Journal of Industrial Microbiology & Biotechnology (2002) 29, 204–208

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COMMENTS

Deep-sea hydrothermal vents: A new source of innovative bacterial exopolysaccharides of biotechnological interest?[†]

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Polysaccharides and, in particular, microbial polysaccharides represent a class of important products of growing interest for many sectors of industry. Although many known marine bacteria produce exopolysaccharides (EPS), continuation in looking for new polysaccharide-producing microorganisms is promising. Hydrothermal deep-sea vents could be a source of novel EPS as indicated by the screening of a number of mesophilic heterotrophic bacteria recovered from different locations. Although originating from such extreme environment, some bacteria were shown to biosynthesize innovative EPS under laboratory conditions. Their specific rheological properties either in the presence or absence of monovalent and divalent ions, biological activities, metal binding capabilities, and novel chemical composition mean that these EPS are expected to find many applications in the near future.
Journal of Industrial Microbiology & Biotechnology (2002) **29,** 204–208 doi:10.1038/sj.jim.7000298

Keywords: hydrothermal vents; bacterial exopolysaccharides

Introduction

Polysaccharides occur as important constituents of plant and microbial cell walls, either as storage polysaccharides or as biopolymers known as exopolysaccharides (EPS) secreted by microorganisms. In recent years, there has been a growing interest in the isolation and identification of new microbial polysaccharides that might have novel uses such as viscosifiers, gelling agents, emulsifiers, stabilizers, and texture enhancers.

Bacterial polysaccharides possess a wide variety of properties that may not be found in more traditional polymers of plant origin. Although they compete with polysaccharides from other sources, e.g., from algae (alginates, carrageenans), crustacean (chitin), or plants, their production is less subject to variability due to marine pollution, crop failure, or climatic impact.

Due to their many interesting physical and chemical properties, e.g., stabilizing, suspending, thickening, gelling, coagulating, film-forming, and water retention capability, polysaccharides have found applications in many industrial sectors, e.g., in detergents, textiles, adhesives, paper, paint, food and beverage industries [28], pharmaceuticals and cancer therapy, drug delivery [3,8], oil recovery and metal recovery in the mining industry and from industrial waste [12], and in the formulation of cell culture media. Some polysaccharides are chemically derivatized to impart or improve desired functional properties [22].

For several years, there has been a growing interest in the recognition of biological activities of microbial polysaccharides [33] such as their antitumor activity and the immunostimulatory activities of some polysaccharides produced by marine bacteria, e.g., *Vibrio* sp. and *Pseudomonas* sp. [16,18]. Antiviral effects of polysaccharides including human immunodeficiency virus activity have been recognized for a decade [2,30]. The anticoagulant activity of these polymers can also be linked to the high sulfate content associated with specific polysaccharides [17,32].

A number of microbial extracellular polysaccharides are produced on an industrial scale. In addition to dextran and xanthan, gellan gum is gaining importance in the food industry [13]. Other well-known polysaccharides of microbial origin include curdlan secreted by *Alcaligenes faecalis* var *myxogenes*, alginates from *Pseudomonas aeruginosa* and *Azotobacter vinelandii*, succinoglycans produced by bacteria of the genera *Pseudomonas*, *Rhizobium*, *Agrobacterium*, and *Alcaligenes*, and others [28].

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 novel biotechnological processes but also as models for investigating how biomolecules are stabilized when subjected to extreme conditions. Deep-sea hydrothermal vents were discovered in 1977 and are characterized by high pressures, high temperature gradients, and high levels of toxic elements such as sulfides or heavy metals. Indeed, deep-sea hydrothermal vents now offer a new source of a variety of fascinating microorganisms well adapted to these extreme environments. Over the past 15 years, an increasing number of new genera and species of both hyperthermophilic and mesophilic bacteria have been isolated from these deep-sea hydrothermal vents. This new bacterial diversity includes strains able to produce novel molecules such as enzymes, polymers, and other bioactive molecules. This may lead to the discovery and characterization of innovative molecules of interest for different industrial sectors.

New bacterial EPS?

Screenings performed on isolates recovered from different oceanographic cruises (Figure 1) led to the discovery of a number of microbial EPS with interesting chemical and rheological properties

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[†]This work is dedicated to Michel Guezennec.
Received 18 March 2002; accepted 13 June 2002

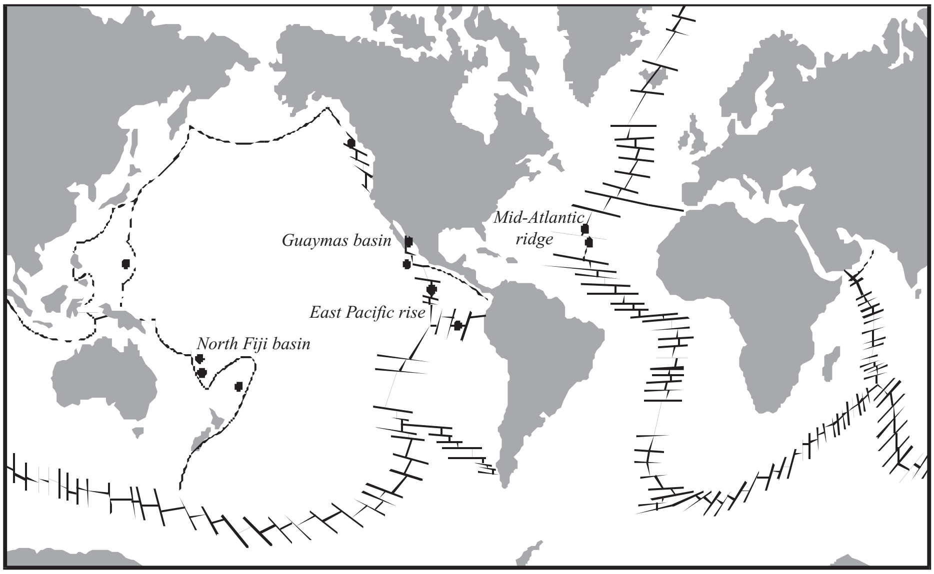

Figure 1 Location of different hydrothermal sites.

[23,24,31]. Bacteria associated with deep-sea hydrothermal conditions have demonstrated their ability to produce unusual extracellular polymers in an aerobic carbohydrate-based medium and, so far, three main EPS producers have been identified, i.e., *Pseudoalteromonas*, *Alteromonas*, and *Vibrio* (Table 1). To date, screenings have been performed mainly on mesophilic heterotrophic bacteria rather than on thermophilic and hyperthermophilic microorganisms, even though the latter microorganisms are considered to be biotechnologically attractive due to their production of thermostable enzymes. Only a few polymers have so far been fully characterized, but considerable information related to the chemical composition of those polymers, and their properties in terms of rheology or metal binding capability, has been determined. Most of them have uronic acid contents from 10% to 40% and high molecular masses up to 10^6 g mol^{-1}.

Alteromonas strain 1545, isolated near a hydrothermal vent from the epidermis of the polychaete *Alvinella pompejana*, produces an anionic EPS, consisting of glucose, galactose, glucuronic, and galacturonic acids, under laboratory conditions [1,9]. Interesting rheological properties have been determined for this polymer, especially as a thickening agent [29]. The chemical structure of this polysaccharide was not completely elucidated, but showed original components as a trisaccharide of uronic acid residues along with a 4,6-*O*-(1-carboxyethylidene)-galactose residue [17].

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. This polysaccharide is composed of four neutral sugars and four acidic sugars. Three of these uronic acid residues form a trisaccharide unit and the last one carries a lactate group on position 3 [7] (Figure 2). In solution, the gel exhibits unusual properties compared with other gelling polysaccharides. 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 [4,5], with the exception for Mg^{2+}.

A polysaccharide was secreted by *Alteromonas macleodii* subsp. *fijiensis*, which is an aerobic, mesophilic, heterotrophic bacterium isolated from a diluted hydrothermal vent fluid at a depth of 2600 m in a rift system of the North Fiji basin (16°59′S, 173°55′W) [19]. The repeating unit of this polymer secreted during its stationary growth phase was characterized by means of chemical analysis and NMR studies and deduced to be a hexasaccharide with three linked uronic acids and with a side chain ended by a 4,6-*O*-(1-carboxyethylidene)-mannose residue [23,24] (Figure 3). This EPS exhibits a significant thickening power and a pronounced shear-thinning behaviour (Figure 4). Gelation properties observed in the presence of calcium can be explained on the basis of intermolecular Ca^{2+} bridges formed between carboxyl oxygen atoms of the glucuronosyl and galacturonosyl residues [24,25]. A high metal-binding maximum capacity (up to 316 mg Pb(II)/g polymer) was observed in a single metal system, indicating that this polymer may have potential for use in applications in wastewater treatment and biodetoxification of heavy metal-polluted water

Table 1 Main characteristics of some microbial EPS of hydrothermal origin

Bacterium	Sampling location (depth)	Chemical composition	References
Alteromonas sp. strain 1545	East Pacific Rise (2600 m)	Glc/Gal/4,6Pyr-Gal/GlcA/GalA	[14,19]
Alteromonas sp. strain 1644	East Pacific Rise (2600 m)	Glc/Gal/GlcA/3Lac-GlcA/GalA	[20,21]
Alt. macleodii subsp. *fijiensis*	North Fiji basin (2600 m)	Glc/Gal/4,6Pyr-Man/GlcA/GlaA	[22,24]
Alt. infernus	Guaymas Basin (2000 m)	Glc/Gal/Man/Rha/GlcA/GalA	[27]
Pseudoalteromonas sp. strain 721	East Pacific Rise (2600 m)	Glc/Gal/[SO_3H]-Man/Rha/GlcA	[30]
V. diabolicus	East Pacific Rise (2600 m)	GlcA/GlcNAc/GalNAc	[32]

Glc: glucose; Gal: galactose; Rha: rhamnose; Man: mannose; GalA: galacturonic acid; GlcA: glucuronic acid; GlcNAc: *N*-acetyl glucosamine; GalNAc: *N*-acetyl galactosamine.

→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-Glc*p*
4
↑
1
αβ?-D-Gal*p*
3
↑
1
3-O-Lac-β-D-Glc*p*A

Figure 2 Repeating unit of the EPS produced by *Alteromonas* sp. strain 1644.

[14,15] (Figure 5). Finally, this hydrophobic bacterial EPS was shown to encourage adhesion of osteoblastic cells during *in vivo* experiments conducted on rat calvaria. Providing surfaces with such a hydrophobic EPS matrix might encourage bone healing [34].

Another new aerobic, mesophilic, and heterotrophic bacterium was isolated from a sample of fluid collected among a dense population of *Riftia pachyptila*, in the vicinity of an active hydrothermal vent of the southern depression of the Guaymas Basin. On the basis of phenotypic and phylogenetic analyses and DNA/DNA relatedness, this strain was recognized as a new species of the genus *Alteromonas* and named *Alt. infernus* [21]. Two unusual polysaccharides were secreted during the stationary growth phase in batch cultures in the presence of glucose. The water-soluble EPS showed a very strong affinity for heavy metals such as lead, cadmium, and zinc [15]. Sulfation and depolymerization of this particular polymer led to highly sulfated, low-molecular-weight fractions with very promising anticoagulant biological properties. The free radical depolymerized fraction with a sulfate content of 40% and a molecular weight of 24 10^3 g mol^{-1} was shown to prolong the clotting assay. The thrombin generation was inhibited in both contact-activated and thromboplastin-activated plasma with a prolonged lag time only in the former [6,10].

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 [25,26] (Figure 6). 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.

→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 Repeating unit of the EPS secreted by *Alt. macleodii* subsp. *fijiensis*.

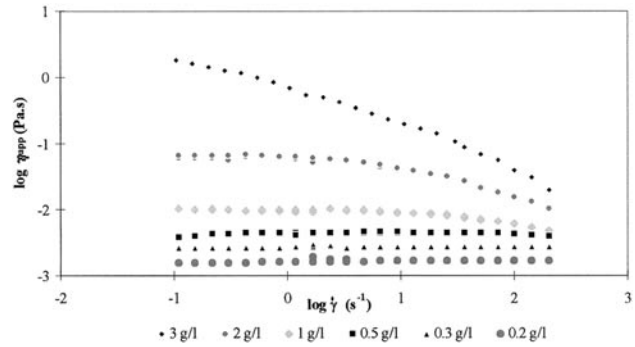

Figure 4 Flow curves of solutions of different concentrations of the *Alt. macleodii* subsp. *fijiensis* EPS (0.1 M NaCl, 20°C).

Intermolecular associations observed with increasing temperature are probably the result of hydrophobic interactions between methyl groups of the rhamnose residues, while additional junction zones occurring upon cooling may be attributed to hydrogen bonds [23,26].

A new facultatively anaerobic, heterotrophic, and mesophilic bacterium was also isolated from a Pompei worm (polychaete *Alv. pompejana*) tube collected from a deep-sea hydrothermal field of the East Pacific Rise and named *Vibrio diabolicus* [21]. During stationary phase growth in batch cultures in the presence of glucose, this bacterium produced an EPS, characterized by equal amounts of uronic acid and hexosamine (*N*-acetyl glucosamine and *N*-acetyl galactosamine), similar to well-known biologically active polysaccharides. Structural studies recently conducted on this polymer demonstrated that it consists of a linear tetrasaccharide repeating unit [27] (Figure 7). The role of this novel bacterial polysaccharide in bone regeneration has recently been successfully investigated [35].

→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 7 Repeating unit of the EPS secreted by *V. diabolicus*.

Recovering EPS producers from biofilms

Screenings have been mostly performed on samples taken from the water column, rocks, sediments, or associated with invertebrates,

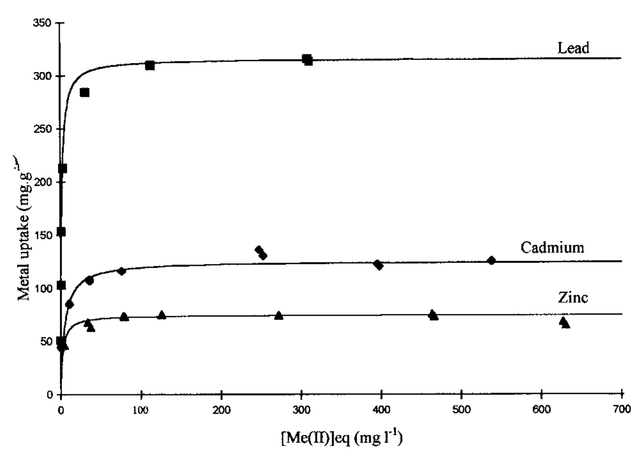

Figure 5 Equilibrium sorption isotherms of lead, cadmium, and zinc by the EPS produced by *Alt. macleodii* subsp. *fijiensis* (room temperature, 3 h).

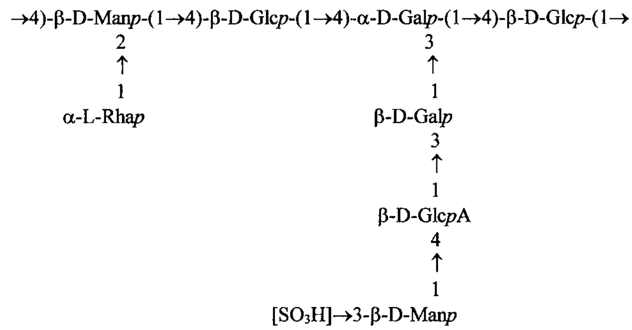

Figure 6 Repeating unit of the EPS secreted by *Pseudoalteromonas* sp. strain 721.

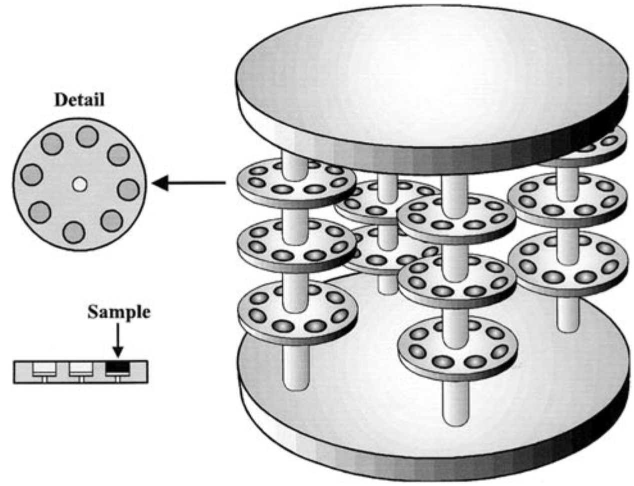

Figure 8 Sample holder used during the "Microsmoke" oceanographic cruise for collecting polysaccharide-producing microorganisms.

clams, mussels, and other animals. During the last oceanographic cruise that took place along the mid-Atlantic Ridge, a new approach for collecting polysaccharide-producing microorganisms was evaluated. The method was based on the biosynthesis of extracellular EPS by microorganisms and their ability to firmly attach onto surfaces exposed in the natural environment. Artificial substrates have been hypothesized to stimulate EPS biosynthesis. Coupons composed of metals (316 L stainless steel, aluminum, titanium, copper, and copper alloy Cu–Ni 90–10) and nonmetallic surfaces (Teflon, nylon, polycarbonate, and polyacrylate) (Figure 8) were deployed in the vicinity of the plumes of deep-sea hydrothermal vents along the mid-Atlantic Ridge. These coupons served as a substratum for colonization of microorganisms adapted to the metal-enriched habitat of the smokers or more generally to the physical and chemical environment of the deep-sea vents [11]. After 1–12 days of contact with the vent environment, the coupons were recovered using appropriate sterile boxes and the attached organisms isolated from subsequent cultures. Complete characterization of the polysaccharides secreted by bacteria constituting the biofilms is in progress, but interesting data on the rheological properties of some polymers have already been determined.

Conclusions

Microbes isolated from extreme environments offer a great diversity in chemical and physical properties of their EPS compared to anywhere else in the biosphere. Bacteria from remote areas still remain virtually unexplored and there is no 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 are expected to find applications in the very near future in different industries. Further screenings are underway as well as research into understanding the structure–function relationships of these unusual polymers.

Those strains that produce EPS exhibiting novel properties will be transferred to private industries interested in further evaluating the polymers for commercial development. However, despite the interesting properties and subsequent applications of these polysaccharides, other factors including the yield, price, and the markets for such new molecules will ultimately determine the commercial development of these polymers.

Acknowledgements

The author is greatly indebted to all contributors to this project — Dr. Hélène Rougeaux, Dr. Martine Loaec, Dr. Sylvia Colliec-Jouault, Dr. Lionel Chevolot, Dr. Philippe Zanchetta, Gerard Raguenes, Corinne Sinquin, Jacqueline Ratiskol, Patricia Pignet, and Olivier Roger. The author also thanks the Britanny province for financial support.

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