Glaciecola arctica sp. nov., isolated from Arctic marine sediment

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A Gram-negative, motile, psychrotolerant, oxidase- and catalase-positive bacterium, designated BSs20135^T, was isolated from Arctic marine sediment. Cells were straight or slightly curved rods and formed circular, convex and yellowish-brown colonies. Buds and prosthecae could be produced. The strain grew at 4–28 °C (optimum 25 °C) and with 1–5 % (w/v) NaCl (optimum 2 %) and hydrolysed aesculin and DNA, but did not reduce nitrate to nitrite. Phylogenetic analysis of 16S rRNA gene sequences indicated that strain BSs20135^T belonged to the genus *Glaciecola* and shared 93.6–97.7 % sequence similarity with the type strains of known species of the genus *Glaciecola*. The major cellular fatty acids of strain BSs20135^T were summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0}, C_{17:1}ω8c and C_{18:1}ω7c. The genomic DNA G + C content was 40.3 mol%. Based on 16S rRNA gene sequence analysis, DNA–DNA hybridization data and phenotypic and chemotaxonomic characterization, strain BSs20135^T represents a novel species, for which the name *Glaciecola arctica* sp. nov. is proposed. The type strain is BSs20135^T (=CCTCC AB 209161^T =KACC 14537^T).

The genus *Glaciecola*, belonging to the class *Gamma-proteobacteria*, was originally proposed by Bowman *et al.* (1998) to accommodate two Gram-negative, psychrophilic, aerobic and seawater-requiring species that were isolated from sea-ice cores collected from coastal areas of eastern Antarctica, i.e. *Glaciecola punicea* (type species) and *Glaciecola pallidula*. Since then, seven more species of this genus have been isolated from diverse marine habitats: *Glaciecola mesophila* from marine invertebrates (Romanenko *et al.*, 2003), *G. polaris* from Arctic seawater (Van Trappen *et al.*, 2004), *G. nitrateducens* from surface seawater (Baik *et al.*, 2006), *G. psychrophila* from Arctic sea-ice (Zhang *et al.*, 2006), *G. chathamensis* from Pacific Ocean sediment (Matsuyama *et al.*, 2006), *G. agarilytica* from marine sediment (Yong *et al.*, 2007) and *G. lipolytica* from coastal surface seawater (Chen *et al.*, 2009). In this study, we report on a novel bacterium, designated BSs20135^T, that was isolated from Arctic marine sediment.

Surface sediment samples (0–5 cm) were collected from the Arctic Ocean (162° 31' 05" W 77° 31' 40" N) at a water

depth of 2200 m during the second Chinese National Arctic Research Expedition cruise of the Chinese icebreaker *Xue Long* into the Canada Basin in August 2003. Samples were stored in sterilized plastic bags (250 ml) and transported to the laboratory at 4 °C. Bacterial strains were isolated from sediment samples and purified as described previously (Zhang *et al.*, 2010) except that sterilized natural Arctic seawater was used. Strain BSs20135^T was routinely cultivated in TYS broth [containing 0.5 % tryptone (Oxoid), 0.1 % yeast extract (Oxoid), artificial seawater (2.75 % NaCl, 0.5 % MgCl₂, 0.2 % MgSO₄, 0.05 % CaCl₂, 0.1 % KCl, 0.0001 % FeSO₄ and distilled water); Smibert & Krieg, 1994; pH 7.0] or on TYS agar (1.5 % agar) and was stored at –80 °C in TYS broth with 16 % (v/v) glycerol. *G. chathamensis* JCM 13645^T (from the Japan Collection of Microorganisms, Saitama, Japan), *G. mesophila* DSM 15026^T and *G. polaris* DSM 16457^T (from DSMZ, Braunschweig, Germany) and *G. psychrophila* 170^T (kept in our laboratory) were used as reference strains in some experiments. The reference strains were routinely cultivated on TYS agar or in TYS broth at 25 °C (12 °C for *G. psychrophila* 170^T).

Genomic DNA extraction and PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Zhang *et al.*, 2010). The obtained 16S

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BSs20135^T is EU365479.

A supplementary table, references and figure are available with the online version of this paper.

rRNA gene sequence of strain BSs20135^T was aligned manually with reference sequences retrieved from GenBank using MEGA version 4.0 (Tamura *et al.*, 2007). The phylogenetic tree was generated using MEGA with the neighbour-joining method (Saitou & Nei, 1987). Evolutionary distances were calculated using the model according to Jukes & Cantor (1969). The topology of the resultant tree was evaluated by bootstrap analysis with 1000 replications (Felsenstein, 1985). Genomic DNA G+C content was determined by the thermal denaturation temperature (T_m) method (Marmur & Doty, 1962) with *Escherichia coli* K-12 genomic DNA as a control. DNA–DNA hybridization experiments were carried out using the thermal denaturation and renaturation method (De Ley *et al.*, 1970) as modified by Huß *et al.* (1983) with a Beckman DU800 spectrophotometer.

Cellular fatty acid analysis was performed using the standard Microbial Identification System (MIDI) at the Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, PR China. The Gram reaction was examined following the non-staining method (Buck, 1982). Motility was examined by light microscopy (CX21; Olympus) using wet mounts. Cell morphology was observed by transmission electron microscopy (JEM-100CX II; JEOL) after cells were negatively stained with 2 % phosphotungstic acid for 5 and 10 s. Colony morphology was observed after incubation on TYS agar at 25 °C for 7–15 days. Growth at 4, 10, 15, 20, 25, 28, 30 and 37 °C was measured in TYS broth. Growth with 0, 1, 2, 3, 4, 5, 6, 8 and 10 % (w/v) NaCl was measured in modified TYS broth. Oxidase activity was determined using commercial oxidase test strips (Merck). Catalase activity was detected by bubble production in a 3 % (v/v) hydrogen peroxide solution. DNase activity was tested using DNase test agar (Oxoid) prepared with artificial seawater. Hydrolysis of casein, starch and Tween 80 was tested on TYS agar supplemented with 1 % (w/v) skimmed milk, 0.2 % (w/v) soluble starch or 1 % (v/v) Tween 80. Susceptibility to antibiotics was tested using commercial filter paper discs with different antibiotics (Tianhe Micro-organism Reagent Co) on TYS agar. Other

enzyme activities and biochemical characteristics were detected using API ZYM, API 20 E, API 20 NE strips (bioMérieux) and GN2 MicroPlates (Biolog) according to the manufacturers' instructions except that cells for inoculation were suspended in artificial seawater. Growth under anaerobic conditions was determined in marine broth 2216 (Difco) for 10 days using an anaerobic chamber (Forma 1029; Thermo Electron).

An almost-complete (1497 nt) 16S rRNA gene sequence of strain BSs20135^T was obtained. Analysis revealed that strain BSs20135^T was affiliated with the genus *Glaciecola*. The isolate showed the highest 16S rRNA gene sequence similarity to *G. psychrophila* 170^T (97.7 %), *G. mesophila* KMM 241^T (97.4 %) and *G. polaris* LMG 21857^T (97.1 %); lower 16S rRNA gene sequence similarities (93.6–96.5 %) were observed with the other members of the genus *Glaciecola*. In the neighbour-joining tree (Fig. 1), strain BSs20135^T and *G. psychrophila* 170^T formed a coherent cluster within the genus *Glaciecola*, which was supported by a high bootstrap level (99 %). DNA–DNA relatedness between strain BSs20135^T and *G. psychrophila* 170^T, *G. mesophila* DSM 15026^T and *G. polaris* DSM 16457^T was 39.3, 37.5 and 28.1 %, respectively. These values were below the 70 % cut-off for species discrimination, which indicated that the isolate represented a novel species in the genus *Glaciecola* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

The DNA G+C content of strain BSs20135^T was 40.3 mol%, which was within the range of G+C contents reported for the genus *Glaciecola* (40–45 mol%). The major fatty acids of strain BSs20135^T were summed feature 3 (comprising $C_{16:1}\omega 7c$ and/or iso- $C_{15:0}$ 2-OH; 40.7 %), $C_{16:0}$ (24.6 %), $C_{17:1}\omega 8c$ (10.0 %) and $C_{18:1}\omega 7c$ (7.5 %), which were similar to those of known members of the genus *Glaciecola* (Supplementary Table S1, available in IJSEM Online).

Cells of strain BSs20135^T were Gram-negative, straight or slightly curved rods and could produce buds and prosthecae (Supplementary Fig. S1). The phenotypic

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain BSs20135^T among members of the genus *Glaciecola* and some other related genera. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

characteristics of strain BSs20135^T are given in the species description and Table 1. Strain BSs20135^T shared some phenotypic characteristics with other members of the genus *Glaciecola*, such as the presence of oxidase and catalase, no growth at or above 37 °C, the requirement of sodium ions for growth and the ability to produce buds and prosthecae. However, strain BSs20135^T could be phenotypically differentiated from other members of the genus *Glaciecola* by a combination of phenotypic characteristics, such as range and optimal temperature for growth, growth with 6 % (w/v) NaCl, nitrate reduction, hydrolysis of different substrates and carbon-source utilization pattern, as shown in Table 1.

In total, phylogenetic analysis of 16S rRNA gene sequences, phenotypic and chemotaxonomic characteristics and DNA–DNA relatedness indicate that strain BSs20135^T should be assigned to the genus *Glaciecola* as a representative of a novel species, for which the name *Glaciecola arctica* sp. nov. is proposed.

Description of *Glaciecola arctica* sp. nov.

Glaciecola arctica (arc'ti.ca. L. fem. adj. *arctica* northern, from the Arctic).

Cells are Gram-negative, straight or slightly curved rods (0.4–0.8 × 1.3–4.8 µm). Motile by a single polar flagellum. Buds and prosthecae can be formed. Colonies on TYS agar are circular, convex and smooth with entire edges. In TYS broth, cell aggregates are yellowish-brown. Oxidase- and catalase-positive. Grows at 4–28 °C (optimum 25 °C) and with 1–5 % (w/v) NaCl (optimum 2 % NaCl); NaCl is required for growth. Hydrolyses aesculin and DNA, but does not hydrolyse casein, Tween 80 or starch (API 20 NE). Does not reduce nitrate to nitrite (API 20 NE). Does not produce indole, acetoin (Voges–Proskauer reaction) or H₂S (API 20 E). Produces alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, esterase (C4) (weak), esterase lipase (C8) (weak), cystine arylamidase (weak) and

Table 1. Differential characteristics of strain BSs20135^T and members of the genus *Glaciecola*

Strains: 1, *Glaciecola arctica* sp. nov. BSs20135^T; 2, *G. psychrophila* 170^T; 3, *G. mesophila* DSM 15026^T; 4, *G. polaris* DSM 16457^T (data for columns 1–4 were taken from this study); 5, *G. chathamensis* JCM 13645^T (Matsuyama *et al.*, 2006); 6, *G. agarilytica* LMG 23762^T (Yong *et al.*, 2007); 7, *G. lipolytica* JCM 15139^T (Chen *et al.*, 2009); 8, *G. nitratireducens* FR 1064^T (Baik *et al.*, 2006); 9, *G. pallidula* ACAM 615^T (Bowman *et al.*, 1998); 10, *G. punicea* ACAM 611^T (Bowman *et al.*, 1998). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Flagellation	Polar, 1	Polar, 1	Polar, 1*	Polar, subpolar†	Polar, 1	Polar, 1	Polar, 1	ND	ND	ND
Buds and prosthecae	+	+	+	+	+	ND	ND	ND	+	+
Temperature for growth (°C)										
Range	4–28	4–15	4–35	4–30	4–30	7–30	4–36	15–30	–2–20	–2–20
Optimum	25	10–12	28–30	25	20‡	ND	30	25	10–15	15
Growth with 6 % (w/v) NaCl	–	+	+	+	+	+	+	+	ND	ND
Nitrate reduction	–	–	–	–	–	–	+	+	–	–
Hydrolysis of:										
Aesculin	+	+	+	+	+	+	+	–	–	+
Urea	–	–	–	–	–	ND	+	–	–	–
DNA	+	+	+	+	+	+	ND	ND	–	–
Starch	–	+	+	+	+	+	+	ND	+	–
Tween 80	–	+	+	+	+	ND	+	–	+	–
Utilization of:										
Glucose	–	+	+	+	+	–	+	–	–	–
Arabinose	–	–	–	–	–	ND	+	+	–	–
Mannose	–	–	–	+	+	+	+	+	–	–
Mannitol	–	–	+	+	+	+	+	–	–	–
N-Acetylglucosamine	–	+	–	–	–	ND	+	+	–	–
Maltose	–	+	+	+	+	–	+	+	–	–
DNA G+C content (mol%)	40.3	42.9	44.8*	44.2†	44.8	45	40.8	44	40.3	43.9

*Data from Romanenko *et al.* (2003).

†Data from Van Trappen *et al.* (2004).

‡Data from this study.

§Sea-salt concentration.

||Data from Zhang *et al.* (2006).

trypsin (weak), but not lipase (C14), α -chymotrypsin, α - or β -galactosidase, β -glucuronidase, α - or β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase or β -fucosidase (API ZYM). Positive for acid production from glucose, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, gelatinase, utilization of citrate and acid production from mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose (API 20 E). Does not assimilate glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, decanoate, adipic acid, malic acid, citrate or phenylacetic acid (API 20 NE). No reactions are observed with GN2 MicroPlates after 7 days at 25 °C. Resistant to tetracycline (30 µg per disc); sensitive to (µg per disc) gentamicin (10), chloromycetin (30), erythromycin (15), kanamycin (30), streptomycin (10), carbenicillin (100), amikacin (30), polymyxin (30) and vancomycin (30). The major cellular fatty acids are summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0}, C_{17:1}ω8c and C_{18:1}ω7c. The DNA G+C content of the type strain is 40.3 mol%.

The type strain, BSs20135^T (=CCTCC AB 209161^T =KACC 14537^T), was isolated from Arctic sediment.

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