CHAPTER – II

REVIEW OF THE LITERATURES
Cyanobacterial Exo-polysaccharide- Productivity, Function, Structure, Biotechnological Potential and Factors Controlling EPS production

Productivity:- Cyanobacterial exopolysaccharides comprise a wide variety of outermost investments differing in thickness, consistency and appearance (De Philippis and Vincenzini 1998). De Philippis et. al. (1998, 2001) reviewed the possible application of ‘Released polysaccharide’ from cyanobacteria and their chemical nature together with their biotechnological potential. They categorized 3 types of cyanobacterial EPS like, i) Sheath- the thin electron dense layer of individual or groups of cell, visible under microscope, ii) Capsule-thick slimy layer intimately associated with cell and , iii) Slime – the mucilagenous material surrounding the organisms. During cell growth, aliquots of polysaccaridic material of both capsule and slime are released into the medium increasing the viscosity of the medium. Cyanobacteria are also able to excrete EPS in the form of tubes, stalks, pads, and mucilage for attachment and motility (Decho 1990; Hoagland et al. 1993). Exocellular polysaccharides may furnish microorganisms with the capability to form biofilms on solid surfaces (Costerton 1981, 1987 and Whitfield 1988). In benthic cyanobacteria, it has been suggested that the attachment of cells to the sediment is modulated by cell hydrophobicity, which is determined by extracellular polymeric substances (Fattom and Shilo 1984).

Exocellular carbohydrate polymer (EPS) production have been reported for a large number of cyanobacteria, e.g. for Anabaena cylindrica (Bishop et al 1954; Bar-Or & Shilo 1987; Lama et al. 1996; Nicolaus et al. 1999; Moreno et al. 2000; Huang et al. 2007), Nostoc spp. (Hough et.al 1952; Moore & Tischer 1964; Mehta & Vaidya 1978; Flaibani et al 1989; Fischer et al. 1997; Huang et al. 1998; Helm et al. 2000; De Philippis et al. 2000; Parikh & Madamwar 2006); Palmella mucosa (Tischer & Moore 1964), and Spirulina spp.etc. (Filali Mouhim et.al 1993; Tseng & Zhao 1994). Works have been also carried out with the taxa like Aphanothece (Sudo et al. 1995), Cyanothece, (De Philippis & Vincenzini 1998), Microcystis (Forni et al. 1997; Huang et al. 2007), Synechococcus (Sangar & Dugan 1972; Panoff et al. 1988), Lyngbya (Gloaguen et al.1995), Microcoleus sp. (Mazor et al. 1996), Oscillatoria (Gloaguen et al. 1995; Nicolaus et al. 1999, Parikh & Madamwar 2006; Huang et al. 2007), Phormidium (Gloaguen et al. 1995; Nicolaus et al. 1999; Bar-Or & Shilo 1987; Hu et al. 2003a; Richert et al. 2005) and others.

It is also found that EPSs are the main fraction of dissolved organic carbon released by freshwater phytoplanktons which are the good source of carbohydrates in natural waters.
Other eukaryotic algae like, marine, estuarine, and freshwater diatoms secrete very large amount of EPS in nature and in cultures, especially in the stationary phase (Hoagland et al.; 1993; Staats et al.; 1999; De Brouwer & Stal; 2002; De Brouwer et al. 2002). Desmids produce EPS in the form of gels, mucilage and slimes that encapsulate cells or used for movement (Domozych et al. 1993, 2005; Coesel 1994)

The cyanobacterial EPS productivities are advantageous due to the reasons that,- (i) being photoautotrophs, they are capable of utilising renewable and cheap substrates and many of them are nitrogen fixers, (ii) many strains can grow in brackish or in waste waters (iii) it is possible to utilize the carbon source from the CO₂ emitted by industrial plants; (iv) the economy of the process could be enhanced by recovering more than one useful compounds, with a multiproduct strategy as already proposed for microalgae (Thepenier et al 1988).

Function:- Cyanobacterial sheath can protect the cells from the detrimental process of biomineralization (Phoenix et al. 2000; Benning & Mountain 2004; Benning et al. 2004). Furthermore, the presence of negatively charged polysaccharidic layers, surrounding the 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, 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.

Polysaccaridic sheath of cyanobacteria are also provided with pigments. The UV-absorbing pigment scytonemin was found in the sheath of a number of cyanobacteria living in environment, 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 presence of mycosporine-like amino acid compounds (MMAs) have also been reported, confirming the role of the sheath in harbouring UV absorbing substances, and thus protecting the cyanobacterial cells from the deleterious effects of UV radiation (Adhikary & Sahu 1998).

It is believed that EPS in cyanobacteria play a major role in protecting cells from various types of stress in severe habitats. Caiola et al. (1996) and De Philippis et al. (1998)
suggested that the extrusion of EPS can serve as a boundary between cells and the surrounding environment, thus fulfilling a protective role against desiccation. EPS might be important for maintaining the structure and functions of biological membranes. Thus EPS protect biological membranes from irreversible and lethal changes during desiccation. It has been reported that the EPS of *N. commune* can prevent fusion of membrane vesicles *in vitro* at low relative humidity in the presence of a reducing sugar such as trehalose or sucrose (Hill 1997). 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, 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 (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. It forms the macroscopic colonies in which the entangled filaments are embedded in massive polysaccharidic structures (Hill et al. 1994; Böhm et al. 1995; Shirkey et al. 2000). The secreted 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). Other authors believed that secretion of EPS probably plays a role in adhesion to facilitate locomotion (Edgar & Pickett-Heaps 1984; Lind et al. 1997 and Wang et al. 1997).

Cyanobacterial exopolysaccharides also create the anaerobic micro-environment within the cell, when it is necessary and protect enzyme like, nitrogenase (the complex responsible for nitrogen fixation) from the deleterious effects of oxygen (Kallas et al. 1983). De Philippis and Vincenzini (1998) have also described the role of EPS in the attachment of cells to sediment in benthic cyanobacteria, facilitating the homogeneous dispersion of trichomes. Exocellular polysaccharides in microorganisms, including cyanobacteria, play major role in protecting cells from stress in extreme habitats and from other harmful conditions. The dense mucilage surrounding the trichomes of many strains could make them less preferred food in comparison with other microalgae that are devoid of capsules.
Chemical Structure: Cyanobacterial EPS are complex heteropolysaccharides, with almost 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 (Hough et al. 1952; Bishop et al. 1954; Moore & Tischer 1965; Dunn & Wolk 1970; Kokyrsta & Chekoi 1972; Sangar & Dugan 1972; Wang & Tischer 1973; Mehta & Vaidya 1978; Painter 1983; De Philippis & Vincenzini 1998).

To date, up to 12 different monosaccharides have been identified in cyanobacterial EPS: 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 more structurally resilient sheaths of Chlorogioeopsis PCC 69 12 (Schrader et al. 1982), Gloeethece PCC 6501 (Jurgens & Weckesser 1985) and Chroococcus minutus SAG B.41.79 (Adhikary et al. 1986) have been shown to contain O-methyl sugars and a protein component, in addition to the typical sugar residues detected in previously studied external layers. 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.

The available data on the monosaccharidic composition of cyanobacterial EPS reveal some distinctive 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. 2008a). 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. The presence of these hydrophobic groups contributes significantly to the emulsifying properties of the polysaccharides (Neu et al. 1992; Shepherd et al. 1995). The production of exopolysaccharides is dependent on the balance between sugar degradation and the sugar nucleotide synthesis. Moreover, the presence of a higher number of different sugars in cyanobacterial EPS suggests that this mechanism may be under a different type of regulation. Typically, the 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 cells surface (Yamazaki et al. 1996; De Vuyst and Degeest 1999; Kleerebezem et al. 1999; Whitfield and Roberts 1999; De Vuyst et al. 2001; Jolly and Stingele 2001; Sutherland 2001). The sequence and compartmentalization of the putative biosynthetic pathway, polymerization and export of EPS in cyanobacteria is shown below (Pereira et al. 2009).
On the basis of chemical characteristics, cyanobacterial exopolysaccharides can be divided into two groups: homopolysaccharides and heteropolysaccharides (Sutherland 2001). The homopolysaccharides are composed of only one type of monosaccharide, on the other hand the heteropolysaccharides consist of high-molecular-mass of hydrated molecules made up of different sugar residues, and are synthesized by the combined action of different types of glycosyltransferase (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 non carbohydrate constituents such as phosphate, lactate, acetate and glycerol (De Vuyst & Degeest 1999; De Vuyst et al. 2001; Ruas-Madiedo et al. 2002; Girard & Schafter-Lequart 2007).

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. 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). 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, especially with the age of the culture (Gloaguen et al. 1995; De Philippis & Vincenzini 1998).

**Biotechnological potential:** - Marine or fresh water cyanobacteria are one of the richest sources of different bioactive compounds. Prokaryotic biopolymers are important materials for their potential use in biotechnology and pharmaceutical fields (Sutherland 1990; Weiner 1997; Selbmann et al. 2002; Pereira 2009). In particular cyanobacteria are considered as a promising source for the production of `new' exopolysaccharides, with interesting activity such as metal removal, adhesion to solid surfaces, cytostatic, antineoplastic effects and antiviral actions (Pulz & Koehler 1994; Sutherland 1990).

For approximately four decades it has been known that sulfated polysaccharides, extracted from cyanobacteria, exhibit a potent broad-spectrum antiviral activity *in vitro* against HIV-1 and HIV-2, HSV, influenza and a series of other enveloped viruses. These compounds interfere with the attachment of the virus to its target cells, thereby inhibiting virus-cell fusion i.e. the entry of the virus into its target cells. Gerber *et al.* (1958) reported that algal polysaccharides exhibited antiviral activity *in vitro* towards mumps and influenza B. More recent reports on antiviral activity of sulfated polysaccharides from algae are given by Feldmann (1999) for fucoidans, by Lee (1999) for rhamnan sulfate, and by Hayashi *et al.* (1996) for Ca spirulan, a sulfated polysaccharide isolated from *Spirulina*. Other sulfated polysaccharides like heparin, dextran sulfate, pentosan polysulfate and others show strong anticoagulant activities, an unfavorable property for the therapeutic use of these antiviral agents. In contrast, the natural algal/cyanobacterial polysaccharides, like the carrageenans, fucoidan and Ca-spirulan appear to have no, or strongly reduced anticoagulant properties (Hayashi et.al.1996, Hayashi and Hayashi 1996).

**Factors controlling EPS production:** Several works are available reporting productivities and biochemical characterization of EPS from different cyanobacterial strains under standard culture conditions (Gloaguen et al. 1995; De Philippis et al. 2000; Nicolaus et al. 1999). During the last three decades, several factors controlling the production of cyanobacterial EPS have been identified. In many species, EPS production rate is directly related to environmental stress conditions including nutritional stress. The type and the amount of
polysaccharide production depend on the species employed and cultivation conditions (Moore & Tischer 1964; Sangar & Dugan 1972). Under nutrient deficient condition, as a consequence of an excess of photo assimilated carbon, EPS is produced and excretion acts as an overflow mechanism to avoid the damage of the photosynthetic apparatus (Fogg 1983; Smith & Underwood 2000). In a different kind of approach, the effects of altered culture conditions on EPS production have also been tested. In this context, different cultural parameters including culture age, source of nitrogen, irradiance, light intensity and cycle, temperature, salinity, airflow and the concentrations of sulfur, phosphorous and potassium have been investigated in relation to EPS production (Vincenzini et al. 1990; Sudo et al. 1995; Lama et al. 1996; Morvan et al. 1997; Moreno et al. 1998; De Philippis and Vincenzini 1998). Gloaguen et al. (1995) reported that the sugar composition may slightly vary, both qualitatively and quantitatively, especially with the age of the culture. The synthesis of EPS may be often related to an impairment of balanced growth both from nutritional point of view and from physical and chemical parameters (De Philippis et al. 1991; Morvan et al. 1997). Among these most of the works on exopolysaccharide production have been focused on the effects of carbon to nitrogen (C: N) ratio and energy availability (De Philippis & Vincenzini 1998; Li et al. 2002). Sutherland (1982) and Roseiro et al. (1992) found that the synthesis of EPS was enhanced in medium containing excess carbohydrate and deficient in other nutrients, particularly nitrogen. Other reports also revealed that nitrogen starved condition had enhanced EPS synthesis in both diazotrophic and non-diazotrophic cyanobacteria (Philips et al. 1989; Fresnedo and Serra 1992; De Philippis et al. 1998; Moreno et al. 1998; Nicolaus et al. 1999). On the other hand, growth and EPS production was higher in the presence of combined nitrogen in different strains of Anabaena (Lama et al. 1996) and Aphanocapsa halophytica (Sudo et al. 1995).

The importance of phosphate supply in regulating the growth of cyanobacteria is widely recognized, especially in aquatic environments. It has been reported that sulfur 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). 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 (Brown 1990; Chen et al. 2006). Generally, under salt stress, cyanobacteria also produce larger amounts of EPS. Many other factors that can
influence EPS production, notably pH, dilution rate, 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). Changes in biochemical composition of exopolysaccharide in relation to nutrient availability have been reported by many other authors also (Myklestad & Haug 1972; Myklestad 1977; Guerrini et al. 1998, 2000; Alcoverro et al. 2000; Corzo et al. 2000; Staats et al. 2000; Haug & Myklestad, 1976; Bhosle et al. 1995; Biersmith & Benner 1998; McConville et al. 1999; Staats et al. 1999).