Carbohydrate Polymers 24 (1994) 287–294
© 1994 Elsevier Science Limited
Printed in Great Britain. All rights reserved
0144-8617/94/$07.00

Preliminary chemical characterization of unusual eubacterial exopolysaccharides of deep-sea origin

J.G. Guezennec, P. Pignet, G. Raguenes,
IFREMER, Centre de Brest, Dept. DRO/EP/LMBH, BP 70, 29280 Plouzané, France

E. Deslandes,
Laboratoire d'Ecophysiologie et Biochimie des Algues Marines, Université de Brest, Brest, France

Y. Lijour & E. Gentric
Université Bretagne Occidentale, Lab. Spectroscopie Moléculaire, 29200 Brest, France

(Received 17 December 1993; revised version received 27 April 1994; accepted 14 May 1994)

Deep-sea hydrothermal vents are characterized by specific physical and chemical parameters including high pressure, high temperature gradients and high concentrations of toxic elements. Bacteria recovered from hydrothermal vents were studied for their capability to produce polysaccharides in normal conditions. A first screening performed on mesophilic aerobic bacterial strains led to the discovery of unusual polymers in terms of chemical composition and rheological properties. Neutral polysaccharides were found, along with uronic-rich exopolymers. In addition, a new sugar was identified in two polymers. Additional studies identified this compound as a hexuronic acid substituted with an ether-linked lactic acid. The sulfate content as estimated by FTIR indicated concentrations ranging from 2 to 21%. These high sulfate concentrations are uncommon in microbial polysaccharides and may lead to new applications in the pharmaceutical area. Uronic-rich exopolymers could be expected to have some heavy metal binding capability and applications in the fields of biodetoxification and wastewater treatment.

INTRODUCTION

The economic potential of marine polysaccharides has been well documented (Curtin, 1985). Marine polysaccharides are increasingly being used in coatings, adhesives, food substrates, pharmaceuticals and biotechnological separations. Most of the polysaccharides from the marine environment are obtained from seaweeds (agar, alginates and carrageenans).

Microbial polysaccharides represent only a small fraction of the current polymer market, but undoubtedly have one of the largest technical potentials for development of novel and improved products. The remarkable properties and commercial success of xanthan gum have stimulated work in many laboratories seeking improved methods of production and other microbial exopolysaccharides. Gellan, dextran, pullulan, steroglucan and alginate are among the best known of microbial exopolysaccharides of industrial interest.

Deep-sea hydrothermal vents are characterized by high pressures, high temperature gradients and high concentrations of toxic elements as sulfides and heavy metals. These extreme conditions raise interesting questions about the survival and growth of microorganisms in these particular environments. Regarding the nature of this environment the presence of unusual microorganisms of biotechnological interest could be expected in terms of thermostable enzymes, hydrocarbon-degrading bacteria, and polysaccharide and polyhydroxyalkanoate producing bacteria.

The general characteristics of polysaccharide function and chemistry have been reviewed (Sutherland & Elwood, 1979; Sutherland, 1985). Uronic acids are the principal charged components of polysaccharides. These compounds, along with hexosamines, ester-linked substituents as sulfates and pyruvate ketals, confer properties of charge to a polymer, which would not be present in a neutral polysaccharide. The degree of pyruvation of xanthan is reported to influence physical properties (Cadmus *et al.*, 1976). It has been suggested that the acetate groups on the mannuronic acid residues of alginic acid control the epimerization which produces the guluronic residues. As guluronic acid is thought to be responsible for gelling, the presence of an acetate group may influence the physical properties of these polymers. The extent of pyruvylation and acetylation of xanthan is thought to influence a number of its solution properties including its filterability (Wernau, 1978), its thermally induced conformational transition (Smith *et al.*, 1978) and its viscosity (Sandford *et al.*, 1977, 1978). Functional groups such as acetyl (indirectly) and pyruvate groups (directly) are associated with metal-binding function (Ford *et al.*, 1990).

The aim of this study was to determine the chemical characterization of exopolysaccharides produced by deep-sea hydrothermal microorganisms. A first selection of polysaccharide-producing bacteria was conducted on mesophilic, aerobic strains isolated from the deep-sea environment. The exopolysaccharides were analyzed for their carbohydrate, proteins and sulfate contents.

MATERIALS AND METHODS

Polymer production

Polysaccharide-producing bacteria were selected on the basis of the appearance of mucoid colonies when grown on solid agar medium supplemented with glucose. Lactose, fructose, sucrose and maltose were sometimes used as substitutes for glucose. Once the polysaccharide producers were identified, sufficient polymers were obtained from cultures in a 2 liter fermentor, using appropriate growth conditions. Exopolysaccharides were isolated from the culture medium after 1 or 2 days. Bacterial cells were removed from the medium by high speed centrifugation, and the polymer was precipitated from the supernatant with pure ethanol. After sequential washes with mixtures of ethanol and water, the resulting polysaccharides were desiccated under nitrogen and stored at room temperature. Detailed procedures related to growth conditions and extraction/purification procedures are reported elsewhere (Talmont *et al.*, 1991; Vincent, 1993).

Chemical analysis

The total neutral carbohydrate and hexuronic acid contents were determined using the orcinol–sulfufic method (Tilmans & Philippi, 1929; Rimington, 1931) and the *meta*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), respectively. Hexosamines were analyzed for using the Elson–Morgan method (Elson & Morgan, 1983). The molar ratio of monosaccharides were determined according to Kamerling *et al.* (1975) and Montreuil *et al.* (1986). The monosaccharides were analyzed after either aqueous hydrolysis or acidic methanolysis of the polymers and subsequent GC analysis as peracetylated derivatives or trimethylsilyl derivatives, respectively. Protein was determined by the method of Lowry *et al.* (1953) with bovine serum albumin as standard.

Infrared spectroscopy

Pellets for infrared analysis were obtained by carefully grinding a mixture of 2 mg of polysaccharide with 200 mg of dry KBr and then pressing in a 16 mm diameter mold. The pellet technique was used because most bacterial polymers were water insoluble and films could not be achieved. Fourier transform infrared (FT-IR) spectra were recorded on a BOMEM MB100 instrument with a resolution of 4 cm^{-1}. Spectra were obtained in the 4000–400 cm^{-1} region.

RESULTS

The gross chemical composition of the nonpurified exopolysaccharides is listed in Table 1. Each value is a mean of three determinations.

Bacterial exopolysaccharides are characterized by a great diversity in terms of neutral, acidic or even amino sugars. Neutral sugars as determined by the orcinol method using the mannose/galactose (1:1) ratio as standard range from 38% (ST 1644) to 66% (ST 1574), while acidic sugars greatly fluctuated from 5% (ST 342) to 40% (ST 1666) of the total sugars. Hexosamines as analyzed for by the Elson–Morgan method were present in very low concentrations. Protein concentrations ranged from 2% to 18%; ST 1666 (2%) and ST 349 (18%).

GC analyses of monosaccharides, following either acidic methanolysis or aqueous hydrolysis, are shown in Figs 1 and 2. The monosaccharide ratios of bacterial exopolysaccharides are listed in Table 2. Glucose, galactose, rhamnose and mannose predominated as neutral sugars, with concentrations ranging from 0 to 29%. It can be observed from this table that mannose predominates for the first eight polysaccharides with concentrations ranging from 8·2 to 17·8%. Rhamnose was also found in the range of 6·7–9·4% of the total sugars for these polysaccharides. Fucose has not been found in any polysaccharides, with the exception of PS 657, PS 708 and PS 722, where concentrations below 2% were determined.

Table 1. Chemical analysis of bacterial exopolysaccharides. For analytical methods see text (Materials and Methods)

	Neutral sugars standard: 1 mann/1 gal (%)	acidic sugars standard: glucuronic acid (%)	Amino-sugars standard: glucosamine (%)	Proteins standard: alb. bovine (%)	Sulfates (%)
Group 1					
721	57	11	0·5	5	21·5
685	61	8	0·9	2	11·1
686	46	8	1·7	10	15·2
719	56	10	2·6	7	18·0
1574	66	9	0·6	3	ND
1579	52	8	3·5	11	13
1582	49	12	3·2	9	16·7
1584	52	10	3·7	8	14
Group 2					
708	49	8	1·0	12	< 10
722	49	8	3·2	8	13
Group 3					
349	42	7	1·7	18	17·2
342	50	5	1·2	16	8·9
Group 4					
1545	49	34	0·2	1	11·2
1666	46	40	1·6	6	9·7
1625	48	36	1·0	4	13·0
Group 5					
1644	38	32	1·0	9	10·1
657	47	26	1·6	10	5·2

ND: not determined.

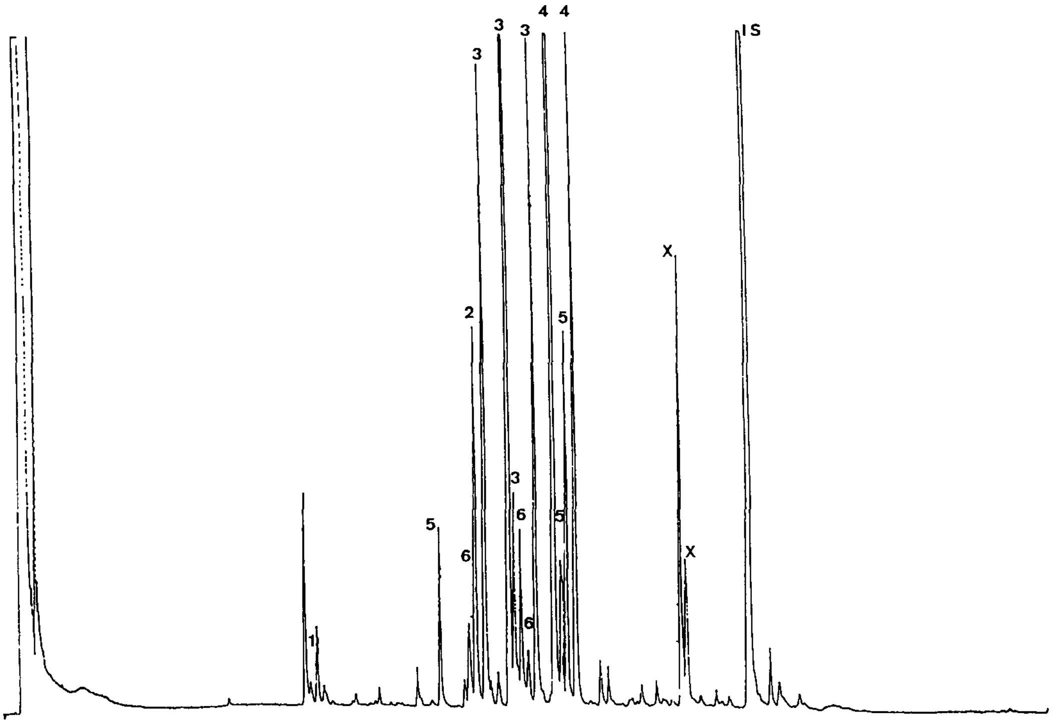

Fig. 1. GLC analysis of monosaccharides from bacterial origin following methanolysis and TMS derivatization. Internal standard (IS): *meso*-inositol; 1: rhamnose; 2: mannose; 3: galactose; 4: glucose; 5: glucuronic acid; 6: galacturonic acid, X: unidentified hexuronic acid.

Glucuronic and galacturonic acids were the only acidic sugars found in these exopolymers with the first acid predominating. No mannuronic acid or guluronic acid were found in any of the exopolysaccharides. In two polymers, GC analysis showed an unidentified peak referenced as 'X' in Table 2. Further studies demonstrated (Dubreucq *et al.*, 1994) that the compound 'X' could be expected to be a diacidic

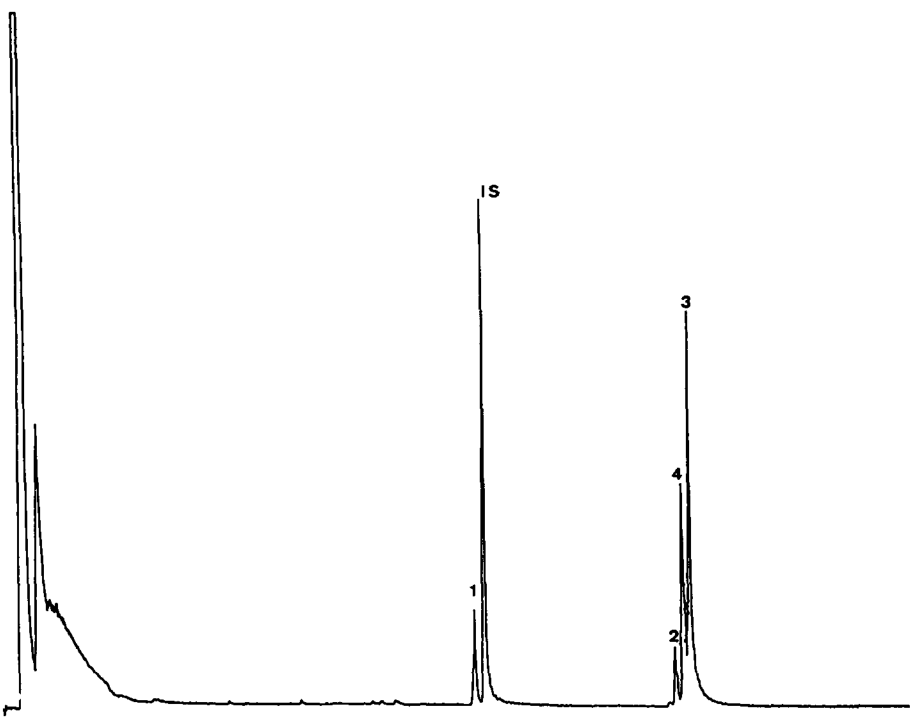

Fig. 2. GLC analysis of monosaccharides from bacterial polymers as alditol acetate derivatives. Internal standard (IS): xylose; 1: rhamnose; 2: mannose; 3: galactose; 4: glucose.

Table 2. Monosaccharide ratios in bacterial exopolysaccharides (see Materials and Methods)

	Rhamnose (%)	Fucose (%)	Mannose (%)	Galactose (%)	Glucose (%)	GAPL (%)	Glucuronic acid (%)	Galacturonic acid (%)	X (%)	*N*-acetylglucosamine (%)	*N*-acetylgalactosamine (%)
721	9·4		17·8	13·9	17·0		6·7				
685	7·8		16·0	14·0	15·4		7·7				
686	9·0		15·7	8·6	11·3		4·3			0·3	0·3
719	8·2		16·8	12·2	13·2		5·8				
1574	6·0		11·3	10·8	11·7		6·3				
1579	7·0		8·2	6·0	6·9		4·2				
1582	9·0		13·7	7·7	10·7		4·3				0·6
1584	6·7		15·6	11·2	12·4		5·1				
708	3·5	1·2	9·6	3·2	14·9		2·2				
722	3·2	1·2	7·3	2·8	17·0		1·7				1·4
349	0·6		0·4	4·9	26·0		0·5				
342	3·9		3·0	2·8	29·1		0·3				
1545	6·6		2·9	7·1	10·2	NQ	4·6	3·1		2·5	
1666	0·6		0·5	13·5	13·2	NQ	7·9	4·9			
1625	0·9		0·6	10·3	14·4	NQ	7·3	4·5			
1644	0·9		1·6	12·4	9·1		4·9	2·4	NQ		
657	3·4	1·7	3·7	17·2	8·9		2·9	3·9	NQ		

GALP: 4,6-(1-carboxy-ethylidene)-galactopyranose.
X: incompletely characterized sugar.
NQ: not quantified.

sugar. Finally, *N*-acetylglucosamine and *N*-acetylgalactosamine were determined as amino sugars in most polysaccharides.

Infrared spectroscopy was performed on all polysaccharides. Figure 3 shows an FTIR spectrum of uronic acid-rich polysaccharide. All the exopolysaccharides considered in this study were characterized by a large band above 3000 cm^{-1} and intense absorbances around 1650 and 1050 cm^{-1}. Absorbance bands were also observed at lower frequencies below 950 cm^{-1}. Sulfate contents as determined both by ionic chromatography (Okutani, 1992) or FTIR spectroscopy (Lijour *et al.*, 1993) showed concentrations ranging from 4 to 21% (Table 1).

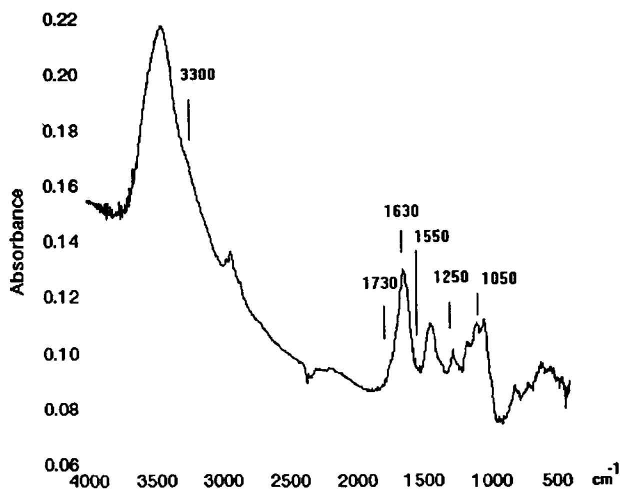

Fig. 3. FTIR spectrum of exopolysaccharide ST 1545.

DISCUSSION

Unusual bacteria associated with deep-sea hydrothermal conditions can be expected to be a potential source of biotechnologically important microorganisms. Several bacterial strains of deep-sea origin have been isolated and have demonstrated their ability to produce extracellular polymers in an aerobic carbohydrate-based medium. Easy growth conditions and subsequent polymer production can make these bacterial strains of great industrial interest.

From Table 1 it can be noted that five of the exopolysaccharides produced by deep-sea hydrothermal bacteria are characterized by their uronic acid contents. Exopolymers from marine bacteria firmly attached to surfaces exposed in seawater have been shown to contain high quantities of galacturonic acid (Fazio *et al.*, 1982). However, with the exception of bacterial alginates and gellan with glucuronic acid contents up to 21%, bacterial exopolysaccharides of industrial interest do not contain large amounts of uronic acids. These uronic acids, along with nonsugar substituents, conferred a net negative charge to the polymers. Uronic acid containing polymers are important and may contribute to adhesion of bacteria to marine sediments. They can also play an important role in the binding capacities of the polymers. Different metal adsorption sites appear to exist on both neutral polysaccharides and anionic polysaccharides. Neutral polysaccharides may bind cations at the hydroxyl group of hexoses, exchanging with the hydrogen ions from water bound by the polymer. Where polymers are anionic polysaccharides, carboxylate groups, along with amino groups, may be the metal binding sites (Mittelman & Geesey, 1985; Geesey & Jang, 1989).

Proteins have been found in large amounts (up to 18%) for some exopolysaccharides. Additional purifications did not remove a significant part of the protein content of the protein-rich polysaccharides. These high protein concentrations are probably responsible for the unfilterability of these polymers. Such protein contents may also sugest a polymer partially linked to the cell wall and detached from the cell due to agitation.

Infrared spectra of carbohydrates and polysaccharides have been described for several years. Band absorbances have been assigned to the different functional groups of the skeleton, i.e. ether, carboxylic, carboxylate and sulfate groups (Morikawa *et al.*, 1974). A more recent work (Sekkal, 1990) reported a comprehensive study of carrageenans by both IR spectroscopy and molecular modelling.

Very few differences can be detected between the polysaccharides analyzed in this study. Significant differences between the exopolysaccharides were observed in the 1750–1650 cm^{-1} region, in the 1200–1250 cm^{-1} region and also at lower frequencies below 900 cm^{-1}. A shoulder at the high frequency side of the 1630 cm^{-1} band could be assigned to the presence of carboxylic groups, which absorb at 1730 cm^{-1} in acids. Upon acid hydrolysis, the 1610 cm^{-1} band decreased while the 1730 cm^{-1} band increased, indicating the conversion from the ionic form towards the acid form.

The band near 1550 cm^{-1} was assigned to the amide II band resulting from the coupling between the NH$_2$ deformation mode and CN stretching vibration. The band centered at 1250 cm^{-1} consisted of two distinct bands, one at 1260 cm^{-1} and the second one at 1230 cm^{-1} (Lijour *et al.*, 1993). These bands have been assigned to the antisymmetric O—S—O stretching vibrations.

Many studies have related the structure of carrageenans to a set of IR bands between 1000 and 800 cm^{-1}. Some represent pseudosymmetric C—O—S stretching vibrations and give structural information on the location of sulfate groups on the saccharide units (Rochas *et al.*, 1986). However, bacterial polysaccharides did not always exhibit well-defined bands in this region, probably because of their more intricate structures (Talmont, 1990; Talmont *et al.*, 1991). When present, they superimposed on a very wide band centered at 600 cm^{-1}, representing the intermolecular vibration mode of adsorbed water.

Galactose, glucose, rhamnose and mannose, together with galacturonic and glucuronic acids, have been found in all the polysaccharides we considered in this study. These neutral hexoses and hexuronic sugars are quite common to all microbial exopolysaccharides (Pace & Righelato, 1980). Methyl pentoses such as L-fucose and L-rhamnose are also present in microbial exopolysaccharides while pentoses are comparatively rare. In the limited number of bacterial polymers of industrial interest that have been characterized, the most common sugar residues are the hexoses with *O*-acetyl or pyruvate ketal groups as common substituents (Sutherland, 1985). Thus, xanthan consists of a cellulosic backbone of α-(1–4) linked β-D-glucan substituted at position 3 on alternate glucose residues with a charged trisaccharide chain. Both acetylated and pyruvylated mannose and glucose form this trisaccharide chain. Gellan is a linear anionic polymer composed of tetrasaccharide repeat units of β-(1–3)-D-glucose, β-(1–4)-D-glucuronic acid, β-(1–4)-D-glucose and α-(1–4)-L-rhamnose. Dextrans produced by several lactic acid bacteria are composed almost entirely of α-(1–6)-linked glucosyl residues. More than 80 types of *Klebsiella* have been found to produce a great variety of exopolysaccharides in terms of neutral, uronic acids and nonsugar substituents (Sutherland, 1970).

Mannose has been found in high concentrations in a certain number of polysaccharides. Mannose is present in xanthan and in some *Klebsiella* exopolysaccharides. An archaebacterium, *Haloferax mediterranei*, has been found in equal amounts with glucose in a halotolerant, thermotolerant polymer-producing bacterium (Pfinner *et al.*, 1986). Rhamnose is present in gellan (O'Neill *et al.*, 1983), polysaccharides (from lactic bacteria) (Cerning *et al.*, 1986) and *Klebsiella* exopolysaccharides (Curvall *et al.*, 1975).

Glucuronic acid and galacturonic acid were the only hexuronic acids found in microbial exopolysaccharides of deep-sea origin considered in this study. No mannuronic acid or guluronic acid were determined even in uronic-acid rich polymers. As was noted above, an unknown peak was found by GC following hydrolysis. Further studies identified this peak referenced 'X' as a furanosyl derivative substituted with two carboxylic functions. The nature of the hexose has not yet been determined. However, one of the acidic substituents has been demonstrated to be an ether-linked lactic acid (Dubrecq *et al.*, 1993). Ether-linked lactic acid residues are rare in microbial exopolysaccharides, but have been found as a substituent of D-glucuronic acid in the *Klebbsiella* type 37 capsular polysaccharide (Lindberg *et al.*, 1976) and joined to D-glucose in the extracellular polysacharide from *Aerococcus viridans* var. *homari* (Kenne *et al.*, 1976). To our best knowledge this sugar appears to be unknown for microbial or algal exopolysaccharides. The fact that only two exopolysaccharides were found having this sugar could be interesting in terms of new physical and rheological properties.

With the analytical properties followed, the pyruvate content of the exopolysaccharides was not accurately determined. However, GC showed, in some polysaccharides, pyruvate associated with galactose. Pyruvates as acetate and succinate are the most common substituents of exopolysaccharides. They play an important role in both the gelling process and in general terms influence the physical and rheological properties of the polymers. They can also contribute to the metal binding capacity of these polysaccharides (Ford *et al.*, 1990).

A considerable part of the polymer composition was not accentuated for by the analytical procedures followed. However, this is not unusual and happens with other bacterial exopolysaccharides. This could be due to a failure in the analytical methods used to determine the correct amounts of components or to the existence of unusual compounds undetectable by the methods used. Owing to the structural complexity of exopolysaccharides, with a variety of monomeric units and linkages having different stabilities towards acid, a single hydrolysis procedure will often not be sufficient to cleave every linkage quantitatively. Some exopolysaccharides are very resistant to acid hydrolysis and the various stabilities of the liberated monosaccharides can require different conditions of hydrolysis (Nevins *et al.*, 1967; Selvendran *et al.*, 1979). Recently, a combined methanolysis and enzyme prehydrolysis was demonstrated to be efficient in the simultaneous determination of acidic and neutral sugars of plant cell wall materials (Quemener *et al.*, 1993). Thus, by using different conditions for both hydrolysis and methanolysis, it is possible to discriminate between the different types of linkages present in the polysaccharides.

The sulfate content of the exopolysaccharides was determined using FTIR or ion chromatography. Based on specific absorbances, the sulfate content of the polysaccharide can be estimated (Lijour *et al.*, 1993). Absorption bands centered at 1250 cm^{-1} are associated with sulfate, and the ratio Abs (1250 cm^{-1})/(1050 cm^{-1}) is correlated with the sulfate content of the polymer. Considering this ratio, sulfate contents were estimated to range from 1 to 21% (Table 1).

Sulfate is present in polysaccharides of marine origin. Carrageenans contain sulfate in concentrations up to 30%. With the exception of cell wall polysaccharides, polymers of bacterial origin are rarely highly sulfated. Marine sulfated polysaccharides offer fascinating applications for the pharmaceutical industry. The degree of sulfation of the polymers has been demonstrated to play an important role in their biological activities. Antiviral efects of polysaccharides including human immunodeficiency virus activity have been recognized for many years (Gonzales *et al.*, 1987). It is also well known that these activities are related to the high sulfate content of these polysaccharides. Recently, experiments were performed to increase the sulfate content of some polysaccharides with the aim of enhancing their biological activities (Nishino & Hagumo, 1992). The anticoagulant activity of these polymers can also be affected by a high content of sulfate in specific polysaccharides. The sulfate groups in heparin play a critical role in its antithrombotic activity (Baba *et al.*, 1988; Petitou *et al.*, 1983).

The sulfate content can also play an important role in the binding capacities of heavy metals with the polymers. In carrageenans, the degree of sulfation was correlated with the metal binding capacity of the polymers (Veroy *et al.*, 1980). Thus, high sulfate containing polymers, along with high uronic acid and amino sugar containing polysaccharides, are of great interest regarding their potential applications in the therapy for metal poisoning and the chelation of heavy metals in wastewater.

Classification of the exopolysaccharides

Regarding the sole chemical composition, exopolysaccharides from deep-sea hydrothermal vents can be divided into several groups (Table 1).

The first group consisted of exopolysaccharides with neutral sugars such as galactose, rhamnose, glucose and mannose predominating, while uronic acids were in the region of 10%. This first group include ST 721, ST 685, ST 686, ST 719, ST 1574, ST 1579, ST 1582 and ST 1584. The second group (ST 708 and ST 722) is characterized by a low content of uronic acids (7–8%) and the presence of fucose along with a high concentration of glucose. These polymers are also characterized by high amounts of proteins. The third group (ST 349 and ST 342) is similar to the second one with the exception of the absence of fucose and a very low uronic acid content (as low as 5%). The fourth group consisted of anionic polysaccharides with high proportions of both galacturonic and glucuronic acids. Pyruvate was also present in these polymers and found to be associated with galactose. Finally, the fifth group was characterized by high concentrations of uronic acids. However, no pyruvate was found in this last group. This last group included the two polysaccharides identified as containing the unusual sugar. The five groups thus classified in terms of chemical compositions have been demonstrated to correlate with a complete physiological description of the strains concerned (Vincent, 1993). Regarding their chemical composition, along with additional interesting rheological properties, most of the polysaccharides included in these groups have been already patented.

CONCLUSION

Undoubtedly, deep-sea microorganisms are interesting in terms of polysaccharide producers. A first screening performed on bacteria recovered from deep-sea hydrothermal vents during different oceanographic cruises led to the discovery of unusual polysaccharides with interesting chemical and rheological properties. These polysaccharides are diverse in terms of monosaccharide ratios ranging from neutral polysaccharides to uronic-rich polymers. Furthermore, one unknown peak was identified following GC analysis. This sugar was found to be a furanic-type hexuronic acid with an ether-linked lactic acid residue. Studies to determine the nature of the hexose are now in progress.

In addition to an unusually high uronic acid content, some of these exopolymers contained significant amounts of sulfate. Applications for these polysaccharides could be expected in various biotechnological fields. High sulfate content can confer biological activities on these exopolymers and provide applications for the pharmaceutical area. Large amounts of uronic acids present in some of these polysaccharides can be useful in the fields of biodetoxification and water treatment. However, more structural and rheological studies of these polymers are required before any industrial applications can be realized.

REFERENCES

Anton, J., Meseguer, I. & Rodriguez-Valera, F. (1988). Production of an extracellular polysaccharide by *Haloferax mediterranei*. *Appl. Environ. Microbiol.*, **54**(10), 2381–6.

Baba, M., Pauwels, R., Balzanini, J., Arnout, J., Desmyter, J. & De Clerq, E. (1988). Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. *Proc. Natl Acad. Sci.*, **85**, 6132–6.

Blumenkrantz, N. & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Anal. Biochem.*, **54**, 484–9.

Cadmus, M.C., Rogovin, S.P., Burton, K.A., Pittsley, J.E., Knutson, C.A. & Jeanes, A. (1976). Colonial variation in *Xanthomonas campestris* NRRL B1459 and characterization of the polysaccharide from a variant strain. *Can. J. Microbiol.*, **22**, 942–8.

Cerning, J., Bouillane, C., Desmazeaud, M.J. & Landon, M. (1986). Isolation and characterization of exocellular polysaccharide produced by *Lactobacillus bulgaricus*. *Biotechnol. Lett.* **8**(9), 625–31.

Curtin, M.E. (1985). Chemicals from the sea. *Bio/Technology*, **3**, 34–7

Curvall, M., Lindberg, B., Lönngren, J & Nimmich, W. (1975). Structural studies of the capsular polysaccharide of *Klebsiella* type 81. *Carbohydr. Res.*, **42**, 73–82.

Dubreucq, G., Fontaine, T., Leroy, Y. & Fournet, B. (1994). Characterization of a new uronic acid from exopolysaccharide produced by bacteria from deep-sea hydrothermal vents. *Carbohydr. Lett.* (submitted).

Elson, L.A. & Morgan, W.T.J (1983). Colorimetric method for the determination of glucosamine and chondrosamine. *Biochem. J.*, **27**, 1824–8.

Fazio, S.A., Uhlinger, D.J., Parker, J.H. & White, D.C. (1982). Estimations of uronic acids as quantitative measures of extracellular polysaccharides and cell wall polymers from environmental samples. *Appl. Environ. Microbiol.*, **43**, 1151–9.

Ford, T., Black, J.P. & Mitchell, R (1990). Relationship between exopolymers and corroding metal surfaces. In *Corrosion/90*, National Association of Corrosion Engineers, Houston, TX, USA.

Geesey, G. & Jang, K.L. (1989). Interactions between metal ions and capsular polymers. In *Metal Ions and Bacteria*, eds T. Beveridge & R. Doyle. John Wiley, New York, Chap. 11, pp. 325–57.

Gonzales, M.E., Alarcon, B. & Carrasco, L. (1987). Polysaccharides as antiviral agents: Antiviral activity of carrageenan. *Antimicrob. Agents Chemother.*, **31**, 1388–93.

Kammerling, J.P., Gerwig, G.J., Vliegenhart, J.F.G. & Clamp, J.R. (1975). Characterization by gas chromatography–mass spectrometry of pertrimethylsilyl glycosides obtained in the methanolysis of glycoproteins and glycolipids. *Biochem. J.*, **151**, 491–5.

Kenne, L., Lindberg, B., Lindquist, B., Lönngren, J., Arie, B., Brown, R.G. & Stewart, R.E. (1976). 4-*O*-[(*S*)-1-carboxyethyl]-D-glucose: a component of the extracellular polysaccharide material from *Aerococcus viridans* var. *homari*. *Carbohydr. Res.*, **51**, 287–90.

Lijour, Y., Gentric, E., Deslandes, E. & Guezennec, J.G. (1993). Estimation of the sulfate content of hydrothermal vent bacterial polysaccharide by Fourier transform infrared spectroscopy. *Analy. Biochem.*, **220**, 244–8.

Lindberg, B., Lindquist, B., Lönngren, J. & Nimmich, W. (1976). 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid: a component of the *Klebsiella* Type 37 capsular polysaccharide. *Carbohydr. Res.*, **49**, 411–7.

Lowry, O.H., Rosenbrough, N.J., Farr, A. & Randall, R.J. (1953). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

Mittelman, M.W. & Geesey, G.G. (1985). Copper-binding characteristics of exopolymers from a freshwater sediment bacterium. *Appl. Environ. Microbiol.*, **49**, 846–51.

Montreuil, J., Bouquelet, S., Debray, H., Fournet, B., Spik, G. & Strecker, G. (1986). Glycoproteins. In *Carbohydrate Analysis: A Practical Approach*, eds M.F. Chaplin & J.K. Kennedy. IRL Press Oxford, Washington, DC, pp. 143–204.

Morikawa, H., Tanizawa, K. & Senda, M. (1974). Infrared spectra of *Nitella* cell walls and orientation of carboxylate ions in the walls. *Agric. Biol. Chem.*, **38**, 343–8.

Nevins, D.J., Albersheim, P. & English, P.D. (1967). The specific nature of the cell wall polysaccharide. *Plant Phytol.*, **229**, 355–62.

Nishino, T. & Hagumo, T. (1992). Anticoagulant and antithrombin activities of oversulfated fucans. *Carbohydr. Res.*, **229**, 255–62.

Okutani, J. (1992). Antiviral activities of sulfated derivatives of a fucosamine-containing polysaccharide of marine bacterial origin. *Nippon Suisan Gakkaishi*, **58**(5), 927–30.

O'Neill, M.A., Selvendran, R.R. & Morris, V.J. (1983). Structure of the extracellular gelling polysaccharide produced by *Pseudomonas eleoda*. *Carbohydr. Res.*, **124**, 123–7.

Pace, G.W. & Righelato, R.C. (1980). Production of extracellular microbial polysaccharides. *Ind. Adr. Biochem. Eng.*, **15**, 41–70.

Petitou, M., Jacquinet, J.C., Duchaussoy, P., Lederman, I., Choay, J. & Sinaÿ, P. (1983). In *Glycoconjugates, Proc. 7th Intl Symp. on Glycoconjugates*, pp. 379–82.

Pfinner, S.M., MacInerney, M.J., Jenneman, G.G. & Knapp, R.M. (1986). Isolation of halotolerant, thermotolerant, facultative polymer-producing bacteria and characterization of exopolymers. *Appl. Environ. Microbiol.*, **51**, 1224–9.

Quemener, B., Lahaye, M. & Thibault, J.F. (1993). Studies of the simultaneous determination of acidic and neutral sugars of plant cell wall materials by HPLC of their methyl glycosides after combined methanolysis and enzymic prehydrolysis. *Carbohydr. Polym.*, **20**, 81–94.

Rimington, C. (1931). The carbohydrate complex of serum protein II: Improved method for isolation and redetermination of structure. Isolation of glucosaminodimannose from protein of glood. *Biochem. J.*, **25**, 1062–71.

Rochas, C., Lahaye, M. & Yaphe, W. (1986). Sulfate content of carrageenan and agar determined by infrared spectroscopy. *Botanica Marina*, **XXIX**, 335–40.

Sandford, P.A., Pittsley, J.E., Knutson, C.A., Watson, P.R., Cadmus, M.C. & Jeanes, A. (1977). Variation in *Xanthomonas campestris* NRRL B-1459: characterization of xanthan products of different pyruvic acid content. In *Extracellular Microbial Polysaccharides*, eds P.A. Sandford & A. Laskin. American Chemical Society, Washington, DC, pp. 192–210.

Sandford, I.H., Watson, P.R. & Knutson, C.A. (1978). Separation of xanthan gums of differing pyruvate content by fractional precipitation with alcohol. *Carbohydr. Res.*, **63**, 253–6.

Sekkal, M. (1990). Utilisation conjointe de la spectrométrie IRTF et de la modélisation moleculaire à l'étude structurale des carraghenanes. PhD Thesis. University of Lille Flandres Artois, 170 pp.

Selvendran, R.R., March, J.F. & Ring, S.G. (1979). Determination of aldoses and uronic acid content of vegetable fiber. *Anal. Biochem.*, **96**, 282–92.

Smith, I.H., Syme, K.C., Lawson, C.J. & Morris, E.R. (1981). The influence of the pyruvate content of xanthan on macromolecular association in solution. *Int. J. Biol. Macromol.*, **3**, 129–34.

Sutherland, I.W. (1970). Structure of *Klebsiella aerogenes* Type 8 polysaccharide. *Biochemistry*, **9**, 2180–5.

Sutherland, I.W. (1985). Biosynthesis and composition of Gram-negative bacterial exocellular and wall polysacharides. *Ann. Rev. Microbiol.*, **39**, 243–70.

Sutherland, I.W. & Elwood, D.C. (1979). Microbial exopolysaccharides—industrial polymers of current and future potential. In *Microbial Technology—Current State and Future Projects*, eds A.T. Bull, D.C. Elwood & C. Ratledge. Cambridge University Press, Cambridge, pp. 107–50.

Talmont, F. (1990). Contribution à l'étude du milieu hydrothermal profond: 1: Etude de tubes d'annélides et de vestimentifères. 2: Etude structurale d'un polysaccharide secrété par une bactérie marine. PhD Thesis. University of Lille Flandres Artois, 201 pp.

Talmont, F., Vincent, P., Fontaine, T., Guezzenec, J., Prieur, D., Aymard, P., Cuvelier, G., Launay, B. & Fournet, B. (1991). Structural investigation of an acidic polysaccharide from a deep-sea hydrothermal vent marine bacterium. *Food. Hydrocoll.*, **5**(1/2), 171–2.

Tilmans, J. & Philippi, J (1929). Uber den gehalt der wichtigsten protein der nahrangsmittel an kohlehydrat and über ein kolorimetrisches verfahren zur quantitativen beshimmung von stuckstoffreiem zucker in elweiss. *Biochem. Z.*, **215**, 36–60.

Veroy, R.L., Montano, N., B de Guzman, M.L., Laserna, E.C. & Cajipe, C.J.B. (1980). Studies of the binding of heavy metals to algal polysaccharides from Philippine seaweeds. I. Carrageenan and the binding of lead and cadmium. *Botanica Marina*, **XXIII**, 59–62.

Vincent, P. (1993). Etude d'eubactéries productrices d'exopolysaccharides, originaires d'un site hydrothermal profond (13°N). PhD Thesis. University of Brest, 336 pp.

Wernau, W.C. (1978). *Br. Pat.* 2 008 600A.