Flavobacterium glaciei sp. nov., a novel psychrophilic bacterium isolated from the China No.1 glacier

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A novel psychrophilic, yellow-pigmented and obligate aerobic bacterium, strain 0499^T, was isolated from the China No.1 glacier. Strain 0499^T displayed the common phenotypic and chemotaxonomic features of the genus *Flavobacterium*, containing menaquinone-6 (MK-6) as the major quinone and C_{15:0}, iso-C_{15:0}, C_{17:1ω6c} and summed feature 3 (C_{16:1ω7c}/iso-C_{15:0} 2-OH) as the major fatty acids. Optimal growth occurred at 21 °C. The genomic DNA G + C content was 36.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain 0499^T was related to members of the genus *Flavobacterium*, sharing the highest sequence similarities with *Flavobacterium succinicans* (97.9%), *Flavobacterium granuli* (97.4%) and *Flavobacterium hydatidis* (97.2%). On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, a novel species *Flavobacterium glaciei* is proposed with strain 0499^T (=CGMCC 1.5380^T=JCM 13953^T) as the type strain.

The genus *Flavobacterium* was proposed by Bergey *et al.* (1923). Since then, the description of the genus has been emended several times (Bernardet *et al.*, 1996). It currently accommodates Gram-negative, non-spore-forming, yellow-pigmented and rod-shaped bacteria that are usually motile by gliding, contain menaquinone 6 (MK-6) as the sole respiratory quinone and have a DNA G + C content in the range of 32–37 mol%. At the time of writing, the genus *Flavobacterium* comprises 37 recognized species (Aslam *et al.*, 2005; Horn *et al.*, 2005; Nogi *et al.*, 2005; Van Trappen *et al.*, 2005; Yi *et al.*, 2005; Wang *et al.*, 2006; Yoon *et al.*, 2006). Members of the genus *Flavobacterium* have been isolated from a wide range of temperate habitats including diseased fish, freshwater and river sediments, seawater and marine sediments, soil and microbial mats. A number of cold-adapted *Flavobacterium* species have also been isolated from glaciers, sea ice and Antarctic lakes.

During a survey of psychrophilic organisms from the China No.1 glacier, located in Xinjiang Uygur Autonomous Region, north-west China, we isolated a novel psychrophilic bacterial strain. Physiological and biochemical features,

cellular fatty acid content, phylogenetic analysis based on the 16S rRNA gene sequence and DNA–DNA hybridization data indicated that the new isolate represents a novel species in the genus *Flavobacterium*.

Strain 0499^T was isolated from frozen soil collected from the China No.1 glacier using previously described media and methods (Zhu *et al.*, 2003). The strain was routinely grown aerobically at 21 °C on PYG medium containing the following components (l⁻¹): 5 g bacto peptone (Difco), 0.2 g yeast extract (Oxoid), 5 g glucose, 3 g beef extract (Oxoid), 0.5 g NaCl and 1.5 g MgSO₄·7H₂O (pH adjusted to 7.0). *Flavobacterium succinicans* DSM 4002^T and *Flavobacterium hydatidis* NBRC 14958^T were obtained from DSMZ and NBRC, respectively. *Flavobacterium granuli* KCTC 12201^T was kindly provided by Dr Sung-Taik Lee. These strains were cultivated as recommended (Bernardet *et al.*, 1996; Aslam *et al.*, 2005) and used as reference strains.

DNA was extracted and purified as described by Sambrook *et al.* (1989). The gene encoding 16S rRNA was amplified by PCR with the forward primer 5'-AGAGTTTGATCCTGG-CTCAG-3' and reverse primer 5'-AAGGAGGTGATCCA-GCCGCA-3' (Liu *et al.*, 2000). The PCR product was sequenced using the ABI BigDye 3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (ABI3730; Applied Biosystems). BLASTn searches with the nearly complete (1424 bp) 16S rRNA gene sequence of strain 0499^T

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Flavobacterium glaciei* sp. nov. strain 0499^T is DQ515962.

A table detailing the cellular fatty acid contents of *Flavobacterium glaciei* sp. nov. and related taxa is available as supplementary material in IJSEM Online.

performed in GenBank and EMBL revealed that the novel isolate shared high sequence similarity ($\sim 97.9\%$) with members of the genus *Flavobacterium*. Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony algorithms with Kimura's two-parameter model (Kimura, 1980) implemented in MEGA version 3.0 (Kumar *et al.*, 2004). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates. Phylogenetic analysis (Fig. 1) based on a consensus 1145 bp length of 16S rRNA gene sequences showed that strain 0499^T grouped with members of the genus *Flavobacterium* and formed a distinct cluster with *F. succinicans* DSM 4002^T (97.9%), *F. granuli* KCTC 12201^T (97.4%) and *F. hydatis* NBRC 14958^T (97.2%). Very similar tree topologies were obtained using the two different algorithms.

Colony morphology was observed on PYG medium after incubation at 21 °C for 24–48 h. Cell morphology was examined under a light microscope (BH-2; Olympus) using bacteria grown under the same conditions. The temperature range for growth was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined in PYG medium adjusted to various pH values with HCl or NaOH (1 M l⁻¹). Catalase, oxidase, arginine dihydrolase, lysine decarboxylase,

ornithine decarboxylase, lecithinase and tryptophan deaminase activities, the Voges–Proskauer and the Simmons' citrate tests and the utilization of various substrates as sole carbon and energy source were examined according to Dong & Cai (2001). Acid production from carbohydrates was determined as described by Leifson (1963). Anaerobic growth was investigated in PYG medium in an anaerobic test tube filled with nitrogen gas. The presence of gliding motility was determined as described by Zhu *et al.* (2003). Congo red adsorption was tested by directly flooding some colonies on agar plates with 0.01% aqueous Congo red solution. The detection of flexirubin-type pigments using 20% (w/v) KOH was performed according to Reichenbach (1989). Other physiological tests were conducted as described by Wang *et al.* (2006). Cells of strain 0499^T were rod-shaped, Gram-negative, non-flagellated and non-gliding. Colonies on PYG agar were yellow, smooth, circular and convex with entire margins. Strain 0499^T was distinguished from its nearest phylogenetic relatives, *F. succinicans*, *F. granuli* and *F. hydatis* by the following phenotypic characteristics: (i) strain 0499^T was an obligate aerobic and non-gliding bacterium, whereas both *F. succinicans* and *F. hydatis* were facultatively anaerobic and motile; (ii) strain 0499^T grew weakly on agar at 25 °C, whereas the optimal growth temperature of its phylogenetic relatives was 25–30 °C; (iii) strain 0499^T did not produce

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain 0499^T and related species. The tree was rooted with *Leeuwenhoekiella marinoflava*. Numbers at nodes represent the bootstrap values (% of 1000 resampled datasets) greater than 50%. GenBank accession numbers are given in parentheses. Bar, 1% sequence divergence.

β -galactosidase, contrary to its phylogenetic relatives. Other phenotypic features that differentiated strain 0499^T from its phylogenetic relatives and from some other psychrophilic *Flavobacterium* species are given in the species description and Table 1.

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989) using menaquinone 6 (MK-6) from *F. granuli* KCTC 12201^T as a reference. Cellular fatty acids were determined from a culture grown in PYG at 21 °C for 3 days and were extracted, methylated and analysed using the standard MIDI (Microbial Identification) procedure (Sasser, 1990). Cells of strain 0499^T contained menaquinone 6 (MK-6) as the major respiratory quinone. The predominant cellular fatty acids of strain 0499^T were C_{15:0} (13.9%), summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH, 10.0%), C_{17:1}ω6c (9.5%), anteiso-C_{15:0} (8.3%), iso-C_{15:0} (8.2%), iso-C_{15:1} (6.2%), iso-C_{15:0} 3-OH (5.2%) and C_{15:1}ω6c (5.2%). The fatty acid profile of strain 0499^T resembled those of other *Flavobacterium* species (Bernardet *et al.*, 1996). Strain 0499^T also contained the unsaturated fatty acid C_{17:1}ω8c

(3.0%) and the hydroxylated fatty acid C_{17:0} 3-OH (1.4%) that are not commonly found in other *Flavobacterium* species. Detailed fatty acid profiles for strain 0499^T, its closest phylogenetic relatives and some other psychrophilic *Flavobacterium* species are available in Supplementary Table S1 in IJSEM Online.

The DNA G + C content of strain 0499^T was determined using the thermal denaturation method (Sly *et al.*, 1986). DNA–DNA hybridization experiments were performed at 65.6 °C using the liquid renaturation method (De Ley *et al.*, 1970) as modified by Huß *et al.* (1983). Both experiments were carried out using a DU800 spectrophotometer (Beckman). The DNA G + C content of strain 0499^T was 36.5 mol%. DNA–DNA relatedness between strain 0499^T and *F. succinicans* DSM 4002^T, *F. granuli* KCTC 12201^T and *F. hydatidis* NBRC 14958^T was 48.4%, 44.1% and 31.9%, respectively.

Based on phenotypic, chemotaxonomic and molecular data, it is concluded that strain 0499^T represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium glaciei* sp. nov. is proposed.

Table 1. Differential characteristics of *Flavobacterium glaciei*, close phylogenetic relatives and some psychrophilic *Flavobacterium* species

Taxa: 1. *F. glaciei* 0499^T; 2. *F. succinicans* DSM 4002^T (Bernardet *et al.*, 1996); 3. *F. granuli* KCTC 12201^T (Aslam *et al.*, 2005); 4. *F. hydatidis* NBRC 14958^T (Bernardet *et al.*, 1996); 5. *Flavobacterium xinjiangense* JCM 11314^T (Zhu *et al.*, 2003); 6. *Flavobacterium omnivorum* JCM 11313^T (Zhu *et al.*, 2003); 7. *Flavobacterium gillisiae* ACAM 601^T (McCammon *et al.*, 2000); 8. *Flavobacterium tegetincola* ACAM 602^T (McCammon *et al.*, 2000); 9. *Flavobacterium gelidilacus* LMG 21477^T (Van Trappen *et al.*, 2003); 10. *Flavobacterium frigidimarum* DSM 15937^T (Nogi *et al.*, 2005); 11. *Flavobacterium antarcticum* JCM 12383^T (Yi *et al.*, 2005). +, Positive; –, negative; (+) weakly positive; v, variable among strains; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Growth on:											
Nutrient agar	+	+	+	+	(+)	+	+	+	+	+	+
Seawater agar	–	–	–	–	–	–	+	+	+	+	+
Trypticase soy agar	+	+	+	+	–	+	+	+	+	+	+
Growth at 25 °C	(+)	+	+	+	–	–	(+)	(+)	(+)	(+)	(+)
Gliding motility	–	+	–	+	–	–	–	+	–	+	–
Flexirubin-type pigments	–	–	–	+	–	–	–	–	–	+	–
Acid from carbohydrates	–	+	–	+	–	–	+	+	–	+	+
Degradation of:											
Aesculin	+	+	ND	+	+	+	+	–	–	+	–
Casein	+	+	–	+	+	+	+	–	+	+	+
Chitin	–	–	–	(+)	+	+	+	–	–	+	–
DNA	–	+	–	+	–	–	–	–	–	ND	+
Gelatin	+	(+)	–	+	+	–	–	–	+	+	+
Pectin	–	ND	ND	+	–	+	–	–	–	+	–
Starch	+	+	–	+	–	+	+	–	+	+	–
Tyrosine	–	–	–	+	–	–	–	–	–	ND	–
H ₂ S production	–	+	–	–	+	–	–	–	–	–	–
Nitrate reduction	+	v	–	+	–	+	–	–	–	–	–
β -Galactosidase activity	–	+	+	+	–	+	–	–	–	+	–
Mean DNA G+C content (mol%)	36	36	36	34	34	35	32	32	30	34	38

Description of *Flavobacterium glaciei* sp. nov.

Flavobacterium glaciei (gla.ci'ei. L. gen. n. *glaciei* of ice, referring to the isolation source, the China No.1 glacier).

Cells are Gram-negative rods, non-flagellated and non-gliding, 0.45–0.55 µm wide and 2.7–6.3 µm long. Colonies are yellow, smooth, circular and convex with entire margins and do not absorb Congo red. Psychrophilic and obligately aerobic. Produce catalase and cytochrome oxidase. Growth occurs at 4–25 °C and pH 6.0–9.0, with optimum growth at 21 °C and approximately pH 6.5–7.5. Growth occurs in the presence of 0–1 % (w/v) NaCl. Nitrate is reduced. Cells do not contain flexirubin-type pigments. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lecithinase and tryptophan deaminase activities are absent. Simmons' citrate and Voges–Proskauer tests are negative. Hydrolyses gelatin, casein, starch and aesculin, but not agar, alginate, carboxymethylcellulose, chitin, pectin, DNA or urea. Acid and gas are not produced from carbohydrates. The following substrates are utilized as sole carbon source: D-glucose, maltose, sucrose, D-trehalose, D-mannose, dextrin, proline, L-glutamic acid and glucose 1-phosphate. The following substrates are not utilized as sole carbon source: fructose, fucose, D-galactose, L-arabinose, D-cellobiose, L-rhamnose, lactose, raffinose, D-melibiose, D-sorbitol, turanose, xylitol, glycerol, inositol, erythritol, serine, threonine, alanine, histidine, leucine, aspartic acid, malonic acid, lactic acid, acetic acid, citric acid, pyruvate, succinate or uridine. Cells contain menaquinone 6 (MK-6). The cellular fatty acids are C_{15:0} (13.9 %), summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH, 10.0 %), C_{17:1}ω6c (9.5 %), anteiso-C_{15:0} (8.3 %), iso-C_{15:0} (8.2 %), iso-C_{15:1} (6.2 %), iso-C_{15:0} 3-OH (5.2 %), C_{15:1}ω6c (5.2 %), iso-C_{17:0} 3-OH (4.2 %), C_{17:1}ω8c (3.0 %), iso-C_{16:0} 3-OH (3.0 %), iso-C_{17:1}ω9c (2.7 %), C_{16:0} (2.1 %), C_{15:0} 3-OH (2.0 %), C_{16:0} 3-OH (1.7 %), anteiso-C_{15:1} (1.4 %) and C_{17:0} 3-OH (1.4 %). The G + C content of the DNA is 36.5 mol%.

The type strain, 0499^T (= CGMCC 1.5380^T = JCM 13953^T) was isolated from the China No.1 glacier in the Xinjiang Uygur Autonomous Region.

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