Extracellular Polymeric Substances from Cyanobacteria: Characteristics, Isolation and Biotechnological Applications-A Review

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Abstract
Cyanobacterial Extracellular Polymeric Substances (EPS) play an important physiological role in mat formation and stress tolerance during adverse conditions. They are highly heterogeneous polymers containing a number of distinct polysaccharides and non-carbohydrate constituents including proteins, phospholipids and nucleic acids. EPSs are therefore regarded as abundant source of structurally diverse polysaccharides. Some of these possess unique properties for special applications in bioremediation, food, and pharmaceutical industries. Their physical and chemical characteristics show little variability and they are not vulnerable to variations in climatic, cultivation, production or pollution conditions and are secreted in harsh environment. For these reasons, they are good candidates for mass production on an industrial scale and therefore show immense biotechnological potential.

Considerable progress has been made in isolation, and characterisation of new cyanobacterial EPSs that possess numerous benefits and are of industrial significance. This paper attempts to highlight these developments with a view to increase the inventory of bioactive compounds whose exploitation will lead to development of clean technologies utilising cheap, desirable and renewable resources such as carbon dioxide and solar energy through photosynthesis that cyanobacteria are capable of.

Key Words- Cyanobacteria, Extracellular Polymeric Substances

INTRODUCTION
Cyanobacteria are photosynthetic prokaryotic organisms which are unicells or filaments. They are cosmopolitan in habitat, being commonly found in fresh and marine water, in soil and in rocks, from the tropics to polar region, and from temperate climates to extremes including deserts [1]. They sometimes participate in the formation of microbial crusts or mats [2, 3]. Some of their great significance biologically is the fact that certain cyanobacteria can fix elemental nitrogen [1]. A number of diazotrophic cyanobacteria grow easily in association with certain green algae, liverworts, waterferns and angiosperms [2].

Cyanobacteria are capable of movement by gliding when in contact with the substrate, (1985)[2]. Some have the ability to survive dessication and extremes of temperature, and grow at high pH and salinity [4]. Cyanobacteria are therefore pioneer photosynthetic communities on all ecosystems including desert ecosystems on earth[5]. Their distribution around the world is surpassed only by the rest of the bacterial taxa put together [6].

Cyanobacteria have been known for a long time to produce large amounts of exopolysaccharide [7]. In his monograph on cyanophyta, Desikachary [8] described ~63.5% morphotypes of cyanobacteria that show thin or thick sheath or mucilaginous film or slime. These exopolymeric investments are known to be largely heteropolysaccharidic composed of 1–10 neutral sugars often associated with amino sugars, proteins and fatty acids [9, 10, 11] Polysaccharide chains are usually formed by using an oligosaccharide as a repeating unit that...
can vary in size depending on the degree of polymerization. EPS are highly heterogeneous polymers containing a number of distinct monosaccharides and non-carbohydrate substituents that are species specific.

**STRUCTURE OF EPS**

A unique property of cyanobacterial EPS is their structural organization. A vast number of EPS from cyanobacteria have been reported over the last decades, and their greatly variable composition, structure, biosynthesis and functional properties have been extensively studied [12, 13, 5].

There are three types of exopolysaccharides-

1. A sheath, a thin uniform structured external layer immediately next to the outer membrane, containing either concentric or radial fibres, according to the strains. Sheaths may differ in the number and volume of layers as well as in the chemical characteristics and packing patterns of the polymer fibrils constituting each layer and in their orientation relative to cell surface. For single layers with polymer fibrils perpendicular to the cell wall, some researchers use the term “capsule,” as in the case of other bacteria. In the absence of a sheath or capsule, oligosaccharide chains (O-antigen) of the outer membrane lipopolysaccharide form a layer similar to the glycocalyx of euakaryotic cells. The cells of many cyanobacterial species that form trichomes, aggregates, or microcolonies have not only individual but also common sheaths or mucous investments.

2. A capsule or slime (capsular or slime polysaccharide, CPS). The capsular polysaccharide is intimately associated with the cell surface and may be covalently bound with well defined limits. In contrast, slime polysaccharide is only loosely associated with the cell surface without sharply defined limits.

3. A soluble polysaccharide (released polysaccharide, RPS), is released by many cyanobacteria as a less organized (amorphous) slime into the media [2, 14, 15]. Recently, this massive production has received increasing attention due to the potential applications of these substances as industrial gums, bioflocculants, soil conditioners and biosorbants, and to their participation in symbiotic processes in plants, in the gliding movement and in the general interaction between microorganisms and their habitats [5, 14, 16, 17, 10].

These exopolysaccharides have found multifarious applications in bioremediation, textiles, detergents, adhesive, food and pharmaceutical industries [10, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25].

**ISOLATION AND EXTRACTION**

EPSs have been isolated from different genera of Cyanobacteria e.g. *Anabaena variabilis* [5], *Nostoc calcicola* [13], *Limnothrix redekei* PUPCCC116 [26] and many others as reviewed by Pengfu et al. 2001 [27].

The soluble and bound EPS can be separated using physical and chemical methods [28]; while the soluble EPS can be extracted by centrifugation alone, the bound EPS require additional chemical treatment for their extraction. Comte et al. [29] have pointed out that not only the EPS yield, but also the chemical composition of EPS depends on the extraction method. Wide spectrums of different sample preparations to loosen EPS from cell surfaces are available. They can be classified as physical (ultrasonication, cation exchange resin, heating, high-speed centrifugation) and chemical (alkaline reagents, ethylenediaminetetraacetic acid (EDTA), aldehyde solutions) methods [29, 30, 31, 32].

**CHEMICAL COMPOSITION**

Extracellular polymeric substances from cyanobacteria are typically composed of polysaccharides, lipids, proteins and DNA in the form of heteropolymers, like lipopolysaccharides or glycoproteins [33]. They are particularly complex in nature due to the presence of proteins, uronic acids (mainly glucuronic and galacturonic), pyruvic acid, and O-methyl, O-acetyl, and sulfate groups (e.g., N-acetylglucosamine) [34, 35, 36]. The high molecular weight heteropolysaccharides consist of linear or branched repeating units comprising 2–10 monosaccharides. Typical monomers are hexoses, pentoses, uronic acids and deoxy-sugars. Phosphate, sulphate, acetate and pyruvate are characteristic substituents; proteins and lipids form side chains. EPS are attached to the cell surface via hydrogen bonds, hydrophobic and electrostatic interactions. Furthermore, phospholipids function as anchor regions, which are covalently bound to the cell wall [28, 37].

A high content of extracellular polymeric substances (EPS) and biomass, embedding the cells in a gelatious matrix, contributes to the formation of an interrelated structure of cyanobacterial mats [38]. Cyanobacterial mats are cohesive consortia of several groups of microorganisms, including cyanobacteria, sulphate-reducing bacteria, colourless sulphur bacteria and anoxygenic phototrophic bacteria.

Cyanobacterial EPS possess in general an anionic character mainly due to the high content of uronic acids. High amounts of neutral sugars like glucose, mannose, arabinose and xylose are observed in the EPS [39, 40, 41]. Furthermore, in some cyanobacterial EPS ester-linked acetyl-groups, peptides and deoxy-sugars like rhamnose and fucose can be found [40, 42]. These components confer hydrophobic properties to the otherwise hydrophilic macromolecules.
Both the soluble and bound EPS derived from activated sludge have shown to be mainly composed of proteins and polysaccharides with the soluble EPS having higher concentration of polysaccharides than the bound EPS [43].

The peculiar features of the polysaccharides released by the cyanobacteria in comparison to those released by other microbial sources are:

1. Many 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 [44].

2. Cyanobacterial released polysaccharide often show the presence of one or two pentose sugar that are usually absent in other polysaccharide of prokaryotic origin[44]. The moiety protects the neighbouring glycosidic bonds from the more common glycan hydrolases.

3. Most released polysaccharides synthesized by cyanobacteria are quite complex, being composed of six or more monosaccharides. This is the striking difference from the polymers synthesized by other bacteria or by microalgae, in which the number of monomers is usually less than four.

**PHYSIOLOGICAL ROLE**

The physiological role of EPS depends on the ecological niches and the natural environment from which microorganisms have been isolated. Most bacteria occur in microbial aggregates whose structural and functional integrity is based on the presence of a matrix of extracellular polymeric substances [28]. They are essential in the aggregate formation, in the mechanism of adhesion to surfaces and to other organisms, adhering to solid surfaces, in the formation of biofilm and in the uptake of nutrients [45, 46]. In particular, studies of sea ice microbial communities have also found bacteria strongly associated to particles and have pointed out that microbial EPSs played an important role in cryoprotection [47]. In fact, EPS production is thus essential to the survival of microorganisms in their natural environment. Exo-polysaccharides are produced by many microorganisms as a strategy for growing successfully, and surviving adverse climatic conditions. EPS possess a protective nature: they form a layer surrounding cells provide an effective protection against high or low temperature and salinity or against possible predators.

Depending on their specific composition, EPS also have multifarious cellular functions, including accumulation of nutrients, diffusion barrier for toxins and heavy metals, cell motility, attachment on surfaces, protection against desiccation, which is important in the natural habitats of cyanobacterial mats. The different roles of EPS are reviewed in detail in Decho [33] and Mancuso Nichols et al.[48]. EPS form the framework for biofilms and microbial mats [33, 49, 50, 38, 51].

Chemical characterisation of EPS has helped to understand the formation or stabilisation of mats and biofilms, and their functions for the producing microorganisms. Gehrke[31] showed that iron species complexed by EPS allow bacteria to attack on pyrite and that Fe(III)-ions complexed by uronic acids in the EPS were needed to dissolve pyrite. Several bacteria produce amphiphilic EPS, which can act as surfactants thereby making hydrocarbons bioavailable [52].

Cyanobacterial EPS have also been shown to play a role in transport of exogenous macromolecules due to their permeability characteristic as well as interaction with the plants as symbiosis [53]

**BIOTECHNOLOGICAL APPLICATIONS**

In recent years, attention towards cyanobacterial EPS has increased because along with the fact that a large number of cyanobacteria are characterized by the presence of polysaccharidic outer investments, these organisms are photosynthetic, easy to culture, some are even N₂ fixers, and are amenable to manipulation of conditions for enhancing growth and/or EPS production [10,54,55].Their physical and chemical characteristic show little variability and they are not vulnerable to variations in climatic, cultivation, production or pollution conditions. For this reason, some microbial polysaccharides are mass produced on an industrial scale and used as raw material for processed foods, in medicine and in industrial preparations.

Studies have shown that EPS play a crucial role in biosorption and binding of heavy metals [56,57,58,59] and that the biosorption capacity of soluble EPS for heavy metals is greater than that of bound EPS [43]. EPS have been used as an effective absorbent for removing organic pollutants such as dyes and pesticides [60,61].

Due to the presence of negatively charged groups ,primarily carboxyl group they have also been shown to have good sorbent capacity towards positively charged metal ions [62,63,64]. These unique properties have endeared them for use as flocculating, gelling, emulsifying, and suspending agents. This make them suitable for varied applications in pharmaceutical, chemical, and food industries. EPS produced by cyanobacteria could therefore be useful in various applications such as water holding capacity of soil and removal of heavy metals and solid materials from water reservoirs and formation of hydrated gels [5,35,36,55,65].
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Also, microbial EPS are preferred in these industries owing to their novel functionality, reproducible physicochemical properties, stable cost and supply [66].

CONCLUSION

It is estimated that EPS production is a process that requires a noticeable energy cost of up to 70% of total energy reserve, representing a significant carbon investment for microorganisms. However, the benefits related to EPS production are significantly higher. [67, 68]

In terms of utilization of cyanobacterial EPS as multiutility biopolymers, further work is needed on biochemical steps of EPS biosynthesis, so that their commercial production is effectively designed in an energy efficient manner.

8. ACKNOWLEDGEMENT

The authors wish to thank Mr Himanshu Gupta, Lecturer, Amity Institute of Biotechnology, Amity University Rajasthan for his kind help in preparation of this manuscript.

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