Salegentibacter flavus sp. nov.

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A yellow-pigmented, non-motile, Gram-negative bacterium, designated Fg 69^T, was isolated from a sediment sample collected in Chazhma Bay (Sea of Japan). The novel organism grew at 10–35 °C, was neutrophilic and required 3–10 % NaCl for optimal growth. Strain Fg 69^T was able to degrade starch and to hydrolyse gelatin and Tween 80 weakly but not casein or agar. Predominant cellular fatty acids comprised n-C₁₅ and n-C₁₆ branched-chain and straight-chain saturated and unsaturated fatty acids, including iso-C_{15:0} (5 %), anteiso-C_{15:0} (11 %), C_{15:0} (9 %), iso-C_{15:1} (5 %), iso-C_{16:0} (8 %), C_{16:0} (5 %) and C_{16:1ω7} (5 %) and iso- and anteiso-branched 2-OH and 3-OH C_{15:0} to C_{17:0} fatty acids (26 % in total). The G + C content of the DNA was 40.4 mol%. 16S rRNA gene sequence data indicated that strain Fg 69^T belonged to the genus *Salegentibacter* but was distinct from recognized *Salegentibacter* species (94–95 % sequence similarity). Based on these results, a novel species, *Salegentibacter flavus* sp. nov., is proposed. The type strain is Fg 69^T (=KMM 6000^T=CIP 107843^T).

The genus *Salegentibacter*, belonging to the family *Flavobacteriaceae*, was created to accommodate moderately halophilic, yellow-pigmented, non-gliding bacteria that were isolated from a hypersaline meromictic lake in Antarctica (McCammon & Bowman, 2000). At the time of writing, the genus comprises three recognized species, *Salegentibacter salegens* (Dobson *et al.*, 1993; McCammon & Bowman, 2000), *Salegentibacter holothuriorum* (Nedashkovskaya *et al.*, 2004) and *Salegentibacter mishustinae* (Nedashkovskaya *et al.*, 2005). We report here the characterization and description of a novel *Flavobacterium*-like bacterium, strain Fg 69^T, isolated from a sediment sample collected in Chazhma Bay (Sea of Japan). On the basis of the results obtained, we propose that this organism be classified as a novel species of the genus *Salegentibacter*.

This work formed part of a taxonomic survey of free-living microbial populations of a coastal area of the north-west Pacific Ocean, where sediments have become contaminated by radionuclides as a result of an accident on a nuclear submarine. Sediment samples were collected from a sub-seafloor depth of 0.5 m in October–November 2000 from Chazhma Bay, Gulf of Peter the Great, Sea of Japan (Ivanova *et al.*, 2005). Samples were kept at 4 °C and processed within 4–8 h. Sample handling and isolation procedures were as described elsewhere (Ivanova *et al.*, 1996, 2004, 2005). Inoculated plates were incubated aerobically at room temperature (about 22–25 °C) for 5–10 days. Strains were stored at –80 °C in marine broth 2216 (Difco) supplemented with 20 % (v/v) glycerol.

Unless otherwise indicated, the phenotypic properties used for characterization of *Flavobacterium*-related species were tested following established procedures (McMeekin *et al.*, 1971; Smibert & Krieg, 1994; Ivanova *et al.*, 1996; Bernardet *et al.*, 2002). To test for spreading growth and gliding

Abbreviation: FAMES, fatty acid methyl esters.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Fg 69^T is AY682200.

motility, strains were grown on medium B (Ivanova *et al.*, 1996) with reduced peptone content (0.2 g l^{-1}). Gliding motility was verified by using phase-contrast microscopy (Nikon) of hanging drop preparations. In order to detect flexirubin-type pigments, the bathochromic shift test utilizing 20 % (w/v) KOH was used (Fautz & Reichenbach, 1980). Cellulose hydrolysis was tested both by using cellulose overlay plates (1 % CM-cellulose) and by examining strips of filter paper in liquid bacterial cultures for dissolution (Smibert & Krieg, 1994). The following physiological and biochemical properties were examined using the methods described by Smibert & Krieg (1994): oxidation/fermentation of glucose; oxidase and catalase activity; gelatin liquefaction; sodium chloride requirement [0, 0.5, 1, 3, 6, 8, 10 and 12 % (w/v) NaCl]; acetoin, urea, indole and H_2S production; and the ability to hydrolyse starch, Tween 80 and casein. Denitrification was assessed using the procedure of Azegami *et al.* (1987). Growth at different temperatures was tested in marine broth 2216 (Difco) at 2, 4, 6, 9, 29, 30, 35 and 37°C . Carbon source utilization was tested on medium containing 0.2 g NaNO_3 , 0.2 g NH_4Cl , 0.05 g yeast extract (Difco) and 0.4 % (w/v) carbon source in 1 litre artificial sea water as described by Nedashkovskaya *et al.* (2003). The following carbon sources were examined: arabinose, glucose, lactose, raffinose, sucrose, inositol, sorbitol, mannitol, rhamnose, salicin, xylose and adonitol.

Phenotypic analysis showed that the organism had characteristics reported for genera of the family *Flavobacteriaceae*: it was Gram-negative, strictly aerobic, oxidase- and catalase-positive, did not produce H_2S or indole and was weakly positive for nitrate reduction. Detailed morphological and physiological properties are shown in Table 1 and are given in the species description.

DNA was isolated following the method of Marmur (1961) and the DNA G+C content was determined using the thermal denaturation method of Marmur & Doty (1962). The G+C content of the DNA of strain Fg 69^T was 40.4 mol%.

For analysis of fatty acids, the strain was grown at 28°C on marine agar 2216. Cells were harvested after 48 h. Bacterial biomass was treated with 5 % HCl in methanol at 80°C for 180 min to produce fatty acid methyl esters (FAMES) (Christie, 1982). FAMES were analysed by flame-ionization detector (FID)-GC (Shimadzu GC-17) with a fused silica capillary column ($30 \text{ m} \times 0.25 \text{ mm}$), coated with Supelco-wax 10, at 210°C . Helium was used as a carrier gas. FAMES were identified by comparing the retention times with those of known standards and using equivalent chain-length measurements. To ensure correct identification, FAMES were further analysed by GC-MS using a model GCMS-QP5050A (Shimadzu) fitted with an MDN-5S capillary column ($30 \text{ m} \times 0.25 \text{ mm}$). The column temperature was programmed as follows: 1 min hold at 170°C , followed by an increase to 240°C at 2°C min^{-1} and a hold at 240°C for 20 min. The temperature of the injector and detector was 250°C . The FAMES formed by the novel organism were (as

Table 1. Major phenotypic characteristics of *Salegentibacter* species

Strains: 1, *S. flavus* sp. nov. Fg 69^T; 2, *S. salegens* DSM 5424^T; 3, *S. holothuriorum* KMM 3524^T; 4, *S. mishustinae* KMM 6049^T. All strains were positive for the following tests: respiratory metabolism, oxidase and catalase activities, hydrolysis of starch and growth at 34°C and at 8 % NaCl. All strains were negative for motility by gliding, hydrolysis of agar and cellulose (CM-cellulose and filter paper), acid production from L-arabinose, L-rhamnose, DL-xylose, adonitol, sorbitol, inositol and mannitol and production of indole and acetoin (Voges-Proskauer reaction). Data are from Dobson *et al.* (1993), McCammon & Bowman (2000), Nedashkovskaya *et al.* (2004, 2005) and this study. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4
DNA G+C content (mol%)	40.4	36.7	36.8	37.5
NaCl required for growth	+	+	–	+
Nitrate reduction	–	+	–	–
Production of H_2S	–	+	+	+
Hydrolysis of:				
Casein	–	–	–	+
Tween 80	w	+	+	+
Gelatin	w	+	+	+
Urea	+	–	–	–
Growth at 37°C	–	–	+	–
Growth at 12 % NaCl	–	+	–	+

percentages of whole-cell fatty acids): $\text{C}_{12:0}$ (1.2 %), iso- $\text{C}_{14:0}$ (1.5 %), $\text{C}_{14:0}$ (2.7 %), iso- $\text{C}_{15:0}$ (5.4 %), anteiso- $\text{C}_{15:0}$ (11.1 %), iso- $\text{C}_{15:1}$ (5.4 %), $\text{C}_{15:0}$ (8.6 %), $\text{C}_{15:1\omega6}$ (2.9 %), iso- $\text{C}_{16:0}$ (7.7 %), anteiso- $\text{C}_{16:1}$ (3.5 %), $\text{C}_{16:0}$ (5.0 %), $\text{C}_{16:1\omega7}$ (5.1 %), $\text{C}_{16:1\omega9}$ (2.4 %), iso- $\text{C}_{17:1}$ (1.8 %), anteiso- $\text{C}_{17:1}$ (1.2 %), $\text{C}_{17:0}$ (0.6 %), $\text{C}_{17:1\omega6}$ (2.8 %), $\text{C}_{17:1\omega8}$ (1.5 %), $\text{C}_{18:0}$ (1.8 %), $\text{C}_{18:1\omega9}$ (1.7 %), $\text{C}_{14:0}$ 3-OH (0.6 %), iso- $\text{C}_{15:0}$ 2-OH (3.6 %), anteiso- $\text{C}_{15:0}$ 2-OH (3.2 %), iso- $\text{C}_{15:0}$ 3-OH (1.0 %), iso- $\text{C}_{16:0}$ 2-OH (4.3 %), iso- $\text{C}_{16:0}$ 3-OH (6.0 %), iso- $\text{C}_{17:0}$ 3-OH (3.2 %) and anteiso- $\text{C}_{17:0}$ 3-OH (4.4 %). The fatty acid profile of the new isolate exhibited features characteristic of the family *Flavobacteriaceae* and of the genus *Salegentibacter*, e.g. the combination of n- C_{15} and n- C_{16} branched-chain saturated and unsaturated cellular fatty acids accounted for 50–70 % of the total. A significantly higher proportion of iso- and anteiso-branched 2-OH and 3-OH n- $\text{C}_{14:0}$ to $\text{C}_{17:0}$ fatty acids (26 % in total) was found for strain Fg 69^T than for *S. salegens*, *S. holothuriorum* or *S. mishustinae* (Nedashkovskaya *et al.*, 2004, 2005).

The small-subunit rRNA gene of Fg 69^T was sequenced (1471 bp) as described elsewhere (Ivanova *et al.*, 2004). The most similar 16S rRNA gene sequences were retrieved using n-BLAST searches (www.ncbi.nlm.nih.gov) and aligned for phylogenetic analysis. Analysis was based on three different approaches: neighbour-joining (Gascuel, 1997), parsimony and maximum-likelihood with global search (from PHYLIP

Fig. 1. Phylogenetic position of *Salegentibacter flavus* sp. nov. according to 16S rRNA gene sequence analysis. Unrooted tree resulting from a neighbour-joining analysis as described in the text. Percentages of 1000 bootstrap replications are indicated for branches that were also supported by parsimony and maximum-likelihood ($P < 0.01$) analyses. Bar, 0.01 nucleotide substitutions per position.

version 3.57c; Felsenstein, 1993). The program DNADIST was used to determine sequence similarities using the Kimura two-parameter correction. Finally, a bootstrap analysis (1000 replications) was performed using neighbour-joining (Felsenstein, 1985). 16S rRNA gene sequence analysis revealed that the new isolate clearly belonged to the genus *Salegentibacter*, as these sequences formed a robust clade (all three methods, bootstrap value of 99.8%; Fig. 1). The 16S rRNA gene sequence of strain Fg 69^T showed <95% similarity with those of the three recognized *Salegentibacter* species. The phylogenetic analysis thus supports the inclusion of Fg 69^T within the genus *Salegentibacter*, while the low level of 16S rRNA gene sequence similarity indicates that it represents a novel species.

Strain Fg 69^T can be readily distinguished from other *Salegentibacter* species by the combination of the following features: ranges of salinity and temperature for growth, inability to reduce nitrate, to hydrolyse casein and to produce H₂S and ability to hydrolyse urea and to hydrolyse Tween 80 weakly (Table 1). Thus, the results indicate that Fg 69^T should be classified as the type strain of a novel species within the genus *Salegentibacter*, for which we propose the name *Salegentibacter flavus* sp. nov.

Description of *Salegentibacter flavus* sp. nov.

Salegentibacter flavus (fla'vus. L. masc. adj. *flavus* golden yellow).

Cells are Gram-negative, microaerophilic, chemo-organotrophic, non-motile, asporogenic rods, 0.5–0.7 mm wide and 2.5–4.0 mm long. Oxidase- and catalase-positive. Colonies are circular, 1–3 mm in diameter and of low convexity when grown on marine agar 2216. Produces non-diffusible yellow pigment. Flexirubin-type pigments are absent. No growth is detected at 8 or 37 °C. Optimal temperature for growth is 22–25 °C. The pH range for growth is 5.0–10.0, with optimal growth between pH 8.0 and 8.5. Growth occurs between 3 and 10% NaCl, with optimal growth at 3% NaCl. No growth is observed without Na⁺. Starch, gelatin and urea are hydrolysed and Tween 80 is weakly hydrolysed. Does not decompose cellulose (CM-cellulose or filter paper), agar or casein. H₂S, indole and acetoin (Voges–Proskauer

reaction) are not produced. Nitrate reduction is negative. Does not utilize citrate, arabinose, glucose, lactose, raffinose, sucrose, inositol, sorbitol, mannitol, rhamnose, salicin, xylose or adonitol. Major cellular fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, C_{15:0}, iso-C_{16:0}, C_{16:0} and a range of C_{14:0} to C_{17:0} hydroxy fatty acids (about 64%). The DNA G+C content is 40.4 ± 0.5 mol%.

The type strain, strain Fg 69^T (= KMM 6000^T = CIP 107843^T), was isolated from sediments from Chazhma Bay, Sea of Japan.

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