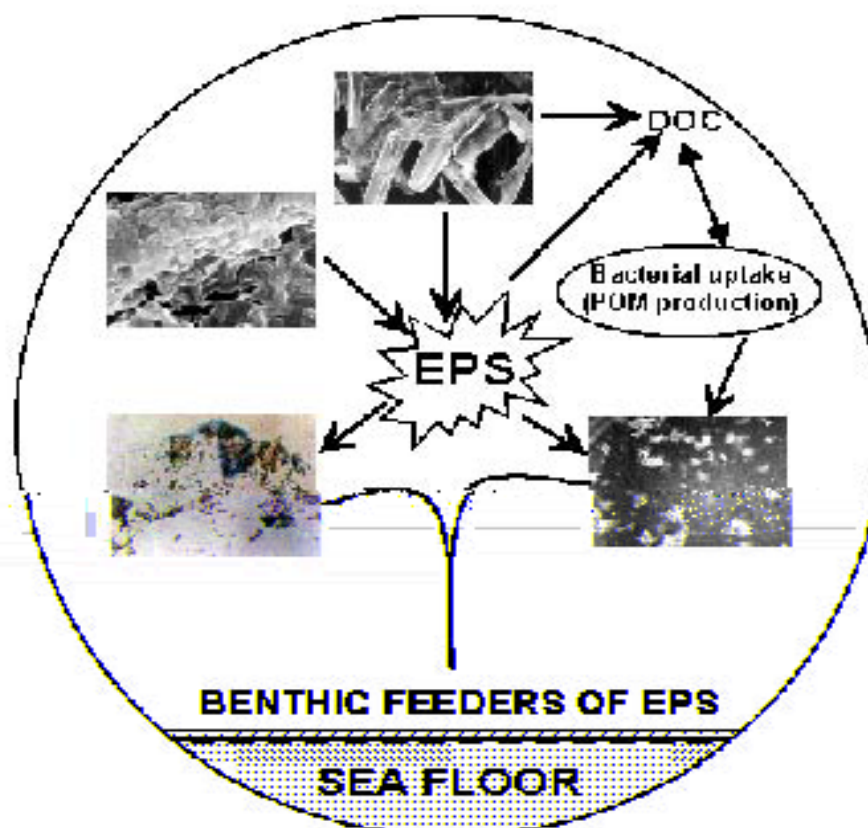


STUDIES ON SOME ASPECTS OF MARINE MICROBIAL EXOPOLYSACCHARIDES

PhD Thesis
BY
P. V. Bhaskar



2003



**STUDIES ON SOME ASPECTS OF MARINE
MICROBIAL EXOPOLYSACCHARIDES**

THESIS SUBMITTED TO GOA UNIVERSITY
FOR THE DEGREE
OF

DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

BY
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UNDER THE GUIDANCE
OF
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*All the corrections has
been incorporated
and certified*

APRIL 2003

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Guide

CERTIFICATE

This is to certify that the thesis entitled "**Studies on some aspects of marine microbial exopolysaccharides**" submitted by **Mr. P. V. Bhaskar** for the award of the degree of Doctor of Philosophy in Microbiology is based on his original studies carried out by him under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any university or institution.



Place: Dona Paula

Date: 9/02/04

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STATEMENT

As required under the University ordinance 0.19.8 (vi), I state that the present thesis entitled "**Studies on some aspects of marine microbial exopolysaccharides**" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.


9/02/2004
(P. V. Bhaskar)

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CHAPTER 1

General Introduction

Chapter 1

1.1 Microbial exopolysaccharides (EPS)

1.1a What is EPS?

Most microorganisms produce varying amounts of extracellular organic polymers that are principally made of polysaccharides with smaller quantities of proteins, lipids, glycoproteins etc (Costerton et al 1981). Such extracellular polymers are called as exopolysaccharides or EPS (Wingender et al 1999). Microbial EPS is produced by both prokaryotes (eubacteria and archaeobacteria) and eukaryotes (phytoplankton, fungi, yeasts, and algae) that form a gel-like matrix around the microorganisms. EPS is produced by microorganisms as free planktonic forms in aquatic environments (Burns 1989, Nielsen et al 1997, Jahn and Nielsen 1998), in association with other organisms (symbiotic existence) (Rosen and Cornford 1971, Costerton et al 1978) or when attached to natural and artificial surfaces (man-made structures) (Decho 1990, Vandivivere and Kirchman 1993, Becker 1996).

Microbial EPS are basically of two types- capsular and slime layer. The capsule is highly hydrated polymeric structure tightly enveloped around the cell and forms discrete extracellular structures (**Fig. 1.1**). These are held to the cell-wall either by linkages between the carboxyl groups of EPS and hydroxyl groups of lipopolysaccharides (LPS) (Sutherland 1977) or through a

covalent bonding through phospholipid (Roberts 1996) and glycoproteins (Chester and Murray 1978). On the other hand, slime layer is a less organized structure, which is loosely held to the cell surface and can easily diffuse into

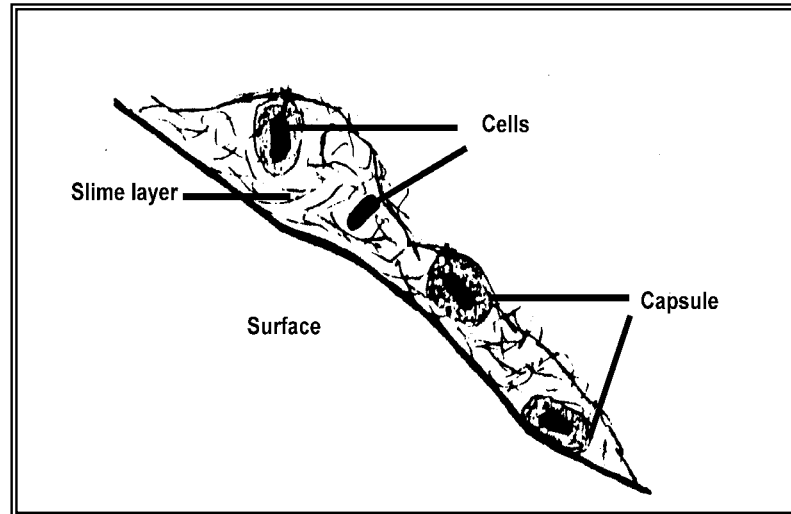


Fig. 1.1 Schematic representation of a biofilm showing the cells enveloped with a capsule and embedded in a matrix of slime EPS. (*Reproduced from Decho (1990)*).

the surrounding waters (Decho 1990) (**Fig. 1.1**). Sheaths are another forms of capsular EPS which envelope a chain of cells (Takeda et al 1998). EPS is highly hydrated polymer and contains up to 99% water. Therefore, EPS is invisible when viewed directly under microscope and need to be stained with either India ink, anion stains like ruthedium red and alcian blue. The electron microscopic images of both capsules and slimes of bacteria reveal a matrix of polysaccharide fibrils. These fibrils in the capsules are tightly wound around the cell-surface (Decho 1990). High-resolution cryo-freeze etching SEM (cryo-FESEM) techniques reveal the fine fibrillar composition of extracellular sheaths in stalked diatoms (Wang et al 2000).

1.1b Chemistry of EPS

Microbial EPS is composed of a long chain of sugars that form the backbone of the polymer. Most of these polymers are generally of high molecular weight polysaccharides (10 to 30 kDa) with variable composition. This chain might be unbranched or branched with side chains of other compounds attached to the polymeric chain (Sutherland 1977). Based upon the composition of the chain, the polymer is classified as homopolysaccharides (having the same monomer) or heteropolysaccharides (different repeating monomer). For example, bacterial cellulose, dextran (**Fig. 1.2a**), levans etc are some of the major homopolymers having glucose as the repeating unit (Sutherland 1983). On the other hand, EPS like bacterial alginate (**Fig. 1.2b**) and most other polymers are heteropolymers with more than two monosaccharides and other additional groups like uronic acid (Sutherland 1977). The monomers of the polysaccharide chain are joined by either α -D or β -D linkages. For example, exopolymers like pullulans are made of glucans linked by β -D (1,3 and 1,6) linkages (Harada 1977) whereas dextran is made of glucans linked by α -D (1,6; 1-4; 1-3 & 1-2) linkages (Jeanes 1977). The monosaccharide composition of the EPS also varies with the source. For example, bacterial EPS are generally rich in hexoses like glucose, galactose, rhamnose, mannose and fucose (Sutherland 1977) than pentoses like xylose (Powell 1979). Apart from monosaccharides, microbial EPS also have carboxylated sugars like uronic acids and non-sugar components like proteins, aminosugars, lipids, pyruvates, ester sulphates and nucleic acid. Generally, these components are present in relatively smaller quantities but are

important in regulating the characteristics of the microbial EPS (Decho 1990). Moreover their contribution to the total EPS varies considerably with the source. One of the major components of the

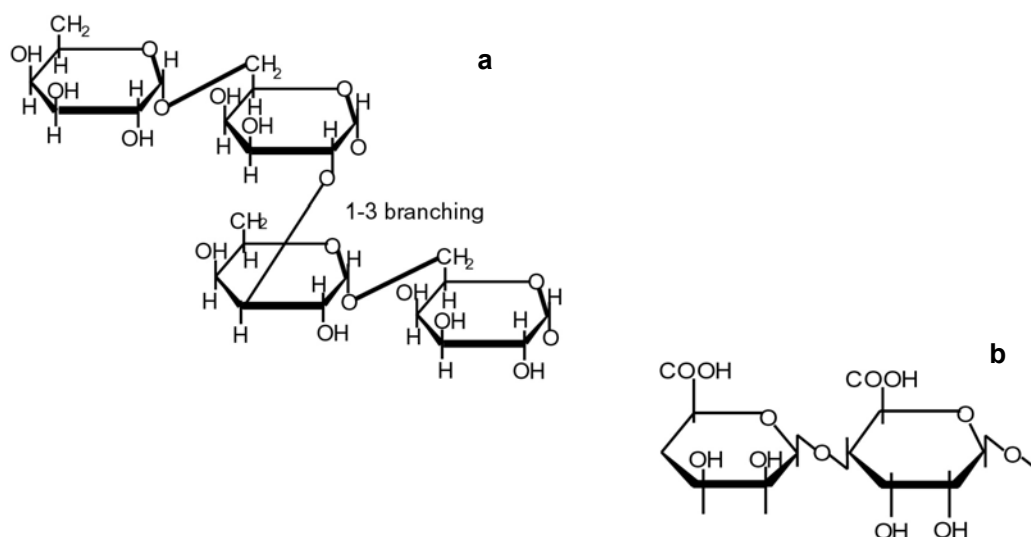


Fig. 1.2 Chemical structure of dextran, a homopolysaccharide made of α -1-6 glucan monomers with 1-3 branched linkages (a) and bacterial alginate, a heteropolysaccharide having a combination of D-mannuronic acid and L-guluronic acid linked by β -1,6 linkage.

EPS is uronic acid that may constitute up to 20-50% of the polysaccharide fraction in marine bacterial EPS (Kennedy and Sutherland 1987, Majumdar et al 1999). Other non-sugar components like phosphates, pyruvates and acyl groups present in the EPS are generally attached to the sugar residues (Sutherland 1979). The presence of pyruvates and acyl groups prevents the epimerisation of uronic acid, thereby ensuring higher content of uronic acid in the polymer (Decho 1990). The higher yield of uronic acid gives an overall negative charge to the polymer, thereby imparting adsorptive properties to the

polymer (Smidsrod 1974). Apart from organic non-sugar moieties, cations like Ca^{2+} and Mg^{2+} have also been widely reported in both bacterial and diatom EPS (Marshall et al 1971, Cooksey 1981). The presence of divalent cations helps in multiple cross-linkages between the sugar molecules of different polysaccharide chains, thereby imparting a high degree of consistency and stability to the EPS (Lewin 1956, Williams et al 1972, Decho and Moriarty 1990).

1.1c Regulation of EPS production

The production of EPS by microorganisms is influenced by various factors including the physiological state of the organism, age of the organism, nutrient levels and nutrient composition (Decho 1990). Bacteria and to a lesser extent phytoplankton produce exopolysaccharides throughout different stages of their life-cycle. However, maximum EPS production generally occurs at the end of the log-phase in most bacteria and phytoplankton species (Myklestad and Haug 1972, Mykelstad 1977, Williams and Wimpenny 1977 & 1978). In both natural environments and laboratory, microorganisms tend to produce more EPS under nitrogen limiting conditions. Increase in carbohydrate concentrations have been reported at the end of phytoplankton blooms in the oceanic environments (Ittekkot et al 1981), mesocosm studies (Mopper et al 1995) and laboratory studies (Henriques-Viera and Myklestad 1986, Fajon et al 1999), coinciding with depletion in nutrients like nitrate and phosphate. Similarly, bacteria also produce maximal EPS in the late log-phase under low nutrient conditions (Williams and Wimpenny 1977 & 1978). Moreover, bacteria

tend to produce more EPS when supplied with higher concentrations of carbohydrates as substrate (Sutherland 1972). Apart from higher concentrations of carbohydrate, the presence of precursors like acetyl CoA, isoprenoids, nucleoside diphosphates sugars and phosphoenol pyruvates have also been shown to enhance the production of EPS (Norval and Sutherland 1969, Sutherland 1979, Whitfield 1988). Other factors that influence the growth of the organisms like pH, aeration and temperature also regulate the production of EPS (Williams and Wimpenny 1977 & 1978). For marine organisms, variation in salinity can also lead to alteration in EPS yield (Allan et al 1972). The genetic regulation of EPS production affects the ability of the organism to produce EPS (Avery et al 1944). Fragments of DNA like transposons and insertion elements are considered important in regulating the production of EPS in some pathogenic bacteria (Bartlett et al 1988). The exact mechanism of genetic control over EPS production is however debatable.

1.1d Biosynthesis of EPS

The biosynthesis of EPS in bacteria can either take place 1) extracellular; utilizing the precursors from the extracellular environment or 2) intracellular, assembled near the membrane and then released outside (Decho 1990). In the extracellular synthesis, precursors like sucrose are used (Sutherland 1977) and the EPS thus produced are characteristically homopolysaccharides. In order to synthesize the polymer, cells produce extracellular transferase but do not require intermediates like nucleoside diphosphate sugars. On the other hand, intracellular production is more common and is found in most bacteria. This type of synthesis yields

heteropolysaccharides. In order to synthesize such polymers, activated precursors like nucleoside diphosphate sugars and carrier molecules like lipid intermediates are required (Norval and Sutherland 1969). The formation and partial assembly of the polymer take place close to cytoplasmic membrane and extruded out into the extracellular environment where further elongation takes place. The synthesis is independent of the substrate and physiological conditions. The site of EPS synthesis in majority cases is at the inner membrane (Osborn et al 1972). The EPS is transported into the external environment through porins located on the membrane (Sutherland 1977). In contrast to bacteria, EPS in diatoms and microalgae are internally synthesized and contained in vesicles produced in Golgi apparatus (Daniel et al 1980). These vesicles are transported to the cell membrane and secreted through longitudinal channels called raphe (Daniel et al 1980).

1.1e Functions of EPS

EPS produced by the microorganisms perform various important functions that help the cells survive in extreme conditions. One of the most important functions of EPS is adhesion of cells to natural and artificial surfaces – animate or inanimate. During adhesion, microorganisms first come closer to the surface where they are held by weak Van der Waal forces of attraction. This is defined as the “reversible sorption” wherein organisms still exhibit Brownian motility and are easily removed from the surface by moderate shear forces (Marshall et al 1971). The reversible sorption depends upon factors like electrostatic forces, surface charges, hydrophobicity of the surface and the

availability of cations. Once the cells are held closer to the surface, they produce EPS that enables the cells to attach irreversibly to the surface and grow, thereby forming biofilms (Costerton et al 1981, Costerton 1984). The production of EPS during the formation of biofilms has many advantages including better access of microorganisms to nutrients, development of microbial consortia, horizontal migration of genetic material, protection against desiccation and toxicity etc (Decho 1990). In the field of medicine, production of capsular EPS can enhance the resistance of pathogens against host immune system (Deighton et al 1996, Pasquier et al 1997). In some microorganisms, the EPS released during the formation of biofilms can produce immunogenic response from the hosts. For example, the production of K-antigen by some *E. coli* serotypes is basically a capsular lipopolysaccharides (LPS) (Whitefield 1988). On the other hand, the formation of biofilms on artificial surfaces results in the conditioning of the surface, thereby serving as a cue for other organisms to colonize the surfaces (Szewzyk et al 1991). Such phenomena are commonly encountered in all submerged structures, affecting their strength and performance. Bacterial biofilms on metal surfaces are also involved in regulation of corrosion. Many bacterial EPS contain acidic moieties like uronic acids, phosphoric acid groups etc (Martin 1971). EPS has the ability to form complex with metal ions (Friedman and Dugal 1968, Martin et al 1972, Bitton and Friehofer 1978, Brown and Lester 1982a & b, Mittleman and Geesey 1985). Although EPS may not be directly involved in inducing corrosion of metals, the presence of EPS in the biofilms helps in creating an ideal environment that supports corrosion processes (Ford and Mitchell 1990).

The adhering properties of EPS also play an important role in cell aggregation and floc formation. EPS produced by bacteria in granular sludge has been considered essential for the formation and maintenance of the microcolony (Tago and Aida 1977, MacCleod et al 1995). In sediments, bacterial EPS help in maintaining anaerobic conditions within the flocs. Such distribution of EPS also helps in spatial distribution of different micro-organisms within the flocs. For example, cell flocs in sediments have aerobes growing at the margins of flocs and anaerobes growing at the center. The presence of aerobes and greater concentration of EPS in the middle portion of cell flocs help maintain the anaerobic environment essential for the anaerobes (Murlidharan et al 1997).

Apart from adhesion, EPS in biofilms have also been implicated for motility in bacteria and diatoms. The role of EPS in motility has been recognised in some gliding bacteria (Decho 1990). Similarly, secretion of EPS by diatoms has been shown to aid motility (Medlin et al 1986, Pickett-Heaps et al 1991). In sediments, motile diatoms use EPS as a vehicle to migrate within the sediment layers in response to changing light and aeration conditions (Harper 1977, Moss 1977). Apart from aiding motility, the hydrated state of EPS (~99% water) provides protection to the cells against desiccation. Such properties enhance the survival of microorganisms in the inter-tidal mudflats that remain exposed during low tides (Decho 2000). The presence of water helps the EPS behave like a sorptive sponge that enables it to sequester nutrients, dissolved organic matter and dissolved metals (Decho 1990, Decho

and Lopez 1993). EPS in biofilms also contain localized enzymes that have been adsorbed from the surrounding environments (Lamed et al 1983, Costerton 1984, Decho 1990). The EPS-enzyme complex protects the enzymes from lysis but still retains its activity and accessibility to substrates. The biofilm EPS can also adsorb extracellular DNA released during cell lysis, thereby preventing the enzymatic degradation of DNA (Decho 1990). The transfer of plasmids between strains has been reported in *E. coli* biofilm (Lebaron et al 1997). The complex structure and chemistry of the EPS has been considered important in its ability to resist easy enzymatic degradation in the guts of animals (Plante 2000). EPS coated cells are frequently ingested by deposit feeders while feeding on sediment biofilms. However, such cells have been found to escape digestion, which are frequently excreted by the grazing community (Decho 1990, Gonzalez et al 1990, Jürgens and Güde 1994).

1.1f Applications of EPS

The unique properties of EPS have found varied applications in diverse fields. Moreover, its easy handling and eco-friendly production has made microbial EPS as a lucrative product for industries. EPS like xanthan gum are widely used as food additives, stabilizer for beverages, reduction of crystallization in, as thinning agent and suspending agent (Sutherland 1998). Another bacterial EPS such as cellulose has found its utility in hospitals and acoustic diaphragms due to its greater water holding capacity, oxygen permissibility and tensile strength (Byrom 1991, Joris and Vandamme 1993). Polymers like β -D-glucans are used as anti-tumor agents. On the other hand, polymers like

heparin and hyaluronic acid are used in surgery, pharmaceutical products and cosmetics (Sutherland 1998). The adhesive properties of the EPS make it a good natural source of gelling agent, binder and glue (Sutherland 1998). EPS are also used in bioremediation of pollutants, in printers, oil recovery, coating of medicines and food items etc. (Sutherland 1998).

1.2 EPS in marine environment

1.2a Sources of EPS

Microorganisms like phytoplankton (diatoms) (Hellebust 1965, Myklestad 1977, Underhill 1981, Goto et al 1999), bacteria (Brophy and Carlson 1989, Goutx et al 1990, Heissenberger et al 1996), cyanobacteria (Singh et al 1999, Rougeaux et al 2001) and dinoflagellates (Guillard and Wangersky 1958, Alldredge et al 1998, Brachvogel et al 2001) release exopolysaccharides in the marine environments. Phytoplankton is considered to be the major source of organic matter in the euphotic waters of marine environments.

The fixing of CO₂ in presence of sunlight and inorganic nutrients like ammonia, nitrate and phosphates and its conversion to different organic biomolecules (both cellular and extracellular) and the subsequent utilization of the organic matter (respiration) is called primary production (PP) (Lalli and Parsons 1997). A major fraction of the primary production is released into the aquatic environments as extracellular polysaccharides (EPS) (Sondergaard et al 1985, Sell and Overbeck 1992). The production of EPS and its contribution

to the dissolved organic matter (DOM) has been reported for both free living (Mykkestad 1977, Smestad et al 1981, Vaarum and Mykkestad 1984, Mykkestad 1995) and benthic forms (Bhosle et al 1995, Becker 1996, Buzelli et al 1997, Smith and Underwood 1998 & 2000, Penna et al 1999, Goto et al 2001) of phytoplankton. Putter (1909) hypothesized DOM as leachate of phytoplankton until Thomas (1971) gave experimental evidence that the production of EPS and its release as DOM was a normal physiological process in phytoplankton. The fraction of primary production released as extracellular organic carbon (EOC), however varies to a large extent and is influenced by method employed for estimation, species used and growth conditions (Eppley and Sloan 1965). Generally, the fraction of PP released as EOC ranges from <3% to more than 90% (Hellebust 1967, Wallen and Geen 1971, Raymond 1980, Gomes et al 1991, Sell and Overbeck 1992). The EOC produced by phytoplankton is principally made of polysaccharides (Wangersky and Guillard 1960, Ittekkot et al 1981, Hama and Yamagi 2001). Other organic compounds like free amino acids and dissolved proteins (Laane 1983, Striquer-Soares and Chevolut 1996) are also found in varying amounts. Moreover, Kirchman et al (2001) have reported that this extracellular polysaccharides released during blooms constituted up to ~50% of the semi-labile DOC, thereby forming a large fraction of the semi-labile DOC pool in oceanic waters.

The other source of EPS in marine environments is bacterioplankton population. These microorganisms range between 10^6 to 10^9 ml⁻¹ in the marine waters (Cho 1991, Ramaiah et al 1998, Bode et al 2001), are

ubiquitously distributed (geographically and spatially) and form the largest living surface in the oceanic environment (Stoderegger and Herndl 1998). Unlike phytoplankton that grows only in the euphotic zone of the oceans (< 1% of the total ocean area), bacteria have been isolated from practically all depths and from extreme environments including hydrothermal vents (Cowen et al 1985, Winn and Karl 1987, Marteinsson et al 1995), deep sea sediments (Deming 1985, Hamamoto et al 1995) and cold seeps (Karl 1987). Moreover, under oligotrophic conditions the bacterial biomass may exceed the phytoplankton biomass even in euphotic zones (Herndl 1991, Fuhrman et al 1989, Cho and Azam 1990). Most bacteria produce capsules as long as they are metabolically active, which is then released into the surrounding waters as DOC (Brovy and Carlson 1989, Tranvik 1993, Heissenberger and Herndl 1994, Stoderegger and Herndl 1998, Heissenberger et al 1996). Bacteria utilize ~70% of phytoplankton EOC of which 45% is converted into EPS (Stoderegger and Herndl 1998). On the other hand, recent studies on EPS of marine bacteria have shown that these organic matter may not be easily degradable unlike phytoplankton EPS and behaved like humic substances (Brovy and Carlson 1989, Tranvik 1993). However unlike phytoplankton EPS, the significance of bacterial EPS in marine environments is not clearly understood and further studies are required to better understand the contribution of bacterial EPS to the DOC pools, its turnover rates and its role in marine food-web and biogeochemical processes.

1.2b Distribution of EPS in marine environments

In the marine environments, microbial EPS exists in various size classes—dissolved, colloidal and particulate forms. In the dissolved form, EPS forms a part of dissolved organic carbon pool ranging from a few kDa to more than 50,000 kDa molecular weight size (Decho 1990). In the form of colloids, EPS exists as particles in the size range of few nm to few μm in length (Cauwet 1978, Hedges 1987, Druffel et al 1992) and account for up to 40% of the organic carbon pool (Benner et al 1992, Ogawa and Ogura 1992, Wells and Goldberg 1992, Kepkay et al 1993). In the particulate form, EPS exists either as free discrete particles ranging from few μm to few hundred μm in length (Alldredge et al 1993, Mari and Kjørboe 1996, Mari and Burd 1998, Schuster and Herndl 1996) or in association with organic particles as in marine snow and other macroaggregates (Alldredge and Silver 1988, Cowen 1992, Herndl 1993, Decho and Herndl 1995, Unanue et al 1998a). EPS in the dissolved form is directly estimated as dissolved carbohydrates. In contrast, EPS associated with cells in cultures have to be extracted using distilled water (Pfetzing et al 2000), saline (Underwood et al 1995), EDTA (Decho 1990) or by alkali treatment (Bhosle et al 1996).

1.2c Transparent exopolymer particles (TEP)

TEP are one type of EPS that are produced by both diatoms and bacteria during their growth (Alldredge et al 1993) (**Fig. 1.3**). These particles are found ubiquitously distributed in the marine environments and are produced from

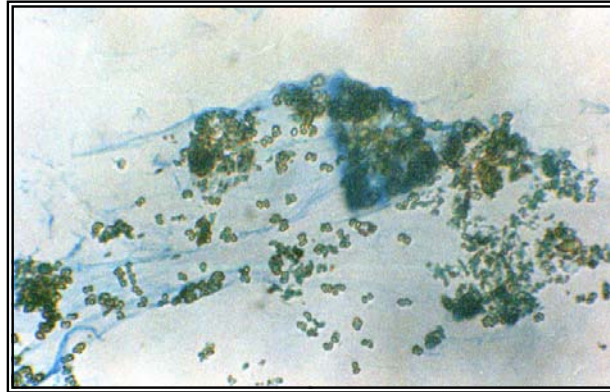


Fig. 1.3 Photograph showing the presence of Transparent exopolymer particles in natural sample. The particles stained blue with alcian blue (pH 2.5) and is associated with cells of *Phaeocystis* sp.

EPS released during the course of a phytoplankton bloom (Alldredge et al 1993, Passow and Alldredge 1994, Passow et al 1995, Schuster and Herndl 1996, Hong et al 1997, Krembs and Engel 2001, Berman and Vinner-Mozzinni 2001, Garcia et al 2002). Recent studies have shown that TEP can be generated from DOC derived from bacteria (Stoderegger and Herndl 1998) and phytoplankton (Passow 2000 & 2002) even in the absence of growing cells. Apart from phytoplankton and bacteria, TEP is also produced from other sources like mucus secreting bivalves (McKee et al 2000) and benthic macroalgae (Ramaiah et al 1999). TEP abundance ranges from 1 to 10^4 ml⁻¹ in near coastal, shelf and open seas. The TEP size varies from $< 2 \mu\text{m}$ to $> 500 \mu\text{m}$ (Passow 2002).

Chemically, these particles are made of acid polysaccharides and are stained by polysaccharide specific anionic stains like alcian blue (pH 2.5) (Alldredge

et al 1993). The amount of TEP varies with source of the EPS (Stoderegger and Herndl 1998, Passow 2000 & 2002), age of the bloom (Kiørboe and Hansen 1993, Passow and Alldredge 1994, Hong et al 1997) and the presence of bacterial heterotrophs (Smith et al 1995) that may degrade the TEP. Due to its ability to coagulate, TEP is also considered important in flocculation of diatoms (Herndl 1993, Passow et al 1994, Dam and Drapeau 1995, Passow and Alldredge 1995, Grossart et al 1997, Engel and Schartau 1999, Brachvogel et al 2001) and their eventual sedimentation at the end of phytoplankton blooms (Crocker and Passow 1995, Logan et al 1995, Reibesell et al 1995, Passow et al 2001, Prieto et al 2001).

1.3 Significance of EPS in the marine environment

The distribution of EPS over a range of size class, its overall contribution to the total organic carbon pool and its unique properties makes it an integral component of biogeochemical processes, cycling of elements (C, N, S) and metals and the food web in the marine environments (**Fig. 1.4**). As evident from the figure, EPS released as DOC by phytoplankton and bacteria plays an important role in the carbon cycling within the water column. Dissolved EPS is involved in the production of TEP, which in turn helps aggregation of cells and formation of marine snow. On the other hand, conversion of dissolved EPS to particulate organic matter (POM) through bacterial uptake and subsequent mineralisation to DOC by particle-associated bacteria forms the microbial loop. The dissolved EPS and TEP can bind and remove dissolved metals from the water column, thereby helping in recycling of metals. On the other

hand, EPS associated with particulate matter including marine snow, large phytoplankton aggregates and its associated bacterial population may serve as a carbon source for grazers in the pelagic layer. Upon sedimentation of aggregates, EPS associated with these aggregates might serve as carbon source for benthic feeders before getting buried into the sediments. All these suggests that understanding the dynamics and properties of microbial EPS may help in better understand the mechanisms controlling the biogeochemical processes and food-web in the marine environments.

1.3a Role of EPS in aggregation

Ever since the discovery of macroaggregates like marine snow (Suzuki and Kato 1953) in the pelagic waters, numerous studies have been carried out to explain the mechanisms of macroaggregate production (McCave 1984, O'Melia 1987, Jackson 1990, Eisma 1993, Kepkay 1994, Zhou et al 1998) and the essentials of aggregation (O'Melia 1987, Eisma 1993, Jackson et al 1994, Stumm et al 1994). Aggregation in the natural environment involves both biotic and abiotic processes. In the biotic process, live cells of phytoplankton and bacteria are the presence of living cells is considered essential for the production of macroaggregates. For example, Biddanda (1985) reported the formation of macroaggregates, which were essentially clumps of bacterial cells glued together by EPS during the microbial degradation of dissolved organic matter. Similarly, aggregate associated bacteria utilize and convert DOM into fibrillar EPS that aids in aggregation (Paerl 1974, Leppard et al 1979, Heissenberger et al 1996). Production of

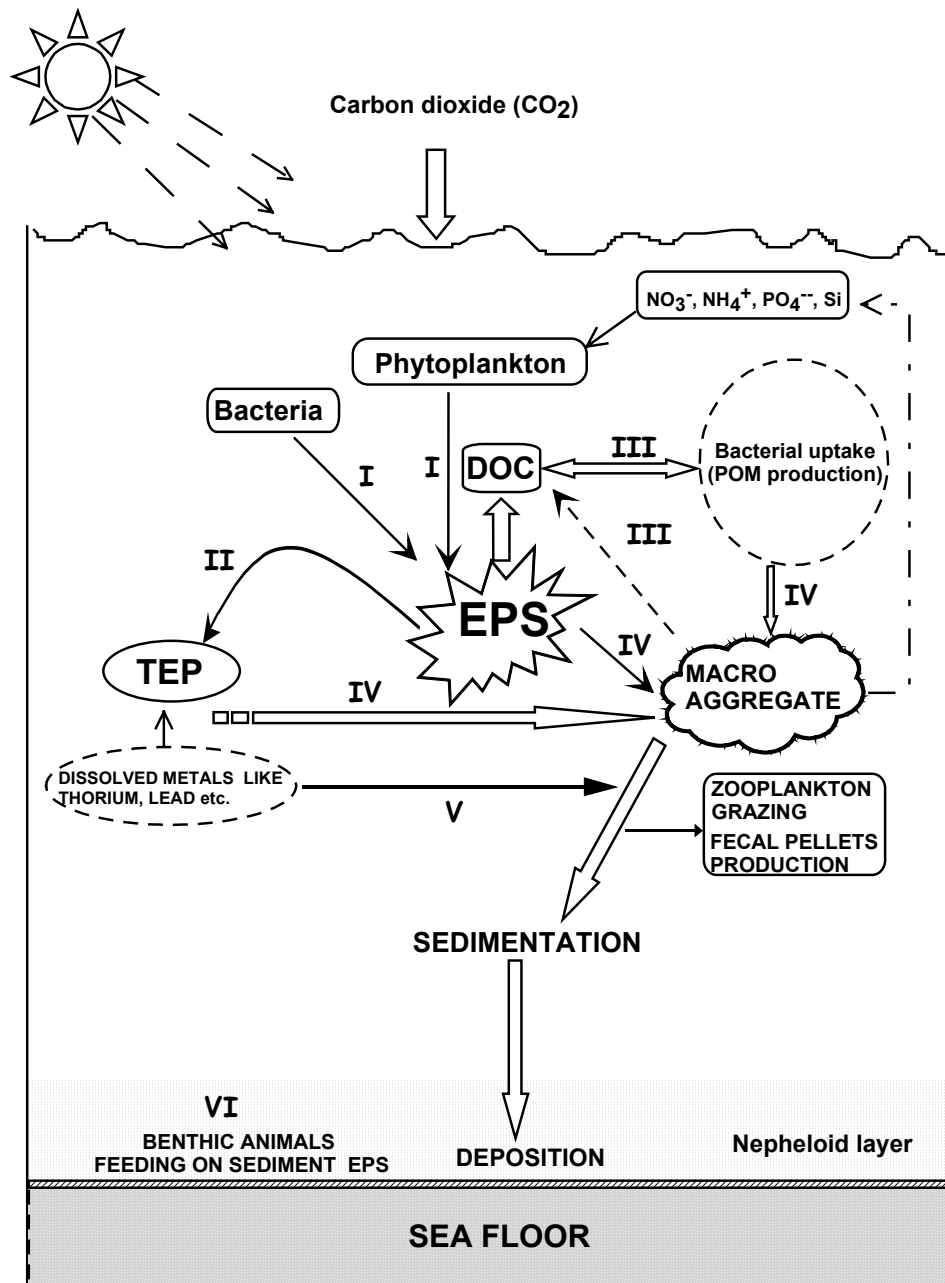


Fig. 1.4 Schematic representation of the various roles of EPS in the marine environment. The numbering indicates the different processes involving EPS.

I- Production of EPS by bacteria and phytoplankton; II- Production of TEP from EPS; III- Microbial loop; IV- Formation of particles; V- Chelation of dissolved metals and VI- EPS as a carbon source for benthic community.

TEP by living microbial cells is also considered an important mechanism of aggregation during phytoplankton blooms (Alldredge et al 1993, Passow and Alldredge 1995).

In contrast to the biotic form of aggregation, EPS in its dissolved form can get converted to form discrete particles in abiotic conditions. Under abiotic aggregation, particles might be produced from dissolved EPS either due to shear (Jackson 1990, Reibesell 1991), surface adsorption of particles (Gibbs 1983, Eisma 1993, Johnson et al 1994), surface coagulation of EPS (Mopper et al 1995, Zhou et al 1998, Mari 1999) and self-coagulation of EPS (Kepkay 1994, Chin et al 1998, Passow 2000). Moreover, free EPS may act as a mesh that entraps particles (Logan and Hunt 1987, Logan and Alldredge 1989, Stolzenbach 1993). EPS may get adsorbed to the surface of colliding particles and allows them to stick together and form macroaggregates. This process is common in the marine environments and is influenced by particle density, collision frequency and the binding properties of the EPS (McCave 1984, Kjørboe et al 1994, Waite et al 1997, Engel 2000).

1.3b EPS in particle sedimentation and carbon flux

The sinking of particulate matter from the surface to the deeper waters is called as sedimentation. This process generally coincides with increase in concentration of EPS at the termination of phytoplankton blooms (Passow and Alldredge 1995). In the natural environments, microbial cells are buoyant in nature and do not sink by themselves. However in presence of extracellular

compounds like EPS, they tend to coagulate along with other particulate matter to form macroaggregates as described earlier, attain a critical mass and start sinking into the deeper waters. Sedimentation of is considered essential for the survival of phytoplankton cells in the marine environments. For example, sedimentation of phytoplankton cells is essential to escape predation, nutrient depleted conditions and transportation to favorable growth conditions (Smetacek 1984). Moreover, spores of diatoms also aggregate and sink into deeper waters, serving as seed for future blooms (Smetacek 1984). The fate of sedimenting particles is also controlled by EPS. In the open ocean, it is possible that only 10% of the photosynthetic carbon escapes the euphotic layer as sinking particles (Fowler and Knauer 1986), of which only 1% reaches the deep-sea sediments (Martin et al 1987, Wefer 1989). One reason for such low organic carbon flux may be the tightly coupled response of grazers and bacterial community to the EPS produced by phytoplankton. For example, the sinking aggregates coated with EPS serve as carbon source for grazers like zooplankton in the water column (Gotschalk and Alldredge 1989, Lampitt et al 1993). Similarly, particle associated bacteria might utilize the EPS coatings using an array of extracellular enzymes and release it in the dissolved form (Azam 1998). Such a response would lead to disintegration of the sinking macroaggregates and most of the organic matter might get remineralized in the euphotic zone.

On the contrary, formation of EPS coated macroaggregates and its sedimentation indicates an uncoupling between the grazers and phytoplankton population. Various factors including EPS chemistry, resistance

to bacterial breakdown of EPS and low grazing of aggregates are attributed to the formation and sedimentation of macroaggregates in marine waters. For example, EPS produced by *Phaeocystis* might resist bacterial mineralisation and lead to the formation of large aggregates (Eberlein et al 1985, Billen and Fontigny 1987). Moreover, depletion of essential nutrients like nitrogen and phosphorus (Pengerud et al 1987) and adsorption of colloids (Johnson and Kepkay 1992) can also limit the bacterial solubilization of phytoplankton EPS. Similarly, presence of EPS may not always support the grazing activity of higher organisms. Studies have shown that TEP produced during blooms of *Thalassiosera* caused aggregation as grazers like copepods did not prefer to consume TEP-coated particles (Preito et al 2001). All these indicate that quality of EPS and its fate is directly linked to the quality of particles reaching the sediments, thereby influencing the benthic community structure and biomass.

1.3c EPS in microbial loop

Since a major fraction of the photosynthetic carbon (> 90%) might be released as soluble EPS (Sell and Overbeck 1992), much of the photosynthetic carbon remains unavailable to higher organisms and escapes the classical food-chain. However, the soluble form of EPS released by the phytoplankton serves as an organic carbon source and is degraded and converted to particulate form by marine bacteria (Decho and Herndl 1995, Azam 1998). On an average about 50% of the photosynthetic carbon is channelised through bacteria within the microbial loop (Fuhrman 1992), although the amount may

vary considerably. The decomposition of aggregates, its dissolution and mineralisation completes the microbial loop (Azam et al 1991, Azam 1998) (**Fig. 1.4**). The bacterial conversion of the dissolved EPS to particulate organic matter (POM) is critical in meeting the carbon demand of secondary consumers. The POM so formed might become a source of carbon for grazers like ciliates, flagellates etc., thus channelizing the organic carbon into the classical food-chain. The significance of microbial loop is not restricted to marine food-web but also in controlling the atmospheric carbon dioxide levels. Globally, the amount of carbon dioxide assimilated into the microbial loop after photosynthesis is reported to be equivalent to 5 times the anthropogenic carbon dioxide emission (Williamson and Holligan 1990). Thus, the quality and the quantity of EPS released by the phytoplankton is important in regulating the efficiency of marine microbial loop.

1.3d Removal of metal ions and its cycling

Microbial EPS are known for their ability to bind a wide range of metals including heavy metals like Pb, Co, Sr, Cr, Th and Cd (Dugan and Pickrum 1972, Corpe 1975, Brown and Lester 1982). Microbial EPS isolated from biofilms have been most often used to study the binding of metals (Harvey 1981, Mittleman and Geesey 1985, Ford et al 1987). Both laboratory and field studies have shown that factors including pH, EPS chemistry and structural orientation of the polymer influence the metal binding efficiency of the EPS in natural environment. In the marine environments, bacterial capsules are known to bind metals in both sediments (Nealson 1983) and in the water

column (Cowen and Silver 1984, Cowen and Bruland 1985). Binding of metal ions by microbial EPS may have several implications. For example, dissolved ions bound by EPS are considered essential in the vertical transport of these ions. EPS in aggregates have been reported to contain metal precipitates that accumulated with depth (Decho 1990). Once the metal ions reach the sediments, it may undergo microbial transformations and leached back into the overlaying waters. On the other hand, particulate EPS like TEP can bind more than 90% of the dissolved thorium ions and thereby alter the rate of removal of thorium from pelagic waters (Quigley et al 2002). Since thorium is used as a proxy to estimate the flux of organic carbon in the open ocean, such binding of thorium to TEP can lead to discrepancy in the estimates of carbon flux (Passow 2002).

1.4 Negative impact of EPS

Production of large aggregates in coastal waters can also be a nuisance factor since it may lead to organic loading in these waters. Mucilaginous polysaccharides of certain phytoplankton form large aggregates that sink to bottom sediments (Herndl and Peduzzi 1988, Stachowitsch et al 1990). Such accumulation of aggregates increases the oxygen demand, causing anoxic conditions in the bottom waters. These anoxic conditions cause large-scale mortality of benthic fauna in these areas (Haumann 1989, Rogers and Lockwood 1991). Similarly, near bottom anoxic waters would lead to anaerobic degradation of organic matter, leading to increased production of greenhouse gases like H₂S and N₂O (Naqvi et al 2000). Since EPS are good

adsorbents of metals and other pollutants, the accumulation of EPS in coastal waters can lead to chelation of these pollutants. The grazing of these aggregates can transfer the bound metals to higher trophic levels in the food chain. Such process can be involved in the accumulation of pollutants in the tissues of higher organisms (Decho 1990). Microbial EPS also play an important role in the fouling and corrosion of economically important submarine structures, thereby causing damage to property and economic losses (Maki 1999).

1.5 Aims of the thesis

Although studies have been conducted to address the importance of microbial EPS in the marine environments, there is no information on the dynamics of EPS and its relationship with heterotrophic activity and production in natural waters, especially in shallow coastal waters. Moreover, there is not much information on the role of bacterial EPS in marine biogeochemical processes and food-web. In this research, an attempt has been made to understand the importance of microbial EPS in various marine processes. The first objective was to understand the dynamics of EPS and its relation to bacterioplankton abundance and productivity, abundance of TEP and extracellular glucosidase activity at a shallow water coastal station at Dona Paula Bay, west coast of India. The second objective was to assess the production of EPS by a marine bacterium and evaluate the chemical characteristics of the EPS produced by the marine bacterium. This chemically characterised EPS was then used to define its role, if any, in the production of macroaggregates. Further, the

bacterial EPS was also assessed to define its role as an organic carbon source to heterotrophs. In this regard, laboratory experiments were conducted using benthic polychaete *Nereis (Hediste) diversicolor* (O F Müller 1876) and natural marine bacterial population. Moreover, the role of EPS in binding of metals like Pb and Cu was also assessed using *Marinobacter* EPS.

The results presented in this study indicate that EPS in the marine waters varied seasonally and supported bacterial production. However, phytoplankton was not the only source of EPS in the shallow water coastal station at Dona Paula Bay, west coast of India. EPS isolated from *Marinobacter* sp. and *S. costatum* were able to form macroaggregates (max. size of 22.7 and 34.5 mm², respectively) within 30 h of incubation in rolling tanks. *Marinobacter* EPS was not ingested and assimilated in significant amounts by benthic polychaete *Nereis (Hediste) diversicolor*. However, natural bacterioplankton was able to rapidly breakdown and assimilate *Marinobacter* EPS within the first 24 h of incubation. Furthermore, the bacterial EPS was found to efficiently bind more of free copper than lead ions.