Flavobacterium marinum sp. nov., isolated from seawater

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A Gram-staining-negative, strictly aerobic, non-gliding, rod-shaped bacterial strain, designated SW105T, was isolated from a seawater sample collected from the Indian Ocean. The strain produced flexirubin-type pigments and grew at 15–45 °C (optimum, 35 °C), at pH 5.5–8.5 (optimum, pH 7.0–7.5) and in the presence of 0–5.0 % (w/v) NaCl (optimum, 1.0–1.5 %). The predominant cellular fatty acids were iso-C₁₅ : 0, summed feature 3 (comprising C₁₆ : 1ω₇c and/or C₁₆ : 1ω₆c), iso-C₁₇ : 1ω₉c and iso-C₁₇ : 0 3-OH. The major menaquinone was menaquinone 6 (MK-6) and the major polar lipids were phosphatidylethanolamine and two unidentified aminophospholipids. The genomic DNA G+C content of strain SW105T was 36.2 mol%.

Phylogenetic analyses based on 16S rRNA gene sequences revealed that the novel isolate was related to members of the genus Flavobacterium, showing the highest similarity to Flavobacterium ummariense DS-12T and Flavobacterium ceti CCUG 52969T (94.3 and 93.0 % sequence similarity, respectively). On the basis of phylogenetic inference and phenotypic characteristics, it is proposed that strain SW105T represents a novel species of the genus Flavobacterium, for which the name Flavobacterium marinum sp. nov. is proposed. The type strain is SW105T (=CGMCC 1.10825T = JCM 18132T).

The genus Flavobacterium, proposed by Bergey et al. (1923) and with its description subsequently emended by Bernardet et al. (1996), is the type genus of the family Flavobacteriaceae. Members of the genus are aerobic, Gram-staining-negative, non-spore-forming rods, non-motile or motile by gliding, and contain menaquinone 6 (MK-6) as the major respiratory quinone. Flavobacterium strains are widely distributed in a variety of habitats such as soil, freshwater and various marine and polar environments (Bernardet & Bowman, 2011).

During a survey of the microbial community in a seawater sample collected from the south-west Indian Ocean (37° 47′ 22″ S 49° 39′ 18″ E) at a water depth of 800 m, we isolated a novel Gram-staining-negative, aerobic strain. Phylogenetically, the strain was affiliated to the genus Flavobacterium; however, it was distantly related to any described member of this genus. In this study, we characterized the new isolate and determined its exact taxonomic position.

Strain SW105T was isolated using the serial dilution and plate-screening method on marine agar 2216 (Difco, Becton Dickinson). Culture purity was checked by microscope examinations. The cultures were preserved in filtered water with 10 % (v/v) glycerol at −80 °C for further study. Strain SW105T was routinely cultivated at 30 °C on nutrient agar (NA; Difco) or in the corresponding broth (NB; Difco).

Total genomic DNA of strain SW105T was extracted and purified by using a Puregene DNA isolation kit (Gentra Systems) in accordance with the manufacturer’s instructions. The almost-complete 16S rRNA gene of strain SW105T was amplified by PCR and sequenced as described previously (Chen & Dong, 2004) and the consensus sequence obtained was submitted to GenBank. For reconstruction of phylogenetic trees, sequences from species of the genus Flavobacterium were selected from the RDP and NCBI databases. On the basis of a consensus sequence of 1357 bp of the 16S rRNA gene sequence of strain SW105T, a phylogenetic tree rooted with Myroides odoratus DSM 2801T was reconstructed (Fig. 1) using the neighbour-joining method implemented in the program MEGA version 4.0 (Tamura et al., 2007). Similar results were obtained using the maximum-likelihood and maximum-parsimony algorithms (not shown). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. Strain SW105T formed a distinct cluster with Flavobacterium ummariense DS-12T (Lata et al., 2012).
and F. ceti CCUG 52969T (Vela et al., 2007), showing 94.3
and 93.0 % sequence similarity, respectively. This
association was supported by high bootstrap values (Fig. 1) and
the large sequence divergence indicated that strain SW105T
represents a novel species of the genus Flavobacterium,
according to the 16S rRNA gene threshold value for
delineation of species proposed by Stackebrandt & Ebers
were obtained from the University of Delhi, grown on NA at
30 °C and used as reference strains for phenotypic tests and
chemotaxonomic analyses.

Cell morphology was examined by electron microscopy
(Hitachi H-600A) after cells were negatively stained with
uranyl acetate. The Gram reaction, the presence of a capsule,
catalase and oxidase activities, hydrolysis of aesculin, casein,
uranyl acetate. The Gram reaction, the presence of a capsule,
and the structure of the cell wall was confirmed by electron
microscopy of ultrathin sections (Fig. S1b). The physio-
ological and biochemical characteristics of strain SW105T
were given in the species description and in Table 1.

For analysis of cellular fatty acids, cells of strain SW105T, F.
ummariense DS-12T and F. ceti CCUG 52969T were
incubated at 30 °C in NB for 48 h and harvested by
centrifugation in the late exponential phase. Cellular fatty
acids were saponified, methylated, extracted according to
Collins (1985) and analysed by reversed-phase HPLC (Wu
et al., 1989). Polyamines of strain SW105T were extracted
and purified as described by Collins (1985) and analysed by
using reversed-phase HPLC (Wu et al., 1989). Polyamines
of strain SW105T were extracted and analysed by HPLC as
described by Flores & Galston (1982) and Hosoya &
Hamana (2004). Polar lipids of SW105T were extracted and
analysed by two-dimensional TLC, as described by
Tindall (1990); 10 % ethanolic molybdophosphoric acid was used
for detection of total polar lipids, ninhydrin for aminoli-
pids, molybdenum blue for phospholipids and 7-naphthol
for glycolipids, as described by Embley & Wait (1994).

The cellular fatty acids of strain SW105T mainly comprised
iso-branched fatty acids, predominantly iso-C15 : 0 (52.7 %),
summed feature 3 (comprising C16 : 1v7c and/or C16 : 1v6c,
according to the manufacturers’ instructions. All tests were
performed in duplicate.

Cells of strain SW105T were rod-shaped, occurring singly
or in pairs, and non-motile (Fig. S1a, available in IJSEM
Online). They stained Gram-negative in the KOH-lysis test;
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Respiratory quinones of strain SW105T were extracted
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15.1%), iso-C17:109c (13.1%) and iso-C17:0 3-OH (11.4%). The three strains shared very similar fatty acid profiles, with only minor differences in the respective proportions of some components (Table 2). The major (>95%) menaquinone detected in strain SW105T was MK-6, in agreement with all members of the family Flavobacteriaceae (Bernardet & Bowman, 2011). The polar lipids of strain SW105T consisted of phosphatidylethanolamine, two unidentified phospholipids, two unidentified aminolipids, two unidentified aminophospholipids and two other unidentified polar lipids (Fig. S2). The major polyamine of strain SW105T was homospermidine.

DNA–DNA hybridization was not performed in this study, since the 16S rRNA gene sequence similarity of strain SW105T to strains of related species was less than 97% (Tindall et al., 2010). The G+C content of the genomic DNA of strain SW105T was determined using HPLC (Mesbah et al., 1989) with the modification of Lee et al. (2005) as 36.2 mol%. This value was similar to those of the two reference strains [37.4 mol% for F. ummariense DS-12T (Lata et al., 2012) and 36.7 mol% for F. ceti CCUG 252969T (Vela et al., 2007)] and within the range reported for species of the genus Flavobacterium (Bernardet & Bowman, 2011).

The chemotaxonomic features of strain SW105T, as well as its DNA G+C content, are in accordance with those of species of the genus Flavobacterium. However, the sequence divergence indicated that strain SW105T cannot be allocated to any described species of the genus Flavobacterium. It may be distinguished from related species by several phenotypic features (Table 1). Hence, strain SW105T represents a novel species of the genus Flavobacterium, for which the name Flavobacterium marinum sp. nov. is proposed.

### Description of Flavobacterium marinum sp. nov.

*Flavobacterium marinum* (ma.ri’num. L. neut. adj. mar-inum of the sea, marine).

Cells are Gram-staining-negative, strictly aerobic rods, 0.3–0.5 μm in diameter and 1.5–3.0 μm long, occurring singly or in pairs. Gliding motility, capsule and endospores are not observed. Growth occurs on Luria–Bertani, trypticase soy, nutrient and marine 2216 agars. Colonies are smooth, entire, light yellow and translucent, 1.5–2.0 mm in diameter after cultivation at 30 °C for 48 h on NA. Flexirubin-type

### Table 1. Differential characteristics of strain SW105T and type strains of closely related species of the genus *Flavobacterium*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony pigmentation*</td>
<td>LY</td>
<td>DY</td>
<td>LY</td>
</tr>
<tr>
<td>Tolerance of 3% NaCl</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Strictly aerobic metabolism</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acid production from (GN2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MicroPlate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-L-Arabinose</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1-L-Rhamnose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+</td>
<td>–</td>
<td>−</td>
</tr>
<tr>
<td>d-Sorbitol</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-d-glucosamine</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Proline</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>−</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>−</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>W</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>W</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

* DY, Dark yellow; LY, light yellow.

### Table 2. Cellular fatty acid contents of strain SW105T and type strains of related species of the genus *Flavobacterium*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>C15:0 2-OH</td>
<td>−</td>
<td>1.1</td>
<td>TR</td>
</tr>
<tr>
<td>C16:0</td>
<td>TR</td>
<td>3.0</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>52.7</td>
<td>31.7</td>
<td>41.7</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>TR</td>
<td>TR</td>
<td>1.7</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>1.1</td>
<td>1.3</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>13.1</td>
<td>9.6</td>
<td>6.2</td>
</tr>
<tr>
<td>iso-C17:109c</td>
<td>2.5</td>
<td>4.8</td>
<td>4.9</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>11.4</td>
<td>17.3</td>
<td>18.7</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>15.1</td>
<td>19.5</td>
<td>20.5</td>
</tr>
</tbody>
</table>

* Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI System. Summed feature 3 comprised C16:1ω7c and/or C16:1ω6c.
pigments are produced and Congo red is not absorbed. The temperature range for growth is 15–45 °C (optimum, 35 °C) and the pH range for growth is 5.5–8.5 (optimum, pH 7.0–7.5). Growth occurs in the presence of 0–5.0 % (w/v) NaCl (optimum, 1.0–1.5 %). Oxidase and catalase activities are present, but arginine dihydrolase and lysine and ornithine decarboxylase activities are absent. Nitrate is not reduced to nitrite. Indole is not produced from tryptophan and H2S is not produced from thiosulfate. Aesculin, casein, CM-cellulose, chitin, DNA, starch, pectin, Tweens 20, 40 and 80, urea and L-tyrosine are not hydrolysed. In the GN2 Microplate and the API 20E system, L-arabinose, D-mannose, lactose, cellobiose, maltose, trehalose, d-mannitol, D-sorbitol, glycerol, D-fructose, D-glucose, L-mannose, lactose, cellobiose, maltose, trehalose, D-mannitol, D-sorbitol, glycerol, D-lactic acid, L-alanine, L-aspartic acid, L-glutamic acid, L-serine, N-acetyl-D-glucosamine and L-proline are utilized, but glycerol, D-fructose, D-glucose, L-rhamnose, D-galactose, melibiose, trehalose, D-sorbitol, inositol, formic acid, acetic acid and propionic acid are not utilized. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, z-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, z-mannosidase and z-fucosidase activities are absent. The predominant cellular fatty acids (>10 %) are iso-C15:0, summed feature 3 (C16:1ω7c and/or C16:1ω6c), iso-C17:0 3-OH and iso-C17:0 3-OH. The major menaquinone is MK-6 and the major polyamine is homospermidine. The major polar lipids are phosphatidylethanolamine and two unidentified aminophospholipids; significant amounts of two unidentified phospholipids, two unidentified aminolipids and two other unidentified polar lipids are also present.

The type strain is SW105T (=CGMCC 1.10825T = JCM 18132T), isolated from a seawater sample collected from the Indian Ocean. The genomic DNA G + C content of the type strain is 36.2 mol% (HPLC).

Acknowledgements

We are grateful to Professor Rup Lal (University of Delhi) for providing us with F. ummariae sp. nov., isolated from hexachlorocyclohexane-contaminated soil, and emended description of Flavobacterium ceti Vela et al. 2007. Int J Syst Evol Microbiol 62, 2674–2679.


References


