Flavobacterium aciduliphilum sp. nov., isolated from freshwater, and emended description of the genus Flavobacterium

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A Gram-stain-negative, rod-shaped, non-motile and yellow-pigmented bacterial strain, designated strain JJ013T, was isolated from an artificial lake in Jeollabuk-do, South Korea, and characterized using a polyphasic approach. The 16S rRNA gene sequence of strain JJ013T indicated that the isolate belonged to the family Flavobacteriaceae and exhibited similarity levels of 96.6 % to the type strains of Flavobacterium cheonanense and Flavobacterium koreense and 96.5 % to the type strain of Flavobacterium chungnamense. Growth was observed at 20–30 °C and pH 5.0–7.0. The major cellular fatty acids of the novel strain were iso-C15:0 (27.5 %), iso-C15:1 G (17.8 %), iso-C17:0 3-OH (9.4 %) and iso-C15:0 3-OH (9.2 %). Flexirubin-type pigments were present. The DNA G+C content of strain JJ013T was 33.9 mol%, the major respiratory quinone was menaquinone-6 (MK-6) and the major polyamine was sym-homospermidine. The polar lipid profile of the strain JJ013T consisted of a phosphatidylethanolamine (PE), two unknown aminolipids (AL1–2), three unidentified lipid (L1–3) and an unknown glycolipid (GL). On the basis of the morphological and physiological properties and biochemical evidence presented, it is concluded that strain JJ013T represents a novel species of the genus Flavobacterium, for which the name Flavobacterium aciduliphilum sp. nov. is proposed; the type strain is JJ013T (=KACC 16594T=JCM 18211T). Since C15:0 which is known as a predominant fatty acid of the genus Flavobacterium was not detected in the novel strain and other reference strains, we propose an emended description of the genus Flavobacterium.

The genus Flavobacterium accommodates Gram-negative, aerobic, rod-shaped and yellow-pigmented bacteria and belongs to the phylum Bacteroidetes. The strains of species of the genus Flavobacterium are found in a variety of environments such as soil, freshwater, seawater, wastewater, Antarctic lakes, gut of earthworm and fish tissues. Most species of the Flavobacterium are sensitive to salts and several species are known to cause diseases in freshwater fish (Bernardet & Bowman, 2006; Wakabayashi et al., 1989).

A bacterial strain JJ013T was isolated from the Lake Yongdam in Jinan, Jeollabuk-do, South Korea. The strain appeared as yellow colonies after 4 days cultivation at 25 °C on R2A agar (Difco) and was selected for characterization and sequence analysis of the 16S rRNA gene. For 16S rRNA gene sequencing and G+C content, chromosomal DNA was extracted by using a bacteria genomic DNA isolation kit (RBC). The 16S rRNA gene was amplified from the DNA by PCR (Marchesi et al., 1998) using universal bacterial primers 27F and 1492R. Because the similarity between selected strain and most related strains was lower than 96.6 %, it was studied further. The novel strain was routinely grown on R2A agar at 25 °C for 3 days and preserved at −70 °C as a suspension in R2A broth supplemented with glycerol [20 % (v/v)].

The closest phylogenetic neighbours were identified using the EzTaxon database (Kim et al., 2012). Phylogenetic analysis was accomplished by using MEGA5 (Tamura et al., 2011), after carrying out a multiple alignment of the data using CLUSTAL_X (Thompson et al., 1997). The clustering was performed by using three different methods, the neighbour-joining Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) methods. Evolutionary distance matrices for the neighbour-joining algorithm were calculated with the Kimura two-parameter model (Kimura, 1980). The topology of the neighbour-joining tree was evaluated by bootstrap analysis on the basis of 1000 replications (Felsenstein, 1985).

Cells of strain JJ013T were collected for morphological observations after cultivation on R2A agar for 3 days at 25 °C. Cell morphology was examined by scanning electron microscope and transmission electron microscope. Gram

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JJ013T is JN712178.

Two supplementary figures are available with the online version of this paper.
reaction was performed using Gram Stain kit (Difco) according to the manufacturer’s instructions. The gliding-motility test was performed as wet mount method and stab culture in the semi-solid R2A medium (Tittsler & Sandholzer, 1936). Oxidase activity was determined using 1 % N,N,N’ ,N’-tetramethyl-1,4-phenylenediamine (Oxidase Reagent, bioMérieux) and catalase activity was examined for bubble production after the addition of 3 % (v/v) hydrogen peroxide. The presence of flexirubin-type pigments was investigated using a 20 % (w/v) KOH solution (Bernardet et al., 2002; Bowman, 2000). Growth on various media such as marine agar, nutrient agar, tryptic soy agar, R2A, Anacker and Ordal’s agar (Anacker & Ordal, 1955), MacConkey agar, MRS agar and Czapek-Doxy medium was evaluated. Moreover, growth on modified Anacker and Ordal’s agar with magnesium sulfate (0.05 g L−1) and/or dipotassium phosphate (0.3 g L−1) or without sodium acetate (0.2 g L−1) was also evaluated. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37 and 40 °C), pH (4.0–12.0 at 0.5 pH unit increments) and NaCl concentrations [0–1 % at 0.1 % intervals and 1–5 % at 1 % intervals (w/v)] was investigated on R2A for up to 4 days. Adjusted pH with hydrochloric acid and sodium hydroxide was readjusted after autoclaving. Hydrolysis of casein (5 %, w/v), starch (1 %, w/v), z-cellulose (1 %, w/v), carboxymethyl-cellulose (1 %, w/v), sodium alginate (1 %, w/v), chitin (1 %, w/v), pectin (1 %, w/v), L-tyrosine (1 %, w/v) and DNA using DNase Test Agar with Methyl Green (Difco) were tested after 4 days. Congo red adsorption was investigated using 0.01 % aqueous with Methyl Green (Difco) were tested after 4 days. Congo red adsorption was investigated using 0.01 % aqueous with Methyl Green (Difco) were tested after 4 days. Congo red adsorption was investigated using 0.01 % aqueous with Methyl Green (Difco) were tested after 4 days.

Polyamines were extracted from strain JJ013T and closely related strains (F. cheonanense ARSA-108T, F. koreense ARSA-42T and F. chungunamense ARSA-103T) and analysis was carried out as described by Scherer & Kniefel (1983), Yang et al. (1993) and Lata et al. (2012). The extracted samples were loaded on TLC plates (Silica gel 20 × 20 cm, Merck, Germany, 105553) and ethylacetate: cyclohexane (2:3) was used as a running solvent. 16S rRNA sequence comparisons via the EzTaxon server showed that strain JJ013T was most closely related to F. cheonanense and F. koreense with similarities of 96.6 %, followed by F. chungunamense with 96.5 % (Lee et al., 2011). Phylogenetic analysis based on 16S rRNA gene sequences suggested that the new isolate JJ013T consisted of a clade with the three most closely related strains within the genus Flavobacterium and simultaneously formed a distinct phyletic lineage (Fig. 1).

The cells of strain JJ013T were Gram-stain-negative, non-motile, rod-shaped and 0.3–0.4 μm wide and 1.0–2.5 μm long (Fig. S1, available in IJSEM Online). Colonies grown on R2A plates for 3 days at 25 °C were circular, 1–2 mm in diameter, convex, smooth, shiny, mucoid and yellow-pigmented. The cells strain JJ013T were slowly growing on Anacker and Ordal’s agar without sodium acetate but did not grow on standard Anacker and Ordal’s agar. Colonies are circular, approximately 1 mm in diameter, convex, shiny, smooth and pale yellow-pigmented on Anacker and Ordal’s agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days. The strain not grew on other media types tested except R2A medium or Anacker and Ordal’s Agar without sodium acetate at 7 days.

The predominant fatty acids were iso-C15:0 (27.5 %), iso-C15:1 G (17.8 %), iso-C17:0 3-OH (9.4 %) and iso-C15:0 3-OH (9.2 %) described in Table 2. C15:0 as a major fatty acid described by (Bernardet et al., 1996) was not detected in the novel strain JJ013T as well as in the closely related type strains (Lee et al., 2011). Because the absence of C15:0 was
shown in our results as well as in some previous reports on other *Flavobacterium* species (Lee et al., 2012; Sheu et al., 2011; Weon et al., 2007), the major compositions of fatty acids of species of the genus *Flavobacterium* were emended. The G+C content of strain JJ013ᵀ was 33.9 mol%. The major respiratory quinone was menaquinone-6 and the major polyamine was sym-homospermidine; this polyamine was also exhibited in three reference strains as in other species of the genus *Flavobacterium* that have been tested. (Bernardet & Bowman, 2006). The polar lipid profile of JJ013ᵀ as well as of the reference strain *F. cheonanense* ARSA-108ᵀ consisted of a phosphatidylethanolamine (PE), two unknown aminolipids (AL1–2) and three unidentified lipids (L1–3). However, an unknown glycolipid (GL) was present in JJ013ᵀ but not in the reference strain *F. cheonanense* ARSA-108ᵀ (Fig. S2). In addition, the reference strain *F. cheonanense* ARSA-108ᵀ contained three more unknown aminolipids (AL3–5) that were absent in the novel strain JJ013ᵀ (Fig. S2).

Taken these results together, strain JJ013ᵀ is considered to represent a novel species of the genus *Flavobacterium*, for which we propose the name *Flavobacterium aciduliphilum* sp. nov.

**Emended description of the genus *Flavobacterium* Bergey et al. 1923 emend. Bernardet et al. 1996 emend. Dong et al. 2013**

The description is as given by Bernardet et al. (1996) and Dong et al., (2013) with the following amendment. The predominant fatty acids are iso-C₁₅:₀, iso-C₁₅:₁G, iso-C₁₅:₀ 3-OH, iso-C₁₆:₀ 3-OH and iso-C₁₇:₀ 3-OH.

**Description of *Flavobacterium aciduliphilum* sp. nov.**

*Flavobacterium aciduliphilum* [a.ci.du.li’phi.lum. L. adj. acidu-lus a little sour, sourish, acidulous; N.L. neut. adj. philum (from Gr. neut. adj. philon) friend, loving; N.L. neut. adj. *aciduliphilum* weak acid-loving].

Cells are Gram-stain-negative, aerobic, rod-shaped, 0.3–0.4 μm wide and 1.0–2.5 μm long and non-motile. Colonies are circular, 1–2 mm in diameter, convex, shiny, smooth, mucoid and yellow-pigmented on R2A agar at 3 days. Colonies on Anacker and Ordal’s agar without sodium acetate are circular, approximately 1 mm in diameter, convex, shiny, smooth and pale yellow-pigmented at 7 days. Growth occurs on R2A or Anacker and Ordal’s agar without
sodium acetate of the tested media. Growth of the cells occurs at pH 5.0 to pH 7.0 with optimum at pH 6.0. Cells grow at temperatures of 20–30 °C and NaCl concentrations of lower than 0.3 % in R2A broth adjusted to pH 6.0, with optimal growth at 25 °C without NaCl. Catalase and oxidase activities are positive. Production of hydrogen sulfide using the SIM medium (MB Cell) and brown diffusible pigment on L-tyrosine medium are negative. The KOH test results indicated that flexirubin-type pigments are present. Congo red is not absorbed by the colonies. Starch, cellulose, carboxymethyl-cellulose, agar, pectin, chitin, alginates and L-tyrosine are not hydrolysed. The result of the DNase assay using DNase Test Agar is negative. Formation of a precipitate on egg yolk agar is negative. In the API 20NE DNase assay using DNase Test Agar is negative. Formation of a red is not absorbed by the colonies. Starch, casein, glycyl-L-aspartic acid, L-ornithine, L-proline, D-serine are not hydrolysed. The result of the lipase (C14) test is positive. The result of the cystine arylamidase test is negative. The result of the α-Chymotrypsin test is negative.

### Table 1. Differential phenotypic characteristics of strain JJ013T and closely related type strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<td><strong>Growth on:</strong></td>
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<tr>
<td>Nutrient agar</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Anacker and Ordal's agar</td>
<td>−</td>
<td>+</td>
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<td><strong>Growth range:</strong></td>
<td></td>
<td></td>
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<tr>
<td>NaCl (%)</td>
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<td>0–1.0</td>
<td>0–0.5</td>
<td>0–0.5</td>
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<tr>
<td>Temp (°C)</td>
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<td>4–37</td>
<td>4–37</td>
<td>10–30</td>
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<tr>
<td>pH</td>
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<td>6.5–9.5</td>
<td>6.0–9.5</td>
<td>6.5–9.5</td>
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<td>Flexirubin-type pigment</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<td>Diffusible brown pigments on tyrosine agar</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td><strong>Hydrolysis of:</strong></td>
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<tr>
<td>Aesculin</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>Gelatin</td>
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<td>−</td>
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<td>+</td>
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<tr>
<td>L-Tyrosine</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td><strong>Assimilation of:</strong></td>
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<tr>
<td>myo-Inositol</td>
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<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Enzymic activity</td>
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<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
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<tr>
<td>α-Chymotrypsin</td>
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<td>−</td>
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<td>+</td>
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<tr>
<td>DNA G + C content (mol%)</td>
<td>33.9</td>
<td>31.4</td>
<td>31.5</td>
<td>33.2</td>
</tr>
</tbody>
</table>

Strains: 1, JJ013T; 2, Flavobacterium cheonanense ARSA-108T; 3, Flavobacterium koreense ARSA-42T; 4, Flavobacterium chungnamense ARSA-103T. All data were obtained from this study except the DNA G + C contents of the three reference strains [taken from Lee et al., (2011)]. +, Positive; −, negative.
The polar lipid profile of the strain JJ013T consists of a phosphatidylethanolamine (PE), two unknown aminolipids (AL1–2), three unidentified lipids (L1–3) and an unknown glycolipid (GL).

The type strain, JJ013T (＝KACC 16594T＝JCM 18211T), was isolated from an artificial lake in Jeollabuk-do South Korea. The DNA G+C content of the type strain is 33.9 mol%.

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References


