Flavobacterium xueshanense sp. nov. and *Flavobacterium urumqiense* sp. nov., two psychrophilic bacteria isolated from glacier ice

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Two Gram-stain-negative, rod-shaped bacteria, designated strains Sr22^T and Sr25^T, were isolated from water of melted ice from the China No.1 glacier, Xinjiang Uygur Autonomous Region, China. Cells formed yellow, circular, convex colonies. 16S rRNA gene sequence analysis indicated that strains Sr22^T and Sr25^T belong to the genus *Flavobacterium*, sharing ≤ 99.1 and ≤ 99.6 % similarity, respectively, with the type strains of recognized species of the genus. Strain Sr22^T shared highest 16S rRNA gene sequence similarity with *Flavobacterium tiangeerense* CGMCC 1.6847^T (98.6 %), *Flavobacterium fryxellicola* LMG 22022^T (98.1 %) and *Flavobacterium omnivorum* CGMCC 1.2747^T (99.1 %). Strain Sr25^T shared highest similarity with *Flavobacterium sinopsychrotolerans* CGMCC 1.8704^T (98.5 %), *Flavobacterium degerlachei* NBRC 102677^T (98.4 %) and *Flavobacterium xinjiangense* CGMCC 1.2749^T (99.5 %). The predominant fatty acids of strain Sr22^T were iso-C_{15:1} G (6.01 %), iso-C_{15:0} (8.93 %), iso-C_{16:1} H (12.68 %), iso-C_{16:0} (10.4 %), C_{15:1}ω6c (8.97 %), C_{17:1}ω6c (5.96 %), iso-C_{16:0} 3-OH (11.14 %) and summed feature 3 (comprising C_{16:1}ω7c and/or C_{16:1}ω6c, 12.33 %). The major fatty acids of strain Sr25^T were iso-C_{15:0} (10.8 %), anteiso-C_{15:0} (5.23 %), C_{15:1}ω6c (11.79 %), C_{17:1}ω6c (5.43 %), iso-C_{16:0} 3-OH (7.04 %) and summed feature 3 (20.42 %). The genomic DNA G+C contents of strains Sr22^T and Sr25^T were 37.2 and 35.1 mol%. On the basis of differential phenotypic and phylogenetic characteristics, these strains are considered to represent two novel species of the genus *Flavobacterium*, for which the names *Flavobacterium xueshanense* sp. nov. (type strain Sr22^T = CGMCC 1.9227^T = NBRC 106479^T) and *Flavobacterium urumqiense* sp. nov. (type strain Sr25^T = CGMCC 1.9230^T = NBRC 106480^T) are proposed.

The genus *Flavobacterium* belongs to the phylum *Bacteroidetes* (formerly the *Cytophaga–Flavobacterium–Bacteroides* group) (Bernardet *et al.*, 2002). It was proposed by Bergey *et al.* (1923) and the description was revised by Bernardet *et al.* (1996). Members of the genus *Flavobacterium* are widespread and have been isolated from a wide range of habitats such as freshwater sediments (Tamaki *et al.*, 2003), glacier ice (Zhu *et al.*, 2003), Antarctic habitats (McCammon & Bowman, 2000), soil (Yoon *et al.*, 2006) and diseased fish (Bernardet & Bowman, 2006). Recently, several bacteria isolated from the China No.1 glacier have been added to the genus *Flavobacterium*, such as *Flavobacterium xinjiangense* and

Flavobacterium omnivorum (Zhu *et al.*, 2003), *Flavobacterium glaciei* (Zhang *et al.*, 2006) and *Flavobacterium tiangeerense* (Xin *et al.*, 2009). In the present study, two novel strains, Sr22^T and Sr25^T, isolated from the China No.1 glacier are characterized.

Strains Sr22^T and Sr25^T were isolated from water melted from glacier ice. They were isolated and cultivated aerobically at 14 °C on PYG agar (Zhang *et al.*, 2006). Reference strains *Flavobacterium degerlachei* NBRC 102677^T, *F. omnivorum* CGMCC 1.2747^T, *Flavobacterium sinopsychrotolerans* CGMCC 1.8704^T, *Flavobacterium fryxellicola* LMG 22022^T, *F. tiangeerense* CGMCC 1.6847^T and *F. xinjiangense* CGMCC 1.2749^T were cultivated under the same conditions.

16S rRNA gene sequences were amplified by using universal primers 27f (5'-AGAGTTTGATCCTGGCTC-AG-3') and 1492r (5'-TACGGCTACCTTGTACGACTT-3')

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Sr22^T and Sr25^T are HQ436466 and HQ436467, respectively.

A supplementary figure is available with the online version of this paper.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strains Sr22^T and Sr25^T and representative species of the genus *Flavobacterium*. The tree was rooted with the type strain of *Leeuwenhoekiella marinoflava*. Numbers at nodes represent bootstrap values (percentages of 1000 resampled datasets); only values >50% are shown. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Bar, 0.01% sequence divergence.

(Lane, 1991). The amplified 16S rRNA gene fragments of strains Sr22^T and Sr25^T were sequenced by using the ABI BigDye3.1 Sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI 3730XL; Applied Biosystems). The program BLASTN was used to compare 16S rRNA gene sequences of strains Sr22^T and Sr25^T (1390 and 1392 nt, respectively) with others available from the GenBank and EMBL databases. Phylogenetic analysis was based on the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony algorithms (Kimura, 1980) available in MEGA version 3.0 (Kumar *et al.*, 2004). Bootstrap analysis (1000 replicates) was used to evaluate the topology of phylogenetic trees.

The results revealed that the novel strains shared 99.5% or less 16S rRNA gene sequence similarity with the type strains of recognized species of the genus *Flavobacterium*. Strains Sr22^T and Sr25^T shared 94.4 and 94.0% 16S rRNA gene sequence similarity, respectively, with *Flavobacterium aquatile* DSM 1132^T (the type species of the genus). Strain Sr22^T shared highest similarity with *F. fryxellicola* LMG 22022^T (98.1%), *F. tiangeerense* CGMCC 1.6847^T (98.6%) and *F. omnivorum* CGMCC 1.2747^T (99.1%); strain Sr25^T shared highest similarity with *F. sinopsychrotolerans* CGMCC 1.8704^T (98.5%), *F. degerlachei* NBRC 102677^T (98.4%) and *F. xinjiangense* CGMCC 1.2749^T (99.5%). The level of similarity between strains Sr22^T and Sr25^T was 96.5%. Fig. 1 shows the phylogenetic relationship between strains Sr22^T and Sr25^T and representative species of the genus *Flavobacterium* based on 16S rRNA gene sequences.

Genomic DNA was extracted according to the method of Marmur (1961). The G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962) by using genomic DNA from *Escherichia coli* K-12 as reference. The DNA G+C content of strains Sr22^T and Sr25^T was 37.2 and 35.1 mol%, respectively.

DNA–DNA hybridization was performed based on the liquid renaturation method (De Ley *et al.*, 1970) to determine the level of relatedness between the new isolates and their closest neighbours. A model Lambda 35 UV/VIS spectrometer equipped with a temperature programme controller (Perkin–Elmer) was used. Levels of DNA–DNA relatedness between strain Sr22^T and *F. fryxellicola* LMG 22022^T, *F. tiangeerense* CGMCC 1.6847^T and *F. omnivorum* CGMCC 1.2747^T were 23.6, 25.9 and 31.5%, respectively; those between strain Sr25^T and *F. sinopsychrotolerans* CGMCC 1.8704^T, *F. degerlachei* NBRC 102677^T and *F. xinjiangense* CGMCC 1.2749^T were 24.6, 34.9 and 40.6%, respectively. These data demonstrated that strains Sr22^T and Sr25^T represent two distinct species of the genus *Flavobacterium*.

Colony morphology of the novel isolates cultivated on PYG agar at 14 °C for 9–10 days was assessed. Gram staining was performed according to the method described by Gerhardt *et al.* (1994). Growth was tested on laboratory-prepared nutrient agar (0.3% beef extract, 1% soy peptone, 0.5% NaCl), trypticase soy agar (1.5% tryptone, 0.5% soy peptone, 0.5% NaCl) and PYG agar at 14 °C after 7 days of

incubation. Gliding motility was investigated by using the hanging-drop technique (Bernardet *et al.*, 2002). Microaerobic and anaerobic growth were tested by using the Oxoid atmosphere generation system. The presence of flagella was observed under a transmission electron microscope (Hitachi H-600 at 75 kV). The temperature range for growth was tested between 0 and 40 °C (1 °C increments at 0–5 and 16–25 °C, and 5 °C increments outside these two ranges). The pH range for growth of the new isolates and reference strains was tested on PYG broth adjusted to pH 4–11 (increments of 0.5 pH units). Tolerance to salinity was determined by using NaCl-free PYG broth supplemented with 0–4 % (w/v) NaCl (at 0.5 % intervals) for 5–7 days at 14 °C. Cells were tested for oxidase activity by determining the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Catalase activity was tested by monitoring the production of oxygen bubbles in a 3 % (v/v) aqueous hydrogen peroxide solution. Congo red adsorption and the detection of flexirubin-type pigments were tested following the methods of Bernardet *et al.* (2002).

The methods used to determine utilization of citrate and hydrolysis of gelatin, urea, DNA and starch followed Smibert & Krieg (1981). Hydrolysis of casein, Tweens 20, 60 and 80, aesculin and tyrosine was investigated according to the methods of Dong & Cai (2001). Hydrolysis of alginate, chitin, pectin and carboxymethylcellulose was performed according to Reichenbach (1992). The production of a precipitate on egg yolk agar was tested on PYG-based medium supplemented with 50 % egg yolk emulsion. Other physiological properties were tested by using the commercial API 20NE, API 20E and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Utilization of carbon and energy sources was investigated by using a basal medium [containing 0.2 % (NH₄)₂SO₄, 0.05 % NaH₂PO₄ · H₂O, 0.05 % K₂HPO₄, 0.02 % MgSO₄ · 7H₂O and 0.01 % CaCl₂ · 2H₂O] supplemented with vitamins, as described by Green & Bousfield (1982). The results of API ZYM tests were read after 4 h of incubation and the results of API 20NE and API 20E tests were read after 48–72 h. Acid production from carbohydrates was determined as described by Hugh & Leifson (1953) over a period of 1 week. All tests were performed at 14 °C and the cells used in the tests were cultivated for 18–24 h. The biochemical and morphological characteristics of strains Sr22^T and Sr25^T are shown in Table 1 and in the species descriptions below.

Cellular fatty acids were tested for and analysed according to the instructions of the standard Microbial Identification (MIDI), Sherlock version 6.0, procedure (Sasser, 1990), with reference to the TSBA 6.0 database. Strains Sr22^T and Sr25^T and reference type strains were incubated on PYG medium at 14 °C for 48 h. The predominant fatty acids of strain Sr22^T were iso-C_{15:1} G (6.01 %), iso-C_{15:0} (8.93 %), iso-C_{16:1} H (12.68 %), iso-C_{16:0} (10.4 %), C_{15:1}ω6c (8.97 %), C_{17:1}ω6c (5.96 %), iso-C_{16:0} 3-OH (11.14 %) and summed feature 3 (comprising C_{16:1}ω7c and/or C_{16:1}ω6c, 12.33 %); those of strain Sr25^T were iso-C_{15:0} (10.8 %), anteiso-C_{15:0} (5.23 %), C_{15:1}ω6c (11.79 %), C_{17:1}ω6c (5.43 %), iso-C_{16:0} 3-OH (7.04 %) and summed feature 3 (20.42 %). The fatty

acid profiles of the novel isolates and of *F. degerlachei* NBRC 102677^T, *F. omnivorum* CGMCC 1.2747^T, *F. tiangeerense* CGMCC 1.6847^T, *F. sinopsychrotolerans* CGMCC 1.8704^T, *F. fryxellicola* LMG 22022^T and *F. xinjiangense* CGMCC 1.2749^T are detailed in Table 2.

Extraction and purification of respiratory quinones were performed as described by Collins (1985). Respiratory quinones were analysed by HPLC (Wu *et al.*, 1989) with *F. xinjiangense* CGMCC 1.2749^T as a reference. Cells of strains Sr22^T and Sr25^T contained MK-6 as the major respiratory quinone, in accordance with data for members of the family *Flavobacteriaceae* (Bernardet & Bowman, 2006). Polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by two-dimensional TLC (Kates, 1986). *Pseudomonas mendocina* CGMCC 1.1804^T was used as a reference strain. In experiments to analyse respiratory quinones and polar lipids, strains Sr22^T and Sr25^T and the reference strains were incubated on PYG medium at 14 °C for 48 h. The major polar lipid of strains Sr22^T and Sr25^T was phosphatidylethanolamine (Fig. S1, available in IJSEM Online), consistent with data for the genus *Flavobacterium*. Strain Sr25^T also contained phosphatidylcholine as a minor component.

The phylogenetic and phenotypic data presented here demonstrate that strains Sr22^T and Sr25^T represent two novel species of the genus *Flavobacterium*, for which the names *Flavobacterium xueshanense* sp. nov. and *Flavobacterium urumqiense* sp. nov. are proposed, respectively.

Description of *Flavobacterium xueshanense* sp. nov.

Flavobacterium xueshanense (xue.sha.nen'se. N.L. neut. adj. *xueshanense* pertaining to the region where the China No.1 glacier is located).

Cells are Gram-stain-negative, non-spore-forming, non-gliding rods, 0.32–0.38 µm wide and 1.04–2.08 µm long. Colonies grown on PYG agar for 9 days are circular, yellow, convex with entire margins and about 1.5 mm in diameter. Growth occurs on PYG, nutrient and trypticase soy agars. Aerobic growth is good and microaerobic growth is weak. Does not grow anaerobically. Growth occurs at 2–18 °C (optimum, 14 °C). The pH range for growth is 6.0–9.0 (optimum, pH 7.0). The salinity range for growth is 0–0.5 % NaCl. Flexirubin-type pigments and brown diffusible pigment on L-tyrosine agar are not produced. Congo red is not absorbed. Catalase- and oxidase-positive. Hydrogen sulfide and indole are not produced. Nitrate is not reduced. No precipitate is formed on egg yolk agar. The methyl red and Voges–Proskauer tests are negative. Does not utilize citrate. Positive for hydrolysis of casein and aesculin, but negative for hydrolysis of gelatin, urea, tyrosine, starch, chitin, pectin, alginate, DNA, carboxymethylcellulose and Tweens 20, 60 and 80. Positive for naphthol-AS-BI-phosphohydrolase, acid phosphatase, alkaline phosphatase, leucine arylamidase, valine arylamidase and *N*-acetyl-β-glucosaminidase activities. Negative for β-glucosidase,

Table 1. Differential characteristics between the novel isolates and the type strains of related species of the genus *Flavobacterium*

Strains: 1, Sr22^T; 2, Sr25^T; 3, *F. sinopsychrotolerans* CGMCC 1.8704^T; 4, *F. xinjiangense* CGMCC 1.2749^T; 5, *F. omnivorum* CGMCC 1.2747^T; 6, *F. fryxellicola* LMG 22022^T; 7, *F. degerlachei* NBRC 102677^T; 8, *F. tiangeerense* CGMCC 1.6847^T. All data are from the present study. All strains were positive for: growth on nutrient agar and trypticase soy agar; hydrolysis of casein; enzyme activity of oxidase, catalase, alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase; and utilization of starch, sodium glutamate and glycogen. All strains were negative for: hydrolysis of urea, alginate, chitin, pectin, DNA, tyrosine, carboxymethylcellulose and Tweens 20, 60 and 80; production of hydrogen sulfide, flexirubin-type pigments and indole; acid production from galactose, lactose, L-arabinose, melibiose, xylose, rhamnose, *meso*-erythritol, dulcitol, sorbitol, sorbose, salicin, mannitol, ribose, methyl α -D-glucoside and glycerol; enzyme activity of cystine arylamidase, α -galactosidase, esterase (C4), esterase lipase (C8), lipase (C14), trypsin, β -galactosidase, β -glucuronidase, α -mannosidase and β -fucosidase; and utilization of D-mannitol, L-rhamnose, xylose, succinic acid, tartaric acid, *meso*-erythritol, fumarate, *myo*-inositol, sorbitol, methyl α -D-glucoside, L-arabinose, melibiose and sodium acetate.

Characteristic	1	2	3	4	5	6	7	8
Growth at 18 °C	+	–	+	+	+	–	+	+
Growth at 19 °C	–	–	+	+	+	–	+	+
Growth on seawater agar	+	+	+	–	+	+	+	–
Growth with 1.0 % NaCl	–	–	+	–	+	–	+	–
Growth at pH 9.5	–	+	+	+	+	+	+	+
Hydrolysis of:								
Gelatin	–	+	–	+	–	–	–	–
Aesculin	–	+	–	+	+	–	+	–
Starch	–	–	+	+	+	+	+	+
Voges–Proskauer reaction (API 20E)	–	–	+	+	+	–	+	–
Assimilation of (API 20NE) <i>N</i> -acetyl-glucosamine	–	–	+	+	–	–	–	–
Acid production from:								
Glucose	+	+	+	+	–	+	+	+
Mannose	+	+	–	+	–	–	+	+
Maltose	+	+	+	+	–	–	+	+
Raffinose	–	–	–	+	–	–	+	–
Amygdalin	–	–	–	–	–	–	+	–
Starch	+	+	+	+	–	–	+	+
Cellobiose	–	–	–	+	–	–	+	–
Trehalose	–	–	–	+	–	+	–	–
Sucrose	–	+	+	+	–	–	–	+
Inulin	–	–	–	+	–	–	–	–
Fructose	–	–	+	–	–	–	–	–
Melizitose	–	+	–	+	–	–	–	–
Enzyme activities								
α -Glucosidase	–	+	+	+	+	+	+	–
β -Glucosidase	–	–	–	+	–	–	–	–
α -Chymotrypsin	–	–	+	–	–	–	–	–
<i>N</i> -Acetyl- β -glucosaminidase	+	–	+	–	–	–	–	–
Utilization of:								
Maltose	+	+	+	+	+	–	+	+
Cellobiose	–	–	–	+	–	–	+	+
α -D-Glucose	–	+	+	+	+	–	+	–
D-Mannose	–	+	+	+	–	–	+	+
D-Raffinose	–	–	–	–	+	–	–	–
Trehalose	–	–	–	+	+	–	+	–
Melizitose	–	+	–	+	–	–	–	–
Glycerol	–	–	–	–	+	–	–	–
Sodium pyruvate	–	+	+	–	+	+	+	–
D-Galactose	–	–	–	–	+	–	–	+
Sucrose	–	+	+	+	+	–	+	+

α -chymotrypsin, trypsin, esterase lipase (C8), esterase (C4), lipase (C14), cystine arylamidase, β -glucuronidase, β -galactosidase, α -mannosidase, β -fucosidase, α -galactosidase,

α -glucosidase and chymotrypsin activities. Acids are produced from starch, glucose, mannose and maltose but not from melizitose, sucrose, cellobiose, sorbitol,

Table 2. Cellular fatty acid compositions (%) of strains Sr22^T and Sr25^T and their closest phylogenetic neighbours in the genus *Flavobacterium*

Strains: 1, Sr22^T; 2, Sr25^T; 3, *F. sinopsychrotolerans* CGMCC 1.8704^T; 4, *F. xinjiangense* CGMCC 1.2749^T; 5, *F. omnivorum* CGMCC 1.2747^T; 6, *F. fryxellicola* LMG 22022^T; 7, *F. degerlachei* NBRC 102677^T; 8, *F. tiangeerense* CGMCC 1.6847^T. All data are from this study. Fatty acids amounting to <1% of the total in all strains are not shown. tr, Trace (<1%); ND, not detected.

Fatty acid	1	2	3	4	5	6	7	8
Saturated straight-chain								
C _{13:0}	ND	tr	tr	tr	tr	1.10	tr	tr
C _{14:0}	tr	tr	tr	1.09	tr	tr	tr	tr
C _{16:0}	1.06	2.53	2.04	2.16	2.05	5.04	4.19	2.60
Saturated branched								
iso-C _{13:0}	tr	tr	tr	1.42	tr	tr	tr	tr
iso-C _{14:0}	4.96	2.37	2.65	3.02	2.68	4.33	1.21	2.90
iso-C _{15:1} G	6.01	3.10	6.51	4.09	7.96	7.16	5.70	7.49
anteiso-C _{15:1} A	tr	tr	tr	tr	tr	tr	1.00	tr
iso-C _{15:0}	8.93	10.80	12.32	12.91	16.10	18.08	8.71	21.36
anteiso-C _{15:0}	1.04	5.23	9.51	4.07	3.99	4.97	10.63	4.97
iso-C _{16:1} H	12.68	3.82	2.78	2.42	4.96	3.60	1.52	2.96
iso-C _{16:0}	10.40	2.13	2.38	2.11	4.73	5.16	1.11	2.14
Unsaturated								
C _{15:1} ω6c	8.97	11.79	7.82	5.05	7.47	6.23	15.81	6.87
C _{15:1} ω8c	ND	tr	ND	1.84	ND	ND	ND	ND
C _{17:1} ω6c	5.96	5.43	8.77	5.69	5.33	4.97	11.02	5.42
C _{17:1} ω8c	tr	tr	tr	1.26	tr	tr	1.36	tr
C _{18:1} ω5c	tr	tr	1.03	tr	1.13	tr	ND	tr
Hydroxy								
C _{15:0} 2-OH	tr	1.38	tr	1.22	tr	tr	tr	tr
C _{15:0} 3-OH	ND	1.54	1.83	1.26	ND	ND	1.49	ND
iso-C _{16:0} 3-OH	11.14	7.04	9.99	5.55	4.93	6.81	3.45	4.62
C _{16:0} 3-OH	1.09	1.08	1.04	1.62	tr	1.74	1.59	1.12
iso-C _{17:0} 3-OH	3.25	3.28	5.50	3.43	5.42	5.44	3.54	4.10
Summed features*								
3	12.33	20.42	7.33	20.42	10.93	11.13	15.18	10.41
4	1.57	ND	ND	ND	1.64	1.09	ND	1.57
9	2.53	2.68	3.36	3.72	6.57	1.41	1.17	2.74

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system (Sherlock version 6.0). Summed feature 3 comprised C_{16:1}ω7c and/or C_{16:1}ω6c; summed feature 4 comprised iso-C_{17:1} I/anteiso B; summed feature 9 comprised iso-C_{17:1}ω9c and/or 10-methyl C_{16:0}.

glycerol, melibiose, sorbose, galactose, rhamnose, manitol, trehalose, lactose, glycogen, methyl α-D-glucoside, meso-erythritol, amygdalin, xylose, L-arabinose, fructose or inulin. Utilizes maltose as sole carbon source. The predominant fatty acids are iso-C_{15:1} G, iso-C_{15:0}, iso-C_{16:1} H, iso-C_{16:0}, C_{15:1}ω6c, C_{17:1}ω6c, iso-C_{16:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or C_{16:1}ω6c). The major polar lipid is phosphatidylethanolamine. The DNA G+C content of the type strain is 37.2 mol%.

The type strain, Sr22^T (=CGMCC 1.9227^T =NBRC 106479^T), was isolated from water of melted ice of the China No.1 glacier, Xinjiang Uygur Autonomous Region, China.

Description of *Flavobacterium urumqiense* sp. nov.

Flavobacterium urumqiense (ur.um.qi.en'se. N.L. neut. adj. *urumqiense* pertaining to the capital of the Xinjiang Uygur Autonomous Region).

Cells are Gram-stain-negative, non-spore-forming, non-gliding rods, 0.42–0.44 µm wide and 1.01–1.80 µm long. Colonies grown on PYG agar for 9 days are circular, yellow, convex with entire margins and about 3.0 mm in diameter. Growth occurs on PYG, nutrient and trypticase soy agars. Aerobic growth is good and microaerobic growth is weak. Does not grow anaerobically. Growth occurs at 2–17 °C (optimum, 14–15 °C). The pH range for growth is

6.0–9.5 (optimum, pH 7.0). The salinity range for growth is 0–0.5% NaCl. Flexirubin-type pigments and brown diffusible pigment on L-tyrosine agar are not produced. Congo red is not absorbed. Catalase- and oxidase-positive. Hydrogen sulfide and indole are not produced. Nitrate is not reduced. No precipitate is formed on egg yolk agar. The methyl red and Voges–Proskauer tests are negative. Does not utilize citrate. Positive for hydrolysis of casein, gelatin and aesculin, but negative for hydrolysis of urea, tyrosine, starch, chitin, pectin, alginate, DNA, carboxymethylcellulose and Tweens 20, 60 and 80. Positive for naphthol-AS-BI-phosphohydrolase, acid phosphatase, alkaline phosphatase, leucine arylamidase, valine arylamidase and α -glucosidase activities. Negative for β -glucosidase, α -chymotrypsin, trypsin, esterase lipase (C8), esterase (C4), lipase (C14), cystine arylamidase, β -glucuronidase, β -galactosidase, α -mannosidase, β -fucosidase, *N*-acetyl- β -glucosaminidase, α -galactosidase and chymotrypsin activities. Acids are produced from starch, melezitose, sucrose, glucose, mannose and maltose but not from cellobiose, sorbitol, glycerol, melibiose, sorbose, galactose, rhamnose, mannitol, trehalose, lactose, glycogen, methyl α -D-glucoside, *meso*-erythritol, amygdalin, xylose, L-arabinose, fructose or inulin. Utilizes D-mannose, cellobiose, α -D-glucose, sucrose, maltose, starch, glycogen, melizitose, amygdalin, salicin, sodium pyruvate and sodium glutamate as sole carbon source; weak growth with trehalose and sodium acetate. The major respiratory quinone is menaquinone-6. The major fatty acids (>5% of the total) are iso-C_{15:0}, anteiso-C_{15:0}, C_{15:1}ω6c, C_{17:1}ω6c, iso-C_{16:0} 3-OH and summed feature 3. The major polar lipid is phosphatidylethanolamine; phosphatidylcholine is present as a minor component. The DNA G + C content of the type strain is 35.1 mol%.

The type strain, Sr25^T (=CGMCC 1.9230^T =NBRC 106480^T), was isolated from water of melted ice of the China No.1 glacier, Xinjiang Uygur Autonomous Region, China.

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References

- Bergey, D. H., Harrison, F. C., Breed, R. S., Hammer, B. W. & Huntoon, F. M. (editors) (1923). Genus II. *Flavobacterium* gen. nov. In *Bergey's Manual of Determinative Bacteriology*, pp. 97–117. Baltimore: Williams & Wilkins.
- Bernardet, J. F. & Bowman, J. P. (2006). The genus *Flavobacterium*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 7, pp. 481–531. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatidis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.
- Bernardet, J. F., Nakagawa, Y. & Holmes, B. for the Subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.
- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Dong, X. & Cai, M. (editors) (2001). *Systematic Bacteriological Manual*. Beijing: Science Press. (in Chinese)
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.
- Green, P. N. & Bousfield, I. J. (1982). A taxonomic study of some Gram-negative facultatively methylotrophic bacteria. *J Gen Microbiol* **128**, 623–638.
- Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J Bacteriol* **66**, 24–26.
- Kates, M. (1986). *Techniques of Lipidology*, 2nd edn. Amsterdam: Elsevier.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. London: Wiley.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- McCammon, S. A. & Bowman, J. P. (2000). Taxonomy of Antarctic *Flavobacterium* species: description of *Flavobacterium gillisiae* sp. nov., *Flavobacterium tegetincola* sp. nov., and *Flavobacterium xanthum* sp. nov., nom. rev. and reclassification of [*Flavobacterium*] *salegens* as *Salegentibacter salegens* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 1055–1063.
- Reichenbach, H. (1992). The order *Cytophagales*. In *The Prokaryotes*, 2nd edn, vol. 4, pp. 3631–3675. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–443. Edited by

P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.

Tamaki, H., Hanada, S., Kamagata, Y., Nakamura, K., Nomura, N., Nakano, K. & Matsumura, M. (2003). *Flavobacterium limicola* sp. nov., a psychrophilic, organic-polymer-degrading bacterium isolated from freshwater sediments. *Int J Syst Evol Microbiol* **53**, 519–526.

Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* [English translation of *Microbiology (Beijing)*] **16**, 176–178.

Xin, Y.-H., Liang, Z.-H., Zhang, D.-C., Liu, H.-C., Zhang, J.-L., Yu, Y., Xu, M.-S., Zhou, P.-J. & Zhou, Y.-G. (2009). *Flavobacterium*

tiangeerense sp. nov., a cold-living bacterium isolated from a glacier. *Int J Syst Evol Microbiol* **59**, 2773–2777.

Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2006). *Flavobacterium soli* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 997–1000.

Zhang, D.-C., Wang, H.-X., Liu, H.-C., Dong, X.-Z. & Zhou, P.-J. (2006). *Flavobacterium glaciei* sp. nov., a novel psychrophilic bacterium isolated from the China No.1 glacier. *Int J Syst Evol Microbiol* **56**, 2921–2925.

Zhu, F., Wang, S. & Zhou, P. (2003). *Flavobacterium xinjiangense* sp. nov. and *Flavobacterium omnivorum* sp. nov., novel psychrophiles from the China No. 1 glacier. *Int J Syst Evol Microbiol* **53**, 853–857.