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Bacterial Exopolysaccharides from Extreme Marine Environments with Special Consideration of the Southern Ocean, Sea Ice, and Deep-Sea Hydrothermal Vents: A Review

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Abstract: Exopolysaccharides (EPSs) are high molecular weight carbohydrate polymers that make up a substantial component of the extracellular polymers surrounding most microbial cells in the marine environment. EPSs constitute a large fraction of the reduced carbon reservoir in the ocean and enhance the survival of marine bacteria by influencing the physicochemical environment around the bacterial cell. Microbial EPSs are abundant in the Antarctic marine environment, for example, in sea ice and ocean particles, where they may assist microbial communities to endure extremes of temperature, salinity, and nutrient availability. The microbial biodiversity of Antarctic ecosystems is relatively unexplored. Deep-sea hydrothermal vent environments are characterized by high pressure, extreme temperature, and heavy metals. The commercial value of microbial EPSs from these habitats has been established recently. Extreme environments offer novel microbial biodiversity that produces varied and promising EPSs. The biotechnological potential of these biopolymers from hydrothermal vent environments as well as from Antarctic marine ecosystems remains largely untapped.

Keywords: EPSs, polymers, Microbial, hydrothermal, ecosystems

BACTERIAL EXOPOLYSACCHARIDES FROM EXTREME MARINE ENVIRONMENTS WITH SPECIAL CONSIDERATION OF THE SOUTHERN OCEAN, SEA ICE AND DEEP-SEA HYDROTHERMAL VENTS – A REVIEW

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ABSTRACT

Exopolysaccharides (EPS) are high molecular weight carbohydrate polymers and make up a substantial component of the extracellular polymers that surrounds most microbial cells in the marine environment. EPS comprise a large fraction of the reduced carbon reservoir in the ocean and enhance the survival of marine bacteria by influencing the physico-chemical environment around the cell. Microbial EPS are abundant in the Antarctic marine environment, for example in sea ice and ocean particles, where they may assist microbial communities to endure extremes of temperature, salinity and

nutrient availability. The microbial biodiversity of Antarctic ecosystems is relatively unexplored. Deep-sea hydrothermal vent environments are characterized by high pressure, temperature and heavy metals. The commercial value of microbial EPS from these habitats has been established recently. Extreme environments offer novel microbial biodiversity that produce varied and promising EPS. The biotechnological potential of these biopolymers from hydrothermal vent environments as well as from Antarctic marine ecosystems remains largely untapped.

INTRODUCTION

The turnover of organic matter by marine microorganisms is an important component of the global carbon cycle (Azam 1998). Organic material in the oceans exists in a heterogeneous continuum from dissolved to particulate matter and this patchiness can support high bacterial diversity (Azam et al. 1994). Dissolved organic matter in the oceans represents one of the largest reservoirs of reduced carbon on earth (McCarthy et al. 1996). Polysaccharides make up a substantial part of oceanic organic matter, especially in surface waters (Benner et al. 1992, Leppard 1995, McCarthy et al. 1996). Studies of bacteria growing in marine sediments, aggregates and detrital particles, show that nearly all the cells are surrounded by extracellular polymeric material (Decho 1990; Costerton 1999) and many of these cells are enclosed within adherent biofilms (White 1986).

Various macromolecules, such as polysaccharides, proteins, nucleic acids and lipids, form the architectural matrix in the intracellular space of microbial biofilms and unattached aggregates in the marine environment

(Wingender et al. 1999). The polysaccharide component is the most abundant of these macromolecules since it generally represents 40-95% of the extracellular polymeric substances (Flemming and Wingender 2001). Abundant microbial polysaccharides present in dissolved organic carbon, particulate material or in biofilms are of major significance in the marine environment. This review provides a brief summary of exopolysaccharide (EPS) biosynthesis, structure-function relationships as well as the role of marine bacterial EPS. We then consolidate what is known so far about the EPS produced by bacteria from two distinct environments, each one experiencing extremes of temperature. There is only a small amount of information in the literature, to the authors' knowledge on the structural elucidation of EPS from Antarctic marine bacteria. As the function is directly related to its chemical structure, structural elucidation will need to be one of the first steps in determining the ecological role of these polysaccharides and establishing whether they are novel or unique. Finally, the commercial potential of EPS produced by bacteria from these two environments is discussed.

REGULATION OF EPS PRODUCTION

Exopolysaccharide (EPS) is a term first used by Sutherland (1972) to described high molecular weight carbohydrate polymers produced by many marine bacteria. Since that time, EPS also has been used to indicate a more broadly define extracellular polymeric substances (Wingender et al. 1999). Within the context of this review, EPS will be used as it was originally defined, that is to mean exopolysaccharide.

EPS can be part of the capsular material that closely surrounds the bacterial cell or released into the surrounding environment as dispersed slime with no obvious association to any one particular cell (Sutherland 1982, Decho 1990). In the natural environment, EPS production seems to be essential for survival since most bacteria occur in microbial aggregates whose structural and functional integrity is based on the presence of a matrix of extracellular polymeric substances (Wingender et al. 1999). Analysis of the polysaccharide component of this matrix from specific bacterial members of natural assemblages is difficult due to the low abundance of any one polymer and the complexity of tracing it back to its source (Christensen 1999). The growth of a single strain under controlled conditions is an approach that is used frequently to examine microbial EPS production in order to form theories about the behaviour of these molecules in the natural environment.

Nutrient limitation

Marine microbes grown in laboratory cultures produce EPS in response to limitation of nutrients such as nitrogen, phosphorus, sulfur and potassium (Sutherland 1982). Exopolymer production may be enhanced in response to physical factors such osmotic stress and temperature (Krembs et al. 2002, Junge et al. 2004). The composition of polysaccharide is generally independent of the nature of the limiting nutrient. When *Pseudomonas* NCIB 11264 was grown in continuous cultures, its polysaccharide varied little in composition irrespective of pH, temperature, nitrogen, carbon or phosphate content of the growth media (Williams and Wimpenny 1978). The yield of this polymer was higher at suboptimal temperatures, high carbon to nitrogen ratios and during stationary phase. A deep sea hydrothermal vent strain of

Alteromonas produced EPS at the beginning of stationary phase and during nitrogen limitation, suggesting EPS synthesis for this strain was also induced by restricted growth conditions (Samain et al. 1997). In fact, most deep-sea bacterial isolates examined to date, produce EPS under these conditions (Guezennec, personal observation)

Most bacteria use carbohydrates as a carbon and energy source and amino acids or an ammonium salt as a nitrogen source (Sutherland 1982). The composition of EPS and the chemical and physical properties of these biopolymers can vary greatly (Decho 1990), but it is generally independent of the carbon substrate (Sutherland 1982). Uptake of substrate is one of the first limitations on EPS production and the presence of a carbohydrate substrate such as glucose results in optimal EPS yields (Sutherland 1979). Marine strain *Hahella chejuensis* produced the highest EPS yield in batch culture when grown on sucrose (Ko et al. 2000). Marine bacteria may also produce EPS during growth in sea water alone (Decho 1990) and during carbon limitation since many species can make use of non-sugar sources for EPS biosynthesis (Sutherland 1979).

Growth phase

Batch cultures of a deep-sea hydrothermal vent strain of *Alteromonas* showed stimulated EPS production during nitrogen limited stationary phase (Samain et al. 1997). Most bacteria release the largest quantity of EPS during stationary growth phase in laboratory culture (Decho 1990, Manca et al. 1996). However, a study by Bozal (1994) examined EPS production by a *Pseudoalteromonas antarctica* NF_3 isolated from glacial marine sludge sampled near the South Shetland Islands and found maximum production

occurred only during exponential phase. The composition of EPS may also vary according to the growth phase of the bacteria (Christensen et al. 1985). Although culture conditions generally do not affect the types of monosaccharides in an EPS, they impact on the functional properties of the polysaccharide such as molecular weight, conformation and monosaccharide ratios (Arias et al. 2003). In natural systems where nutrients levels in close proximity to the bacterial cell may vary considerably, shifts in the physiological state of the cell probably result in variable EPS compositions (Geesey 1982).

BIOSYNTHESIS

One of the first steps in the biosynthesis of EPS takes place when the substrate enters the cell unaltered or after phosphorylation (Sutherland 1977). EPS is synthesized near the cytoplasmic membrane using activated precursors and carrier molecules. Uridine diphosphate-glucose pyrophosphorylase is a key enzyme producing a precursor for both cell wall polymers and exopolysaccharide biosynthesis in many organisms (Sutherland 1977). Several enzymes involved in nucleotide synthesis are membrane bound. Therefore, it is not clear whether their products occur freely within the cytoplasm or whether they are produced in close proximity to the enzymes that require them for polymer synthesis (Sutherland 1977).

The construction of the repeating units is dependant on the transfer of the appropriate monosaccharides from sugar nucleotides to a carrier lipid–isoprenoid alcohol phosphate. The requirement for this carrier lipid in exopolysaccharides biosynthesis is also common to other polymers containing glycan repeating-units located external to the cell membrane

including peptidoglycan, teichoic acids and lipopolysaccharides (Sutherland 1977, Sutherland 1982). After polymerisation, the polysaccharide chain may be hydrolysed from the isoprenoid carrier lipid by a highly specific enzyme to produce slime (Sutherland 1977). At the same time, the polysaccharide is transported through the inner and outer membranes (Sutherland 1982). In capsule-producing strains, a ligase reaction may remove the polymer chain from the carrier lipid and attach it covalently to an outer membrane protein (Sutherland 1982) or to phospholipid or lipid-A molecules on the cell surface (Roberts 1996). Capsular exopolymer may only be loosely attached (Costerton et al. 1992) to the peptidoglycan layer of the cell wall via S-layers, non-covalently associated proteins or glycoproteins cell and can be shed as amorphous slime (Sidhu and Olsen 1997).

STRUCTURE-FUNCTION RELATIONSHIPS

Most EPS produced by marine bacteria are heteropolysaccharides consisting of three of four different monosaccharides arranged in groups of ten or less to form repeating units (Decho 1990). The monosaccharides may be pentoses, hexoses, amino sugars or uronic acids. Most polymers are linear overall and of varying lengths with an average molecular weight of 1 x 10^5 to 3 x 10^5 daltons (Sutherland 1977). Branches of one or more monosaccharides are often attached at regular intervals (Decho 1990). Organic or inorganic (sulphate, phosphate) substituents may also be present. Components most commonly found in marine EPS are listed in Table 1 (adapted from Kenne and Lindberg 1983)

Influence of functional groups

The frequency and type of functional groups present in the EPS impact on the tertiary structure and over-all physicochemical characteristic of the polymer in the surrounding aqueous environment (Decho 1990). Exopolymers are highly hydrated molecules (up to 99% water, Decho 1990, Sutherland 1977). EPS possess hydroxyl and carboxyl groups, which can have a hydrophilic character in aqueous solutions. EPS produced by marine bacteria may contain up to 20-50% of their EPS as uronic acids (pK_a 3.2-3.4, Kennedy and Sutherland 1987). These are carboxylated sugars and they confer a net negative charge and acidic properties to the EPS (Corpe 1970) at the pH of seawater (pH ~ 8). Depending on their interaction with other organic and inorganic material in the marine environment, microbial exopolymer may be present in dissolved form or as biofilms and aggregates in a gel-like slime matrix (Flemming et al. 1997). Three types of weak interactions provide cohesive forces, and these include: dispersion forces, electrostatic interactions and hydrogen bonding. Weak interactions are significant when the frequency of the functional groups involved and the size of the polymers are considered (Flemming et al. 1997).

Phylogenetic similarities and differences

Taxonomic relatedness does not necessarily ensure similarity of EPS structure. Analysis of 32 closely related *Halomonas* strains, isolated from a hypersaline environment, showed that when grown under the same conditions, EPS yield, chemical composition and physical properties varied from strain to strain (Bouchotroch et al. 2000). The EPS produced by several deep-sea hydrothermal vent bacterial isolates have been well characterized

(Rougeaux et al. 1996), including two from genus the *Pseudoalteromonas*. Despite the strains belonging to two different species, the EPS produced by these deep-sea isolates were very similar with respect to crude chemical and monosaccharide composition. Sulfate content was noted as the only structural difference between the polymers in these two hydrothermal vent strains and this component may have influenced the intrinsic viscosity, which also varied (Table 2).

The results noted above contrast with those presented in other studies (Mancuso Nichols et al. 2004; Mancuso Nichols et al. in press) in which EPS produced by closely related Antarctic strains in the genus *Pseudoalteromonas* vary substantially in terms of crude chemical composition. Another study found two closely related hydrothermal vent bacteria from different subspecies of *Alteromonas macleodii*, and isolated from different sites, produced very different EPS under the same growth conditions (Cambon-Bonavita et al. 2002). These EPS show a high metal binding capacity (Loaec et al. 1998) and are thought to aid in attaching bacteria to the hydrothermal chimney as well as lowering the concentrations of toxic heavy metals in the microenvironment (Table 2). EPS produced by the Antarctic marine isolates examined by Mancuso Nichols et al. (2004; in press), included carboxyl groups present in uronic acids, amides present in amino sugars, sulfates and hydroxyl groups, which are abundant in all monosaccharides. The authors suggested metal binding as one potential ecological role for these polymers (Table 2).

ROLES IN THE MARINE ENVIRONMENT

Exopolymer production may require an energy expenditure of up to 70% and this amounts to a significant carbon and energy investment for the bacterial cell (Harder and Dijkhuizen 1983, Wolfaardt et al. 1999). However benefits derived from exopolymer production enhance the growth and survival of microbes and the complex communities in which they are found (Wolfaardt et al. 1999). Extracellular polymers augment the ability of microbes to compete and survive in changing environmental conditions by altering the physical and biogeochemical micro-environment around the cell (Costerton 1974). In the marine environment, bacterial exopolymers and EPS are essential in the production of aggregates (Biddanda 1985, Harris and Mitchell 1973, Alldredge and Silver 1988), adhesion to surfaces and other organisms (Marshall 1985, Fletcher and Floodgate 1973; Paerl 1975, Paerl 1976, Vincent et al. 1994, Holmstrom and Kjelleberg 1999), biofilm formation (Sutherland 2001, Sutherland 1999), sequestering of nutrients (Decho and Herndl 1995), as well as providing protection (Decho and Lopez 1993, Bitton and Friehofer 1978) and ecosystem stability (Uhlinger and White 1983, Dade et al. 1990). The role of microbial exopolymers in the ocean has been reviewed extensively (Decho 1990, Wolfaardt et al. 1999) and is summarized briefly below and in Table 2. Where information relates to EPS, specific mention is made.

Adhesion to and colonization of surfaces.

Surfaces exposed to seawater quickly adsorb and concentrate dissolved organic compounds. Attachment to these surfaces by bacteria provides the opportunity for growth in dilute solutions that would otherwise be

unavailable (Zobell 1943, Paerl 1975). Charged substrates including amino acid, sugars, fatty acids and glycoproteins are often the first concentrated on surfaces (Marshall 1985). Many bacterial cells possess a capsule prior to attachment. Capsular and slime heteropolysaccharides that contain uronic acids (pKa ~ 3) confer a net negative charge to the cell above pH 3 (Sutherland 1980). Since surfaces and cells both tend to be anionic, the presence of positive ions such as Ca^{2+} is important.

Exopolymers including capsular polysaccharides and proteins are important in bacterial adhesion to surfaces (Wolfaardt et al. 1999). The initial attachment can be reversible and is also related to the electrostatic interactions and cell wall hydrophobicity (van Loosdrecht et al. 1990, van Loosdrecht et al. 1987). Irreversible binding may occur since some bacteria, in close proximity to a surface, secrete large amounts of EPS-slime (Costerton 1984). Additional cross-linking of adjacent EPS chains enable permanent attachment to occur (Marshall 1980). This process is influenced by electrolyte concentration (Fletcher 1988). Bacteria may reversibly attach by secreting an exopolymer allowing them to stick to a surface and use surface-associated nutrients (Hermannson and Marshall 1985). This is followed by the secretion of a second polymer, which releases the attached bacterium.

Facilitates biochemical interactions

Biochemical interactions between the bacteria and surrounding cells and tissues may be made possible by exopolymer material (Logan and Hunt 1987; Decho 1990). Exopolymer slime and capsular material provide a biofilm matrix around the cell. This is a hydrated layer, which can provide a buffering against sudden changes in the adjacent osmotic environment (Dudman

1977). Such a stable environment may aid in the localisation of secreted exoenzymes, which are essential in the cycling of both organic and inorganic material in the marine environment (Decho 1990). The hydrated exopolymer matrix retains the exoenzymes activity in close proximity to the cell thereby facilitating cellular uptake of small molecules for metabolic conversion to energy and biomass (Decho and Herndl 1995).

Symbiotic relationships may also occur between bacteria and other organisms. Bacteria adhere to the site of nitrogen fixation on cyanobacterial heterocysts (Paerl 1976) via the heterocyst-produced EPS (Lupton and Marshall 1984). These microzones around cells facilitate the transfer of nutrients from one organism to another (Paerl 1976). The heavy metal binding properties of an EPS produced by a hydrothermal vent strain was thought to be advantageous to the tubeworm host. (Vincent et al. 1994). Members of the genera *Pseudomonas* and *Alteromonas* produce polysaccharide-containing exopolymers that potentially benefit the survival of other marine organisms by facilitating attachment to surfaces (Szewzyk et al. 1991; Holmstrom and Kjelleberg 1999).

Provides a protective barrier around the cell.

Exopolymer may act as a physical barrier to grazers. In a study by Caron (1987), microflagellates grazed more readily on freely suspended bacteria than on those on surfaces or enclosed in aggregates. The EPS may have provided protection to cells within the aggregates, since the microflagellates were only able to graze the bacteria on the surface of the aggregates. Slime exopolymer from one bacterial strain may be preferred by

consumers to the capsular exopolymer of the same strain (Decho and Lopez 1993).

Changes in pH and salinity over a wide range had little effect on the viscosity and stability of EPS produced by marine bacteria in a study by Boyle and Reade (1983). Such results suggest that these EPS may provide some buffering against shifting environmental conditions in the natural environment. Bacteria isolated from deep-sea hydrothermal vent communities displayed resistance to heavy metals (Jeanthon and Prieur 1990) and the purified EPS produced by these strains in laboratory cultures showed very good metal binding properties (Loaec et al. 1997; Loaec et al. 1998). Capsular polysaccharide may also provide the bacterial cells with a protective barrier against toxic substances in the water column (Bitton and Friehofer 1978).

In biofilm studies involving removal of organic and heavy metal pollutants, exopolymeric substances removed the majority of organic pollutants while heavy metals were taken up by the cellular fraction. These results indicated an important role for cell wall components such as proteins in metal binding in complex biofilm systems (Spaeth et al. 1998). These findings were confirmed in a more recent study that showed heavy metals were bound by cellular sorption as well as extracellularly by polymeric substances such as polysaccharides in bacterial biofilms and microbial flocs (Wuertz et al. 2000).

Cells imbedded in the gel matrix of a biofilm are well protected from biocidal treatments (Brown and Gilbert 1993; McBain and Gilbert 2001). Current strategies to eliminate unwanted biofilms involve the design of antimicrobial agents that can penetrate the gel matrix and target slow or

dormant cells. Some success has been achieved by incorporating transition metal catalysts into the substratum. These generated biocidal species and killed the biofilm from the inside overcoming the protection provided by the exopolymer matrix (Wood et al. 1998).

Acts as a sponge for sequestering dissolved organic material.

In natural aquatic environments, the nutrients required to support maximal microbial growth rarely are present in sufficient quantities in the water column. Microbial attachment to fixed surfaces, other cells and aggregates is a likely strategy to increase the rate of substrate uptake (Logan and Hunt 1987). Microbial cells surrounded by a porous matrix of exopolymer sequester and concentrate dissolved organic compounds (Decho and Lopez 1993). The highly hydrated exopolymer matrix act as a sponge to trap and concentrate nutrients in flowing liquids (Decho 1990).

EPS IN THE ANTARCTIC MARINE ENVIRONMENT

Antarctic sea ice

Bacteria and exopolymers are abundant

Bacteria contribute significantly to secondary production in sea ice communities and to the overall carbon cycle in the Antarctic environment. Annual sea-ice is a microhabitat for a complex community of marine bacteria often in close association with microalgae. These assemblages are essential components of carbon and energy transfers in the Southern Ocean (Sullivan and Palmisano 1984). Abundant bacterial populations have been found in thick annual pack ice, with psychrophilic bacteria being particularly common in

samples of brown ice and pore waters (Delille 1992). Studies of both the Arctic (Krembs and Engel 2001) and Antarctic (Sullivan and Palmisano 1984) sea-ice communities suggest that exopolymer production by both microalgae and bacteria contribute to organic carbon in the sea-ice and ice-water interface. In thick pack ice, bacterial secondary production even exceeds primary production as the light supply to the bottoms layers of ice is reduced (Grossmann and Dieckmann 1994).

Sea ice bacteria maintained in laboratory culture secreted large amounts of mucous (Helmke and Weyland 1995). In a more recent *in situ* study of bacterial-algal interactions in melting sea ice in the Weddel Sea, it was suggested that bacterial mucous contributed to particulate organic carbon sustaining microbial growth in the sea ice crack pools (Gleitz et al. 1996). Bacterially-produced EPS may provide a means by which bacteria can adhere to the microalgal cells (Sullivan and Palmisano 1984). During ice formation, microalgal cells are scavenged by sea ice crystals floating up to the sea surface (Gleitz and Thomas 1993) and bacteria attached to algal cells may be incorporated into new ice in conjunction with some algal species (Grossmann and Dieckmann 1994).

High salinity, low temperature environments

Bacteria are found in abundance in the bottom layers of the ice or in brine channels, and are often attached to detrital particles or to living microalgal cells (Sullivan and Palmisano 1984, Archer et al. 1996, Delille and Rosier 1996). Delille and Rosier (1996) also suggested that the high numbers of particle associated bacteria found in sea ice may explain observations of underlying seawater being enriched in bacterial biomass relative to the open

ocean (Grossmann and Dieckmann 1994). More recently, studies of Arctic sea ice in winter showed that even at temperatures as low as –20^{o}C and salinity of 209 ppt, active bacteria were found in the brine channels and were particle associated (Junge et al. 2004). The same authors showed that high concentrations of exopolymeric substances were found in brine channels and could have been produced by the abundant bacteria or diatom populations present.

A study of Arctic sea ice demonstrated that photosynthesis rates by phytoplankton from under the ice were stimulated to similar levels by sea ice extracts as they were by the chelator, ethylenediamine tetra-acetic acid, and trace metals (Apollonio et al. 2002). From those results, the authors suggested that a natural 'conditioning agent' is produced within the bottom-ice algal layer that enhances phytoplankton growth. Sea ice bacterial communities and high amounts of exopolymer are concentrated in these layers (Krembs and Engel 2001; Krembs et al. 2002). It is yet not clear that in addition to the availability of sufficient light, whether the abundance of trace metals is a limiting nutrient for primary production in sea ice microbial communities.

EPS as a cryoprotectant

Arctic studies (Krembs and Engel 2001; Krembs et al. 2002) have shown that large quantities of microbially produced exopolymeric substances occur in sea ice and at the ice-water interface. This material was positively correlated to bacterial abundances, although diatoms were thought to dominate the exopolymer production in this system. These authors suggested high concentrations of exopolymer with its high polyhydroxyl content would

decrease the freezing point of water in the low temperature, high salinity brine channels, especially near the cell, where concentration of exopolymer are highest (Krembs et al. 2002). Exopolymer in the brine channels might have provided buffering against harsh winter conditions and high salinity as well as cryoprotecting the microbes living there against ice crystal formation by depressing the ice nucleation temperature of water (Krembs et al. 2002).

In a recent study (Mancuso Nichols et al. in press), EPS produced by sea ice isolates were shown, by molecular weight analysis to be between 5 and 50 times larger than the average observed for other marine EPS (1 – 3 x 10^5 Daltons, Decho 1990). The structure and properties of EPS are influenced by the length of the polymer chain, that is the molecular weight (Christensen 1999). As the length of the polymer increases, there is a greater opportunity for complex entanglement of the chains and intramolecular associations, and these contribute to the tertiary structure and physical behavior of the polymer (Sutherland 1994). A fungal strain, *Phoma herbarum,* isolated from Antarctic soil produced a homosaccharide of glucose with a molecular weight of 7.4 x 10^6 Da (Selbmann et al. 2002). The authors of this study suggested that the fungal EPS could have provided a cryoprotective role in the harsh Antarctic environment where the availability of liquid water and temperatures were extremely low. Similarly, the freezing processes in sea ice result in brine channels where temperature is very low and salinity is high due to brine. EPS may be providing a cryoprotectant role in these environments of high salinities and low temperature (Krembs et al. 2002)

In a study by Mancuso Nichols et al. (2004), a strain of Antarctic *Pseudoalteromonas* isolated from sea ice, produced 30 times as much EPS at

-2 and 10°C compared to 20°C in liquid culture. Previous studies have shown that many *Pseudoalteromonas* strains are psychrotrophic bacteria with a temperature growth range from 4°C to 30°C (Bozal et al. 1997; Bowman 1998), and show optimal growth at 22°C to 25°C (Bowman 1998). Members of this genus are among the dominant bacteria generally found in this environment as determined by cultivation dependent and independent techniques (Bowman et al. 1997; Staley and Gosink 1999; Brown and Bowman 2001; Brinkmeyer et al. 2003). In the Mancuso Nichols et al. (2004) study, the consumption of glucose per mg of EPS produced was highest at -2°C, well below the expected optimal growth temperature for this genus (20°C). This finding supports the proposed hypothesis that EPS production by psychrotolerant bacteria may play an important role in the sea ice microbial community. Whether this increased EPS production at low temperature is a specific cold adaptation mechanism for this strain requires further investigation. Bacterial EPS production in brine channels and perhaps other cold, high salinity ecosystems may provide a barrier against the environmental extremes experienced by the bacterial cell by modifying water properties near the cell.

Arctic sea ice studies (Krembs and Engel 2001; Krembs et al. 2002) also demonstrated that the neutrally buoyant polymeric material was carried large distances by prevailing under-ice currents and ice drifts. Studies in more temperate waters showed marine bacterial exopolymer production was important in the aggregate formation process (Biddanda 1986; Decho 1990). When released into the water column, a combination of biological, chemical and physical forces caused this colloidal material to form aggregates

(Alldredge and Jackson 1995; Passow 2000; Kiorboe 2001), which became centers of high microbiological heterotrophic activity (Kiorboe 2001).

EPS in the Southern Ocean

Marine Snow

In natural aquatic systems, when exopolymer is associated with particulate material, it exists in particulate form, or it is present in its colloidal form, which is operationally defined as part of the dissolved organic matter (DOM) because it can pass through a filter with a given pore size (less than 0.1 μm to 0.46 μm, Chin et al. 1998). In the oceans, exopolymer exuded by phytoplankton and bacteria coalesce to form transparent exopolymer particles (TEP) that range in size from microns to hundreds of microns (Sullivan and Palmisano 1984; Passow and Alldredge 1994). The aggregation of TEP, phytoplankton, bacteria, faecal pellets, zooplankton and other organic debris form larger particles (>0.5 mm in diameter), which are known as marine snow (Fowler and Knauer 1986, Logan and Hunt 1987; Mueller-Niklas et al. 1994).

Marine snow in the Southern Ocean

Marine snow has been shown to include highly concentrated and diverse microbial communities (Rath et al. 1998) engaged in photosynthesis, microbial decomposition (Biddanda and Pomeroy 1988, Biddanda 1988) and remineralization of carbon at elevated levels relative to the surrounding sea water (Alldredge and Silver 1988; Simon et al. 1990; Smith et al. 1992). Marine snow particles therefore make a significant contribution to the carbon cycle in the euphotic zone and to the ‘biological pump’, which transports fixed carbon to deep waters (Alldredge 2000, Kiorboe 2001). The flux of

aggregates in the Ross Sea, near the Antarctic peninsula, was found to dominate the vertical export of organic matter from the euphotic zone (Asper and Smith 2003). In another study, the abundance of marine snow particles in samples taken near Australian Antarctic bases Davis and Mawson was approximately 100 times lower that observed in the Ross Sea (Marchant et al. 2000). Spatial and temporal variation in particle production and sedimentation has been observed previously in the Antarctic marine environment (Karl et al. 1991). This variation was also consistent with findings from temperate and tropical waters (Alldredge and Silver 1988).

Bacteria in marine aggregates are at an advantage compared to free living cells (Logan and Hunt 1987). Their proximity to other cells and surfaces provides opportunities for interaction and nutrient uptake. Bacterial polysaccharides form the fibrillar frame work, act as glue in the ultrastructure and provide the structural network for microbial associations within marine aggregates (Biddanda 1986; Decho and Herndl 1995; Heissenberger et al. 1996; Lewis 2000; Flemming and Wingender 2001). Microscopic and laboratory studies have shown that bacterially produced EPS have a major role in aggregate formation (Biddanda 1986, Heissenberger and Herndl 1994; Leppard 1995*).*

EPS as organic ligands

The ability of bacterial EPS to accumulate metals has been known for some time (Bitton and Friehofer 1978; Brown and Lester 1979; Loaec et al. 1997) and at the pH of ambient seawater (pH ≈ 8), anionically charged EPS can remove >99% of Zn and Ag (Harvey and Luoma 1985). Exopolymer complexation with trace metals may impact strongly on the availability of

these micronutrients to marine organisms and may be important in the downward transport of trace metals and micronutrients in the ocean (Decho 1990). Microbial EPS may also be a major component of the colloidal matter which has been proposed to bind trace metals within an 'onion'-like matrix of metal oxides/hydroxides and organic compounds (Mackey and Zirino 1994).

Most (99%) dissolved iron in the ocean is bound to organic ligands with a high affinity for iron (Rue and Bruland 1995). Wu et al.(2001) examined the soluble and colloidal iron in the oligotrophic North Atlantic and North Pacific and showed that soluble iron and iron-binding organic ligands were depleted at the surface and enriched at depth. The authors suggested that iron, which was once thought to be dissolved and available to phytoplankton, might be tied up as colloidal material, which eventually aggregates and settles out of the photosynthetic zone. In another study in the subarctic Pacific Ocean, Maldonno and Price (1999) showed that heterotrophic bacteria play a significant role in dissolved iron uptake and that the iron bound to strong organic ligands, the most predominant form of iron in the sea, is available to phytoplankton in these environments.

Trace metal enrichment experiments, conducted in ship-board ultra-clean facilities showed that iron may be the most important trace metal controlling phytoplankton development in the Southern Ocean (Scharek et al. 1997) and this has recently been confirmed by field experiments where iron was added to a patch of seawater (Boyd et al. 2000). The iron-stimulated diatom bloom was succeeded by an increase in diatom associated silica particulates in sediment traps at depth after 3 weeks and then by increased particulate organic carbon export to deeper waters. Bacterial remineralization

and mesozooplankton grazing of this particulate material, which may have included exopolymer material accounted for over half the increased particulates associated with addition of iron to the system. Geider (1999) argued organic ligands produced by microbes keep iron in solution and that microbes are important in the conversion of iron from the particulate to the dissolved phase. As iron is essential for nitrogen fixation, photosynthesis and respiration, the importance in these microbially produced organic ligands to the biogeochemical iron cycle and the broader issue of climate change should not be overlooked.

Implications for primary chemical structure

Although actual content may vary based upon extraction and purification method used (Neilsen and Andreas 1999), exopolysaccharides produced by marine bacteria may contain up to 20-50% of the polysaccharide as uronic acid (Kennedy and Sutherland 1987). The presence of uronic acids contributes a negative charge to the overall polymer (Decho 1990). Sulfate was thought to occur only in polysaccharides produced by Archaea and Cyanobacteria until recently, and its presence in polymers produced by prokaryotes is seen as uncommon (Arias et al. 2003). When sulfate is present as a functional group, it also contributes to the anionic quality of these EPS in seawater (Leppard et al. 1996). The overall negative charge gives the molecule a ‘sticky’ quality. This anionic charge is important in terms of the affinity of these EPS for binding to cations such as dissolved metals (Brown and Lester 1982).

Korstgens et al (2001) studied biofilms formed by *Pseudomonas aeruginosa* that were dominated by polysaccharides with carboxyl groups.

Calcium, copper and iron provided stability to the network by acting as bridging ions. The presence of calcium and magnesium salts resulted in increased viscosity in solutions of polysaccharide from marine bacteria stored at low pH (Boyle and Reade 1983). Divalent cations provide stability to the polysaccharide gel matrix (Decho 1990). Recent work with the purified EPS from *Ps. aeruginosa* showed that there were strong electrostatic interactions between divalent cation manganese and the carboxylate groups occurring along the EPS chain. This study provides insight into the molecular geometry of the stability provided by divalent cations such as calcium, which are present in marine systems (Emmerichs et al. 2004).

Bacteria isolated from Southern Ocean particulate material produced EPS in liquid culture (Mancuso Nichols et al. 2004). Preliminary characterizations show that the structure of the EPS includes sulfate as well as high levels of uronic acids as galacturonic acid, along with acetyl groups. In addition, the EPS was shown by NMR data to include a succinyl group. These features convey an overall polyanionic quality to the EPS in the marine environment, since at the pH of seawater many of the acidic groups present on these polymers are ionized (Decho 1990). This ‘stickiness’ is important in terms of the affinity of these EPS for binding of cations such as dissolved metals (Brown and Lester 1982). The EPS produced by Antarctic bacterial isolates examined by Mancuso Nichols et al. (in press) appear to be polyanionic and, therefore, ‘sticky’ with respect to cations such as trace metals. The availability of iron as a trace metal is of critical importance in the Southern Ocean where it is known to limit primary production (Scharek et al. 1997). Since 99% of dissolved iron in the ocean is bound to organic ligands

(Wu et al. 2001), implications for the role of these bacterial polysaccharides in the Antarctic marine environment require further investigation.

Monosaccharide analyses of the ten EPS produced by Antarctic marine bacteria (Mancuso Nichols et al. in press) showed that the sugars present were generally similar to sugars typically found in bacterial EPS (Table 1, Kenne and Lindberg 1983) and more specifically in marine bacterial EPS (Kennedy and Sutherland 1987). Arabinose was present, to varying degrees, in all EPS produced by Antarctic bacteria examined in this study and xylose was present as a minor component in several strains. Arabinose and xylose are not commonly found in bacterial EPS but are components of the lipopolysaccharide layer of some microbes (Kenne and Lindberg 1983). The significance of these finding requires further investigation.

An exopolysaccharide, known as mauran, is produced by the halophilic bacterium, *Halomonas maura* (Arias et al. 2003). When this strain was grown in media containing salt (2.5%, w/v), it produced a high molecular weight (4.5 x 10^6 Daltons) EPS that contained glucose, mannose and galactose as well as high amounts of glucuronic acid (8%, w/w) and sulfates (6.5%, w/w). This polysaccharide was able to bind a range of heavy metal cations. The authors also noted the stability of muran under different conditions of stress including high salt concentrations and during freezing/thawing. There are similarities between muran and several of the EPS produced by the Antarctic marine isolates in terms of chemical composition.

Further research is necessary to more accurately define the structure of the Antarctic marine bacterial EPS and to relate these findings to the function of these molecules in the natural environment. As yet, it is unclear

how these polysaccharides may be acting mechanistically as organic ligands, protectants against low temperature or high salinity, or whether the size of these EPS is related to their ecological role. An increased understanding of these structural and functional roles is also a prerequisite to potential biotechnological exploitation of Antarctic bacterial EPS.

EPS FROM THE DEEP-SEA

Hydrothermal vent communities

The oceans constitute more that 70% of the earth's surface. The deep sea (>1000 m) was once thought to be a biological desert until submarine hydrothermal systems were discovered along mid-ocean ridges at depths greater than 2200 m (Snelgrove and Grassle 1995). Geological formations include hot fumaroles, springs and sediments and deep-sea vents (Stetter 1998). In these environments, hydrostatic pressure averages 25 x 10^6 Pascals, and temperatures can range from 380^{o}C within the fumarole to 2^{o}C in the surrounding sea-water (Yayanos 1998). Hot anaerobic waters rich in hydrogen sulfide and heavy metals escape the vents and blend with cold oxygenated seawater. The presence of heavy metals is a characteristic feature of the hydrothermal vent environment (Jeanthon and Prieur 1990). Despite these environmental extremes, a complex food web based on chemosynthesis including dense invertebrate populations supported by a rich microbial community of heterotrophic and autotrophic bacteria were found in the vicinity of the vents (Antoine et al. 1995).

The selective pressures imparted on the inhabitants of this ecosystem result in hydrothermal vent environments being centres of unusual biological

communities. Vent environments are now considered to be an enormous source of genetic and metabolic microbial biodiversity (Deming and Baross 1998). Innovations in microbiological culturing techniques recently have been employed to gain insight into microbial biochemical processes and microbial by-products used for growth and survival in these hydrothermal vent environments (Deming 1998).

Microbial EPS from hydrothermal vents

Deep-sea hydrothermal vents offer a new source of a variety of fascinating microorganisms well adapted to these extreme environments. Over the past 17 years, an increasing number of new genera and species of both hyperthermophilic and mesophilic bacteria have been isolated from these vents communities (Guezennec 2002). Bacteria associated with deep-sea hydrothermal vent communities have demonstrated their ability to produce unusual extracellular polymers in an aerobic carbohydrate-based medium and so far, three main EPS producing genera have been identified *i.e.*, *Pseudoalteromonas*, *Alteromonas* and *Vibrio*. To date, only a small number of EPS have been fully characterized, since they hold some biotechnological promise (see below). Information related to the chemical composition of these polymers reveals potential commercial usefulness at the same time as providing insight into their role in the deep-sea vent ecosystem.

BIOTECHNOLOGICAL POTENTIAL OF MICROORGANISMS

Biotechnology is recognised as one of the most promising technologies for the 21st century considering its potential to ameliorate major global problems (disease, malnutrition and environmental pollution), achieve

industrial sustainability (optimising use of renewable resources, slowing global warming and developing cleaner products and processes) and achieve economic competitiveness (Bull et al. 2000). Since biotechnology is based on the discovery of exploitable biology, the recognition that only a very small fraction of the earth's microbial biodiversity has been identified implies a great potential for innovation. Knowledge of the interaction of microbes in their environment is critical in accessing both the microbe itself and processes it employs to survive, both of which hold biotechnological promise (Bull et al. 2000).

Biotechnological potential of EPS

The species-specific structural heterogeneity and the many roles EPS play in the natural environment are reflected in the numerous existing and potential applications for these bio-polymers (Weiner 1997). Xanthan gum, the most well known microbial polysaccharide, is produced by the plant-pathogen *Xanthomonas campestris* pv. campestris. Because of its physical properties it is commonly used as a thickener in both food and non-food industries (Becker et al. 1998). Bacterial cellulose, produced by *Acetobacter xylinum* and other, mainly Gram-negative bacterial species, has a high water binding capacity. This EPS is used to make a type of wound dressing for patients with burns, chronic ulcers or extensive tissue loss (Sutherland 1998). Several *Agrobacterium* and *Rhizobium* species produce curdlan and this improves the texture of tofu, bean jelly and fish pastes in Japan (Sutherland 1998). The study of EPS produced by bacteria from the marine environment provides additional opportunities for novel uses of these biopolymer

EPS from deep-sea hydrothermal vents

In recent years, there has been a growing interest in the isolation and identification of new microbial polysaccharides that may have novel applications such as viscosifiers, gelling agents, emulsifiers, stabilizers and texture enhancers. In the course of the discovery of novel polysaccharides of biotechnological interest, it is now widely accepted that extremophilic microorganisms will provide a valuable resource not only for exploitation in biotechnological processes but also as models for investigating how biomolecules are stabilized when subjected to extreme conditions. Deep-sea hydrothermal vents offer a new source of novel bacteria. Several have been found to produce exopolymers with exploitable properties.

EPS-producing thermophilic and mesophilic strains have been sourced from vent environments and the EPS produced by these strains in laboratory culture have been examined for a range of applications. Several bacterial exopolymers were found to be novel with significant biotechnological potential (Guezennec et al. 1994, Raguénès et al. 1997b). To date, investigations generally have been performed on mesophilic heterotrophic bacteria rather than on thermophilic and hyperthermophilic microorganisms. This is despite the biotechnological appeal of microorganisms adapted to life at high temperature that may produce thermostable enzymes (Guezennec 2002).

The structure of the EPS produced by *Pseudoalteromonas* strain 721 has been investigated. The repeating unit of this polymer shows some irregularities but can be defined as an octasaccharide with two side-chains (Figure 1, Rougeaux et al. 1999). This exopolymer exhibits a gelation following thermal treatment. The viscoelastic behaviour of the HYD721/NaCl

system under varying temperatures suggests that two effects contribute to the creation of the gel network. Intermolecular associations observed with increasing temperature are probably the result of hydrophobic interactions between methyl groups of the rhamnose residues (Guezennec 2002).

Alteromonas strain 1545, isolated near a hydrothermal vent from the epidermis of the polychaete *Alvinella pompejana*, produces an anionic EPS under laboratory conditions (Talmont et al. 1991). A polysaccharide secreted by a bacterium (*Alteromonas* strain 1644) isolated from *Alvinellidae* samples, collected near a hydrothermal vent of the East Pacific Rise showed an original chemical structure and unique rheological behaviour (Figure 2). This polymer shows strong selectivity between monovalent and divalent ions and exhibits a great affinity for the divalent ions, higher than predicted by electrostatic theories (Bozzi et al. 1996a; Bozzi et al. 1996b) with the exception for Mg^{2+}. *A. macleodii* subsp. *fijiensis* is an aerobic, mesophilic, heterotrophic bacterium isolated from a diluted hydrothermal vent at a depth of 2600m in a rift system of the North Fiji basin (16°59'S, 173°55' W). This strain produces an EPS with a high metal-binding maximum capacity (up to 316 mg Pb(II) / g polymer (Loaec et al. 1997; Loaec et al. 1998). Proposed uses for this polymer include water treatment and removal of heavy metal pollutants (Table 2). This EPS also holds promise as a food-thickening agent since it has many chemical similarities to xanthan (Figure 3, Rougeaux et al. 1996). This hydrophobic bacterial exopolysaccharide was also shown to encourage adhesion of osteoblastic cells during in vivo experiments conducted on rat calvaria. Results suggested that hydrophobic EPS matrix added to bone surfaces might encourage healing (Zanchetta and Guezennec 2001). Other studies of

EPS produced by bacteria from these vent environments either in their native state or following chemical modifications also suggested clinical applications in the area of cardio-vascular diseases (Colliec-Jouault et al. 2001; Matou et al. submitted) and bone healing.

A facultatively anaerobic, heterotrophic and mesophilic bacterium was also isolated from a Pompeii worm (polychaete *Alvinella pompejana*) tube collected from a deep-sea hydrothermal field of the East Pacific Rise and named *Vibrio diabolicus.* This was the first member of the genus *Vibrio* to be isolated from a deep-sea ecosystem. Novel EPS produced by this strain is characterized by equal amounts of uronic acid and hexosamine (*N*-acetyl glucosamine and *N*-acetyl galactosamine (Figure 4, Raguénès et al. 1997a).The role of this novel bacterial polysaccharide in bone regeneration has been recently successfully investigated (Zanchetta et al. 2003a; Zanchetta et al. 2003b).

There is not doubt that extreme environments such as deep-sea hydrothermal vents are a rich source of microorganisms of biotechnological importance. A number of interesting and unique polysaccharides have been isolated from these microorganisms and these are expected to find industrial applications in the very near future. Further screenings are underway as well as research into understanding the structure-function relationships of these unusual polymers (Guezennec 2002).

Cool prospects: EPS from Antarctica

The Antarctic marine environment is perennially cold, in some cases it is permanently ice covered. Extremes of temperature, salinity, and water activity govern microbial life in enriched microenvironments for example,

hypersaline sea ice brine channels, the pelagic water column and particles. Spatial heterogeneity combined with extreme seasonal fluctuations such as those experienced during annual sea ice formation events results in a high diversity of microbial habitats and therefore, microbial communities (Karl 1993). Very few bacterial species isolated from the Antarctic marine environment have been described to date (Nichols et al. 2001). An opportunity clearly exists for the search and discovery of novel microbial products with biotechnological potential.

Ecological studies examining the role of EPS in marine habitats now provide evidence that these substances are abundant in the Antarctic marine environment (Helmke and Weyland 1995; Krembs and Engel 2001; Krembs et al. 2002, Mancuso Nichols et al. 2004; Mancuso Nichols et al. in press). However, few studies focusing on the biotechnological potential of EPS produced by bacteria from the Antarctic marine environment are available from the literature to date. *Pseudoalteromonas antarctica* NF_3 produces a exopolymeric compound of glycoprotein character that displays the ability to coat liposomes and provides protection against surfactants (Cocera et al. 2000; Cocera et al. 2001). A study by Mancuso Nichols et al. (in press) has shown that, even among closely related strains, EPS produced by Antarctic bacteria commonly found in the marine environment were diverse. The full subunit structure of EPS produced by Antarctic marine bacteria remains to be elucidated. This information will facilitate assessment of possible commercial applications. These initial studies reveal largely untapped reservoir of biotechnological potential is waiting to be accessed. Whether these EPS will

become useful as cryoprotectants, chelators of heavy metals or in some other form remains to be established.

CONCLUSIONS

Bacterial exopolymers and their EPS components are abundant and ubiquitous in the marine environment where they serve essential functions that enhance microbial survival. In the Antarctic marine environment, exopolymers may provide cryoprotection in high salinity, low temperature brine channels. In the Southern Ocean iron limits primary productivity and the resulting draw-down of carbon dioxide, an important green house gas, from the atmosphere. Microbial EPS in suspended aggregates of marine snow, may influence the availability of dissolved iron for primary production in Antarctic waters. In hydrothermal vent environments of the deep-sea, where bacteria have adapted to physical stresses such as extremes of temperature and pressure, exopolymers have been found that produce biochemically interesting EPS. The two environments highlighted in this review provide examples of reservoirs of microbial biodiversity that are relatively untapped. Several EPS produced by microbes from these extreme environments are showing biotechnological promise. By examining the chemical characteristics of these carbohydrate polymers, it is possible to begin to understand the ecological role of these natural products as well as to gain insight into their commercial potential.

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FIGURES

Figure 1 Repeating unit of the exopolysaccharide secreted by *Pseudoalteromonas* sp. strain 721 (Guezennec 2002).

Figure 2 Repeating unit of the exopolysaccharide produced by *Alteromonas* sp. strain 1644 (Guezennec 2002).

Figure 3 Repeating unit of the exopolysaccharide secreted by *Alteromonas. macleodii* subsp*. fijiensis* (Guezennec 2002).

Figure 4 Repeating unit of the exopolysaccharide secreted by *Vibrio diabolicus* (Guezennec 2002).

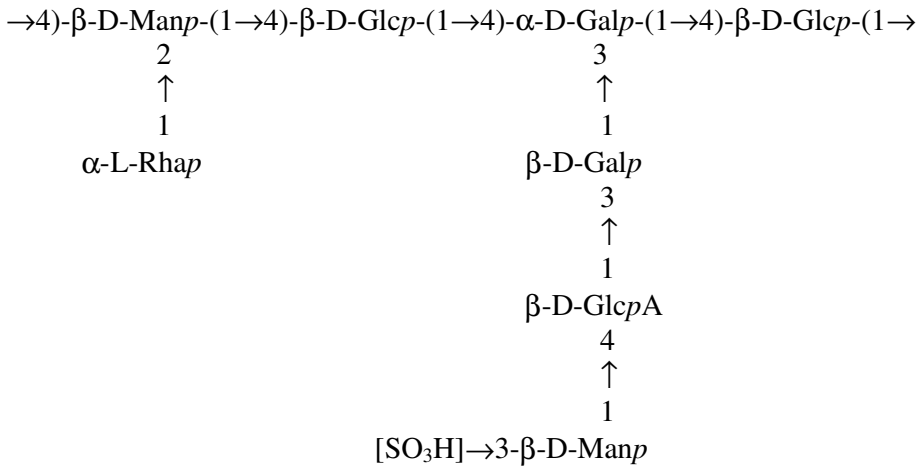
→4)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→
2
↑
1
α-L-Rha*p*
3
↑
1
β-D-Gal*p*
3
↑
1
β-D-Glc*p*A
4
↑
1
[SO3H]→3-β-D-Man*p*

Figure 1

→4)-α-D-Glc*p*A-(1→3)-β-D-Glc*p*A-(1→3)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*-(1→?)-Hexose(1→

4

↑

1

β-D-Glcp

4

↑

1

αβ?-D-Galp

3

↑

1

3-O-Lac-β-D-GlcpA

Figure 2

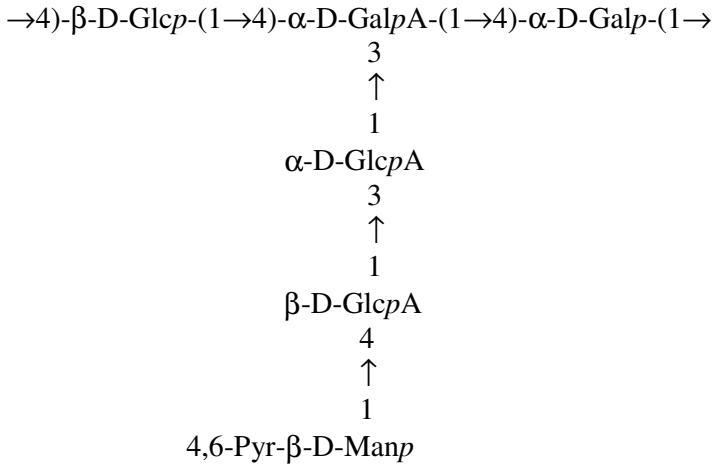
→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*-(1→
3
↑
1
α-D-Glc*p*A
3
↑
1
β-D-Glc*p*A
4
↑
1
4,6-Pyr-β-D-Man*p*

Figure 3

→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*A-(1→4)-α-D-Gal*p*NAc-(1→

Figure 4

Table 1. Sugar and non-sugar components of bacterial exopolysaccharides*

Type	Component	Example	Mode of linkage
Sugar	Pentoses	D-Arabinose	
		D-Ribose	
		D-Xylose	
	Hexoses	D-Glucose	
		D-Mannose	
		D-Galactose	
		D-Allose	
		L-Rhamnose (6-Deoxy-L-mannose)	
		L-Fucose (6-Deoxy-L-galactose)	
	Amino sugars	D-Glucosamine (2-Amino-2-deoxy-D-glucose)	
		D-Galactosamine (2-Amino-2-deoxy-D-galactose)	
	Uronic acids	D-Glucuronic acid	
		D-Galacturonic acid	
Non-sugar	Acetic acid		*O*-Acyl, *N*-Acyl
	Succinic acid		O-Acyl
	Pyruvic acid		Acetal
	Phosphoric acid		Ester, diester
	Sulfuric acid		Ester

* adapted from Kenne L, Lindberg B (1983) Bacterial Polysaccharides.
In: Aspinall GO (ed) The Polysaccharides. Academic Press, New York, pp 287-363

Table 2. Examples of characterized marine bacterial exopolysaccharides from Antarctic and deep-sea hydrothermal vent sources

Microorganism	Source environment	Description of EPS	Distinguishing characteristics	Suggested ecological role	Biotechnological application	Reference
Pseudoalteromonas sp. (strain CAM025, bacteria)	Filtered sea ice particulates, **Antarctica**	Sulfated heteropolysaccharide, high in uronic acids with acetyl groups	High* molecular weight (5.7 x 10^6 Da)	Cryprotection in sea ice brine channels	_^	Mancuso Nichols et al. 2004
Pseudoalteromonas sp. (strain CAM036, bacteria)	particulates from **Southern Ocean**	Sulfated heteropolysaccharide, high in uronic acids with acetyl and succinyl groups	High* molecular weight (1.7 MDa)	Trace metal binding in iron depleted Southern Ocean	–	Mancuso Nichols et al. 2004
HYD-1545 (bacteria)	Tissue of marine polychaete from **deep-sea hydrothermal vent (HTV)** habitat	Sulfated heteropolysaccharide, high in uronic acids, with pyruvate	High uptake of heavy metals	–	–	Vincent et al. 1994
Alteromonas macleodii subsp. f*ijiensis* (bacteria)	Seawater, **deep-sea HTV**, North Fijian Basin	Sulfated heteropolysaccharide, high in uronic acids, with pyruvate	High uptake of lead, cadmium and zinc	–	Thickening agent in food-processing industry, biodetoxification and wastewater treatment, bone healing, treatment of cardio-vascular deseases	Rougeaux et al. 1996, Loaec et al. 1997, Colliec-Joult et al. 2001
Pseudoalteromonas sp. (strain GY 768, similar to *P. carrageenovora,* bacteria)	Invertebrate tissues, d**eep-sea HTV**, Guaymus Basin	Sulfated (13%) heteropolysaccharide, high in uronic acids, with pyruvate and acetate	Polyelectrolyte character	–	Biodetoxification and wastewater treatment, bone healling	Rougeaux et al. 1996, Zanchetta and Guezenec 2001
Pseudoalteromonas sp. (strain GY 786, similar to *P. undina, bacteria*)	Invertebrate tissues, **deep-sea HTV**, Guaymus Basin	Sulfated (6.5%) heteropolysaccharide, high in uronic acids, with pyruvate and acetate	Polyelectrolyte character	–	Biodetoxification and wastewater treatment	Rougeaux et al. 1996
Vibrio sp. (bacteria)	Invertebrate tissues, **deep-sea HTV** 9°N East Pacific Rise	Heteropolysaccharide high in uronic acids and amino sugars, traces of neutral sugars (EPS 800)	Similar to heparin	–	Anticoagulant activity, anti-HIV activity, pharmaceutical activity	Rougeaux et al. 1996
Alteromonas infernus (strain GY 685, bacteria)	Seawater from *Riftia pachyptila*, **deep-sea HTV**, Guaymas Basin	Two EPS, EPS-1 associated with cells, rich in uronic acid and protein; EPS-2: heteropolysaccharide with uronic acids	–	–	Biodetoxification and wastewater treatment	Raguénès et al. 1997

* average molecular weight of most marine bacterial EPS: 0.1 - 0.3 x 10^6 Da (Decho et al 1990)
^ none mentioned

Table 3. Some of the roles of microbial exopolymeric material in the marine environment

Role of exopolymer	Examples	References
Assists in attachment to surfaces	Exopolymers of marine *Vibrio* MH3 were involved in reversible attachment	Hermannson and Marshall 1985
	Cross-linking of adjacent polysaccharide chains aided in permanent adhesion	Marshall 1980
Facilitates biochemical interactions between cells	Exopolymer matrix localized secreted exoenzymes	Decho and Herndl 1995
	Exopolymer mediate bacterial attachment to the polar end of bluegreen N2-fixing alga. EPS aided attachment to symbiotic host such as vent tube worm to absorb metals and detoxify microenvironment.	Paerl 1974, Vincent et al. 1994
	Exopolymer buffered against sudden osmotic changes	Dudman 1977
Provides protective barrier around the cell.	Bacteria in aggregates were less preferred by grazers that freely suspended bacteria	Caron 1987
	EPS-producing deep-sea hydrothermal vent bacteria showed resistance to heavy metal. Metal binding involves cell wall components as well as polysaccharides	Jeanthon and Pieur 1990, Spath et al. 1998
	Exopolymer in sea-ice brine channels provided cryoprotection by interacting with water at low temperature to depress freezing point	Krembs et al. 2002
	Nutrient uptake by bacteria in aggregates was higher than for free-living cells in low nutrient systems	Logan and Hunt 1987
Absorbs dissolved organic material	Porous and hydrated matrix acts like a sponge and sequestered and concentrated dissolved organics	Decho 1990, Decho and Lopez 1993