**Mucilaginibacter koreensis** sp. nov., isolated from leaf mould

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A Gram-staining-negative, strictly aerobic, rod-shaped, pale-pink pigmented bacterial strain, designated TF8T, was isolated from leaf mould in Cheonan, Republic of Korea. Its taxonomic position was determined through a polyphasic approach. Optimal growth occurred on R2A agar without NaCl supplementation, at 25–28 °C and at pH 6.0–7.0. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain TF8T belongs to the genus *Mucilaginibacter* in the family *Sphingobacteriaceae*. The sequence similarity between 16S rRNA genes of strain TF8T and the type strains of other species of the genus *Mucilaginibacter* ranged from 92.1 to 94.7 %. The closest relatives of strain TF8T were *Mucilaginibacter lutimaris* BR-3T (94.7 %), *M. soli* BR-18T (94.5 %), *M. litoreus* BR-18 (94.5 %), *M. rigui* WPCB133T (94.0 %) and *M. daejeonensis* Jip 10T (93.8 %). The major isoprenoid quinone was MK-7 and the major cellular fatty acids were iso-C15 : 0 (33.0 %), summed feature 3 (C16 : 1ω7c and/or C16 : 1ω6c; 24.8 %) and summed feature 8 (C18 : 1ω7c and/or C18 : 1ω6c; 13.0 %). The major polar lipids of TF8T were phosphatidylethanolamine and three unidentified aminophospholipids. The G+C content of the genomic DNA was 46.2 mol%. On the basis of the data presented here, strain TF8T is considered to represent a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter koreensis* sp. nov. is proposed. The type strain is TF8T (=KACC 17468T=JCM 19323T).

The genus *Mucilaginibacter*, a member of the family *Sphingobacteriaceae* (phylum *Bacteroidetes*) was first proposed by Pankratov et al. (2007) along with the description of two novel species, *Mucilaginibacter paludis* (type species) and *Mucilaginibacter gracilis*. The description of the genus was emended by Urai et al. (2008) and Baik et al. (2010). The genus groups oxidase-positive, strictly aerobic or facultatively anaerobic bacteria that contain MK-7 as the major respiratory quinone and straight and branched-saturated fatty acids, and have a DNA G+C content in the range 42.4–49.8 mol%. At the time of writing, the genus *Mucilaginibacter* comprised 22 species with validly published names: *M. paludis* (type species) and *M. gracilis* (Pankratov et al., 2007), *M. kameinonensis* (Urai et al., 2008), *M. daejeonensis* (An et al., 2009), *M. ximonensis* (Luo et al., 2009), *M. oryzae* (Jeon et al., 2009), *M. rigui* (Baik et al., 2010), *M. gosyppii* and *M. gosyppicola* (Madhaiyan et al., 2010), *M. frigitoritolerans*, *M. lappiensis* and *M. mallensis* (Männistö et al., 2010), *M. myungsuensis* (Youn & Joh, 2011), *M. composti* (Cui et al., 2011), *M. borygensis* (Kang et al., 2011), *M. dorajii* (Kim et al., 2011), *M. polysacchara* (Han et al., 2012), *M. soli* (Jiang et al., 2012), *M. angelicae* (Kim et al., 2012a), *M. lutimaris* (Kim et al., 2012b), *M. litoreus* (Yoon et al., 2012) and *M. jinjuensis* (Khan et al., 2013). Members of the genus *Mucilaginibacter* have been isolated from various sources including peat bog, rice straw, wetland freshwater, marine sand, soil and tidal flat sediment (Pankratov et al., 2007; Urai et al., 2008; An et al., 2009; Baik et al., 2010; Yoon et al., 2012; Jiang et al., 2012; Kim et al., 2012b; Han et al., 2012; Khan et al., 2013). Strain TF8T was isolated from leaf mould, collected at the campus of Dankook University, Cheonan, Republic of Korea (36° 83' 85.72" N 127° 16' 87.58" E). Analysis using a polyphasic approach was carried out and the results suggested that strain TF8T is a representative of a novel species of the genus *Mucilaginibacter*.

Strain TF8T was isolated using the dilution-plating technique on R2A agar (Difco) after 3 days of incubation at 25 °C. After isolation, the strain was routinely cultured on

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TF8T is J0966281. One supplementary figure is available with the online version of this paper.
R2A agar, and suspended in distilled water containing 20% glycerol (w/v) and preserved at −80 °C. *M. lutimaris* KCTC 23461T, *M. soli* KCTC 32028T, *M. litoreus* KCTC 23697T, *M. rigui* KCTC 12534T and *M. daejeonensis* KCTC 12639T were obtained from the Korean Collection for Type Culture (KCTC, Korea) and used as reference strains in most analyses.

Gram staining was performed using a bioMérieux Gram stain kit according to the manufacturer’s instructions. The presence of a capsule was assessed using Anthony’s staining (Anthony, 1931). Cellular morphology of strain TF8T was examined by light microscopy. Gliding motility was assessed using wet mounts as described by Bowman (2000). Growth was evaluated at 25 °C on R2A agar, tryptic soy agar (TSA), nutrient agar (NA) and plate count agar (PCA) (all from Difco). Growth was tested at 4, 10, 15, 20, 25, 28, 30, 32, 37 and 42 °C and at pH 4.0–9.5 (at intervals of 0.5 pH unit). The pH of the medium was adjusted as described by Gomori (1955). NaCl tolerance was tested in R2A broth and on R2A agar supplemented with 0, 0.5, 1, 2, 3, 4 and 5% (w/v) NaCl at 25 °C. The bathochromic shift test with 20% KOH was Table 1. Differential characteristics of strain TF8T and related species of the genus *Mucilaginibacter*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<td>Range for growth</td>
<td></td>
<td></td>
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<tr>
<td>Temperature (°C)</td>
<td>4–32</td>
<td>4–37</td>
<td>4–32</td>
<td>10–40</td>
<td>4–37</td>
<td>15–37</td>
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<tr>
<td>pH</td>
<td>5.0–8.0</td>
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<td>5–10</td>
<td>4.5–8.5</td>
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<td>NaCl (% w/v)</td>
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<td>0–1.0</td>
<td>0–1.0</td>
<td>0–2.0</td>
<td>0–1.0</td>
<td>0–3.0</td>
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<tr>
<td>Hydrolysis of casein</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Assimilation of (API 20NE):</td>
<td></td>
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<td></td>
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<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Potassium gluconate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Acid production from (API 50CH):</td>
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<tr>
<td>Fructose</td>
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<td>+</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<td>Rhamnose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Methyl D-glucoside</td>
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<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Arbutin</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Salicin</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Raffinose</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Gentioisobiose</td>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Turanose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Lyxose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Enzyme activity (API ZYM):</td>
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<td>Esterase (C4)</td>
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<td>–</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>46.2</td>
<td>49.8**</td>
<td>47.2b</td>
<td>42.4c</td>
<td>47.0d</td>
<td>40.4e</td>
</tr>
</tbody>
</table>

*Data from: *a*, Kim et al. (2012b); *b*, Jiang et al. (2012); *c*, Yoon et al. (2012); *d*, Baik et al. (2010); *e*, An et al. (2009).
conducted to study the presence of flexirubin-type pigments (Bernardet et al., 2002). Growth of strain TF8<sup>T</sup> under anaerobic conditions was evaluated on R2A agar incubated at 25 °C for 15 days in an anaerobic jar with a GasPak (BD) system. Catalase activity was determined by oxygen bubble production in a 3% (v/v) hydrogen peroxide solution and oxidase activity was determined using oxidase reagent (bioMérieux). Hydrolysis of casein, DNA, starch, gelatin, aesculin and Tweens 20, 40, 60 and 80 was investigated as described by Lányi (1987) using R2A agar as the basal medium. Utilization of various substrates, enzyme activities and other physiological and biochemical properties were tested using API 20E, API 20NE, API 50CH and API ZYM kits (bioMérieux) according to the manufacturer’s instructions. The five reference strains were grown under the same conditions as strain TF8<sup>T</sup> and included in the hydrolysis test and in the API strips. Selected characteristics that differentiate strain TF8<sup>T</sup> from related species of the genus Mucilaginibacter are shown in Table 1.

Genomic DNA of strain TF8<sup>T</sup> was extracted using an InstaGene Matrix kit (Bio-Rad) and the 16S rRNA gene was amplified using universal primers (Yoon et al., 1998) and a GeneAmp PCR system 9700 (Applied Biosystems). The 16S rRNA gene was sequenced using an ABI3730XL automated sequencer (Applied Biosystems) and the primers 27F, 518F, 97F, 1087F, 1492R and 1951R (Lane, 1991) at Macrogen (Korea). The amplified 16S rRNA gene sequence of strain TF8<sup>T</sup> was 1417 bp in length. Sequence similarity with species of the genus Mucilaginibacter was determined using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012c). Sequences were aligned using CLUSTAL W (Thompson et al., 1994) and the BioEdit program (Hall, 1999) and evolutionary distances were calculated based on the Kimura two-parameter model (Kimura, 1983). Finally, a phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) using the software program MEGA 5.1 (Tamura et al., 2011) and the bootstrap analysis was based on 1000 replications (Felsenstein, 1985). The topology of the maximum-likelihood (Felsenstein, 1981) tree that was also reconstructed was essentially the same (data not shown).

Phylogenetic inference showed that strain TF8<sup>T</sup> belongs to the genus Mucilaginibacter in the family Sphingobacteriaceae (Fig. 1). Sequence similarity between strain TF8<sup>T</sup> and its closest relatives, M. lutimaris BR-18<sup>T</sup>, M. soli R9-65<sup>T</sup>, M. litoreus BR-18<sup>T</sup>, M. rigui WPCB113<sup>T</sup> and M. daejeonensis Jip 10<sup>T</sup>, was 94.7, 94.5, 94.5, 94.0 and 93.8 %, respectively. These results suggest that strain TF8<sup>T</sup> is a novel species in the genus Mucilaginibacter.

Strain TF8<sup>T</sup> and the five reference strains were cultured on R2A agar at 25 °C for 3 days for whole-cell fatty acid

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**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences indicating the position of strain TF8<sup>T</sup>, other species of the genus Mucilaginibacter and other representatives of the family Sphingobacteriaceae. Bootstrap values >70% (as percentages of 1000 replicates) are shown at branching points. Flexibacter flexilis NBRC 15060<sup>T</sup> was used as an outgroup. Bar, 0.02 substitution per nucleotide position.
Mucilaginibacter koreensis sp. nov.

Mucilaginibacter koreensis (ko.re.en’sis. N.L. masc. adj. koreensis of Korea).

Cells are Gram-staining-negative, strictly aerobic, non-motile and non-spore-forming rods, surrounded by a thin capsule, approximately 0.4–0.5 μm in diameter and 1.2–2.5 μm in length. Colonies are pale pink, circular, convex, smooth, entire and approximately 3 mm in diameter after 3 days of incubation on R2A agar. Good growth occurs on R2A and PCA, and weak growth occurs on NA and TSA. Growth occurs at 4–32 °C (optimum, 25–28 °C), at pH 5.0–8.0 (optimum pH 6.0–7.0) and in R2A broth supplemented with 0–0.5 % (w/v) NaCl (optimum, without NaCl supplementation). Large amounts of extracellular polymeric substances are produced as shown by the increased viscosity of broth cultures. Catalase- and oxidase-positive. Flexirubin-mentation). Large amounts of extracellular polymeric substances are produced as shown by the increased viscosity of broth cultures. Catalase- and oxidase-positive. Flexirubin-positive. Aesculin is hydrolysed, but starch, casein, gelatin, DNA, and Tween 20, 40, 60 and 80 are not hydrolysed. In the API 50 CH strip, acid is produced from

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Table 2. Cellular fatty acid contents (%) of strain TF8T and related members of the genus Mucilaginibacter

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Straight-chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C14:0</td>
<td>0.9</td>
<td>0.6</td>
<td>TR</td>
<td>TR</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>C16:0</td>
<td>5.4</td>
<td>1.7</td>
<td>1.6</td>
<td>9.7</td>
<td>3.3</td>
<td>1.7</td>
</tr>
<tr>
<td>C17:0</td>
<td>TR</td>
<td>TR</td>
<td>0.5</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>Branched</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>iso-C11:0</td>
<td>–</td>
<td>TR</td>
<td>0.5</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>33.0</td>
<td>22.9</td>
<td>22.7</td>
<td>26.2</td>
<td>22.4</td>
<td>23.3</td>
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<tr>
<td>iso-C15:1</td>
<td>–</td>
<td>–</td>
<td>0.7</td>
<td>–</td>
<td>TR</td>
<td>–</td>
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<tr>
<td>iso-C16:0</td>
<td>0.6</td>
<td>1.4</td>
<td>TR</td>
<td>TR</td>
<td>1.0</td>
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<td>TR</td>
<td>2.4</td>
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<td>TR</td>
<td>TR</td>
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<tr>
<td>anteiso-C15:0</td>
<td>0.7</td>
<td>1.3</td>
<td>0.7</td>
<td>TR</td>
<td>0.9</td>
<td>0.8</td>
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<tr>
<td>Unsaturated</td>
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<td>1.2</td>
<td>1.2</td>
<td>TR</td>
<td>TR</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>C16:015:0</td>
<td>6.9</td>
<td>6.4</td>
<td>6.8</td>
<td>5.1</td>
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<td>14.0</td>
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<tr>
<td>C17:015:0</td>
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<td>–</td>
<td>0.5</td>
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<td>0.5</td>
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<tr>
<td>C17:016:0</td>
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<td>0.8</td>
<td>1.6</td>
<td>TR</td>
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<td>0.5</td>
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<td>0.6</td>
<td>TR</td>
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<td>0.5</td>
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<tr>
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<td>TR</td>
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<td>1.8</td>
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<tr>
<td>iso-C15:0 3-OH</td>
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<td>1.8</td>
<td>1.5</td>
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<tr>
<td>iso-C16:0 3-OH</td>
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<td>0.8</td>
<td>–</td>
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<td>8</td>
<td>13.0</td>
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<td>TR</td>
<td>TR</td>
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<tr>
<td>9</td>
<td>1.6</td>
<td>2.4</td>
<td>12.4</td>
<td>2.9</td>
<td>4.9</td>
<td>1.8</td>
</tr>
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</table>

*As indicated by Montero-Calasanz et al. (2013), summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete equivalent chain lengths (ECLs) as well as those where the ECLs are not reported separately. Summed feature 3 was listed as C16:015:0 and/or C16:107:0; summed feature 8 was listed as C15:016:0 and/or C18:107:0; summed feature 9 was listed as C16:0 10-methyl and/or iso-C17:019:0.

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3 days of incubation on R2A agar. Good growth occurs on R2A and PCA, and weak growth occurs on NA and TSA. Growth occurs at 4–32 °C (optimum, 25–28 °C), at pH 5.0–8.0 (optimum pH 6.0–7.0) and in R2A broth supplemented with 0–0.5 % (w/v) NaCl (optimum, without NaCl supplementation). Large amounts of extracellular polymeric substances are produced as shown by the increased viscosity of broth cultures. Catalase- and oxidase-positive. Flexirubin-type pigments are not produced. Nitrate is not reduced to nitrite. Casein and aesculin are hydrolysed, but starch, gelatin, DNA, and Tween 20, 40, 60 and 80 are not hydrolysed. In the API 50 CH strip, acid is produced from...
galactose, glucose, mannose, methyl z-D-mannoside, methyl z-D-glucoside, N-acetylgalactosamine, aesculin, salicin, cello-
biose, maltose, lactose, melibiose, sucrose, trehalose, gentio-
biose and turanose; acid is not produced from the other
substrates. In the API ZYM strip, alkaline phosphatase,
esterase lipase (C8), leucine arylamidase, valine arylamidase,
cystine arylamidase, acid phosphatase, z-galactosidase, z-
galactosidase, z-glucosidase and N-acetyl-
2278
beta-glucosaminidase activities are present, but esterase (C4),
lipase (C14), trypsin, z-chymotrypsin, naphthol-AS-BI-
phosphohydrolase, z-glucuronidase, z-mannosidase and z-
fucosidase activities are absent. In the API 20E and 20NE
strips, positive for beta-galactosidase activity; utilization of
citrate; and assimilation of glucose, mannose, N-acetyl-
galactosamine and maltose; but negative for arginine dihydrob-2279
alse, lysisin decaxyblose, ornithine decaxyblose, urease, gela-
tinase and tryptophan deaminase activities; production of
H2S, indole and acetoin; nitrate reduction; oxidation of
mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose,
amygdalin and arabinose; fermentation of glucose; and
assimilation of arabinose, mannitol, potassium gluconate,
capric acid, adic acid, malic acid, trisodium citrate and
phenylacetic acid. The major isoprenoid quinone is MK-7
and the major cellular fatty acids (>10%) are iso-C15:0
summed feature 3 (C16:1<ω7c and/or C16:1<ω6c) and summed
feature 8 (C18:1<ω7c and/or C18:1<ω6c). The major polar
lipids are phosphatidylyethanolamine, unidentified aminophos-
pholipids unidentified aminolipids and unidentified lipids.

The type strain is TF8T (=KACC 17468T=JCM 19323T),
isolated from a leaf mould sample in Cheonnan, Republic
of Korea. The DNA G+C content of the type strain is
46.2 mol%.

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