum [3]. 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 [3, 9, 18].

VII. POTENTIAL USES OF CYANOBACTERIAL EPS

BIOFLOCCULANT ACTIVITY:

According to the IUPAC definition, flocculation is "a process of contact and adhesion whereby the particles of dispersion form larger-size clusters." Flocculation is synonymous with agglomeration and coagulation / coalescence; either spontaneously or due to the addition of a clarifying agent. Despite the effective flocculation performance and low cost of the synthetic organic polymeric flocculants and the inorganic flocculants their usages have become a source of concern as some adverse health effects have been associated with them. Furthermore, the synthetic organic polymeric flocculants are environmental problem as they are not biodegradable in nature. Owing to the hazardous nature of inorganic and organic synthetic flocculants, microbial flocculants are attracting attention as no known pathologic association has been ascribed to them, hence they are topical and ecosystem friendly. Biofloculants are kinds of extracellular biopolymer (like the EPS from *Nostoc*.sp.) containing glycoprotein, polysaccharide, protein, cellulose, lipid, glycolipid and nucleic acid [5]. Subsequently, it is anticipated that flocculants from deep sea microorganisms can have a special adaptability to low temperature and high salinity conditions and can be used as effective biofloculants for low temperature and highly saline effluent treatments [5].

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BIOSURFACTANT ACTIVITY:

Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant contains both a water insoluble (or oil-soluble) component and a water-soluble component. Surfactants will diffuse in water and adsorb at interfaces between air and water or at the interface between oil and water, in the case where water is mixed with oil. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. The water insoluble hydrophobic group may extend out of the bulk water phase, into the air or into the oil phase, while the water-soluble head group remains in the water phase. Biosurfactant is another property of the EPS that is produced by cyanobacteria. It is a diverse group of surface active molecules/chemical compounds synthesized by microorganisms [21]. Biosurfactants reduce surface tension and critical micelle dilution (CMD) in both aqueous solution and hydrocarbon mixtures [7]. Nostoc sp. has been reported to produce polymeric biosurfactants [7]. The most commonly isolated and the best studied groups of biosurfactants are mainly glycolipids and phospholipids in nature. Biosurfactants are eco-friendly and have potential industrial and environmental applications [7].

VIII. FACTORS THAT AFFECTS EPS PRODUCTION, BIOFLOCCULLANT, BIOSUFFACTANT PROPERTY

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 [3]. During the last three decades, several main factors controlling the production of the cyanobacterial EPS have been identified [3]. These include energy availability and the C-N ratio [17]. 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 [3].

IX. THE POSSIBLE FACTORS THAT INFLUENCE ON THE EPS PRODUCTION

1. CHEMICALS IN THE MEDIUM

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, the atmospheric fixed nitrogen source. 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. In some cyanobacteria, the amount of polymer produced varied according to the nitrogen source used [3,16].

TABLE III.
AMOUNT OF EPS PRODUCED AND GROWTH RATE OF CYNOBACTERIA FOR DIFFERENT NITROGEN SOURCE.

Nitrogen source	Specific growth rate (d^{-1})	Exo-polysaccharide production (gL^{-1})
N ₂	1.87 ± 0.2	17.2 ± 1.4
KNO	2.26 ± 0.2	2.0 ± 0.1
NH	1.75 ± 0.1	2.7 ± 0.2

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, results in the increased cyanobacterial bloom. Generally absence of phosphate had no significant effect an increase in

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phosphate concentration in the growth medium has little effect on the amount of exopolymers [3].

SALT (NaCl): Generally, under salt stress (about 0.5 M), cyanobacteria produce larger amounts of EPS [3].

2. 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 [3,8].

3. 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 [3].

4. IRRADIATION: The synthesis and release of EPS are particularly light dependent, even though different light regimens do not seem to have a significant effect on the quality of the polymer [15]. 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 photo- protective pigments [3].

5. OTHER FACTORS: Any 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, but not consistently evaluated [3].

X. FACTORS THAT AFFECT BIOFLOCCULLANT AND BIOSURFACTANT ACTIVITY

1. Calcium (Ca^{2+}) concentration: Cations can neutralize negatively charged functional groups of both bioflocculant molecules and suspended particles, which increased the adsorption of flocculant to suspended particles [5].

2. pH: pH is another important factor that must be considered but these properties depends on the individual strain and also the working condition as well. Generally at the neutral pH the flocculant and surfactant activity is very high [5,7].

3. TEMPERATURE: Temperature is also another criterion which depends on the strain, medium used. Biosurfactant activity is at its maximum at 37.5C for EPS produced by *Nostoc* sp. [7].

4. SALT (NaCl): Effect of NaCl on Biosurfactant production is that a very little change was observed up to 10% (w/v) NaCl [7].

XI. APPLICATION OF EPS IN INDUSTRY

One of the most important prerequisites of a polysaccharide, which determines many of the properties generally considered useful for its industrial utilization (i.e. high viscosity of its aqueous solutions, capability of forming gels with good tensile strength, stabilizing emulsions, etc); is that it must possess, together with an adequate composition and structure, a high molecular mass (MW). In this respect, only seven cyanobacterial polysaccharides have been tested, results showing MWs higher than 1000 KDa. The highest MW values, in the range of 1400-2800 KDa, depending on the analytical techniques utilized for molecular weight determination, have been reported for the RPS produced by *C.capsulata* [4]. The major utilization of EPS would be as flocculants, surfactants, single cell protein etc.

XII. CONCLUSION

EPS that is produced by cyanobacteria has an edge over other EPS as they can use

- Cheap carbon source
- They can with stand extreme condition
- Easy production, no toxic substances present.

There exist lot of work that deals with the characterization of the components in EPS and also the potential use of EPS in industries as well as their application point. But the major drawback is that they lack the examination of rheology and structural property.

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XIII. FUTURE STUDY FROM THIS REVIEW

- To maximize the production of EPS so that that would become possible for economical use in the commercial scale.
- To study the rheology and structural property of EPS.
- Molecular study of the mechanism involved in the EPS production.

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BIOGRAPHY



C. S. Jeba Samuel was born and brought up in Nagercoil, Kanyakumari. She believes that almost every soul on this world is so much excited about the promises made by life science. So she pursued her masters in the field of Biotechnology from Udaya School of Engineering, Nagercoil. She believes that a person who can make hard things easy is the educator, so after her graduation she started her career as Assistant professor in Department of Biotechnology, Rajalakshmi Engineering College. She also has a major contribution to the research field. She has done a lot of projects from her under grad level and also been a mentor to encourage students who actively involved in projects. As a gift for her dedication in work, she received a grant worth around two lakhs from Union Grants Commission, Government of India to carry out a minor research project in the field of Marine Biotechnology. She has also published various research articles in the reputed journals in different field of biotechnology.