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Carbohydrate Polymers 42 (2000) 375–383

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

A cellulosic exopolysaccharide produced from sugarcane molasses by a *Zoogloea* sp.

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Received 1 February 1999; received in revised form 28 March 1999; accepted 20 September 1999

Abstract

An extracellular polysaccharide producing bacterium *Zoogloea* sp. was isolated from an agro-industrial environment in the north-eastern region of Brazil. The extracellular polysaccharide produced from sugarcane molasses was hydrolysed with trifluoroacetic acid (mild and strong conditions) giving 88% of soluble material. The main monosaccharides present in the soluble fraction were glucose (87.6%), xylose (8.6%), mannose (0.8%), ribose (1.7%), galactose (0.1%), arabinose (0.4%) and glucuronic acid (0.8%). Methylation analysis of the polysaccharide showed mainly 2,3,6-tri-*O*-methylhexitol (74.7%) and 2,3,-di-*O*-methylhexitol (17.7%). Enzyme hydrolysis of the polysaccharide with a cellulase confirmed the presence of (1 → 4)-β-D-glucopyranosyl residues.

Keywords: Exopolysaccharide; *Zoogloea* sp; (1 → 4)-β-D-Glucopyranosyl residues

1. Introduction

Many microorganisms have the ability to synthesise extracellular polysaccharides and excrete them out of the cell either as soluble or insoluble polymers (Sutherland, 1990). In recent years, there has been a growing interest in the isolation and identification of new microbial polysaccharides that might have novel uses.

Cellulose is produced by *Acetobacter xylinum* and possibly by a range of other, mainly Gram-negative, bacterial species (Masaoka, Ohe & Sakota, 1993; Sutherland, 1990). In these bacteria, cellulose is an exopolysaccharide that is excreted into the medium, where it rapidly aggregates as microfibrils. Bacterial cellulose has long been used in foods in Asian countries (Okiyama, Shirae, Kano & Yamanaka, 1992a; Okiyama, Motoki & Yamanaka, 1992b) and its different properties from wood-derived cellulose opens new industrial applications. Bacterial cellulose possesses high crystallinity, high degree of polymerization, high tensile strength and tear resistance, and high hydrophilicity that distinguish it from other forms of cellulose (Yamanaka & Watanabe, 1994). Various potential industrial applications for bacterial cellulose include acoustic diaphragms, artificial skin or wound healing, filter membranes, ultra strength paper and paper additives (Takai, 1994; Yamanaka & Watanabe, 1994; Yamanaka et al., 1989). In food processing, suspensions of disintegrated bacterial cellulose have been found useful as thickening and binding agents and as a dietary fibre supplement (Yamanaka & Watanabe, 1994).

Floc-forming bacteria play an important role in the purification of activated sludge or water with a high content of organic material. In purification process, the exopolysaccharide synthesised by *Zoogloea ramigera* and related species play important roles. Some of these polysaccharides are claimed to have an unusually high affinity for metallic ions and for amino acids (Geddie & Sutherland, 1993; Sutherland, 1990).

Zoogloea ramigera produces zooglan, a polysaccharide composed of D-glucose, D-galactose and pyruvic acid in an approximate ratio of 11:3:1.5 (Ikeda, Shuto, Saito, Fukui & Tomita, 1982). It is a long chain polysaccharide consisting of mainly 1,4-β-linked glucose residues and 1,4-β- and 1,3-β-linked galactose residues with branches of glucose residues at the C-3 or C-6 positions of the galactose residues. The pyruvic acid residues, the acidic component, are linked to the non-reducing end and/or 1,3-linked glucose residues through 4,6-ketal linkages (Ikeda et al., 1982). It has also been shown that the polysaccharide contains *O*-acetyl and *O*-succinyl groups, in addition to glucose, galactose and pyruvate (Troyana, Lee, Rha & Sinskey 1996). The structure of zooglan may vary with fermentation conditions (Lee, Yeomans, Allen, Gross & Kaplan, 1997). The polysaccharide

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PII: S0144-8617(99)00179-4

has an irregular structure with a composition that varies due to slight differences in the growth conditions of the organism.

Here, we present recent studies on the composition and structure of an exopolysaccharide produced from sugarcane molasses by a *Zoogloea* sp.

2. Experimental

2.1. Materials

The following materials were obtained from sources indicated: sugarcane molasses (Estaçâo Experimental de Cana-de-Açucar, Brazil); yeast extract, peptone and agar (Merck, USA); Celluclast 1.5 L (cellulase) (Novo Nordisk, Denmark); cellulose powder (Sigma, UK); monosaccharide standards (myo-inositol, glucose, mannose, arabinose, xylose, fucose, galactose, ribose and glucuronolactone) (Sigma, UK); trifluoroacetic acid (Janssen Chimica, UK); formic acid, diethylamine, 1-methyl imidazole, dimethyl sulphoxide and acetic anhydride (BDH, UK); barium carbonate (Sigma, UK); sulphur dioxide (Fluka, UK), methyl iodide (Aldrich, UK). Other chemicals of analytical grade were obtained commercially.

2.2. Microorganism

An extracellular polysaccharide producing bacterium *Zoogloea* sp. was isolated from an agro-industrial environment in the north-eastern region of Brazil. The identification of the microorganism was carried out at the Instituto de Antibióticos, Universidade Federal de Pernambuco, Brazil. The *Zoogloea* sp. was maintained as slant cultures at 4°C. The culture medium consisted of glucose (20.0 g/l), yeast extract (5.0 g/l), peptone (3.0 g/l), agar (15.0 g/l) in deionised water and the pH was adjusted to 6.8 before sterilisation (120°C, 20 min).

2.3. Production of the exopolysaccharide sample

For the exopolysaccharide production, cells were transferred from agar slants to 100 ml of sterilised medium (120°C, 40 min), consisting of sugarcane molasses (15°Brix, pH 5.0), in 250 ml Erlenmeyer flasks. Cultures were incubated at 30°C for seven days. The polysaccharide pellicles, a gel-like material, were formed at the air–liquid interface. The pellicles were washed in deionised water, sterilised (120°C, 40 min) and dried at 60°C on glass plates to form solid polysaccharide sheets.

2.4. Determination of the water soluble material of the polysaccharide sheet

The polysaccharide sheet (211.04 mg) was cut into small particles (ca. 4 mm^2) and transferred to a centrifuge tube. Deionised water (6 ml) was added, the material was mixed for 1 min using a vortex mixer, left to stand for 15 min, mixed again for a further 1 min and centrifuged at 8000*g* for 15 min. The supernatant was transferred to a round bottom flask. The residue was washed with deionised water (6 ml) and centrifuged at 8000 g for 15 min. This procedure was repeated twice. The supernatants were combined, lyophilised and dried using an Abderhalden dryer at 39°C. The residue was dried using an Abderhalden dryer and the dried weight of the residue determined.

2.5. Mild acid hydrolysis of the polysaccharide sample

The water insoluble residue (47.51 mg) was suspended in trifluoroacetic acid (2 M, 4 ml) in a round bottom flask, allowed to stand for 45 min and refluxed at 97°C for 3 h. The hydrolysate was left at ambient temperature for 20 h. Trifluoroacetic acid was removed by drying the sample using a rotor evaporator. The hydrolysate was washed with deionised water (2 ml) which was also removed by evaporation. This procedure was repeated six times. The residue was transferred to a centrifuge tube and deionised water (1.3 ml) was added. The sample was centrifuged at 8000*g* for 30 min. This procedure was repeated three times and the supernatants were combined and stored at −20°C prior to anion exchange high performance liquid chromatography analysis. The acid insoluble residue was dried using an Abderhalden dryer (39°C) and the dry weight determined.

2.6. Strong acid hydrolysis of the polysaccharide sample

Trifluoroacetic acid (99%, 3 ml) was added to the dried acid insoluble residue (33.5 mg) and allowed to stand at ambient temperature (16 h) to swell. The sample was refluxed for 2 h, at 97°C, diluted to 80% trifluoroacetic acid with deionised water and refluxed for 30 min. The sample was diluted again to 30% trifluoroacetic acid with deionised water and refluxed for a further 4 h. The trifluoroacetic acid was removed and the samples worked up as for the mild acid hydrolysates.

2.7. Monosaccharide analysis using anion exchange high performance liquid chromatography

The trifluoroacetic acid hydrolysates were neutralised with sodium hydroxide solution prior to the analysis. The analysis of monosaccharides of the hydrolysates was performed on a Dionex DX-500 system consisting of a GP40 gradient pump and a Dionex ED40 electrochemical detector. The standard sequence of potentials for carbohydrate detection (50 mV for 200 ms; 750 mV for 200 ms and −150 mV for 400 ms) was applied to the Au ED40 working electrode for pulsed amperometric detection. The columns used were a CarboPac PA1 analytical column (250 × 4.0 mm ID) and a CarboPac PA1 guard columns (50 × 4.0 mm ID). All eluents and reagents were prepared using water purified to 18.2 MΩ using a UHQPS, system (Elga). Eluents A, B and C were deionised water, sodium acetate (1 M) and sodium hydroxide (1 M), respectively.

Sodium hydroxide (300 mM) at 0.5 ml/min was added as a post column reagent. After equilibrating the system with deionised water for 30 min a sample (50 μl) was injected. The sodium acetate concentration was then increased, linearly, to 200 mM over the following 10 min and held at this level for 10 min. The columns were regenerated by flushing with sodium hydroxide (300 mM) for 20 min and then the system was equilibrated by flushing with deionised water for 20 min. Sugars were identified by comparison of the retention times with those of standards and relative amounts were calculated.

2.8. Oligosaccharide analysis using anion exchange high performance liquid chromatography—method 1

The analysis of the oligosaccharide component of the water soluble extract and the mild trifluoroacetic acid hydrolysate was performed on a Waters 625-LC system. It was fitted with a non-metallic flow path, a 464 pulsed amperometric detector (PAD), fitted with a gold working electrode and a base stable reference electrode, and a Whisp 712 injector. A single piston reciprocating pump with two metres of polymer tubing was used to add sodium hydroxide (300 mM), at a flow rate of 0.7 ml/min, to the eluent stream between the columns and detector. For the analysis of the oligosaccharides the PAD was operated in the cathodic mode with the following sequence of potentials: 50 mV for 200 ms; 800 mV for 200 ms; and −600 mV for 500 ms. The columns used were a CarboPac PA1 analytical column (250 × 4.0 mm ID) and a CarboPac PA1 guard column (50 × 4.0 mm ID). All eluents and reagents were prepared using water purified to 18.2 MΩ quality using a UHQPS system (Elga). Eluents A, B, C, and D were sodium hydroxide (100 mM), sodium hydroxide (100 mM) containing sodium acetate (800 mM), sodium hydroxide (300 mM), and sodium hydroxide (500 mM), respectively. Eluent C was used as the post column reagent. After equilibrating the system with sodium hydroxide (100 mM) a sample (200 μl) was injected. The sodium acetate concentration was then increased, linearly, to 800 mM over the following 60 min and held at this level for 5 min. The columns were regenerated by flushing with sodium hydroxide (500 mM) for 80 min and then the system was equilibrated by flushing with sodium hydroxide (100 mM) for 10 min before the next sample was injected. The flow rate was 0.7 ml/min throughout.

2.9. Oligosaccharide analysis using anion exchange high performance liquid chromatography—method 2

The method used for the analysis of the oligosaccharide component of the strong trifluoroacetic acid hydrolysate and the enzyme hydrolysates was similar to method 1. The only difference was that the concentration of sodium acetate was increased, linearly, to 400 mM over 60 min.

2.10. Methylation analysis

The water insoluble polysaccharide was methylated by three cycles according to method described by Isogai, Ishizu and Nakano (1985).

2.10.1. Preparation of polysaccharide suspension

The water insoluble polysaccharide (32.4 mg) was dispersed in dimethyl sulphoxide (2.84 ml). Concentrated sulphur dioxide–methyl sulphoxide solution (ca. 0.3 g/ml) was prepared as follows: sulphur dioxide (15 g) was bubbled into methyl sulphoxide (50 ml). Sulphur dioxide–methyl sulphoxide solution (165 μl) was added to the sample. Then, diethylamine (61 μl) was added. The suspension was stirred at ambient temperature for 20.3 h. Complete dissolution was not obtained.

2.10.2. Methylation of polysaccharide

Freshly powdered sodium hydroxide (389 mg) was added to the sample, at ambient temperature, and the mixture stirred for 1 h under helium. Methyl iodide (390 μl) was added dropwise, at ambient temperature, and the mixture was stirred for 1 h, and then kept at 40°C for 0.5 h, at 50°C for 0.5 h and at 60°C for 1 h. The methylation procedure was repeated three times, starting from the preparation of polysaccharide suspension (from the addition of sulphur dioxide–methyl sulphoxide solution). When solidification occurred during stirring, methyl sulphoxide was added to the sample to regenerate the slurry (1 ml prior to the second methylation and 2 ml prior to third methylation). The methylated products were isolated by dialysis and subsequently lyophilised.

2.10.3. Formic acid and sulphuric acid hydrolysis

The dry methylated product (11.8 mg) was allowed to swell in formic acid solution (90%, 1.18 ml) overnight, at ambient temperature. The sample was then refluxed during 2 h, the formic acid evaporated, the residue washed once with deionised water (1 ml) and then evaporated. Sulphuric acid solution (0.125 M, 2.95 ml) was added to the sample and refluxed during 10 h, neutralised with barium carbonate (ca. 236 mg) and the insoluble barium sulphate removed by centrifugation (7500 g, 15 min). The partially methylated sugars present in the supernatant were then used for the preparation of alditol acetates.

2.11. Preparation of alditol acetates

The resulting partially methylated aldoses were converted to partially methylated alditol acetates as described by Blakeney, Harris, Henry and Stone (1983). Ammonia (80 μl) was added to the hydrolysate (containing ca. 2 mg methylated monosaccharides in 0.5 ml). myo-Inositol solution (4 mg/ml, 100 μl) was added as an internal standard. Sodium borohydride (ca. 70 mg) was added and the sample was allowed to stand at 40°C for 1.5 h. Excess borohydride

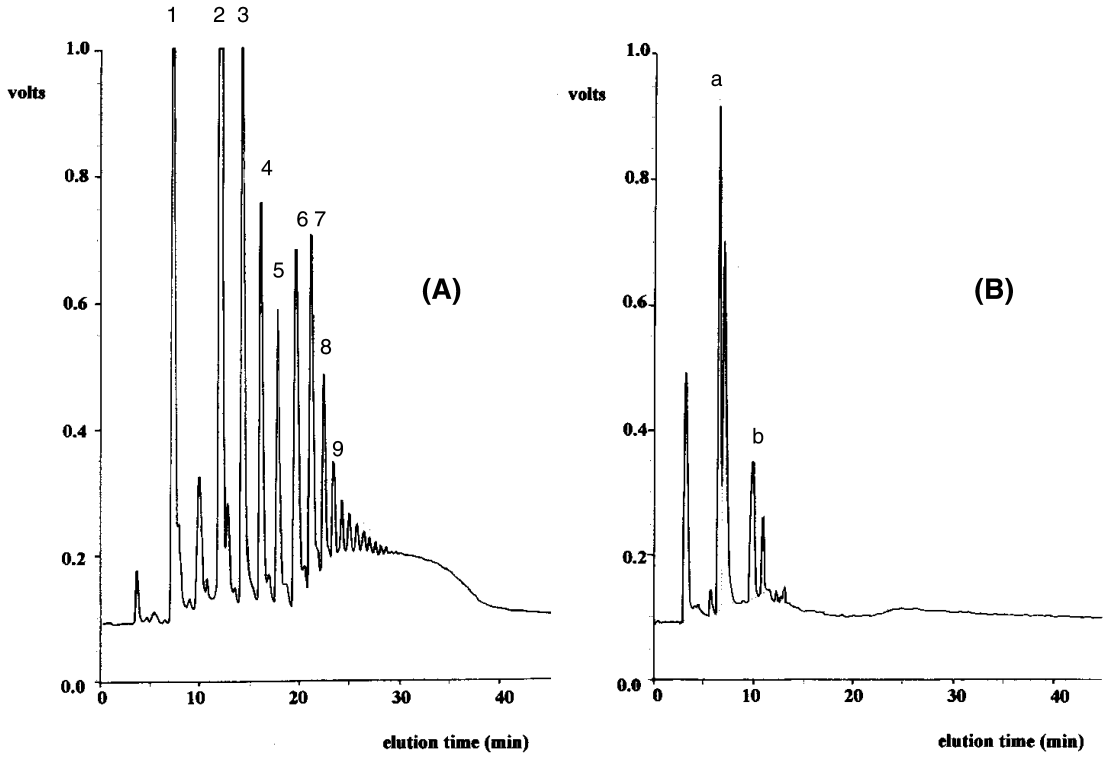

Fig. 1. (A) Oligosaccharide profiles of a starch hydrolysate; and (B) of the water soluble component of the polysaccharide sheet.

was destroyed by addition of glacial acetic acid (0.2 ml). After cooling to ambient temperature, 1-methyl imidazole (1.5 ml) was added and the sample shaken vigorously to ensure it was in solution. Acetic anhydride (5 ml) was added cautiously (in a bath containing a mixture of ice and cold water), causing an immediate rise in temperature. The sample was allowed to react for 20 min, with frequent gentle agitation. The excess anhydride was hydrolysed by addition of deionised water (12 ml) and shaken to thoroughly mix the sample. It was cooled for 5 min in a bath containing a mixture of ice and water, then extracted twice with dichloromethane (1 ml). The dichloromethane (lower layer) was removed by pipette; the extracts from the sample were combined and submitted to gas liquid chromatography analysis.

2.12. Gas liquid chromatography analysis

Quantification of the partially methylated alditol acetates was carried out on a Carlo Erba GC 8000 series gas chromatograph fitted with a BPX 70 (70% biscyanopropyl-polysilphenylene-siloxane, 25 m × 0.33 mm, 0.25 μm film thickness) capillary column (supplied by SGE, UK) and a flame ionisation detector. The column pressure was 150 kPa and the flow of helium was 2 ml/min. The initial temperature of the oven was 150°C and was increased at a rate of 10°C/min to 190°C and maintained for 2 min. The temperature was then increased at a rate of 5°C/min to a final temperature of 250°C and maintained for 10 min. The injection temperature was 260°C and the detector temperature was 300°C.

2.13. Gas chromatography and mass spectrometry

Gas chromatography and mass spectrometry (GC/MS) analyses were carried out on a Prospec (VG Company) equipped with a BPX 70 (70% biscyanopropyl-polysilphenylene-siloxane, 50 m × 0.33 mm, 0.25 μm film thickness) capillary column (supplied by SGE, UK). The initial temperature of the oven was 150°C and was increased at a rate of 10°C/min to 190°C and maintained for 2 min. The temperature was then increased at a rate of 5°C/min to a final temperature of 250°C and maintained for 5 min.

2.14. Enzymatic hydrolysis of the polysaccharide sample

Particles of the water insoluble fraction of the polysaccharide sheet (4.3 mg) were suspended in sodium acetate buffer (0.4 M, pH 5.0, containing Celluclast 1.5 L, 5 μl/ml, 860 μl), to give a concentration of 5 mg sample/ml. Cellulose powder (3.5 mg) was also suspended in sodium acetate buffer (0.4 M, pH 5.0, containing Celluclast 1.5 L, 5 μl/ml, 700 μl). The samples and control (sodium acetate buffer containing Celluclast 1.5 L) were mixed and incubated in a water bath at 50°C for 68 h and then at 100°C for 10 min to deactivate the enzyme. After centrifugation at 10 000*g* for 30 min, the supernatants were decanted and deionised water (same volume used for the enzymatic

Table 1
Monosaccharide compositions of the soluble fractions produced by trifluoroacetic acid hydrolysis, under mild and strong conditions, determined by anion exchange high performance liquid chromatography

Monosaccharide	Mild conditions (%, w/w)	Strong conditions (%, w/w)	Total (%, w/w)
Fucose	0.01	0.01	0.01
Arabinose	1.91	0.26	0.37
Rhamnose	0.22	–	0.01
Galactose	1.58	0.03	0.13
Glucose	55.41	89.88	87.57
Xylose	29.71	7.06	8.58
Mannose	1.96	0.74	0.82
Ribose	2.37	1.63	1.68
Glucuronic acid	6.83	0.39	0.83
Total	100.00	100.00	100.00

hydrolysis) was added to the residues. The samples were centrifuged at 10 000*g* for 30 min. The supernatants were combined and stored at −20°C prior to anion exchange high performance liquid chromatography analysis.

3. Results and discussion

3.1. Composition analysis

The contents of the water soluble and insoluble components of the polysaccharide sheet were 12 and 88% (w/w), respectively. The profile of the oligosaccharides present in the water-soluble component of the polysaccharide sheet, determined by anion exchange high performance liquid chromatography analysis, is shown in Fig. 1(B). This profile was compared with an oligosaccharide fingerprint of a starch hydrolysate reference material, Fig. 1(A), where the peaks are labelled according to their degree of polymerisation. Peak (a), a doublet in the sample chromatogram elutes in the region associated with neutral monosaccharides and the group of peaks labelled (b) elutes in the neutral disaccharide region. This indicates the presence of more than one neutral monosaccharide and that there are a number of disaccharides with different monosaccharide composition or linkage position or type.

Polysaccharides are hydrolysed under acid conditions to their component monosaccharides by cleavage of the glycosidic linkage at the bond between the anomeric carbon atom and the glycosidic oxygen. Conditions chosen for acid hydrolysis of polysaccharides are always a compromise between release and destruction of the component monosaccharides. Depending on the type of structural information required, conditions can be selected to give optimum release for monosaccharides or to favour release of oligosaccharide fragments. In this study two acid hydrolysis conditions (mild and strong) were chosen to release the monosaccharides.

The percentages of soluble and insoluble components obtained from the trifluoroacetic acid hydrolysis of the polysaccharide sheet, under mild conditions, were 15.8 and 84.2% (w/w), respectively. The percentages of soluble and insoluble components obtained from the strong trifluoroacetic acid hydrolysis of the acid insoluble fraction of the polysaccharide sheet (obtained using mild conditions) were 72.2 and 12.0% (w/w), respectively. The total percentage of soluble material obtained from the trifluoroacetic acid hydrolysis, using mild and strong conditions, was 88% (w/w) of the water insoluble fraction.

The monosaccharide compositions of the soluble components obtained from the trifluoroacetic acid hydrolysis of the polysaccharide sheet, under mild and strong conditions, determined by anion exchange high performance liquid chromatography analysis, are shown in Table 1. Glucose accounted for 87.6% of the total monosaccharides present in the acid hydrolysates (mild and strong conditions). Galactose was present in a very small amount (0.1%). These results differ from those obtained in the literature for zooglan, which showed glucose:galactose ratios of 3.7 (Ikeda et al., 1982) and 2.4 (Troyana et al., 1996). The content of xylose (8.6%) was higher than the other monosaccharides present in the hydrolysates.

The percentage of monosaccharides present in the acid hydrolysates, obtained using strong and mild conditions, were 14.4 and 43.7% (w/w), respectively. The total percentage of monosaccharides released by the acid hydrolysis was only 38.4% of the total acid soluble component.

The oligosaccharide profile of the trifluoroacetic acid hydrolysate, under mild conditions, is shown in Fig. 2 together with the reference material, hydrolysed starch. The main peak in the trifluoroacetic hydrolysate (a) is in the monosaccharide region and the other small peaks are in the region of di- and tetra-saccharides. It should be noted that the ratio of the two peaks in the monosaccharide component is significantly different for the trifluoroacetic acid hydrolysate produced using the mild conditions and the water-soluble component (Fig. 1(B)). This would suggest that there are differences in the solubility of sample according to the monosaccharide composition.

The trifluoroacetic acid hydrolysate, obtained using strong conditions, was analysed for oligosaccharide content at two different concentrations. Fig. 3 shows the separation

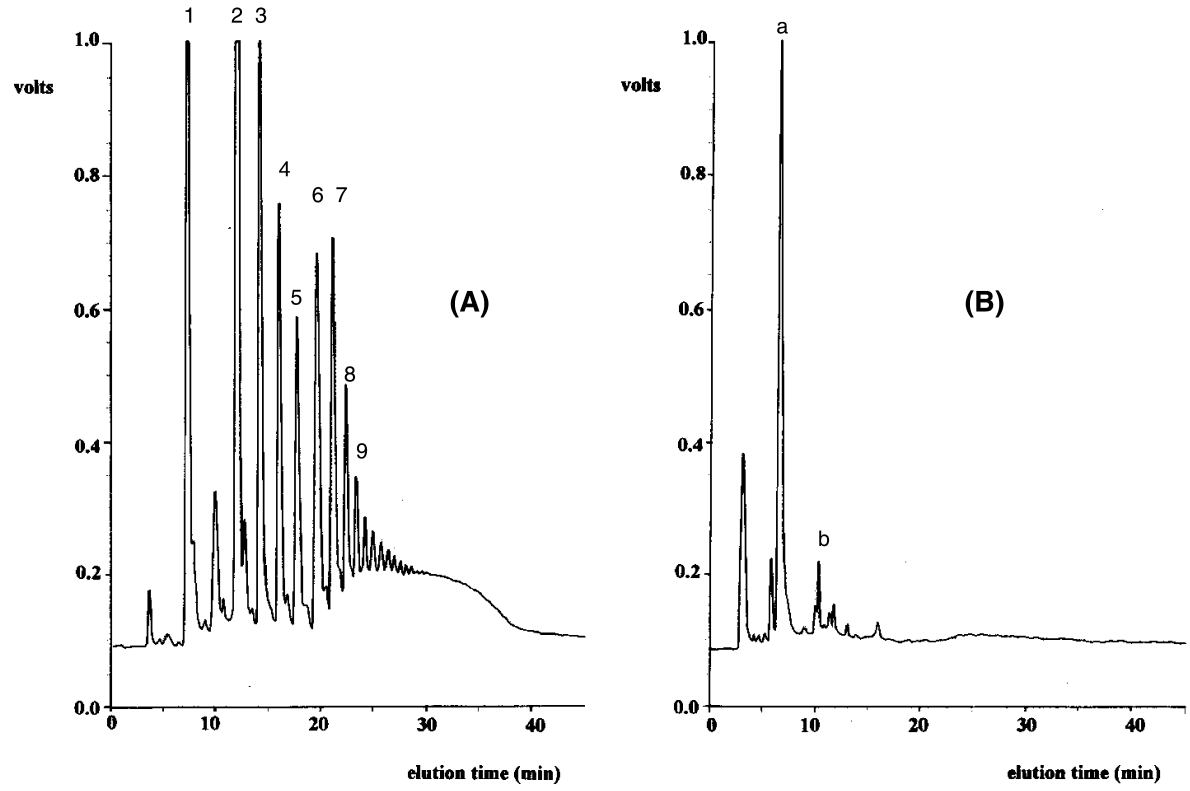

Fig. 2. (A) Oligosaccharide profiles of a starch hydrolysate; and (B) of the trifluoroacetic acid hydrolysate of the polysaccharide sheet obtained using the mild conditions.

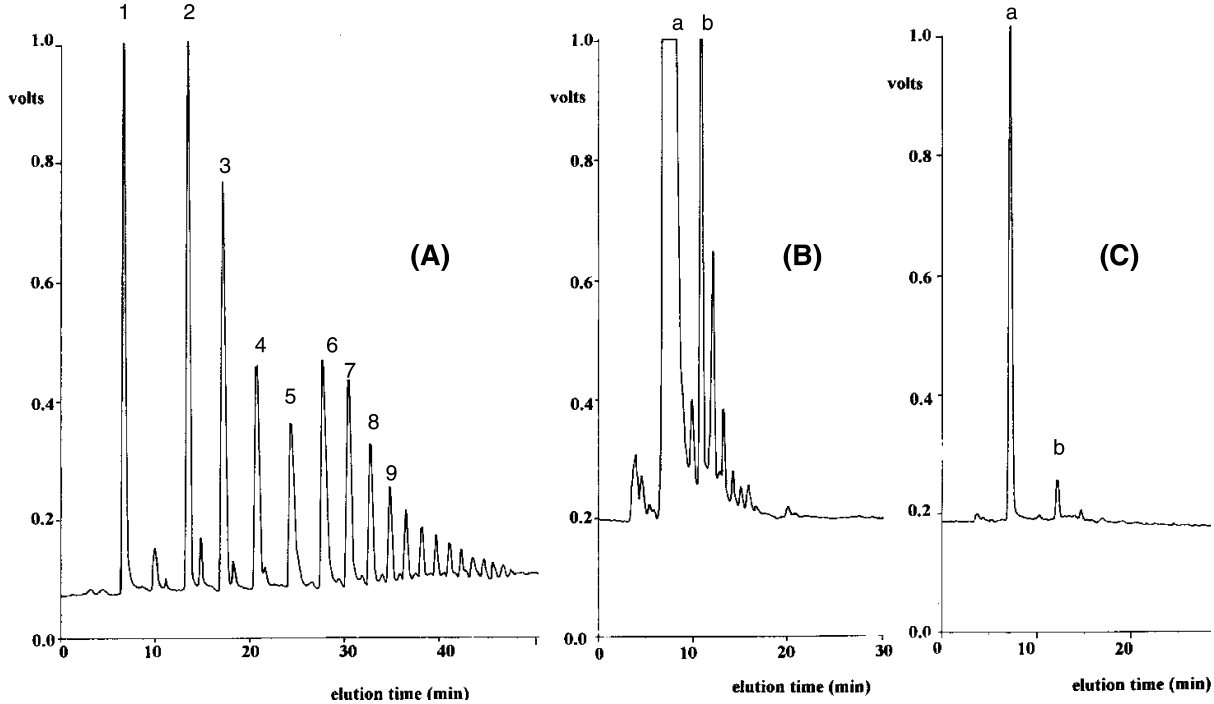

Fig. 3. (A) Oligosaccharide profiles of a maltodextrin reference sample; (B) of the trifluoroacetic acid hydrolysate of the polysaccharide sheet obtained using the strong conditions, concentrated sample; and (C) diluted sample.

Table 2
Partially methylated alditol acetates from the polysaccharide sheet obtained from *Zoogloea* sp. Peaks were assigned by comparison of the fragmentation patterns (GC-MS) with published data (Jansson, Kenne, Liedgren, Lindberg & Lonngren, 1976)

Compound	Type of linkage	Molar ratio	%
2,3,4,6-tetra-*O*-methyl hexitol	Terminal hexose	2.0	0.73
2,3,5-tri-*O*-methyl pentitol	(1–4) pentose	6.2	2.23
2,3,6-tri-*O*-methyl hexitol	(1–4) hexose	208.7	74.69
2,6-di-*O*-methyl hexitol	(1–3–4) hexose	1.1	0.41
3,6-di-*O*-methyl hexitol	(1–2–4) hexose	1.0	0.36
2,3-di-*O*-methyl hexitol	(1–4–6) hexose	49.6	17.74
2-*O*-methyl hexitol	(1–3–4–6) hexose	3.6	1.28
3-*O*-methyl hexitol	(1–2–4–6) hexose	7.2	2.56

of a maltodextrin reference material (A), the concentrated hydrolysate (B) and the diluted hydrolysate (C). The main peak (a) is in the monosaccharide region and its elution position correlates with peak (1), the monosaccharide, glucose, in the maltodextrin material. It should be noted that even with the higher column load the monosaccharide is a single peak unlike the doublet which was observed in the oligosaccharide profiles of the mild trifluoroacetic acid hydrolysate and of the water soluble component of the polysaccharide sheet. However, the disaccharide peak in the hydrolysate, peak (b) (Fig. 3), does not correlate with the elution position of maltose, the disaccharide present in the maltodextrin reference material, which would indicate that although the monosaccharide residue is glucose the polysaccharide linkage is different.

As expected, these results confirm that a higher amount of material was released from the polysaccharide sheet by the strong acid conditions compared with the mild conditions.

Table 3
Approximate composition of sugarcane molasse (Chen & Chou, 1993)

Component	Normal range (%, w/w)
Water	17–25
Sucrose	30–40
Glucose	4–9
Fructose	4–12
Gums, starch, pentosans, traces of hexitols and uronic acids	2–5
Ash	7–15
Nitrogen compounds	2.5–4.5
Protein	0.5–4.5
Amino acids	0.3–0.5
Non-nitrogenous acids (e.g. aconitic, citric, malic, oxalic acids)	1.5–6.0
Wax, sterols and phosphatides	0.1–1.0
Vitamins (vitamin A, biotin, niacin, pantothenic acid, riboflavin, thiamine)	Varying amounts

3.2. Structure analysis

Methylation analysis was carried out to study further the polysaccharide sheet. Methylation of the polysaccharide sheet demonstrated the presence of the sugar residues shown in Table 2. The main components of the polysaccharide are (1–4) linked glucose (74.7%) and (1–4–6) linked glucose (17.7%). Other linkages detected are (1–3–4), (1–2–4), (1–3–4–6) and (1–2–4–6) linked branch points. A small amount (2.2%) of (1–4) linked pentose is also present. The monosaccharide analysis, using trifluoroacetic acid hydrolysis, showed that glucose and xylose accounted for 33.6% and 3.2% (w/w), respectively, of the total acid soluble component of the polysaccharide sheet. An approximate composition of sugarcane molasses is shown in Table 3, which shows that the pentosans are in the range of 2–5% (w/w).

3.3. Enzymatic hydrolysis

In order to confirm the monosaccharide linkage position and type in the polysaccharide sheet, hydrolysis using an enzyme with a known specificity was used. The commercial enzyme preparation Celluclast 1.5 L hydrolyses cellulose, (1 → 4)-β-D-glucopyranose residues in a polysaccharide. The oligosaccharide profiles of the maltodextrin reference material and of the sodium acetate buffer, containing the enzyme preparation used for the hydrolysis, are shown in Fig. 4(A) and (B), respectively. It is clear from these chromatograms that there is a component in the sodium acetate buffer which elutes at the column breakthrough position but nothing which would interfere in the identification of the mono- and/or oligo-saccharide components.

A sample of cellulose powder and of the polysaccharide sheet were incubated with the enzyme preparation Celluclast 1.5 l and the hydrolysates were subjected to oligosaccharide analysis, Fig. 5(A) and (B), respectively. The two oligosaccharide profiles are very similar. The buffer peak is evident in both profiles as is the monosaccharide peak, peak (1), which elutes in the position identified as glucose from the analysis of the maltodextrin reference material. The smaller components, and possibly the dimer cellobiose, are common to both hydrolysates. From these analyses it can, therefore, be concluded that the polysaccharide sheet contains some (1 → 4)-β-D-glucose residues which are hydrolysed to glucose by the enzyme.

4. Conclusions

The contents of the water soluble and insoluble components of the polysaccharide sheet were 12 and 88%, respectively. The total percentage of soluble material obtained from trifluoroacetic acid hydrolysis of the water insoluble fraction of the polysaccharide sheet, using mild and strong conditions, was 88% (w/w). The main monosaccharides present in the soluble fraction were glucose

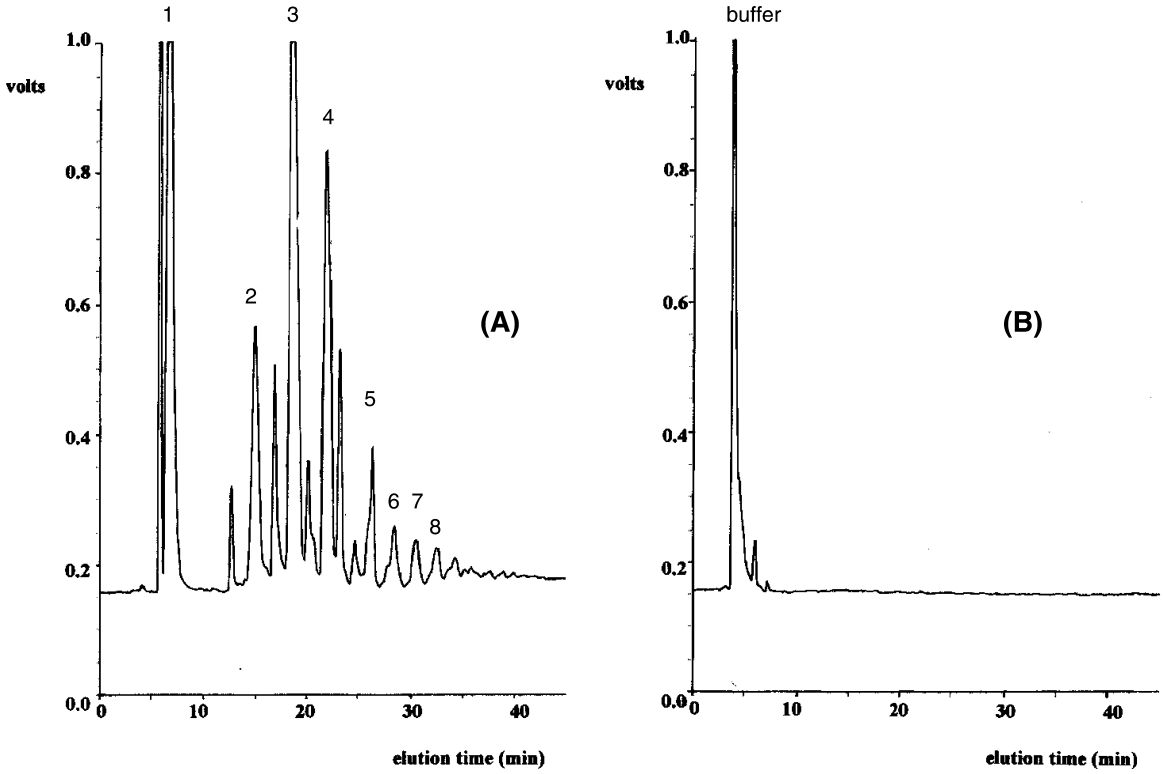

Fig. 4. (A) Oligosaccharide profiles of a maltodextrin reference sample in sodium acetate buffer (0.2 M, pH 5.0); and (B) of the sodium acetate buffer (0.2 M, pH 5.0) containing Celluclast 1.5 L used for the enzymatic hydrolysis.

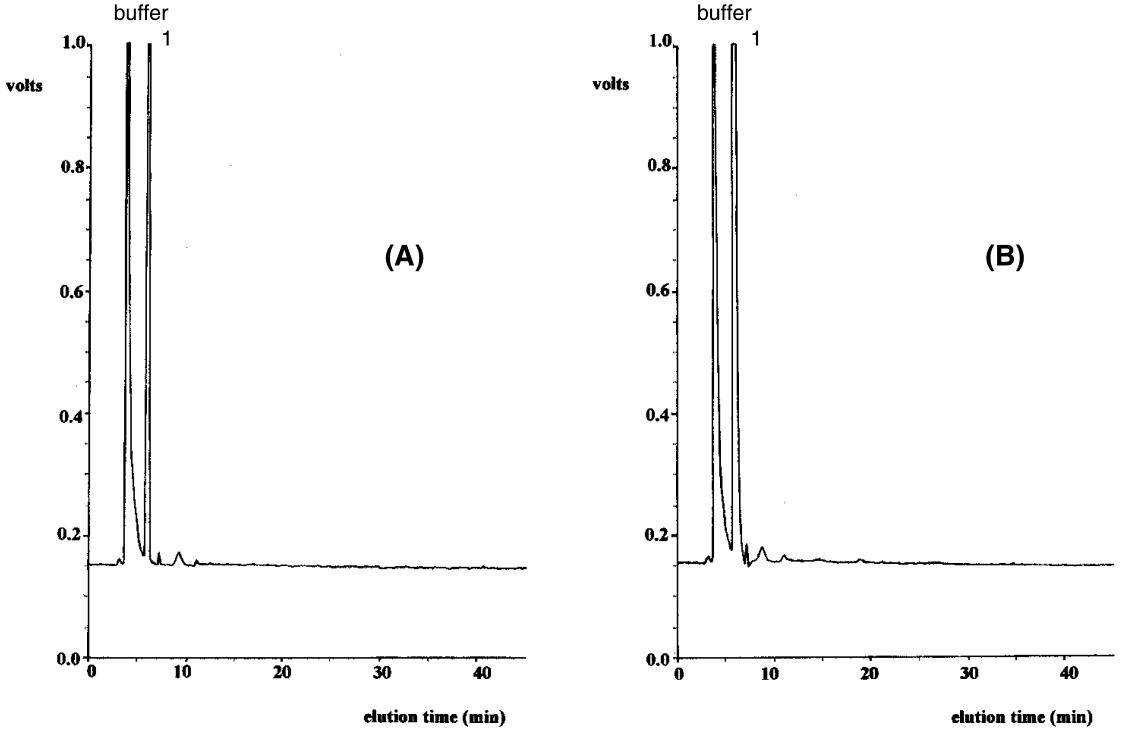

Fig. 5. (A) Oligosaccharide profiles of cellulose powder sample treated with Celluclast 1.5 L in sodium acetate buffer (0.2 M, pH 5.0); and (B) of the polysaccharide sheet treated with Celluclast 1.5 L in sodium acetate buffer (0.2 M, pH 5.0).

(87.6%), xylose (8.6%), mannose (0.8%), ribose (1.7%), galactose (0.1%), arabinose (0.4%) and glucuronic acid (0.8%). Methylation analysis of the polysaccharide showed mainly 2,3,6-tri-*O*-methylhexitol (74.7%) and 2,3-di-*O*-methylhexitol (17.7%). Enzyme hydrolysis of the polysaccharide with a cellulase (Celluclast 1.5 L) confirmed the presence of (1 → 4)-β-D-glucopyranosyl units.

Acknowledgements

We thank Prof. J. Otamar, from the Instituto de Antibióticos, Universidade Federal de Pernambuco, Recife, Brazil, for his assistance in the identification of the *Zoogloea* sp. We also thank Mr G. Burns, from the School of Chemistry, University of Birmingham, UK, for his assistance in the monosaccharide analysis.

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