Glaciecola chathamensis sp. nov., a novel marine polysaccharide-producing bacterium

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Two novel exopolysaccharide-producing bacteria, strains S18K6^T and S18K5, were isolated from Pacific Ocean sediment. The isolates were Gram-negative, motile, strictly aerobic chemoheterotrophic bacteria. The DNA G + C contents of strains S18K6^T and S18K5 were 44.8 and 46.3 mol%, respectively. DNA–DNA relatedness between the two strains was 70 %. Major fatty acids were hexadecanoic acid (C_{16:0}), hexadecenoic acid (C_{16:1}ω7c) and octadecenoic acid (C_{18:1}ω7c). 16S rRNA gene sequence, chemotaxonomic and morphological data indicated that these strains clearly belonged to the genus *Glaciecola*. Based on phenotypic properties and DNA–DNA hybridization data, strains S18K6^T and S18K5 are considered to represent a novel species of the genus *Glaciecola*, for which the name *Glaciecola chathamensis* sp. nov. is proposed. The type strain is S18K6^T (=JCM 13645^T=NCIMB 14146^T).

There have been many reports of micro-organisms that produce exopolysaccharides (EPSs). Some bacterial polysaccharides are produced on an industrial scale and are used as raw materials in food processing and in medical and industrial preparations. It remains possible that new polysaccharide-producing bacteria will be found in various habitats. In attempts to find new polysaccharide-producing bacteria from ocean bottom sediment we have successfully isolated numerous bacteria that produced polysaccharide in our laboratory. We describe here two polysaccharide-producing bacterial strains that were considered to belong to the genus *Glaciecola*. The genus *Glaciecola* is a member of the *Gammaproteobacteria* (Bowman *et al.*, 1998). The physiological and biochemical features, chemotaxonomic characteristics and phylogeny of the two strains, designated S18K6^T and S18K5, were examined. DNA–DNA relatedness data showed that the strains should be classified as a novel species of the genus *Glaciecola*.

Sediment samples were collected during the R/V *Hakuho-Maru* cruise (KH-05-2) in 2005. Strains S18K6^T and S18K5 were isolated by selective enrichment from a sediment sample taken from the Pacific Ocean floor near Chatham Rise at a water depth of 4627 m (39° 59' 57" S 169° 59' 85"

W). The sediment sample was inoculated in 10 ml mineral salts medium containing (per litre) 5 g peptone, 10 g glucose, 0.008 g Na₂HPO₄, 0.0016 g NH₄NO₃, 23.4 g NaCl, 0.7 g KCl, 10.6 g MgCl₂·6H₂O, 1.1 g CaCl₂, 3.9 g Na₂SO₄, 0.2 g NaHCO₃, 1.0 g (NH₄)₂SO₄, 0.01 g K₂HPO₄ and 6.05 g Tris, pH 7.8. This medium was incubated at 15 °C with shaking at 150 r.p.m. After being incubated for several days, a portion of the suspension was transferred into 10 ml fresh medium and the medium was then re-incubated. After three successive transfers, the suspension was plated onto solid medium to isolate pure cultures. The isolates were checked for their ability to produce EPSs by adding ethanol to the broth supernatant. Of the strains isolated, S18K6^T and S18K5, which showed good EPS production, were selected for further study. To investigate their morphological and physiological characteristics, strains S18K6^T and S18K5 were cultivated aerobically at 15 °C on marine agar 2216 (Difco). Cell morphology was examined via transmission electron microscopy. Cells were fixed with 1 % (v/v) glutaraldehyde and negatively stained with 4 % (w/v) aqueous uranyl acetate and carbon film. Samples were examined under a JEOL JEM-1210 transmission electron microscope.

Strains S18K6^T and S18K5 were also examined for a range of phenotypic properties using standard procedures (Komagata, 1985). Growth at different temperatures (4–40 °C) and pH values (5.0–10.0) was tested using marine broth 2216. Additional biochemical tests with the

Abbreviation: EPS, exopolysaccharide.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of S18K6^T and S18K5 are AB247623 and AB247624, respectively.

API 20NE test kit (bioMérieux) and the Biolog GN MicroPlate method were performed as described by the manufacturers, except that strains were suspended in 3 % NaCl.

Whole-cell fatty acids were extracted from 100 mg freeze-dried cells, which were cultivated on marine broth 2216, and esterified by acid methanolysis. The methyl esters were analysed using a gas–liquid chromatograph equipped with a flame-ionization detector (model GC 353; GL Sciences) and an SP-2560 column (0.25 mm × 100 m, 0.2 µm film; Supelco) at an oven temperature of 140 °C for 15 min. These methyl esters were then identified by comparing their retention times with those of fatty acid methyl ester standards purchased from Supelco and GL Sciences, and by using a GC/MS system (model INCOS 50; Finnigan MAT) connected to a gas–liquid chromatograph (model 3400; Varian).

Fig. 1. Transmission electron micrograph of a negatively stained cell of strain S18K6^T. Bar, 1 µm.

DNA was prepared from bacterial cells according to the method of Marmur (1961). The G + C content of the DNA was determined according to the method of Tamaoka &

Table 1. Phenotypic characteristics of strains S18K6^T and S18K5 and of recognized *Glaciecola* species

Taxa: 1, strain S18K6^T; 2, strain S18K5; 3, *Glaciecola mesophila*; 4, *Glaciecola polaris*; 5, *Glaciecola punicea*; 6, *Glaciecola pallidula*. Data are taken from Bowman *et al.* (1998), Romanenko *et al.* (2003), Van Trappen *et al.* (2004) and this study. Symbols: +, positive; –, negative; V+, variable between strains, type strain positive; V–, variable between strains, type strain negative; W, weak or delayed; ND, not determined. All were positive for the following tests: motility, sodium ion requirement for growth, oxidase, catalase, growth at 7–20 °C and growth in 1–6 % (w/v) NaCl. All were negative for growth at 37 °C, the indole reaction, arginine dihydrolase, and for utilization of citrate, L-arabinose, L-histidine, L-threonine and N-acetylglucosamine.

Characteristic	1	2	3	4	5	6
Pigmentation	None	None	None	None	Pink–red	Pale pink
Growth at 25 °C	+	+	+	+	+	–
Growth in 10 % (w/v) NaCl	+	+	V–	+	–	–
Nitrate reduction	–	–	+	–	–	–
Hydrolysis of:						
Urea	–	–	–	–	W	–
Gelatin	–	+	–	–	–	–
Aesculin	+	+	W	+	V+	–
β-Galactosidase	+	+	+	+	+	–
Production of EPS	+	+	+	W	ND	ND
Utilization of:						
Glycogen	–	+	+	+	–	+
Dextrin	+	+	+	+	–	+
D-Mannitol	+	+	+	+	–	–
Glycerol	–	–	–	–	–	+
Sucrose	+	+	V+	+	–	–
D-Mannose	+	+	V+	+	–	–
L-Rhamnose	+	+	–	–	–	–
D-Fructose, D-trehalose	+	+	V+	+	–	–
L-Leucine	+	+	–	+	–	–
L-Proline	+	+	–	W	+	–
Acetate	–	–	–	+	–	+
L-Glutamate	+	+	V+	+	–	+
DL-Lactate	–	–	–	–	–	+
Succinate	W	W	–	–	+	–
Propionate	W	W	–	+	–	–
DNA G + C content (mol%)	44.8 (HPLC)	46.3 (HPLC)	44.6–44.8 (T _m)	44.2 (HPLC)	44–46 (T _m)	40 (T _m)

Komagata (1984). Levels of DNA–DNA relatedness were determined fluorometrically according to the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microplates.

The 16S rRNA gene was amplified by using the PCR method with primers 9F (5'-GAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCC-3'). The PCR product, approximately 1.5 kb in size, was sequenced by the dideoxynucleotide chain-termination method, using a BigDye terminator cycle sequencing ready kit (version 3.0; Applied Biosystems) and a DNA sequencer (ABI Prism 3100). Primers 9F, 339F, 686F, 1099F and 357R were used in the gene-sequencing reaction. Multiple alignments of the sequences were performed and the nucleotide-substitution rate (K_{nuc} value) was calculated. A phylogenetic tree was constructed according to the neighbour-joining method (Kimura, 1980; Saitou & Nei, 1987) by using the CLUSTAL W program (Thompson *et al.*, 1994). Sequence similarity was calculated by using the GENETYX computer program (Software Development).

Cultural properties, cell morphology (Fig. 1), motility and the results of some physiological tests of strain S18K6^T are given in the species description. Cells were motile with a single polar flagellum. The strains required sodium ions for

growth and grew in 1–10 % NaCl; they grew at 4–30 °C but not at or above 37 °C. The phenotypic properties of strains S18K6^T and S18K5 and reference members of the genus *Glaciecola* are compared in Table 1.

Strains S18K6^T and S18K5 possessed similar whole-cell fatty acid profiles and predominant fatty acids. The fatty acid profile of strain S18K6^T is as follows: 27.2 % C_{16:0}, 22.7 % C_{16:1ω7c}, 14.6 % C_{18:1ω7c}, 9.5 % C_{16:1ω5c}, 8.3 % C_{17:1ω8c}, 4.6 % C_{12:0}, 4.1 % C_{17:0}, 2.8 % C_{15:0}, 2.0 % C_{14:0}, 1.6 % C_{17:1ω6c}, 1.6 % C_{18:1ω5c} and 1.2 % C_{18:0}. The profile of strain S18K5 is as follows: 24.3 % C_{16:0}, 21.1 % C_{16:1ω7c}, 14.0 % C_{18:1ω7c}, 8.9 % C_{16:1ω5c}, 9.5 % C_{17:1ω8c}, 7.3 % C_{12:0}, 4.6 % C_{17:0}, 2.4 % C_{15:0}, 2.4 % C_{14:0}, 2.0 % C_{17:1ω6c}, 1.5 % C_{18:1ω5c} and 1.9 % C_{18:0}. The fatty acid profiles of strains S18K6^T and S18K5 clearly resemble those determined for other marine genera of the *Gammaproteobacteria*, e.g. *Alteromonas*, *Pseudoalteromonas* and *Glaciecola* (Ivanova *et al.*, 2000).

The almost-complete 16S rRNA gene sequence of strain S18K6^T (1496 nt) was compared with all other sequences in the database, and a phylogenetic tree was constructed using related taxa. The phylogenetic tree indicated that strains S18K6^T and S18K5 fall within the evolutionary radius of the genus *Glaciecola* (Fig. 2). 16S rRNA gene sequence

Fig. 2. Phylogenetic tree derived from 16S rRNA gene sequence data of strains S18K6^T and S18K5, recognized species of the genus *Glaciecola* and other related organisms based on the neighbour-joining method. Numbers indicate bootstrap values of greater than 500. Bar, 0.1 K_{nuc} units.

similarities of strain S18K6^T to S18K5, *Glaciecola mesophila* LMG 21855, *G. mesophila* KMM 241^T and *Glaciecola polaris* LMG 21857^T were 99.9, 99.1, 98.7 and 98.5 %, respectively. Accordingly, strains S18K6^T and S18K5 are considered to belong within the genus *Glaciecola*.

Genomic relatedness between strains S18K6^T and S18K5 and their closest phylogenetic relatives, *G. mesophila* LMG 21855, *G. mesophila* KMM 241^T and *G. polaris* LMG 21857^T, was determined by DNA–DNA hybridization experiments. On this basis, strain S18K6^T is closely related to S18K5, having a DNA–DNA reassociation value near 70 %, which is generally accepted as the threshold for species delineation (Wayne *et al.*, 1987). Levels of hybridization between strain S18K6^T and *G. mesophila* LMG 21855, *G. mesophila* KMM 241^T and *G. polaris* LMG 21857^T were 22, 23 and 10 %, respectively. On the basis of the above results, strains S18K6^T and S18K5 were considered to represent a novel species.

The DNA G + C contents of S18K6^T and S18K5, which were determined using an HPLC method, were 44.8 and 46.3 %, respectively. These values are consistent with those of recognized members of the genus *Glaciecola*, which range between 40 and 46 mol% (Bowman *et al.*, 1998).

On the basis of this polyphasic taxonomic analysis, strains S18K6^T and S18K5 are considered to represent a novel species of the genus *Glaciecola*, for which the name *Glaciecola chathamensis* sp. nov. is proposed.

Description of *Glaciecola chathamensis* sp. nov.

Glaciecola chathamensis (chat.ham.en'sis. N.L. fem. adj. *chathamensis* pertaining to Chatham Rise, from where the type strain was isolated).

Aerobic, Gram-negative, oxidase- and catalase-positive, non-pigmented, non-spore-forming, ovoid or curved rod-shaped cells, 1.2–2.0 µm long and 0.6–1.0 µm in diameter, motile by means of a single polar flagellum. On marine agar, forms smooth, convex, non-pigmented colonies. The temperature range for growth is 4–30 °C; no growth occurs at or above 37 °C. Growth is observed on marine agar with up to 10 % (w/v) NaCl, indicating that cells are moderately halophilic and psychrotolerant. The pH range for growth is 5.0–9.0. Produces EPS. Positive for hydrolysis of aesculin, and for activity of β-galactosidase. Able to utilize α-cyclodextrin, dextrin, Tweens 40 and 80, D-fructose, D-galactose, α-D-glucose, myo-inositol, α-D-lactose, maltose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, sucrose, D-trehalose, turanose, L-alanine, methyl pyruvate, L-glutamic acid, L-leucine and L-proline. Negative for indole production, hydrolysis of urea and nitrate reduction.

Negative for arginine dihydrolase activity. Cannot utilize citrate, L-arabinose, L-histidine, L-threonine, N-acetylglucosamine, adonitol, cellobiose, erythritol, L-fucose, D-sorbitol, xylitol, acetic acid, formic acid, D-gluconic acid, itaconic acid, DL-lactic acid, malonic acid, D-alanine, inosine or glycerol. Predominant fatty acids are C_{16:0}, C_{16:1}ω7c and C_{18:1}ω7c.

The DNA G + C content of the type strain is 44.8 % (HPLC method). The type strain, S18K6^T (= JCM 13645^T = NCIMB 14146^T), was isolated from sediment of the Pacific Ocean floor near Chatham Rise.

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