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Production of exopolysaccharides by Antarctic marine bacterial isolates

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Abstract: This study was undertaken to examine and characterize Antarctic marine bacterial isolates and the exopolysaccharides (EPS) they produce in laboratory culture.

Methods and Results: Two EPS-producing bacterial strains CAM025 and CAM036 were isolated from particulate material sampled from seawater and sea ice in the southern ocean. Analyses of 16S rDNA sequences placed these isolates in the genus *Pseudoalteromonas*. In batch culture, both strains produced EPS. The yield of EPS produced by CAM025 was 30-fold higher at –2 and 10°C than at 20°C. Crude chemical analyses showed that these EPS were composed primarily of neutral sugars and uronic acids with sulphates. Gas chromatographic analysis of monosaccharides confirmed these gross compositional findings and molar ratios of monosaccharides revealed differences between the two EPS.

Conclusions: The EPS produced by Antarctic bacterial isolates examined in this study appeared to be polyanionic and, therefore, 'sticky' with respect to cations such as trace metals.

Significance and Impact of the Study: As the availability of iron as a trace metal is of critical importance in the southern ocean where it is know to limit primary production, the role of these bacterial EPS in the Antarctic marine environment has important ecological implications.

Keywords: Antarctic marine bacteria, exopolysaccharides, particulate organic material, Pseudoalteromonas spp., sea ice.

INTRODUCTION

The production of exopolysaccharides (EPS) by bacteria in natural systems has been described as a strategy for growth (Costerton 1999). Studies of bacteria growing in aquatic systems, such as marine sediments, aggregates and detrital particles, show that nearly all the cells are surrounded by EPS (Decho 1990, Costerton 1999) and many of these cells are enclosed with adherent biofilms (White 1986).

In the oceans, EPS exuded by phytoplankton and bacteria coalesce to form transparent exopolymer particles (TEP), which range in size from microns to hundreds of microns (Sullivan and Palmisano 1984, Passow and Alldredge 1994). The aggregation of TEP, phytoplankton, bacteria, faecal pellets, zooplankton and other organic debris form larger particles (> 0.5 mm in diameter), which are known as marine snow (Fowler and Knauer 1986). Marine snow has been shown to include highly concentrated and diverse microbial communities Rath et al. (1998) engaged in photosynthesis, microbial decomposition (Biddanda 1988) and remineralization of carbon at elevated levels relative to the surrounding sea water (Smith et al. 1992). Marine snow particles therefore make a significant contribution to carbon cycle in the euphotic zone and to the 'biological pump', which transports fixed carbon to deep waters (Alldredge 2000).

Microbial communities have been found associated with deep-sea hydrothermal vents. These ecosystems are characterised by extremely high temperatures and pressures as well as high concentrations of toxic elements (*e.g.* sulphides and heavy metals). EPS-producing thermophilic and

mesophilic strains have been isolated from vent environments. Several bacterial exopolymers were found to be novel with significant biotechnological potential (Guezennec et al. 1994).

In the Antarctic marine environment, annual sea-ice is a microhabitat form a complex community of marine bacteria often in close association phytoplankton. These assemblages are essential components of carbon and energy transfers in the Southern Ocean (Sullivan and Palmisano 1984). Abundant bacterial populations have been found in thick annual pack ice with psychrophilic bacteria being particularly common in samples of brown ice and pore waters (Delille 1992). Bacterially produced EPS may provide a means by which bacteria can adhere to the microalgal cells (Sullivan and Palmisano 1984). During ice formation microalgal cells are scavenged by sea-ice crystals floating up to the sea surface (Gleitz and Thomas 1993) and bacteria attached to algal cells may be incorporated into new ice in conjunction with some algal species (Grossmann and Dieckmann 1994).

Studies of both the Arctic (Krembs and Engel 2001) and Antarctic (Sullivan and Palmisano 1984) sea-ice communities suggest that exopolymer production by both phytoplankton and bacteria make a significant contribution to organic carbon in the sea-ice and ice-water interface. Sea-ice bacteria maintained in laboratory culture are reported to secrete copious amounts of mucous (Helmke and Weyland 1995). Little is known about EPS produced by Antarctic marine bacteria or about its role in this extreme environment. In this study, bacterial strains were isolated from the Antarctic marine environment. Two of these isolates and the EPS they produce were examined and results are presented. The authors of this study suggested the fungal EPS could

provide a cryoprotective role in the harsh Antarctic environment where the availability of liquid water and temperatures are extremely low.

MATERIALS AND METHODS

Isolation of bacteria

Samples were obtained during the November/December 2001 voyage of RSV *Aurora Australis* (approximate location: 66°52'41"S, 139°41'47"E). Sea-ice pieces were collected with a long handled sieve. Small chunks of approximately 250 ml to 500 ml of bottom ice (distinguished by algal pigment coloration in bottom 1 cm) were melted at 2°C in 500 ml artificial seawater (2.2% w/v, Sigma) and stored at 2°C for two weeks. Upon return to Hobart, aliquots of 200 ml of this liquid were passed through a Nucleopore 0.8 μm filters placed over a glass fibre filter (Schleicher & Schuell), which had been preheated to 450°C for one hour. The 0.8 μm filter was placed in a glass McCartney bottle containing 10 ml seawater nutrient broth (SNB) described previously (Bowman and Nichols 2002). These enrichment cultures containing media and filters were incubated for 24 hrs at 2°C, then mixed and 200 μl aliquots were removed and spread on SNAgar (SNB with 12 g l^{-1} agar added prior to sterilization) and SNAgar + Glucose (2%w/v). Agar plates were incubated at 4°C for four weeks. Additional isolates were obtained from a plankton net (20 μm) trawled through the Southern Ocean (approximate location: 65°32'06"S, 143°10'16"E). Aliquots of 20 μl of material from the cod end of the plankton net were spread onto SNAgar and SNAgar + Glucose (2%w/v) and the plates were incubated at 2°C.

After initial isolations, both strains were subcultured onto marine agar (MA, 1 g yeast extract, Oxoid L21; 5 g bacteriological peptone, Oxoid L37; 32 g artificial sea salts, Sigma S9883; 15 g agar; 1000 ml distilled water) and MA supplemented with 3% (w/v) glucose (MA+Glu). A glucose solution was prepared and autoclaved separately before being combined with MA. Strains were selected if they displayed a mucoid morphology when grown on MA+Glu

Characterization of bacterial isolates

16S rDNA sequence analyses

The 16S rDNA genes of CAM025 and CAM036 were amplified by PCR according to procedures described by (Bowman et al. 1996) using DNA primers 10F and 1519R. The PCR products were purified by Prep-A-Gene purification (Bio-Rad, CA, USA) and the concentration of purified DNA in each sample was measured using a Smart Spec 3000 (Bio-Rad, Regent Park, NSW, Australia). The 16S rDNA sequences were obtained with a Beckman Coulter CEQ 2000 automated sequencer for electrophoresis and data collection after preparation according to protocol specified by manufacturer (Beckman Coulter, Inc, Fullerton, CA, USA).

Sequences were manipulated and aligned using BioEdit v. 5.0.9 (Hall 1999). Sequences were compared to 16S rDNA genes available in the GenBank library by BLAST searching (Atschul et al. 1990) through the National Center for Biotechnology Information (U.S. National Institute of Health) Internet site as described by Bowman et al. (1997). Sequences were aligned to their closest related sequences determined from the BLAST searches. PHYLIP (version 3.57c) (Felsenstein 1993) was used to analyse the sequence data and sequence similarities with the maximum likelihood

algorithm option were determined using DNADIST. Phylogenetic trees were constructed by the neighborliness method with the program NEIGHBOR. The sequence for *Escherichia coli* (J01695) was included as an outgroup. Partial sequences for these two isolates were deposited into GenBank and assigned the following numbers: AY243365 (CAM025) and AY243366 (CAM036).

Whole-cell fatty acid analyses

Growth temperatures may affect whole cell fatty acid profiles, therefore similar incubation temperatures to those used in other studies (Bozal et al. 1997, Bowman 1998) were used in this study so that whole cell fatty acid profiles could be compared. Isolates CAM025 and CAM036 were grown on MA at 12^{o}C for four weeks. Whole cell fatty acids were extracted from cell material according the MIDI protocol (Sasser 1990). Fatty acid methyl esters (FAME) were treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide to convert hydroxy acids to their corresponding trimethylsilyl (TMSi) ethers for analysis by gas chromatography (GC) and GC-mass spectrometry. Double bond position and geometry of monounsaturated FAME were determined after the formation of dimethyl-disulfide (DMDS) adducts prepared according to methods described previously (Nichols et al. 1986). Determination of the *cis* and *trans* geometry in the original monounsaturated FAME was also possible (Skerratt et al. 1991).

GC analyses were performed on a Hewlett Packard 5890A GC fitted with an HP-5 cross-linked methyl silicone fused capillary column (50 m X 0.32 mm i.d.) and flame ionization detector (FID) and an HP 7673A auto sampler. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 50^{o}C. After one minute, the oven temperature was raised

to 150°C at 30°C min^{-1} then to 250°C at 2°C min^{-1} and finally to 300°C at 5°C min^{-1}. GC-MS analysis of the FAME was performed using a Finnigan GCQ Plus GC/MS System fitted with on-column injection set at 45°C. Samples were injected using an AS2000 auto sampler into a retention gap attached to a HP 5 Ultra 2, (50 m X 0.32 mm i.d., and 0.17 μm film thickness column using helium for the carrier gas. The chromatograms and mass spectra were manipulated using Excalibur software. Peaks were identified by comparison to known standards, the library included with the software, and by consideration of the mass spectra.

Fatty acids are designated by the total number of carbon atoms:number of double bonds, followed by the position of the double bond from the terminal (ω) end of the molecule. The suffixes c and t indicate *cis* and *trans* geometry; the prefixes I and a refer to iso and anteiso branching.

EPS production and characterization analysis

Growth of CAM025 and CAM036 in batch cultures for EPS production

A McCartney bottle containing 20 ml Marine broth supplemented with 3 % (w/v) glucose (MB+Glu) was inoculated with approximately ten colonies of either CAM025 or CAM036 from an agar plate (MA+Glu) inoculated ten days earlier and incubated at 20°C. This incubation temperature was chosen as it approximates the optimum growth temperature measured for other *Pseudoalteromonas* strains isolated from the same environment (Bowman 1998). The 20 ml culture was shaken for 24 hr (200 rpm) at 20°C. This 20 ml culture was used to inoculate 200 ml of the same media and the resulting culture was shaken (200 rpm) and incubated at 20°C. After 48 hr, the purity of

this culture was checked by subculturing onto a MA+Glu plate and a 10 ml aliquot was removed to measure pH. The remaining broth was used to inoculate 500 ml MB+Glu (pH 7) in a 2 l Schott bottle.

This 500 ml broth culture was bubbled with compressed air (20 lb in^{-2}). Inlet and outlet air was filtered through a 0.2 μm Midisart filter (Sartorius Australia Pty. Ltd, VIC). Broth cultures were shaken (150 rpm) at 20°C for one week. Purity of this broth was checked by subculturing onto MA+Glu.

The CAM025 isolate was also grown in duplicate 250 ml MB + Glu (pH 8) broth cultures at -2°C, 10°C and 20°C. The baffled flasks were incubated in oscillating water baths (Ratek Pty Ltd, Australia) fitted with refrigeration units, which cooled and circulated antifreeze liquid at the desired temperature. The batch cultures at –2°C, 10°C were harvested after 2 weeks incubation. The cultures at 20°C were harvested after 1 week. The final pH was measured for each culture at time of harvest.

Isolation and purification of CAM025 and CAM036 EPS

Culture broth was centrifuged at 30000 g for 2 hr at 4°C. The cell pellets were freeze-dried and weighed. The supernatants were pressure filtered successively through cellulose nitrate filters with the following pore sizes: 8.0 μm, 3.0 μm, 1.2 μm, 0.8 μm and 0.45 μm (Sartorius Australia Pty. Ltd., VIC). EPS were precipitated from the final filtrate after the addition of cold ethanol (filtrate 60 ml / ethanol 40 ml) and the solution was chilled to 2°C over night. The resulting precipitate was recovered by vacuum filtration through scintered glass. An additional 100 ml cold ethanol was added to the filtrate and the solution was placed at –20°C overnight. The precipitate was recovered as above. The precipitates were washed with 70% to 100% ethanol

– water mixtures. After washing with ethanol, the EPS were combined and dried in a desiccator and stored at room temperature. To remove excess salts, the EPS were redissolved in distilled water and dialyzed (molecular weight cut off of 100,000 daltons, Spectra/Por, Spectrum Laboratories, CA, USA) against distilled water for 2 days at room temperature (approx 25^{o}C). Excess water was removed under vacuum before lyophilization. The EPS were stored at room temperature until analysis.

EPS Characterization

Colorimetric Analyses

Uronic acid content of the EPS was determined by the meta-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen 1973, Filisetti-Cozzi and Carpita 1991). Protein content was determined by the Lowry protein assay (Lowry et al. 1951) with bovine serum albumin as the standard. The total neutral carbohydrate content was determined by the orcinol-sulfuric acid method modified by Rimington (1931).

Monosaccharide analyses

To a solution containing 250 μg total EPS, 50 μg erythritol was added as an internal standard. Samples for GC analysis were prepared in triplicate. The polymer was hydrolysed by the addition of methanolic HCl (3N, Supelco, PA, USA) and heating for 16 hr at 80^{o}C (Kamerling et al. 1975, modified by Montreuil et al. 1986). The monosaccharides were converted to their trimethylsilyl derivatives by the addition of Bis-(trimethylsilyl)trifluoroacetamide:trimethylchlorosilane / 99:1 (Supelco, PA, USA) and pyridine followed by incubation at room temperature for 2 hr. The

samples were then dried under nitrogen and redissolved in dichloromethane prior to analysis.

Gas Chromatography

Analyses of the monosaccharides as trimethylsilyl derivatives were performed on a GC8000 (Fisons, France) gas chromatograph (GC) fitted with an automatic injector, a flame ionization detector (FID) and a CP-Sil-5CB glass capillary column (0.32 mm X 60 m, Chrompac, Varian, France). Hydrogen was the carrier gas. The GC oven was temperature programmed as follows: 50^{o}C for 1 min then an increase of 20^{o}C/min until 120^{o}C, followed by a gradient of 2^{o}C/min until 240^{o}C.

FT-IR Spectroscopy

Pellets for infrared analysis were obtained by grinding a mixture of 2 mg polysaccharide with 200 mg dry KBr, followed by pressing the mixture into a 16 mm diameter mold. The Fourier transform-infrared (FT-IR) spectra were recorded on a Bruker Vector 22 instrument with a resolution of 4 cm^{-1} in the 4000-400 cm^{-1} region. Sulfate content was determined by FT-IR spectroscopy according to the method of Lijour et al. (1994).

NMR Spectroscopy

NMR spectra were obtained on a Bruker AMX-500 (500 MHz for ^{1}H and 125MHz for ^{13}C) at 55°C. Samples were exchanged three times with D_2O with intermediate lyophilization and finally dissolved in 500 µl D_20 to a final concentration close to 30 mg. Chemicals shifts were reported in parts per million relative to sodium 2,2,3,3-d_4-(trimethylsilyl) propanoate for ^{1}H and CDCl3 for ^{13}C NMR spectra.

RESULTS

Isolation and characterization of bacterial isolates

Cultivation of isolates

Bacterial strain CAM025 was isolated from filtered sea-ice particulates, while CAM036 was isolated from diatom-rich trawled material. Both strains showed enhanced growth on MA+Glu and, after 14 days of growth at 20^{o}C on this medium, produced opaque, circular, convex or pulvinate, off-white colonies 1-2 mm in diameter with a mucoid texture and an entire margin. Both were Gram-negative curved rods (2-5 μm X 0.5 μm).

Whole cell fatty acid analyses

Table 1 contains a list of the whole cell fatty acids present in CAM025 and CAM 036 as the percentage of the total fatty acids. Major whole cell fatty acids in CAM025, in decreasing order of abundance, included 17:1ω8c, 16:1ω7c, 16:0, 17:1ω8t, 17:0, 15:0 and 15:1ω8c. For CAM036, the major whole cell fatty acids included 16:1ω7c, 17:1ω8c, 16:0, 16:1ω7t, βOH-12:0, 17:0, and 17:1ω8t. These results are generally consistent with those reported for *Pseudoalteromonas* strains from other studies (Bozal et al. 1997, Bowman 1998.

16S rDNA sequences

Analysis of partial 16S rDNA sequences from CAM025 (1488 base pairs) and CAM036 (1485 base pairs) also indicated that both isolates belonged to the genus *Pseudoalteromonas,* with the closest species *P. haloplanktis* (sequence similarity CAM025: 99.3%, CAM036: 99.4%) and *P.*

nigrifaciens (sequence similarity CAM025: 99.6%, CAM036: 99.8%). Figure 1 shows the phylogenetic relationship of *Pseudoalteromonas* species and two Antarctic isolates (CAM025 and CAM036) based on 16S rDNA sequences. The tree was created using maximum-likelihood distanced clustered by the neighbor-joining method (Bar: 0.1 changes per mean nucleotide position). Numbers in parentheses are GenBank nucleotide accession numbers. Outgroup for the analysis was *Escherichia coli* (J01695). Precise species identification of CAM025 and CAM036 will require further investigation by DNA-DNA hybridization.

Characterization of exopolysaccharides

Colorimetric analyses

The gross chemical composition of bacterial EPS is presented in Table 2. Protein content for EPS from CAM025 and CAM036 was low (2-3%). Neutral sugars dominated in both EPS and accounted for 74% and 50% of EPS from CAM025 and CAM036, respectively. Both EPS contained significant amounts of uronic acids (CAM025 – 22%; CAM036 – 25%) according to colorimetric analyses.

FT-IR analyses

FT-IR spectra of the CAM025 and CAM036 EPS (Figure 2) displayed a broad O-H stretching band above 3000 cm^{-1} and intense absorptions between 1650 and 1050 cm^{-1} characteristic of polysaccharides. An absorbance at approximately 1730 cm^{-1} indicated the presence of carboxyl groups (Lijour et al. 1994). In addition, a small absorption at 1550 cm^{-1}, indicative of either amino sugars or proteins was present in CAM036 EPS, but extremely small in

CAM025 EPS. GC data confirmed the presence of amino sugars in the CAM036 EPS (Table 3) and minor amounts in the CAM025 EPS. Sulfate content in each EPS, determined by the presence of a doublet at 1230-1250 cm-1, was calculated to be 5% for each of the EPS samples (Table 2).

Monosaccharides analyses

Molar ratios of monosaccharides are listed in Table 3. The EPS secreted by the bacterial strain CAM025 was composed of five major monosaccharides with glucose predominating over other neutral sugars (arabinose, galactose and rhamnose). Traces of ribose, fucose and mannose were also present. The presence of galacturonic acid was also substantial, accounting for approximately one-quarter of the monosaccharides in the EPS. The EPS produced by bacterial isolate CAM036 was composed of five monosaccharides with galacturonic acid predominating over the other sugars accounting for one third of the monosaccharides present. Mannose and glucose were the most abundant neutral sugars along with arabinose while galactose was present as traces. N-acetyl galactosamine was a relatively abundant aminyl-sugar in this EPS.

NMR Spectra

From the ^{1}H and ^{13}C NMR spectra of the native EPS, it is possible to extrapolate some information. The ^{1}H spectrum showed essentially five and seven anomeric signals for the EPS produced by isolates CAM025 and CAM036 respectively. The presence in both EPS of acetyl groups linked to different sugars was confirmed by a signal at δ 2.02 ppm. Interestingly, the signals at δ 2.55 ppm and δ 2.68 ppm, indicative of a succinyl group, were

present only for the EPS produced by isolate CAM036. The ^{1}H NMR spectra for the EPS from CAM036 are presented in Figure 3.

EPS yield determinations for CAM025

The average change in pH over the period of incubation of two CAM025 cultures at each of the three temperatures was calculated. After two weeks at –2°C and 10°C, the average decrease of 0.63 and 0.93 pH units respectively, was observed. After one week at 20°C, the average decrease in pH for these two cultures was 1.69 pH units. The final yields of EPS for CAM025 incubated at –2°C, 10°C and 20°C (Figure 4) were calculated by dividing the final weight of the freeze-dried polymer for each culture by the weight of the corresponding freeze-dried cell pellets. Results show that the yield of EPS for CAM025 grown at -2°C and at 10°C was approximately 100 mg EPS per gram dry weight of cells. The yield of EPS for the same strain grown at 20°C was approximately thirty fold lower.

DISCUSSION

The bacterial strain CAM025 was obtained from particles from melted Antarctic sea ice retained on a filter with a pore size of 0.8 μm. CAM036 was isolated from particles captured by a plankton net (20 μm) towed through the Southern Ocean. Both psychrotolerant strains (growth at 4°C and 25°C) displayed an enhanced mucoid morphology on marine agar supplemented with glucose. Results from 16S rDNA sequencing and whole cell fatty acid analyses indicate that these two isolates were closely related and belonged to the genus *Pseudoalteromonas.*

Previous studies have shown that many *Pseudoalteromonas* strains are the psychrotrophic bacteria with a temperature growth range from 4°C to 30°C (Bozal et al. 1997, Bowman 1998), and show optimal growth at 22°C to 25°C (Bowman 1998). Members of this genus are the bacteria most frequently isolated from sea ice and underlying sea water (Bowman et al. 1997, Bowman 1998;Delille 1992). In liquid culture amended with glucose, CAM025 and CAM036 produced EPS, which when chemically analysed by colorimetric techniques were shown to have similarly low amounts of protein and abundant neutral sugars and uronic acids. FT-IR and NMR analyses confirmed the presence of acetyl groups and low amounts of ester sulfate groups in both polysaccharides. Similar biochemical compositions were observed in previous studies of EPS from *Alteromonas* species isolated from hydrothermal vent communities (Rougeaux et al. 1996, Cambon-Bonavita et al. 2002, Raguénès et al. 2003).

Arctic studies (Krembs and Engel 2001, Krembs et al. 2002) have shown that large quantities of microbially produced EPS occur in sea ice and at the ice-water interface. This material was positively correlated to bacterial abundances although diatoms were thought to dominate the EPS production in this system. These authors suggest high concentrations of EPS in the brine channels may provide buffering against harsh winter conditions and high salinity as well as cryoprotect the microbes living there against ice crystal formation. In our study, the EPS yield data suggest that there is a decreased production of EPS at higher temperature (20°C) for the Antarctic sea ice strain tested. This finding supports the proposed hypothesis that EPS

production by psychrotolerant bacteria may play an important role in the sea ice microbial community.

The Arctic sea ice studies (Krembs and Engel 2001, Krembs et al. 2002) also demonstrated that the neutrally buoyant polymeric material was carried large distances by prevailing under-ice currents and ice drifts. Studies in more temperate waters show marine bacterial EPS production plays a major role in the aggregate formation process (Biddanda 1986, Decho 1990). When released into the water column, a combination of biological, chemical and physical forces causes this colloidal material to form aggregates (Alldredge and Jackson 1995, Passow 2000, Kiorboe 2001), which become centers of high microbiological heterotrophic activity (Kiorboe 2001).

Preliminary characterizations show that the structure of the EPS from CAM025 and CAM036 includes sulfate as well as high levels of uronic acids as galacturonic acid, along with acetyl groups. In addition, the EPS from CAM036 was shown by NMR data to include a succinyl group. These features convey an overall polyanionic or ‘sticky’ quality to the EPS in the marine environment, since at the pH of seawater (pH 8.0) many of the acidic groups present on these polymers are ionized (Decho 1990). This ‘stickiness’ is important in terms of the affinity of these EPS for binding to other cations such as dissolved metals (Brown and Lester 1982).

The EPS were subjected to further analytical characterization and based on relative molar ratios of monosaccharides (normalized to arabinose), there were several similarities between these two EPS including the presence of the sole acidic sugar, galacturonic acid in significant proportion. Glucose was a major neutral sugar present in both polysaccharides, albeit to varying

degrees. There were also several differences between the EPS from the two Antarctic strains. Rhamnose and fucose were present in the CAM025 EPS and absent in CAM036 EPS. Mannose accounted for a large proportion in the CAM036 EPS while being present at low levels in the EPS produced by CAM025, based on molar ratios. Another significant difference is the presence of aminyl sugars in only one polysaccharide (CAM036) as determined by both the GC and NMR analyses.

In this study we have demonstrated that two Antarctic marine bacterial isolates; one from Southern Ocean particulate material and the other from melted sea ice, belong to the genus *Pseudoalteromonas* and produce different EPS in laboratory culture. This feature is also found in bacteria from deep-sea hydrothermal vents (Rougeaux et al. 1996, Raguénès et al. 1997) and other aquatic microbial communities (Nicolaus et al. 1999). The EPS produced by Antarctic bacterial isolates examined in this study appear to be polyanionic and, therefore, 'sticky' with respect to cations such as trace metals. The availability of iron as a trace metal is of critical importance in the Southern Ocean where it is know to limit primary production (Scharek et al. 1997). Since 99% of dissolved iron in the ocean is bound to organic ligands (Wu et al. 2001), implications for the role of these bacterial polysaccharides in the Antarctic marine environment require further investigation.

Biotechnological uses for microbially produced EPS include environmental, clinical, nutritional and cosmetic applications, to name a few (Guezennec 2000, Gutnick and Bach 2000, Sutherland 2001). Increased knowledge of the role of Antarctic bacterial EPS will also provide insight into possible commercial uses for these novel polymers.

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FIGURE LEGENDS

Figure 1

Phylogenetic relationship of *Pseudoalteromonas* species and two Antarctic isolates (CAM025 and CAM036) based on 16S rDNA sequences. The tree was created using maximum-likelihood distanced clustered by the neighbor-joining method. Bar 0.1 changes per mean nucleotide position. Numbers in parentheses are GenBank nucleotide accession numbers. Outgroup for the analysis was *Escherichia coli* (J01695).

Figure 2

FT-IR spectra of EPS from CAM025 and CAM036, two Antarctic marine bacteria grown in laboratory culture. Absorbances at 3000-3600cm^{-1} are indicative of OH stretch; 1730 cm^{-1}**,** carboxyl groups; 1650 cm^{-1} and 1050 cm^{-1}**,** polysaccharides; 1550 cm^{-1}, amino sugars and/or proteins**;** 1230-1250 cm^{-1}, sulfate.

Figure 3

^{1}H NMR spectra of EPS produced in laboratory culture by Antarctic marine bacterialisolate, CAM036. Signal at δ 2.02 ppm indicates acetyl group**,** the signals at δ 2.55 ppm and δ 2.68 ppm, indicate a succinyl group.

Figure 4

Yield of EPS (mg EPS per g dry weight of cells) from batch cultures CAM025 incubated at –2^{o}C, 10^{o}C and 20^{o}C.

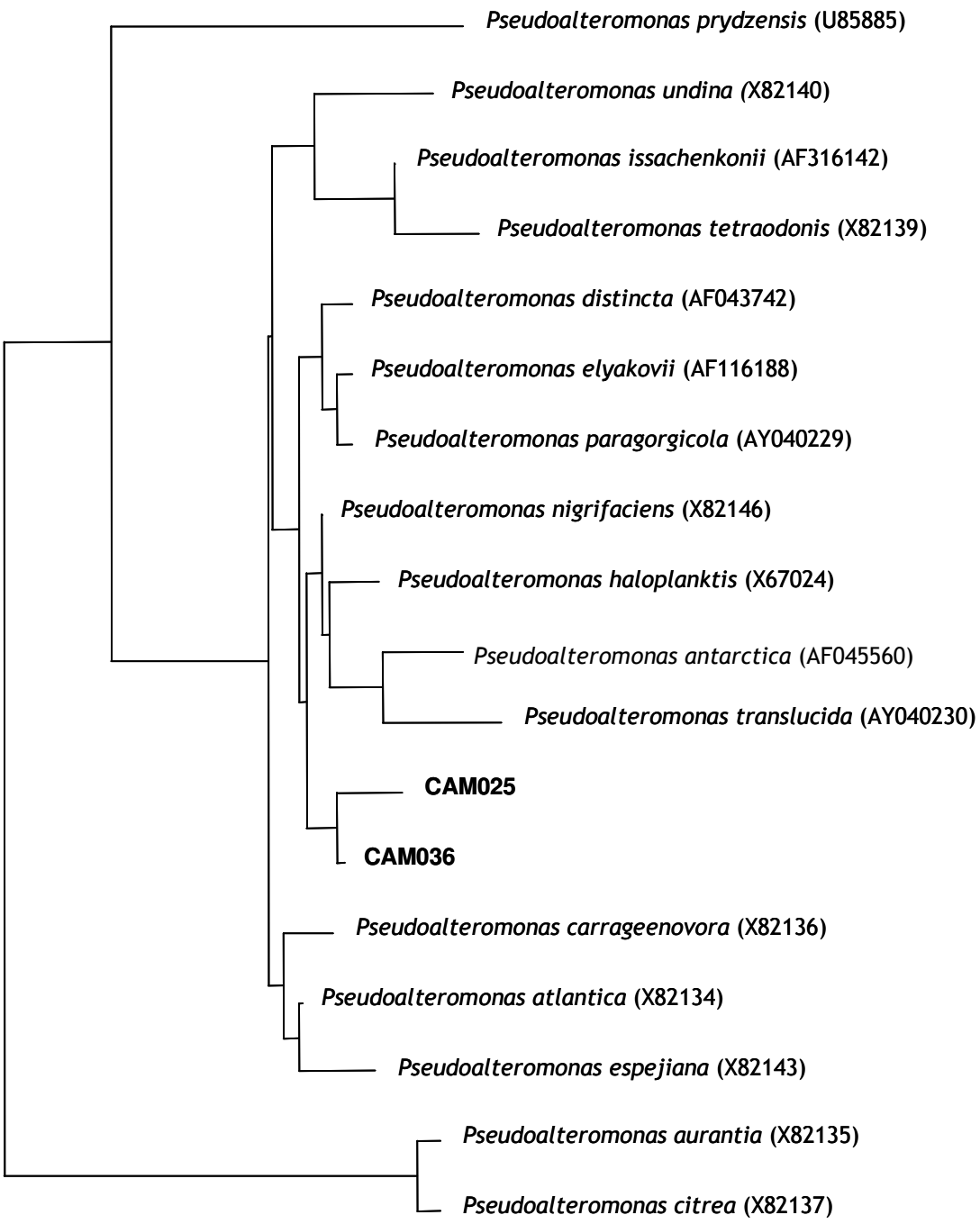
Pseudoalteromonas prydzensis (U85885)
*Pseudoalteromonas undina (*X82140)
Pseudoalteromonas issachenkonii (AF316142)
Pseudoalteromonas tetraodonis (X82139)
Pseudoalteromonas distincta (AF043742)
Pseudoalteromonas elyakovii (AF116188)
Pseudoalteromonas paragorgicola (AY040229)
Pseudoalteromonas nigrifaciens (X82146)
Pseudoalteromonas haloplanktis (X67024)
Pseudoalteromonas antarctica (AF045560)
Pseudoalteromonas translucida (AY040230)
CAM025
CAM036
Pseudoalteromonas carrageenovora (X82136)
Pseudoalteromonas atlantica (X82134)
Pseudoalteromonas espejiana (X82143)
Pseudoalteromonas aurantia (X82135)
Pseudoalteromonas citrea (X82137)

Figure 1

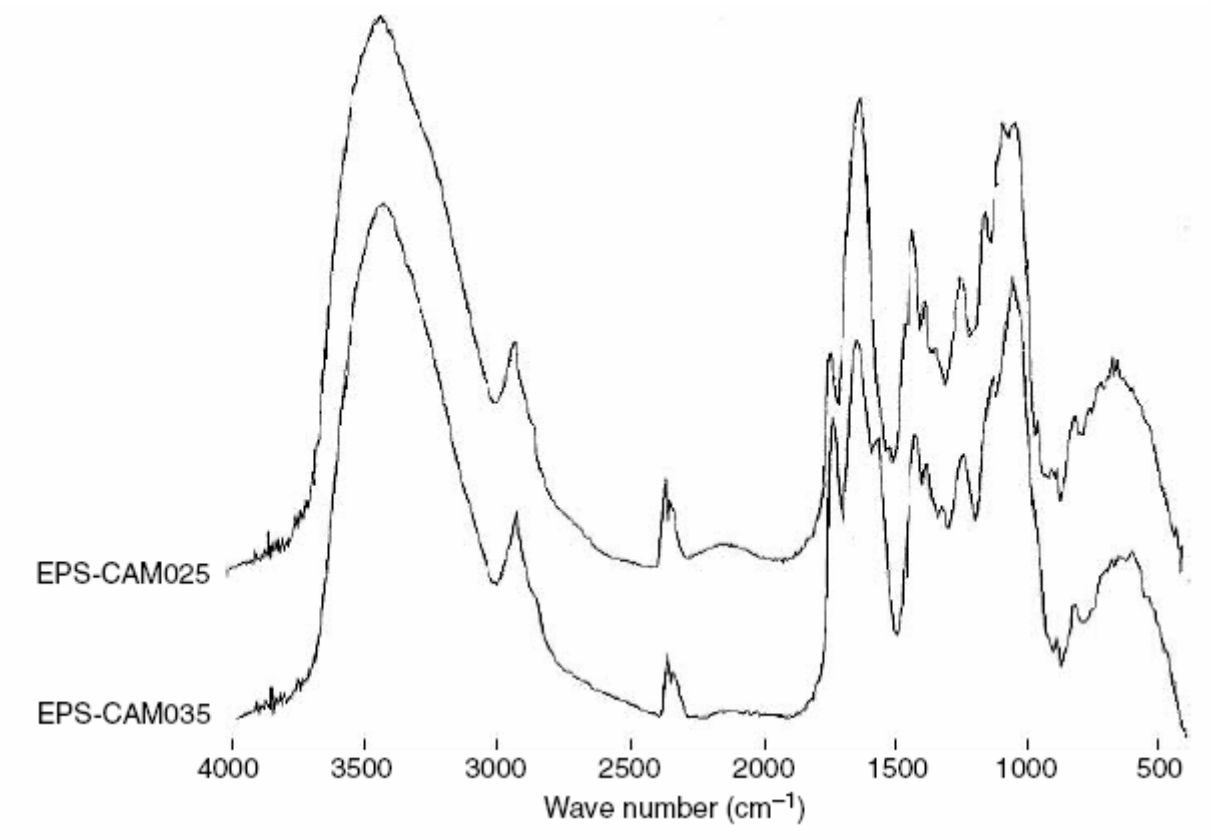

Figure 2

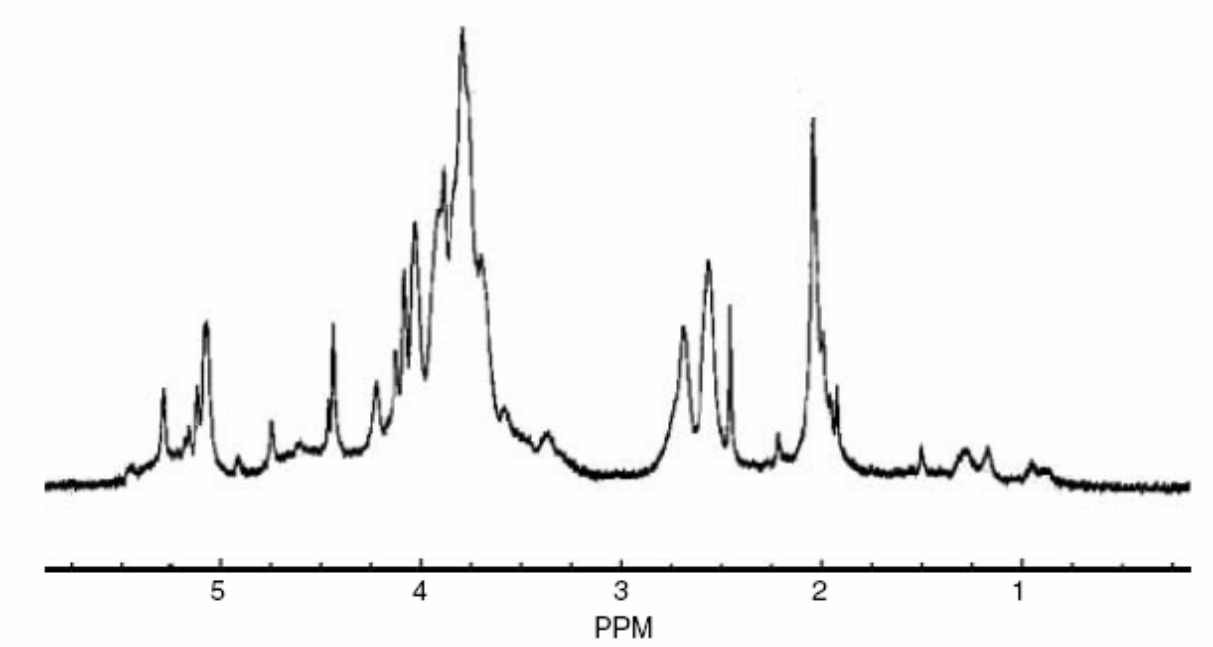

Figure 3

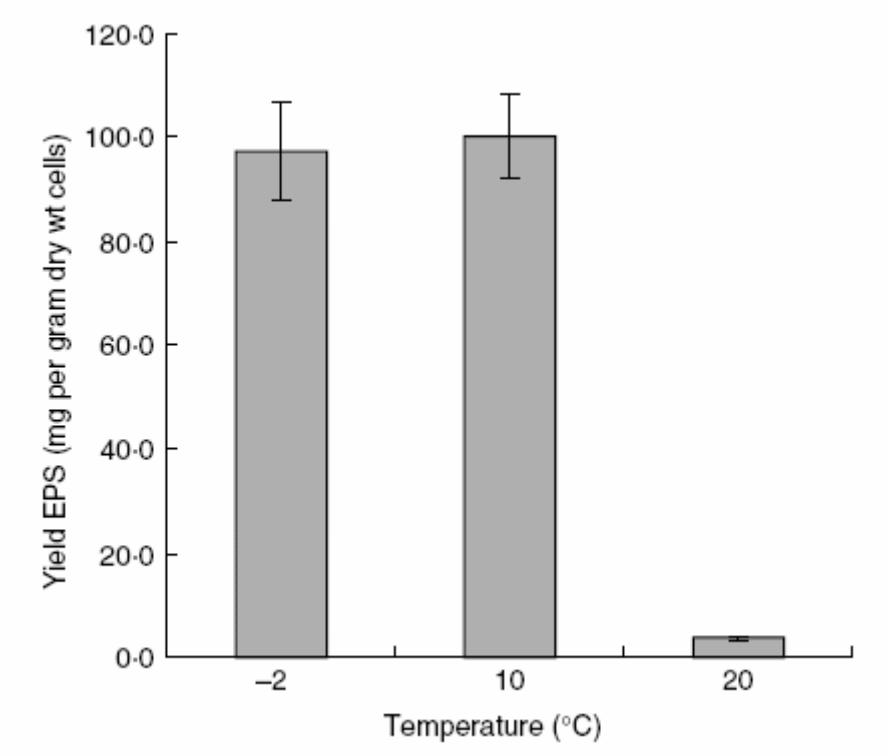

Figure 4

Table 1 Whole-cell fatty acids of two Antarctic marine bacterial isolates* reported as per cent of total area

Fatty acid	CAM025	CAM036
i14:0 + β-OHi12:0	1·5	2·3
14:0	tr	tr
β-OH12:0	3·5	6·5
i15:1	1·9	2·1
15:1ω8c	4·0	3·3
15:1ω8t	1·5	1·8
15:0	4·7	3·3
β-OH 13:0	2·2	3·3
i16:0	tr	tr
16:1ω9c	tr	tr
16:1ω7c	21·4	26·2
16:1ω7t	7·5	6·7
16:0	7·5	7·3
17:1ω8c	29·3	24·2
17:1ω8t	6·8	4·1
17:0	4·8	5·6
18:1ω7c	3·5	3·3
Total	100	100

*Grown at 12°C for 4 weeks on marine agar. tr: %≤0·5% total area.

Table 2 Chemical analysis of exopolysaccharides from two Antarctic bacterial marine isolates (g·100 g^{-1} total EPS)

Isolate	Uronic acids (%)	Neutral sugars (%)	Proteins (%)	Sulphates (%)
CAM025	22	74	2	5
CAM036	25	50	3	5

Table 3 Molar ratios of EPS monosaccharides determined by gas chromatography-flame ionization detection (GC-FID) analysis after acid methanolysis and trimethylsilyl derivatization

	Isolate	
Monosaccharides	CAM025	CAM036
Ara	1·0	1·0
Rib	0·3	0·0
Rha	1·1	0·0
Fuc	0·4	0·0
Gal A	6·4	7·2
Man	0·3	5·8
Gal	1·3	0·4
Glc	12·8	6·1
Gal N Ac	0·1	2·6

Values have been normalized to Ara = 1·0Ara, arabinose; Rib, ribose; Rha, rhamnose; Fuc, fucose; Xyl, xylose; Gal A, galacuronic acid; Glc A, glucuronic acid; Man, mannose; Gal, galactose; Glc, glucose; Gal N Ac, N-acetyl-galactosamine.