Flavobacterium limi sp. nov., isolated from forest mud

Gabriela Moya,† Zheng-Fei Yan,† Kyung-Hwa Won,† Jung-Eun Yang,† Moo-Chang Kook2 and Tae-Hoo Yi†,*

Abstract

A Gram-stain-negative, aerobic, non-motile, yellow and rod-shaped bacterial strain was isolated from forest mud located at Kyung Hee University, South Korea. Strain THG-AG6.4T grew at 10–35 °C, pH 6.0–9.5 and in the presence of 0–1.5% (w/v) NaCl. Phylogenetic analysis, based on 16S rRNA gene sequencing, showed that strain THG-AG6.4T was most closely related to Flavobacterium gyeonganense HME 7524T (97.66%), Flavobacterium defluvi EMB 117T (96.93%) and Flavobacterium arsenitoxidans S2-3H7T (96.80%). The DNA G+C content of strain THG-AG6.4T was 30.2 mol%. The DNA–DNA relatedness values between strain THG-AG6.4T and its closest phylogenetic neighbour, F. gyeonganense HME 7524T, were below 61.0%. The predominant quinone of strain THG-AG6.4T was MK-6. The major polar lipids were phosphatidylethanolamine, an unidentified phospholipid, five unidentified glycolipids, phosphatidylserine, an unidentified lipid, an unidentified aminophospholipid, two unidentified aminolipids and two unidentified aminoglycolipids. The major fatty acids were C16:0, C18:1 ω7c. The major polyamine was homospermidine. On the basis of the phenotypic, genotypic and phylogenetic characterization of strain THG-AG6.4T, it is concluded that the isolate represents a novel species of the genus Flavobacterium, for which the name Flavobacterium limi sp. nov. is proposed, with THG-AG6.4T as the type strain (=KACC 18851T=CGMCC 1.16060T).

The genus Flavobacterium is the type genus of the family Flavobacteriaceae in the phylum Bacteroidetes. Since the first description of the genus Flavobacterium by Bergey et al. [1], later emended by Bernardet et al. [2], Dong et al. [3], Kang et al. [4] and Kuo et al. [5]. The genus has expanded extensively due to the description of novel species so that at the time of writing it comprises a total of 162 species with validly published names (http://www.bacterio.net/flavobacterium.html). Members of the genus Flavobacterium are usually Gram-staining-negative, straight or slightly curved, single rods, non-motile or motile by gliding, aerobic and produce yellow carotenoid and/or flexirubin pigments. Most species are chemoorganotrophic, and positive for catalase and oxidase [5–7]. Menaquinone MK-6 is the only or the predominant respiratory quinone, phosphatidylethanolamine is the common major polar lipid and the DNA G+C contents range from 30 to 52 mol% [5]. The genus Flavobacterium contains species that have been isolated from many different habitats, including soil, sediment, freshwater, marine environments, glaciers, Antarctic lakes, diseased fish and microbial mats [2, 6–16]. In the present study, the taxonomic characterization of a novel species, Flavobacterium limi sp. nov., using a polyphasic approach, is reported.

Strain THG-AG6.4T was isolated from forest mud, collected at Kyung Hee University (37°16’33” N 127°10’40” E), Yongin, Gyeonggi, South Korea. One gram of sample was suspended in 10 ml sterile water containing 0.85% NaCl (w/v). Serial dilutions of up to 10⁻⁵ were prepared using the same solution. Consequently, 100 µl of each dilution was plated onto nutrient agar plates (NA; Difco). The plates were incubated at 28 °C for 2 days. Single colonies were purified, transferred to fresh NA and were incubated once again. One isolate, THG-AG6.4T, was cultured routinely on NA at 28 °C and preserved as a suspension in nutrient broth (NB; Difco) with glycerol (25%, w/v) at −80 °C. The reference strains Flavobacterium gyeonganense KACC 17688T, Flavobacterium defluvi EMB 11877T, Flavobacterium arsenitoxidans KCTC 22507T and Flavobacterium aquatile KACC 11692T were obtained from the Korean Agricultural Culture Collection (KACC) and the Korean Collection for Type Cultures (KCTC). These strains were cultured under the same optimum conditions as strain THG-AG6.4T.

Author affiliations: †Department of Oriental Medicinal Material and Processing, College of Life Science, Kyung Hee University Global Campus, 1732 Deokyoungdaero, Giheung-gu, Yongin-si, Gyeonggi-do 17104, Republic of Korea; Department of Food Nutrition, Baewha Women’s University, Seoul 03039, Republic of Korea.
*Correspondence: Tae-Hoo Yi, drhoo@khu.ac.kr
†These authors contributed equally to this work.
Keywords: Flavobacterium limi; mud sample; phenotypic analysis.
Abbreviation: PE, Phosphatidylethanolamine.
The GenBank accession number for the 16S rRNA gene sequence of strain THG-AG6.4T is KX263231.
The genomic DNA of strain THG-AG6.4<sup>T</sup> was extracted using a commercial genomic DNA extraction kit (Biofact). The 16S rRNA gene was amplified from the chromosomal DNA with the universal bacterial primer pair, 27F and 1492R [17], and the purified PCR products were sequenced by Biofact (South Korea). The identification of phylogenetic neighbours was determined using the EzBioCloud server (http://www.ezbiocloud.net/identify) [18]. Multiple alignments were performed by using the CLUSTAL_X program [19]. Gaps were edited using the BioEdit program [20] and evolutionary distances were calculated using Kimura’s two-parameter model [21]. The phylogenetic trees were reconstructed using the neighbour-joining method [22] and the maximum-likelihood method in the MEGA 5 Program [23], with bootstrap values based on 1000 replications [24].

The 16S rRNA gene sequence of strain THG-AG6.4<sup>T</sup> was a continuous stretch of 1451 bp. According to the EzBioCloud server analysis, the novel strain was closely related to F. gyeonganense HME 7524<sup>T</sup> (97.66 %), F. defluvii EMB 117<sup>T</sup> (96.93 %) and F. arsenitoxidans S2-3H<sup>T</sup> (96.80 %). The relationship between strain THG-AG6.4<sup>T</sup> and other members of the genus Flavobacterium was also supported by phylogenetic trees (Fig. 1).

Gram staining was determined using a bioMérieux Gram stain Kit according to the manufacturer’s instructions. The growth of strain THG-AG6.4<sup>T</sup> was tested on several bacterial media including Nutrient agar (NA, Oxoid), Trypticase soy agar (TSA; Oxoid), Reasoner’s 2A agar (R2A; Oxoid), Luria-Bertani agar (LA; Oxoid), Marine agar (MA; Difco) and Macconkey agar (MCA; Oxoid), at 28 °C. Adherence of colonies to the agar was tested by trying to collect colonies on NA with a loop and determining the consistency. Growth at different temperatures (4, 10, 15, 25, 30, 35 and 42 °C) and pH values (pH 5.0–10.0, at intervals of 0.5 pH unit) were tested in NB for 5 days at 28 °C. Two different buffers were used (final concentration, 100 mM) to achieve different pH values: acetate buffer was used for pH 5.0–6.5 and phosphate buffer was used for pH 7.0–10.0. Salt tolerance was tested in NB supplemented with 0–5.0 % (w/v) NaCl (at 0.5 % intervals) for 5 days of incubation at 28 °C. Growth was estimated by monitoring optical density at 600 nm. Anaerobic growth was tested at 28 °C in serum bottles containing NB supplemented with thioglycolate (1 g l<sup>−1</sup>), in which the air was substituted with nitrogen for 5 days. Cell morphology was observed with a transmission electron microscope (JEM1010; JEOL; ×11 000) using cells grown for 2 days at 28 °C on NA. Colony morphology was observed on NA [25]. Motility was assayed in sulphide-indole-motility medium (SIM; Difco). The presence of gliding motility was assayed using the method described by Bernardet et al. [26]. Production of flexirubin-type pigments was determined by procedures outlined by Fautz and Reichenbach [27]. Catalase activity was determined by bubble production in 3 % (v/v) H<sub>2</sub>O<sub>2</sub> (Daejung) and oxidase activity was determined using 1 % (w/v) N<sub>N</sub>, N<sub>N</sub>, N<sub>N</sub>′-tetramethyl-1,4-phenylenediamine reagent (Sigma). H<sub>2</sub>S production from thiosulfate was tested according to Tindall et al. [28]. Tests for degradation of starch [1 % (w/v), Difco], casein [2 % (w/v) skimmed milk, Oxoid], DNA (DNase agar, Oxoid), Tween 20 [1.0 % (w/v), Sigma], Tween 80 [1.0 % (w/v), Sigma], L-tyrosine [0.5 % (w/v), Sigma], carboxy methyl cellulose (CMC, 0.1 % (w/v), Sigma) and chitin [1.0 % (w/v), Sigma] were evaluated for 5 days of incubation at 28 °C. F. gyeonganense KACC 17688<sup>T</sup>, F. defluvii KACC 11877<sup>T</sup>, F. arsenitoxidans KCTC 22507<sup>T</sup> and F. aquatile KACC 11692<sup>T</sup> were included as reference strains in biochemical tests under the same laboratory conditions as the novel isolate. Carbon-source utilization and constitutive enzyme activities of strain THG-AG6.4<sup>T</sup> and the closest reference strains were tested using API 20NE, API 32 GN and API ZYM test kits (bioMérieux) according to the manufacturer’s instructions. In addition, aerobic acid production from different carbohydrates was determined by employing the API 50 CH system (bioMérieux), according to the manufacturer’s instructions. The API kits were incubated at 28 °C, and results were obtained after 24–48 h. Phenotypic characteristics of THG-AG6.4<sup>T</sup>, are given in the species description and in Fig. S1 (available in the online Supplementary Material), plus a comparison of the selected characteristics of strain THG-AG6.4<sup>T</sup> and related type strains is presented in Table 1.

For determination of the DNA G+C content, genomic DNA was extracted, purified as described by Moore and Dowhan [29] and degraded enzymatically into nucleosides, which were analyzed using a reversed-phase HPLC system (Alliance 2690, Waters), as described previously [30], using the reversed-phase column, SunFireTM C18 (4.6×250 mm×5 µm), at a flow rate of 1.0 ml min<sup>−1</sup>. The solvent mobile phase consisted of 200 mM (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub>/acetonitrile (97:3, v/v), and detection was at 270 nm. Escherichia coli was used as the standard. The DNA G+C content of strain THG-AG6.4<sup>T</sup> was 30.2 mol%, which lies in the range for G+C contents of members of the genus Flavobacterium (30–52 mol%) [5].

DNA–DNA hybridization was performed fluorometrically, according to Ezaki et al. [31], using photobiotin-labelled DNA probes and micro-dilution wells. DNA–DNA hybridization experiments were performed between strain THG-AG6.4<sup>T</sup> and reference strains. The optimum hybridization temperature for strain THG-AG6.4<sup>T</sup> was 26.4 °C, which was determined by the following calculation: [(0.51×G+C content)+47]–36 [32]. Hybridization was performed with five replicates for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were converted to percentage DNA–DNA relatedness values. The DNA–DNA relatedness values for strain THG-AG6.4<sup>T</sup> with respect to F. gyeonganense KACC 17688<sup>T</sup>, F. defluvii KACC 11877<sup>T</sup> and F. arsenitoxidans KCTC 22507<sup>T</sup> were 61.0 % (40.2 % reciprocal analysis), 44.8 % (33.5 %) and 31.4 % (24.5 %), respectively. These DNA relatedness values were well below the 70 % threshold value [33], suggesting that THG-AG6.4<sup>T</sup> represents a novel species of the genus Flavobacterium.
Flavobacterium dengelachovi/LMG 21915T (AJ557886)
Flavobacterium gilliae ACAM 601T (J885889)
Flavobacterium fricensi LMG 21922T (AJ557887)
Flavobacterium sputii LMG 8756T (FJ654474)
Flavobacterium unciflavum JCM 15935T (AF431173)
Flavobacterium omnorum JCM 15113T (AF431174)
Flavobacterium frigulicola LMG 22002T (AJ811981)
Flavobacterium frigidarium A21T (AF162266)
Flavobacterium algaica TC2T (AB455265)
Flavobacterium psychrilyminae LMG 22018T (AJ585428)
Flavobacterium yushanense 92T (HQ435446)
Flavobacterium tangerense O563T (EU386219)
Flavobacterium fluvii H1T (EU109724)
Flavobacterium reichenbachi/LMG 25512T (JPR01000002)
Flavobacterium limosoa ST-82T (AB075230)
Flavobacterium flaveolum DSM 1076T (AM230486)
Flavobacterium oxyae JY-05T (HE997081)
Flavobacterium tegehiocola DSM 2377T (AJDN01000041)
Flavobacterium antarcticum DSM 19726T (FIM13401)
Flavobacterium segatis AT1048T (AY851115)
Flavobacterium weaverensis AT1042T (AY581114)
Flavobacterium myungsaeanum HM1033T (GG148878)
Flavobacterium branchiophorus FL-1T (FM895180)
Flavobacterium aquatile 294T (JRNH01000003)
Flavobacterium terrigena DS-20T (D0989724)
Flavobacterium swingsi WB 2.3-68T (AM344561)
Flavobacterium psychrophilum IO1 15942T (AB078060)
Flavobacterium psychrophilum JP028968T (AM3988681)
Flavobacterium restrictum BD365T (EF755653)
Flavobacterium succinicans LMG 10402T (JAT01000001)
Flavobacterium glaciei (GD015962)
Flavobacterium granuli Kw05T (AD910738)
Flavobacterium ydvalis DSM 2063T (AM320487)
Flavobacterium peclinorum DSM 6368T (AM230490)
Flavobacterium mastainense T01T (KF857167)
Flavobacterium hibernum DSM 12611T (JPRK01000008)
Flavobacterium chungangense LMG 26729T (JASY01000008)
Flavobacterium chilense LM-09-FpT (FR774915)
Flavobacterium chungbukense LMG 21919T (AJ557888)
Flavobacterium plectroturum 1126-1H-08T (HE612094)
Flavobacterium piscisis 4129-09T (HE612010)
Flavobacterium friguridis LA-1T (AB183888)
Flavobacterium pancticarum DCY69 (JX233806)
Flavobacterium aquiduresis WB 1.1-06T (AM177392)
Flavobacterium saccharophorum DSM 1811T (AM320491)
Flavobacterium micromai DLM 1919T (AJ557888)
Flavobacterium pluviosum 1126-1H-08T (HE612094)
Flavobacterium piscis 4129-09T (HE612010)
Flavobacterium friguridis LA-1T (AB183888)
Flavobacterium pancticarum DCY69 (JX233806)
Flavobacterium aquiduresis WB 1.1-06T (AM177392)
Flavobacterium saccharophorum DSM 1811T (AM320491)
Flavobacterium trucetiae 483-08T (HE812100)
Flavobacterium spirantis T16T (IU287799)
Flavobacterium limi THG-AG84T (X2363212)
Flavobacterium georgianense HME7524T (KC690142)
Flavobacterium seoulense EM1T (JNCA01000002)
Flavobacterium palustre S44T (KJ157269)
Flavobacterium nitratireducens N1T (FR927898)
Flavobacterium glycines Gm-149T (EU672803)
Flavobacterium daejeonense GH1-10T (DO222427)
Flavobacterium chungbukense CS150T (HM327393)
Flavobacterium banpanikense 15F3T (GQ288770)
Flavobacterium avaricinus ATCC 23107T (M62799)
Flavobacterium johnsoniae UW101T (CP003086)
Flavobacterium ginsengleri DCY55 (HM776706)
Flavobacterium compostarboris 15C3T (GQ281769)
Flavobacterium arsenoxidans 92T (JX001187)
Flavobacterium defluvi GB11T (DO297966)
Flavobacterium tlapiae Rev-71T (HQ111525)
Flavobacterium ginsengsi DCY54T (HM776705)
Flavobacterium dentihirundis EDO9T (AJ318907)
Flavobacterium kyongnyangense THG-107T (JN196130)
Flavobacterium anhuiense D3T (EU046269)
Flavobacterium nitrigenilgens NKU-44T (KPT11654)
Flavobacterium phragmitis BLN4T (EU564326)
Flavobacterium sol DSM 19725T (AUGO0100020)
Flavobacterium suzhouense XIN-1T (KM089833)

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain THG-AG64T and related species of the genus Flavobacterium. Filled circles at nodes indicate branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) over 70 % are shown at the branching points. Flavobacterium suzhouense XIN-1T (KM089833) was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.
For fatty acid methyl ester analysis, fatty acids were extracted, methylated and saponified, as described by the Sherlock Microbial Identification system (MIDI) and were analyzed by capillary GC (model 6890; Hewlett Packard) using the Microbial Identification software package with the Sherlock system MIDI 6.1 and the Sherlock Aerobic Bacterial database (TSBA 6.1; Sasser) [34]. The fatty acid profiles of strain THG-AG6.4\textsuperscript{T} and related strains of species of the genus Flavobacterium are shown in Table 2. The major cellular fatty acids were identified as C\textsubscript{16:0} (34.4%), C\textsubscript{18:0} 10-methyl (13.0%), summed feature 3 (C\textsubscript{16:0}ω7c and/or C\textsubscript{16:1}ω6c; 12.1%) and C\textsubscript{18:1}ω9c (10.0%). However, the novel strain was distinguishable from other species of the genus Flavobacterium due to the presence of C\textsubscript{18:0} 10-methyl and C\textsubscript{18:1}ω9c.

The polar lipids of strain THG-AG6.4\textsuperscript{T} were extracted from freeze-dried cells [35, 36]. Polar lipids were examined by 2-dimensional TLC (2D-TLC) using TLC Kieselgel 60 F254 (Merck) plates (10×10 cm) with (1) chloroform/methanol/
Table 2. Cellular fatty acid profiles of strain THG-AG6.4T and its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0</td>
<td>1.0</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>anteiso-C12:0</td>
<td>2.6</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.6</td>
<td>TR</td>
<td>1.5</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C16:0</td>
<td>34.4</td>
<td>8.2</td>
<td>12.5</td>
<td>7.6</td>
<td>7.2</td>
</tr>
<tr>
<td>C17:0</td>
<td>TR</td>
<td>8.2</td>
<td>12.5</td>
<td>7.6</td>
<td>1.1</td>
</tr>
<tr>
<td>C17:0 10-methyl</td>
<td>1.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C17:1ω8c</td>
<td>1.5</td>
<td>TR</td>
<td>1.1</td>
<td>TR</td>
<td>1.3</td>
</tr>
<tr>
<td>C17:1ω9c</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C18:0</td>
<td>9.8</td>
<td>5.5</td>
<td>6.9</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>C18:0 10-methyl (TBSA)</td>
<td>13.0</td>
<td>TR</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>10.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C18:1ω9c (6, 9, 12)</td>
<td>2.0</td>
<td>TR</td>
<td>1.3</td>
<td>TR</td>
<td>1.2</td>
</tr>
<tr>
<td>C19:0</td>
<td>3.1</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C20:0</td>
<td>1.5</td>
<td>2.1</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>12.1</td>
<td>3.2</td>
<td>10.5</td>
<td>12.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Summed feature 9</td>
<td>TR</td>
<td>TR</td>
<td>3.1</td>
<td>TR</td>
<td>TR</td>
</tr>
</tbody>
</table>

*As indicated by Montero-Calasanz et al. [46] 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 ECLs, as well as those where the ECLs are not reported separately. Summed feature 3 was listed as C18:1ω7c and/or C16:1ω6c; summed feature 9 was listed as iso-C17:1ω9c.

The polyamines of strain THG-AG6.4T were extracted as described by Busse and Auling [41], and by Taibi et al. [42]. Homospermidine was the major polyamine, which was consistent with other members of the genus Flavobacterium [5].

In summary, the characteristics of strain THG-AG6.4T were consistent with descriptions of members of the genus Flavobacterium with respect to morphological, biochemical and chemotaxonomic properties. The results of this polyphasic analysis of strain THG-AG6.4T and its closest phylogenetic neighbours indicates that strain THG-AG6.4T should be assigned to the genus Flavobacterium as a novel species, for which the name Flavobacterium limi sp. nov. is proposed.

**DESCRIPTION OF FLAVOBACTERIUM LIMI SP. NOV.**

*Flavobacterium limi* (li’ mi. L. gen. n. limi of mud, the source of the type strain).

Cells are Gram-stain-negative, rod-shaped, 1.7–2.0×0.4–0.5 μm, aerobic and non-motile. Colonies are yellow, slightly sticky, bright, flat, circular and 1.5–4.0 mm in diameter on NA. Flexirubin-type pigments are produced. Growth occurs on R2A, TSA and NA, but not on LA, MCA and MA. Can grow at 10–35 °C (optimum 25–35 °C), at pH 6.0–9.5 (optimum 7.0–8.0) and in the presence of 0–1.5% (w/v) NaCl (optimum 1.0%). Catalase- and oxidase-positive. Is able to hydrolyze CMC, but not DNA, starch, casein, chitin, L-tyrosine, and Tween 20 and 80. H₂S is not produced. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, negative for lipase (C14), acid phosphatase, β-glucuronidase, α-mannosidase and α-fucosidase. Positive for the reduction of nitrates to nitrogen, arginine dihydrolase, hydrolysis of aesculin, PNPG, assimilation of D-glucose, L-arabinose, D-mannose and D-maltose; weakly positive for glucose acidification and the assimilation of N-acetyl-glucosamine; negative for indole production, the hydrolysis of

---

Table 2. Cellular fatty acid profiles of strain THG-AG6.4T and its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0</td>
<td>1.0</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>anteiso-C12:0</td>
<td>2.6</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.6</td>
<td>TR</td>
<td>1.5</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C16:0</td>
<td>34.4</td>
<td>8.2</td>
<td>12.5</td>
<td>7.6</td>
<td>7.2</td>
</tr>
<tr>
<td>C17:0</td>
<td>TR</td>
<td>8.2</td>
<td>12.5</td>
<td>7.6</td>
<td>1.1</td>
</tr>
<tr>
<td>C17:0 10-methyl</td>
<td>1.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C17:1ω8c</td>
<td>1.5</td>
<td>TR</td>
<td>1.1</td>
<td>TR</td>
<td>1.3</td>
</tr>
<tr>
<td>C17:1ω9c</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C18:0</td>
<td>9.8</td>
<td>5.5</td>
<td>6.9</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>C18:0 10-methyl (TBSA)</td>
<td>13.0</td>
<td>TR</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>10.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>C18:1ω9c (6, 9, 12)</td>
<td>2.0</td>
<td>TR</td>
<td>1.3</td>
<td>TR</td>
<td>1.2</td>
</tr>
<tr>
<td>C19:0</td>
<td>3.1</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C20:0</td>
<td>1.5</td>
<td>2.1</td>
<td>ND</td>
<td>ND</td>
<td>TR</td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>12.1</td>
<td>3.2</td>
<td>10.5</td>
<td>12.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Summed feature 9</td>
<td>TR</td>
<td>TR</td>
<td>3.1</td>
<td>TR</td>
<td>TR</td>
</tr>
</tbody>
</table>

*As indicated by Montero-Calasanz et al. [46] 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 ECLs, as well as those where the ECLs are not reported separately. Summed feature 3 was listed as C18:1ω7c and/or C16:1ω6c; summed feature 9 was listed as iso-C17:1ω9c.

The polyamines of strain THG-AG6.4T were extracted as described by Busse and Auling [41], and by Taibi et al. [42]. Homospermidine was the major polyamine, which was consistent with other members of the genus Flavobacterium [5].

In summary, the characteristics of strain THG-AG6.4T were consistent with descriptions of members of the genus Flavobacterium with respect to morphological, biochemical and chemotaxonomic properties. The results of this polyphasic analysis of strain THG-AG6.4T and its closest phylogenetic neighbours indicates that strain THG-AG6.4T should be assigned to the genus Flavobacterium as a novel species, for which the name Flavobacterium limi sp. nov. is proposed.

**DESCRIPTION OF FLAVOBACTERIUM LIMI SP. NOV.**

*Flavobacterium limi* (li’ mi. L. gen. n. limi of mud, the source of the type strain).

Cells are Gram-stain-negative, rod-shaped, 1.7–2.0×0.4–0.5 μm, aerobic and non-motile. Colonies are yellow, slightly sticky, bright, flat, circular and 1.5–4.0 mm in diameter on NA. Flexirubin-type pigments are produced. Growth occurs on R2A, TSA and NA, but not on LA, MCA and MA. Can grow at 10–35 °C (optimum 25–35 °C), at pH 6.0–9.5 (optimum 7.0–8.0) and in the presence of 0–1.5% (w/v) NaCl (optimum 1.0%). Catalase- and oxidase-positive. Is able to hydrolyze CMC, but not DNA, starch, casein, chitin, L-tyrosine, and Tween 20 and 80. H₂S is not produced. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, negative for lipase (C14), acid phosphatase, β-glucuronidase, α-mannosidase and α-fucosidase. Positive for the reduction of nitrates to nitrogen, arginine dihydrolase, hydrolysis of aesculin, PNPG, assimilation of D-glucose, L-arabinose, D-mannose and D-maltose; weakly positive for glucose acidification and the assimilation of N-acetyl-glucosamine; negative for indole production, the hydrolysis of
urea and gelatin, and the assimilation of D-mannitol, glucose, caprate, adipate, malate, trisodium citrate and phenylacetate. Positive for L-rhamnose; weakly positive for D-sucrose; negative for salicin, D-melibiose, L-fucose, D-sorbitol, propionate, valerate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, D-ribose, inositol, itaconate, suberate, sodium malonate, sodium acetate, D,L-lactate, L-alanine, 5-ketogluconate, glycophenol, 3-hydroxy-benzoate and L-serine. Positive for galactose, cellobiose, lactose, D-xylose, D-fructose, amygdalin, arbutin, starch and β-gentiobiose and negative for the other tests.

The predominant polar lipids are phosphatidylethanolamine, an unidentified phospholipid, five unidentified glycolipids, phosphatidylserine, an unidentified lipid, an unidentified aminophospholipid, two unidentified aminolipids, phosphatidylcholine, 

The predominant menaquinone is MK-6. C16:0, C18:0 10-methyl, summed feature 3 (C16:1ω7c and/or C16:1ω6c) and C18:1ω9c are the major components of cellular fatty acids. The predominant polar lipids are phosphatidylethanolamine, an unidentified phospholipid, five unidentified glycolipids, phosphatidylserine, an unidentified lipid, an unidentified aminophospholipid, two unidentified aminolipids and two unidentified aminoglycolipids. Homospermidine is the major polyamine.

The type strain, THG-AG6.4T (=KACC 18851T=CGMCC 1.16060T), was isolated from forest mud, collected at Kyung Hee University, Yongin, Gyeonggi, South Korea. The DNA G+C content of the type strain is 30.2 mol%.

Funding information
This work was conducted under the industrial infrastructure program for fundamental technologies which is funded by the Ministry of Trade, Industry and Energy (MOTIE), Korea (no. N0000888).

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
It is the original work of the authors. The work described has not been submitted elsewhere for publication, in whole or in part, and all authors listed carried out the data analysis and manuscript writing and This article does not contain any studies with human participants or animals performed by any of the authors. Moreover, all authors read and approved the final manuscript.

References
11. Kacagan M, Inan K, Belduz AO, Canacsi K. Flavobacterium anatoliense sp. nov., isolated from forest mud, collected at Kyung Hee University, Yongin, Gyeonggi, South Korea. The DNA G+C content of the type strain is 30.2 mol%.
Tindall BJ, Sikorski J, Smibert RM, Krieg NR.

27. Bernardet JF, Nakagawa Y, Holmes B.


Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.