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

Hidetoshi Matsuyama,1 Toshikazu Hirabayashi,1 Hirokazu Kasahara,1 Hideki Minami,2 Tamotsu Hoshino2 and Isao Yumoto3

1,2Department of Bioscience and Technology1 and Department of Marine Science and Technology2, School of Engineering, Hokkaido Tokai University, Minamisawa, Minami-ku, Sapporo 005-8601, Japan
3Research Institute of Genome-based Biofactory, National Institute of Advanced Industrial Science and Technology (AIST), Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan

Two novel exopolysaccharide-producing bacteria, strains S18K6T 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 S18K6T 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 (C16:0), hexadecenoic acid (C16:1ω7c) and octadecenoic acid (C18:1ω7c). 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 S18K6T 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 S18K6T (=JCM 13645T =NCIMB 14146T).

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 (Bowman et al., 1998). The physiological and biochemical features, chemotaxonomic characteristics and phylogeny of the two strains, designated S18K6T 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 S18K6T 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, 0.016 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, 0.016 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, S18K6T and S18K5, which showed good EPS production, were selected for further study. To investigate their morphological and physiological characteristics, strains S18K6T 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 S18K6T 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
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).

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 S18K6T and S18K5 and of recognized Glaciecola species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigmentation</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Pink–red</td>
<td>Pale pink</td>
</tr>
<tr>
<td>Growth at 25 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 10% (w/v) NaCl</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>W</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
<td>V+</td>
<td>-</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Production of EPS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dextrin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>V+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>+</td>
<td>V+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Fructose, D-trehalose</td>
<td>+</td>
<td>+</td>
<td>V+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>L-Proline</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>W</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acetate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>+</td>
<td>+</td>
<td>V+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>DL-Lactate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Succinate</td>
<td>W</td>
<td>W</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Propionate</td>
<td>W</td>
<td>W</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>44.8 (HPLC)</td>
<td>46.3 (HPLC)</td>
<td>44.6–44.8 (Tm)</td>
<td>44.2 (HPLC)</td>
<td>44.4–46 (Tm)</td>
<td>40 (Tm)</td>
</tr>
</tbody>
</table>
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'-AAGGAGGTGATCCAGGCC-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 S18K6T 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% C16:0, 22.7% C16:1ω7c, 14.6% C18:1ω7c, 9.5% C16:1ω5c, 8.3% C17:1ω8c, 4.6% C12:0, 4.1% C17:0, 2.8% C15:0, 2.0% C14:0, 1.6% C17:1ω6c, 1.6% C18:1ω5c and 1.2% C18:0. The profile of strain S18K5 is as follows: 24.3% C16:0, 21.1% C16:1ω7c, 14.0% C18:1ω7c, 8.9% C16:1ω5c, 9.5% C17:1ω8c, 7.3% C12:0, 4.6% C17:0, 2.4% C15:0, 2.4% C14:0, 2.0% C17:1ω6c, 1.5% C18:1ω5c and 1.9% C18:0. 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
similarities of strain S18K6T to S18K5, Glacieola mesophila LMG 21855, G. mesophila KMM 241T and Glacieola polaris LMG 21857T were 99-9, 99-1, 98-7 and 98-5 %, respectively. Accordingly, strains S18K6T and S18K5 are considered to belong within the genus Glacieola.

Genomic relatedness between strains S18K6T and S18K5 and their closest phylogenetic relatives, G. mesophila LMG 21855, G. mesophila KMM 241T and G. polaris LMG 21857T, was determined by DNA–DNA hybridization experiments. On this basis, strain S18K6T 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 S18K6T and G. mesophila LMG 21855, G. mesophila KMM 241T and G. polaris LMG 21857T were 22, 23 and 10 %, respectively. On the basis of the above results, strains S18K6T and S18K5 were considered to represent a novel species.

The DNA G+C contents of S18K6T 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 Glacieola, which range between 40 and 46 mol% (Bowman et al., 1998).

On the basis of this polyphasic taxonomic analysis, strains S18K6T and S18K5 are considered to represent a novel species of the genus Glacieola, for which the name Glacieola chathamensis sp. nov. is proposed.

**Description of Glacieola chathamensis sp. nov.**

Glacieola 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, Tween 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, D-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 C₁₆:0, C₁₆:1ω7c and C₁₈:1ω7c.

The DNA G+C content of the type strain is 44.8 % (HPLC method). The type strain, S18K6T ( = JCM 13645T = NCIMB 14146T), was isolated from sediment of the Pacific Ocean floor near Chatham Rise.

**References**


