J Ind Microbiol Biotechnol (2003) 30: 95–101
DOI 10.1007/s10295-002-0019-8

ORIGINAL PAPER

V. Schiano Moriello · L. Lama · A. Poli
C. Gugliandolo · T.L. Maugeri · A. Gambacorta
B. Nicolaus

Production of exopolysaccharides from a thermophilic microorganism isolated from a marine hot spring in flegrean areas

Received: 8 July 2002 / Accepted: 4 November 2002 / Published online: 15 January 2003

Abstract A thermophilic strain isolated from sea sand at Maronti, near Sant' Angelo (Ischia), is described. The organism grows well at an optimal temperature of 60 °C at pH 7.0. The thermophilic bacterium, named strain 4004, produces an exocellular polysaccharide (EPS) in yields of 90 mg/l. The EPS fraction was produced with all substrates tested, although a higher yield was obtained with sucrose or trehalose as sole carbon source. During growth, the EPS content was proportional to the biomass. Three fractions (EPS1, EPS2, EPS3) were obtained after purification. Quantitative monosaccharide analysis of the EPSs revealed the presence of mannose:glucose:galactose in a relative ratio of 0.5:1.0:0.3 in EPS1, mannose:glucose:galactose in a relative ratio of 1.0:0.3:trace in EPS2, and galactose:mannose:glucosamine:arabinose in a relative ratio of 1.0:0.8:0.4:0.2 in EPS3. The average molecular mass of EPS3 was determined to be 1×10^6 Da. From comparison of the chemical shift values in ^1H and ^{13}C spectra, we conclude that EPS3 presents a pentasaccharide repeating unit.

Keywords Exopolysaccharide · Marine hydrothermal vent · *Bacillus* · *Geobacillus* · Lipid · Fatty acids · NMR

V. Schiano Moriello · L. Lama · A. Poli · A. Gambacorta
B. Nicolaus (✉)
Istituto di Chimica Biomolecolare (ICB),
CNR, via Campi Flegrei 34, Comprensorio Olivetti, 80078,
Pozzuoli, (Na), Italy
E-mail: bnicolaus@icmib.na.cnr.it
Tel.: +39-81-8675245
Fax: +39-81-8041770

C. Gugliandolo · T.L. Maugeri
Dipartimento di Biologia Animale ed Ecologia Marina, Sez.
Ecologia Microbica e Biotecnologie, Univ. Messina,
salita Sperone 31, 98166 Messina, Italy

Introduction

Many microorganisms produce polysaccharides which are located in the cell wall (LPS), attached to the cells forming capsules (CPS), or secreted into the extracellular environment in the form of slime (exopolysaccharide, EPS) [1, 5, 9, 12, 14]. Increasing attention has been focused on these biopolymers due to their commercial application. EPS and CPS are incorporated into food as thickeners, and in the pharmaceutical industry they can be used as hydrophilic matrix. Moreover, there are numerous studies on their participation in pathogenic and symbiotic processes in plants and animals and in general interactions between microorganisms and their environment [6, 13, 20].

During a screening for marine thermophilic strains capable of producing new and interesting polysaccharides [14], a thermophilic strain, 4004 (DSM 14422), isolated from geothermal sea sand in Italy (Ischia island, locality Maronti) was selected for further study. This strain formed mucoid colonies on Bacto Marine Agar (Difco, Ann Arbor, Mich., USA) and produced exocellular polysaccharides.

In this work, we describe the phenotypic characterization of the thermophilic isolate 4004, its EPS production, and the partial characterization of purified EPS.

Materials and methods

Microbial strain

Strain 4004 (DSM number 14422) was enriched using sediment samples collected from a marine hot spring near the seashore of Maronti (Ischia island, Italy) as reported previously [14].

Morphological and physiological studies

The methods for the morphological, biochemical, and physiological tests used for the characterization of strain 4004 were carried out as reported by Maugeri [10].

The following thermophilic *Geobacillus* strains [11] were included as reference strains: *G. stearothermophilus* DSM 22^T, *G. thermocatenulatus* DSM 730^T, *G. thermodenitrificans* DSM 465^T [7], *G. thermoleovorans* DSM 5366^T, *G. kaustophilus* M7263 and *G. thermoglucosidasius* DSM 2542^T.

The isolate was cultivated using Bacto Marine Broth 2216 (Difco) at the optimal temperature of 60 °C at pH 7.2. The above medium was solidified by adding 2% agar to the broth culture. Colonies grown on plates were observed with a Leica M8 stereomicroscope, which showed the presence of a mucilaginous layer.

Growth on minimal media was carried out in batch cultivation. The minimal media had the same salt composition of Bacto Marine Broth 2216; yeast extract (0.02% w/v) and peptone (0.01% w/v) were reduced and different sugars (1% w/v) were added as a carbon source (galactose, glucose, fructose, xylose, mannose, cellobiose, sucrose, and trehalose).

Growth and polysaccharide production by strain 4004 were carried out in a 3-l fermenter (Chemap) using sucrose added to the minimal medium, with low-level mechanical agitation (100 rpm) and an aeration flux of 20 ml min^{-1} per liter of broth at 60 °C and pH 7.2. Cell density was estimated by measuring absorbance at 540 nm. Electron microscopy was performed at the Centre of Research of Biological Structures, Faculty of Science University Federico II, Naples, Italy.

Lipid and fatty acid analysis

Lipids were extracted from dried cells grown on Bacto Marine Broth 2216 (Difco) at the optimal temperature of 60 °C at pH 7.2, using $CHCl_3$:CH_3OH:H_2O (65:25:4, v/v/v). The lipids were analyzed by thin-layer chromatography (TLC) on silical gels (0.25 mm, F_{254}, Merck) eluted with $CHCl_3$:CH_3OH:H_2O (65:25:4, v/v/v) and were detected by spraying the plates with specific reagents for phospholipids, amino-lipids and glycolipids according to the procedure of Nicolaus [15].

Fatty acid methyl esters were obtained from complex lipids by acid methanolysis [15]. They were detected using a Helwett-Packard 5890A gas chromatograph fitted with an FID detector and equipped with an HP-V column with a flow rate of 45 ml/min using the temperature program of 120 °C (1 min), from 120 °C to 250 °C at 2 °C/min [17].

EPS production

Microbial growth and EPS production were monitored quantitatively by sampling 20 ml of culture broth at 0, 4, 8, 12, 16, 20, 24, 48 h. Biomass production was monitored by reading the absorbance at 520 nm. EPS in cell-free culture broth was tested by the phenol-sulphuric acid method with glucose as standard [4].

On reaching the stationary phase, the cells were harvested by centrifugation. The supernatant was treated with the same volume (3 l) of cold ethanol added drop-wise with stirring. The alcoholic solution was kept at 18 °C overnight and then centrifuged at 8,000 *g* for 40 min. The precipitated EPS was dissolved in distilled water, dialyzed against tap water (72 h), and lyophilized. This sample was tested for carbohydrate, protein, and nucleic acid content by the methods of Dubois [4] and Bradford [2], and by reading the absorbance at 260 nm, respectively.

Polysaccharide purification

Polysaccharide was purified by gel chromatography (Sephadex G-50; 2.5×50 cm) using H_2O/pyridine/glacial acetic acid (500/5/2,v/v/v) as eluant, with a flux of 6 ml/h (5 ml each fraction). Fractions containing polysaccharides were further purified by anion-exchange chromatography (Sepharose DEAE CL-6B; 1.5×40 cm) eluted with 0.1 l of H_2O and 1 l of NaCl gradient from 0 to 1 M with a flux of 12 ml/h. The volume of each fraction was 10 ml. Fractions were tested for carbohydrate qualitatively by spot test on TLC sprayed with α-naphthol [8] and quantitatively by the Dubois method [4]. The α-naphthol-positive fractions were saved. The material obtained at each purification step was estimated for protein content and for nucleic acid content. Saved fractions were exhaustively dialyzed against water, freeze-dried and weighed. This material was used for all analytical work.

Polysaccharide characterization

The molecular masses of the samples were estimated by gel filtration on a Sepharose CL-6B column and density-gradient centrifugation using a sucrose gradient from 0 to 50% (w/v) [8]. In both experiments, 10 mg of EPS and a mixture of dextrans (10 mg each of T-700; T-400, T-150) was used for calibration curves.

Sugars were analyzed by hydrolysis of polysaccharide fractions with 2 M trifluoroacetic acid (TFA) at 120 °C for 2 h. The resulting sugar mixture was identified by high-pressure anion-exchange pulsed amperometric detection (HPAE)-PAD using standards for identification and calibration curves. HPAE-Pad Dionex equipped with a Carbopac PA 1 column was eluted isocratically with: (a) 15 mM NaOH for neutral sugars, (b) a buffer consisting of 100 mM NaOH and 150 mM NaOAc for acidic sugars [16].

Alditol acetates were obtained after acid hydrolysis followed by reduction with $NaBH_4$, and by acetylation with Ac_2O/pyr (1:1, v/v) at 120 °C for 3 h. Sugars were identified by gas chromatography (GC) using standards. GC was carried out on a Hewlett-Packard 5890–5970 instrument equipped with a HP-V column and with a flow rate of 50 ml min^{-1}; the temperature program used was 170 °C for 1 min and from 170 to 250 °C at 3 °C min^{-1} increments [18].

Ultraviolet spectra of polysaccharides were obtained by reading the absorbance of aqueous solutions (3 mg ml^{-1}) from 350 to 210 nm on a Varian DMS-90 instrument. Optical rotation values were obtained on a Perkin-Elmer 243 B polarimeter at 25 °C in water.

Viscosity measurements were done at 25 °C in a Cannon-Ubbelohde 75 suspended level viscometer. Polysaccharide solutions (0.8%, w/v) were prepared in water, in 1% (w/v) $CaCl_2$, and 1% (w/v) NaCl aqueous solutions and the viscosity was measured.

NMR spectra were obtained on a Bruker AMX-500 (500.13 MHz for ^1H and 125.75 MHz for ^{13}C) at 70 °C. Samples were exchanged twice with D_2O with intermediate lyophilization and then dissolved in 500 μl D_2O to a final concentration of 30 mg/ml. Chemical shifts were reported in parts per million relative to sodium 2,2,3,3-d_4-(trimethylsilyl) propanoate for ^1H and $CDCl_3$ for ^{13}C-NMR spectra [8, 19].

Results

Characteristic of the isolate

A thermophilic strain, 4004, was isolated from sea sand from Maronti near Sant'Angelo (Ischia, Italy) [14]. The vegetative cells of the isolate are motile, gram-positive, rods (0.8–0.9×1.8–2.5 μm), and are spore forming (Fig. 1). On agar plates, colonies appear mucoid, opaque, and circular, with an intact margin.

Strain 4004 grew aerobically from 50 °C to 75 °C with an optimal temperature of 60 °C. The pH range for growth was 6–9, with an optimum of pH 7.2. The isolate grew in a range 0–5% NaCl, with optimal growth at 2%. it was resistant to nalidixic acid and sensitive to streptomycin, tetracycline, novobiocin, penicillin G, chloramphenicol, erythromycin, kanamycin, bacitracin, and

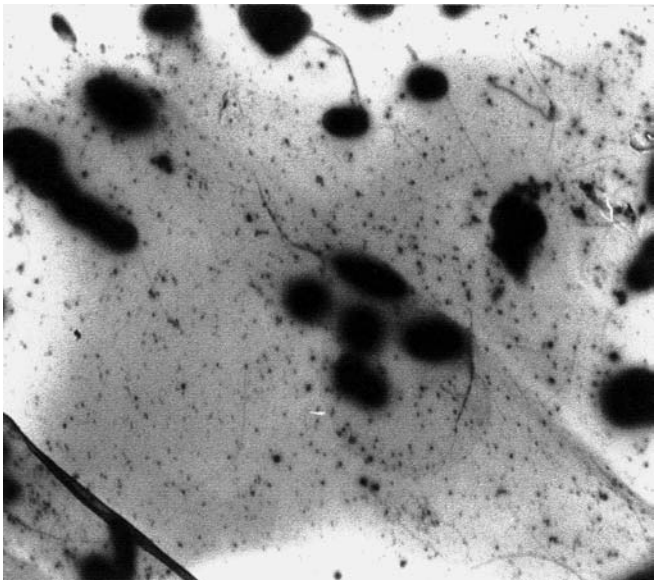

Fig. 1 Electron micrograph of strain 4004

polymyxin B. Positive tests for the strain were: hydrolysis of gelatin, esculin, and Tween 60; reduction of nitrate, β-galactosidase; acidification of glycerol, galactose, glucose, fructose, mannose, inositol, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, xylitol, and turanose. Negative tests were: arginine dehydrolase; lysine and ornithine decarboxylase; urease; tryptophan deaminase; production of hydrogen sulfide, indole, and acetoin; assimilation of caprate, adipate and citrate; acidification of erythritol, arabinose, ribose, xylose, sorbose, rhamnose, dulcitol, mannitol, sorbitol, *N*-acetyl glucosamine, amygdaline, arbutine, salicine, cellobiose, inuline, glycogene, β-gentiobiose, lyxose, tagatose, fucose, arabitol.

Lipid analyses revealed the presence of one glycolipid and three phosphoglycolipids. The isolate contained a high portion of branched fatty acids. Within the branched fatty acids, the portion of the *anteiso*-branched acids was significantly lower than that of the *iso*-branched acids (*iso* 86%, *anteiso* 10%, straight chain 4%) (Fig. 2).

On the basis of physiological characteristics and the results of lipid analysis, we have classified isolate 4004 in the genus *Geobacillus* [11]. The differential phenotypic properties of the isolate in comparison with those of four related *Geobacillus* reference strains are shown in Table 1.

Production of exopolysaccharides

Strain 4004 produces an EPS, which can be obtained from the supernatant of liquid cultures by cold-ethanol precipitation, in yields of 30 mg/l. The EPS fraction was recovered with all substrates tested (Fig. 3), although a higher yield was obtained with sucrose as sole carbon source. During growth in sucrose medium, the EPS content was proportional to the biomass as obtained by using a 3-l fermenter and 1-l batch culture. The yield of EPS reached its maximum, 90 mg/l, at the beginning of the stationary phase in the fermenter; this was three-fold higher than obtained in batch culture (Fig. 4). The increased yield of the EPS was due to better aeration.

Isolation and characterization of the polysaccharides

The EPS produced by strain 4004 in a sucrose medium was isolated via ethanol precipitation of the cell-free medium. The polysaccharide fraction was tested for carbohydrate (85%), protein (10%), and nucleic acid (2%) content. The polysaccharide was purified by gel filtration chromatography on Sephadex G-50 with a yield of 80% and then chromatographed on DEAE-Sepharose CL-6B with a yield of 70%. The elution profile is shown in Figure 5. Three fractions were obtained, EPS1 (5.5% eluted at O M NaCl), EPS2 (11% eluted at 0.2 M NaCl), and EPS3 (80% eluted at 0.4 M

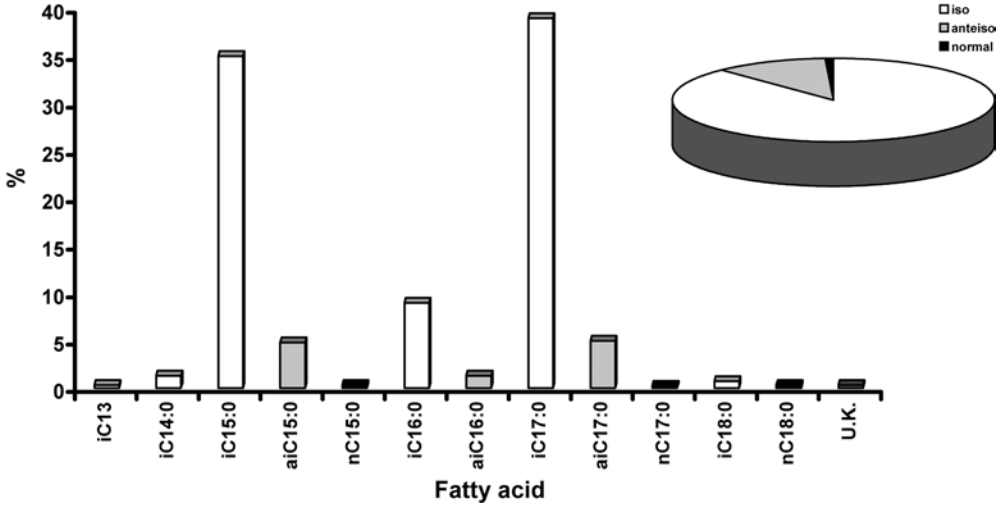

Fig. 2 Fatty acid compositions of strain 4004

Table 1 Phenotypic properties of strain 4004 and reference type strains of *Geobacillus* species. *v* Variable, *w* weak, *nd* not determined

Microrganisms	Catalase	Oxidase	Growth temperature	Growth with salt (%)	Acid from:													Production of:					Hydrolysis of:		
					glucose	sucrose	mannose	fructose	maltose	cellobiose	rhamnose	glycerol	galactose	sorbitol	raffinose	mannitol	acetoin	H2S	Urease	Citrate-utilisation	Nitrate reduction	esculin	gelatin	starch
Strain 4004	+	+	55–75	0–5	+	+	+	+	–	–	+	+	–	–	+	–	–	–	–	–	+	+	+	+
G.thermodenitrificans	+	+	45–70	0–5	+	+	+	+	+	v	w	–	–	–	+	–	–	–	v	+	+	+	+	+
G.thermoleovorans	v	v	35–78	0–2	+	+	+	+	+	+	nd	+	–	+	+	–	–	–	v	+	+	+	+	+
G.stearothermophilus	–	–	45–75	0–3	+	+	+	+	–	–	+	w	–	+	–	+	–	–	–	+	+	+	+	+
G.thermoglucosidasius	+	+	42–69	0–2	+	+	+	+	+	+	+	–	+	+	+	–	+	+	v	+	–	–	–	+

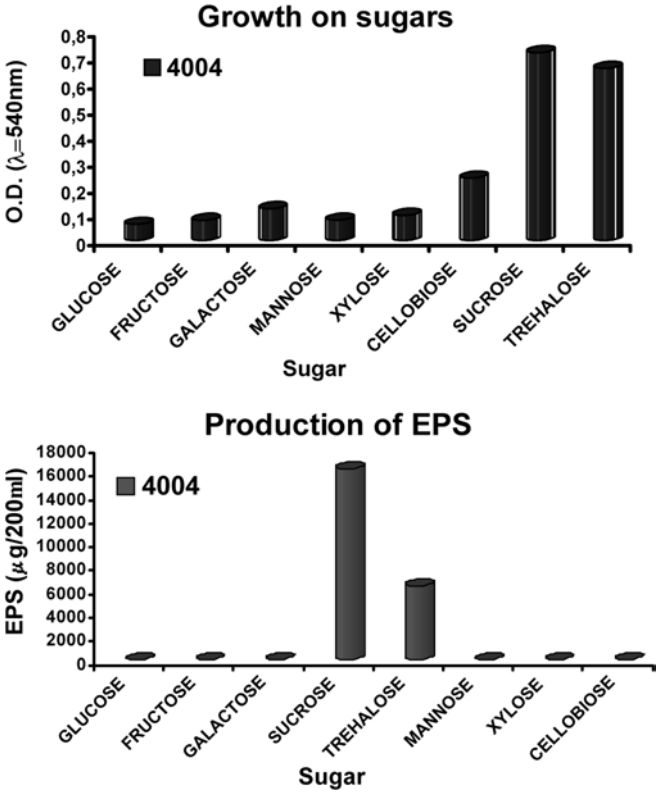

Fig. 3 Biomass and exopolysaccharide (EPS) production of strain 4004 grown on different sugars

NaCl). The three fractions were assayed for carbohydrate and protein content. EPS2 and EPS3 contained at least 95% sugars while EPS1 contained a trace amount of carbohydrate (Table 2). Quantitative monosaccharide analysis of the EPSs, including determination of the absolute configuration, revealed the presence of D hexoses, mannose:glucose:galactose in a relative ratio of 0.5:1.0:0.3 in EPS1, mannose:glucose:galactose in a relative ratio of 1.0:0.3:trace in EPS2, and galactose:mannose:glucosamine:arabinose in a relative ratio of 1.0:0.8:0.4:0.2 in EPS3 (Table 2). The average molecular mass of EPS3 was 1×10^6 Da. The protein content of the purified EPS3 was ca. 2%. EPS3 was used for further NMR spectroscopic analysis.

The ^{1}H and ^{13}C-NMR spectra of EPS3 (Fig. 6) are quite complex, but it is possible to extrapolate some information. In fact, the ^{1}H spectrum showed, inter alia, five anomeric signals at δ 5.17($J_{H1,H2}$ 3–4), 5.04($J_{H1,H2}$ 0.5–1), 4.90($J_{H1,H2}$ 0.5–1), 4.65($J_{H1,H2}$ 0.5–1) and 4.62($J_{H1,H2}$ 7–8); this signal latter partially overlapped with the previous signal (δ 4.65). Another remarkable signal was at δ 2.02, attributable to an acetyl group. The ^{13}C spectrum of EPS3 contained essentially five anomeric signals at δ 102.7, 103.9, 104.5, 105.3 and 106.2. The signals at δ 25.8, indicative of an *N*-acetyl group, and a signal at δ 54.8, representative of carbon-carrying nitrogen, were present. Finally, signals at δ 187.8 and 178.1, attributable to carbonyl groups, were found.

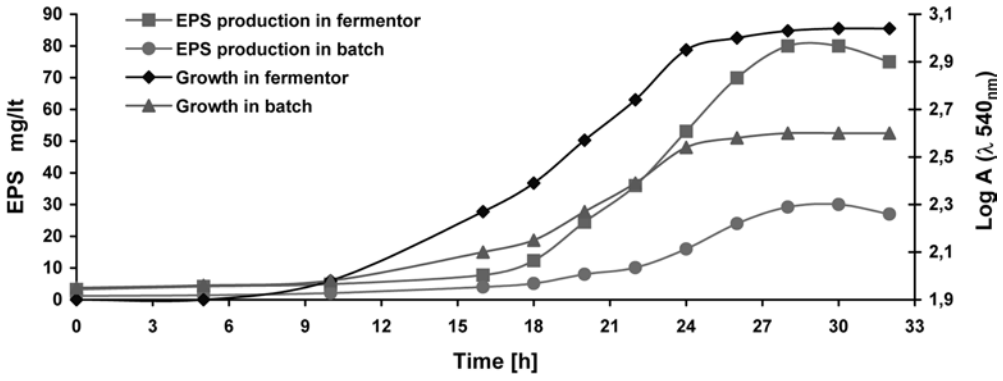

Fig. 4 EPS production of strain 4004 grown in batch culture and in a fermenter

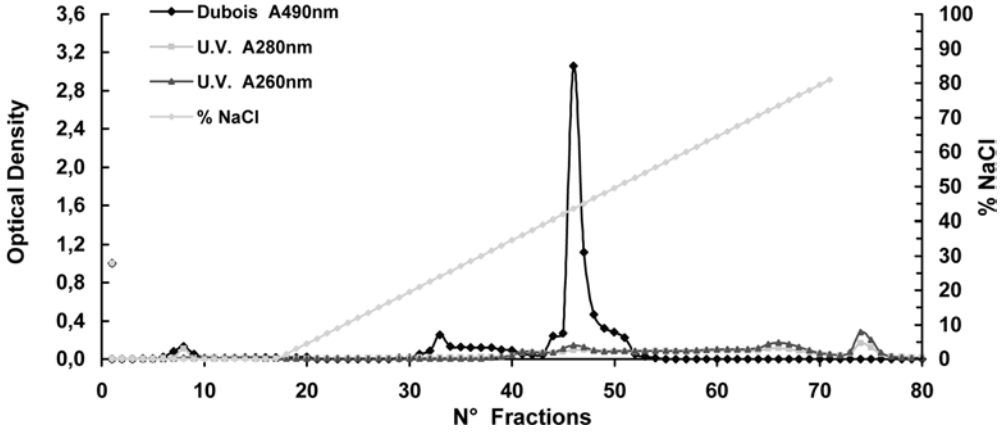

Fig. 5 Elution profiles from DEAE-Sepharose CL-6B of the carbohydrate fraction

Table 2 Chemical characterization of exopolysaccharides (*EPSs*) from strain 4004. *nd* Not determined

Properties	EPS1	EPS2	EPS3
Fraction (% weight)	5.5	11	80
NaCl (M)	0	0.2	0.4
Carbohydrate content (% w)	5	20	75
Proteincontent (% w)	6	2	2.6
Sugar composition relative ratio	Man:Glu: Gal:0.5:1.0:0.3	Man:Glu:Gal: 1.0:0.3:tracer	Gal:Man:GlcN: Arab1.0:0.8:0.4:0.2
Minorcompounds	nd	nd	nd
Molecular weight	nd	nd	> 1,000,000
$[a]^{25}D$	nd	nd	−199.77
Absoluteconfiguration	nd	nd	Gluco/galactoand manno
Repeating unit	nd	nd	5

These data showed that there are five different residues, two of them with a *gluco*/*galacto* configuration and three with a *manno* configuration. One of the residues is an acetamidosugar and at least one uronic acid is present.

To gain insight into the viscometric properties, specific viscosity (μ) was studied using different concentrations of EPS3 in water, in 1% $CaCl_2$ and 1% NaCl. With increasing concentrations of EPS, there was an increase in μ in all the solutions. The viscosity was maximum at 1,08 dl/g in 1% $CaCl_2$.

Discussion

In recent years, increasing attention has been paid to microbial EPSs, which are of widespread occurrence and can readily be prepared by fermentation [21]. Such molecules are of interest because of their bio-active roles and their wide range of commercial applications, including as food additives and in biopharmaceutical industries [1].

There exists relatively little information concerning polysaccharide production by thermophiles. Until now, it was not clear whether these organisms were likely to be useful sources of polymers.

Some extremophiles, including *Methanosarcina, Haloferax, Haloarcula, Sulfolobus* and *Bacillus* species, and more recently *Thermotoga marittima* and *Thermococcus litoralis*, produce EPSs [8, 9]. The results of the preliminary study reported here indicate that, from a taxonomic point of view, isolate 4004 may be considered a member of the genus *Geobacillus*. It forms large and

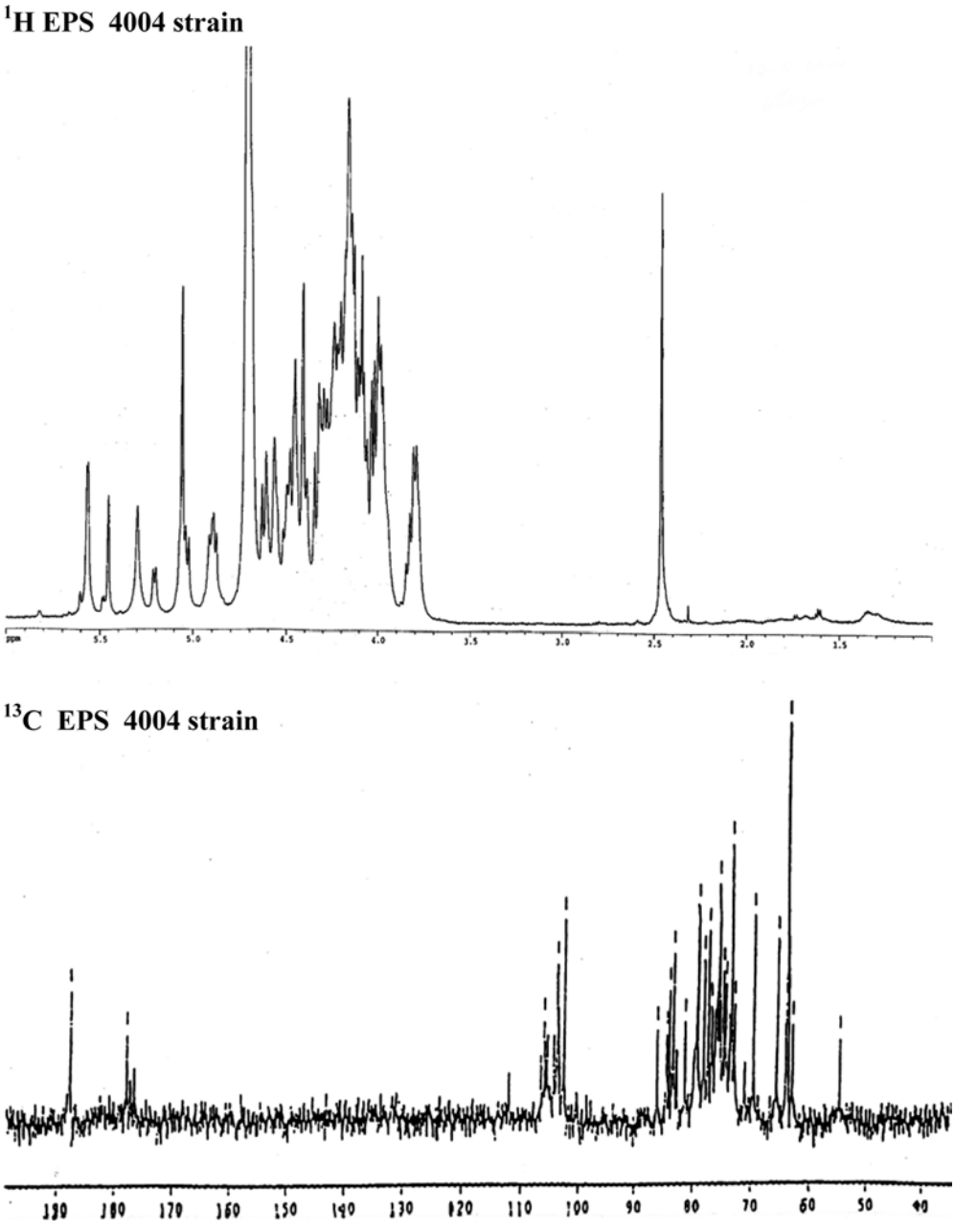

Fig. 6 ^{1}H and ^{13}C NMR spectra of EPS3 from strain 4004

viscous colonies containing large amounts of sugars on solid media, and has been selected and cultured in sugar-rich liquid media. Saccharose or trehalose, in a minimal medium, resulted in the best EPS formation.

These results are therefore encouraging, because by modulating the conditions of growth we were able both to increase the yield of the biopolymer and to induce the biosynthesis of new polysaccharides. Biomass production was directly proportional to EPS production. Optimal production occurred during the stationary phase of growth in fermenters.

The increased yield is essential to define the primary structure of the polymer as determined by NMR analyses [18, 19]. The proton spectrum of EPSs produced by strain 4004 was indicative of complex structures. However the downfield signals, representative of an anomeric region, and signals up-field indicated the presence of deoxysugars and acetaminosugars. From the comparison of the chemical shift values in ^{1}H and ^{13}C spectra, we conclude that EPS3 of strain 4004 presents a pentasaccharide repeating unit essentially consisting essentially of two sugars having a *gluco-galacto* configuration and three with *manno* configuration.

The polydisperse nature, evidenced by the large molecular size, and the presence of acidic residues allows this polysaccharide to bind divalent ions, strongly promoting gel formation. It has been hypothesized that the synthesis of EPSs in microorganisms plays a major role in protecting cells from stress in extreme habitats. Therefore, the production of EPSs by thermophilic

marine microorganisms could serve to form a boundary between the bacterial cell and its immediate environment.

Acknowledgements This study was partly funded by European Commission project MAS3-CT95-0034, which was coordinated by Prof. Daniel Prieur, University of Brest, France. The authors thank Eduardo Pagnotta for assistance in lipid analyses and Vincenzo Mirra for NMR service.

References

1. Boyle CD, Reade AE (1983) Characterization of two extracellular polysaccharides from marine bacteria. Appl Environ Microbiol 46:392–399
2. Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein using the principles of protein–dye binding. Anal Biochem 72:248–254
3. Degryse E, Glansdorff N, Pièrard A (1978) A comparative analysis of extreme thermophilic bacteria belonging to genus *Bacillus*. Arch Microbiol 117:189–196
4. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric methods for determination of sugars and related substances. Anal Chem 28:350–356
5. Guezennec JG, Pignet P, Raguénès G, Deslandes E, Lijour Y, Gentric E (1994) Preliminary chemical characterization of unusual eubacterial exopolysaccharides of deep-sea origin. Carbohyd Polym 24:287–294
6. Loaëc M, Olier R, Guezennec J (1998) Chelating properties of bacterial exopolysaccharides from deep-sea hydrothermal vents. Carbohydr Polym 35:65–70
7. Manachini PL, Mora D, Nicastro G, Parini C, Stackebrandt E, Pukall R, Fortina MG (2000) *Bacillus thermodenitrificans* sp. nov., nom. rev. Int J Syst Evol Microbiol 50:1331–1337
8. Manca MC, Lama L, Improta R, Esposito E, Gambacorta A, Nicolaus B (1996) Chemical composition of two exopolysaccharides from *Bacillus thermoantarcticus*. Appl Environ Microbiol 62:3265–3269
9. Maugeri TL, Gugliandolo C, Caccamo D, Panico A, Lama L, Gambacorta A, Nicolaus B (2002) A halophilic thermotolerant *Bacillus* isolated from a marine hot spring able to produce a new exopolysaccharide. Biotechnol Lett 24:515–519
10. Maugeri TL, Gugliandolo C, Caccamo D, Stackebrandt E (2001) A polyphasic taxonomic study of thermophilic bacilli from shallow, marine vents. Syst Appl Microbiol 24:572–587
11. Nazina TN, Tourova TP, Poltaraus AB, Novikova EV, Grigoryan AA, Ivanova AE, Lysenko AM, Petrunyaka VV, Osipov GA, Belyaev SS, Ivanov MV (2001) Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus, Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans, G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. Int J Syst Evol Microbiol 51:433–446
12. Nicolaus B, Lama L, Esposito E, Manca MC, Improta R, Bellitti MR, Duckworth AW, Grant WD, Gambacorta A (1999) *Haloarcula* spp. able to biosynthesize exo-endopolymers. J Ind Microbiol Biotechnol 23:489–496
13. Nicolaus B, Lama L, Manca MC, Gambacorta A (1999) Extremophiles: polysaccharides and enzymes degrading polysaccharides. Recent Res Devel Biotechnol Bioeng 2:37–64
14. Nicolaus B, Lama L, Panico A, Schiano Moriello V, Romano I, Gambacorta A (2002) Production and characterization of exopolysaccharides excreted by thermophilic bacteria from shallow, marine hydrothermal vents of flegrean areas (Italy). Syst Appl Microbiol 25:319–325
15. Nicolaus B, Manca MC, Lama L, Esposito E, Gambacorta A (1995) Effects of growth temperature on the polar lipid pattern and fatty acid composition of seven thermophilic isolates from the Antarctic continent. Syst Appl Microbiol 18:32–36
16. Nicolaus B, Panico A, Manca MC, Lama L, Gambacorta A, Maugeri T, Gugliandolo C, Caccamo D (2000) A thermophilic *Bacillus* isolated from an eolian shallow hydrothermal vent, able to produce exopolysaccharides. Syst Appl Microbiol 23:426–432
17. O'Leary WM, Wilkinson SJ (1988) Gram-positive bacteria. In: Ratledge C, Wilkinson SJ (eds) Microbial lipids, vol. I. Academic, New York, pp 117–185
18. Pazur JK (1994) Neutral polysaccharides. In: Chaplin MF, Kennedy F (eds) Carbohydrate analysis, 2nd edn. IRL, Oxford, pp 73–124
19. Perlin AS, Casu B (1982) Spectroscopic methods. In: Aspinal GO (ed) The polysaccharides, vol. 1. Academic, London, pp 133–193
20. Segerer AH, Burggraf S, Fiala G, Huber G, Huber R, Pley U, Stetter KO (1993) Life in hot springs and hydrothermal vents. In: Origin of life and evolution of biosphere. Orig Life Evol Biosph 23:77–90
21. Sutherland IW (1990) The properties and potential of microbial exopolysaccharides. Chim Oggi 9:9–14