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Flavobacterium ureilyticum sp. nov., a novel urea hydrolysing bacterium isolated from stream bank soil

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Abstract A novel bacterium designated S-42^T was isolated from stream bank soil. Cells were found to be aerobic, Gram staining-negative, oxidase-positive, catalase-negative, non-motile, non-spore-forming, rod-shaped, and yellow-pigmented. The strain can grow at 15–35 °C, pH 6.0–10.0, and at 0.5% (w/v) NaCl concentration. Urea was hydrolysed. Flexirubin-type pigments were absent. Phylogenetic analysis based on its 16S rRNA gene sequence revealed that strain S-42^T formed a lineage within the family *Flavobacteriaceae* of the phylum *Bacteroidetes* that is distinct from various species of the genus *Flavobacterium*, including *Flavobacterium maotaiense* T9^T (97.6% sequence similarity), *Flavobacterium hibernum* ATCC 51468^T (97.4%), and *Flavobacterium granuli* Kw05^T (97.1%). The 16S rRNA gene sequences identity between strain S-42^T and other members of the genus *Flavobacterium* were $< 97.0\%$. Strain S-42^T contains MK-6 as sole respiratory quinone. The major polar lipids were identified as phosphatidylethanolamine and an unidentified aminolipid. The major cellular fatty acids were identified as iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ 3-OH, and iso-$C_{15:1}$ G. The DNA G + C content of the strain was 35.8 mol%. The polyphasic characterization indicated that strain S-42^T represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium ureilyticum* sp. nov. is proposed. The type strain is S-42^T (= KEMB 9005-537^T = KACC 19115^T = NBRC 112683^T).

Keywords *Flavobacterium ureilyticum* sp. nov · *Flavobacteriaceae* · Urea hydrolysis · Stream bank soil

Introduction

The genus *Flavobacterium* was proposed by Bergey et al. (1923) for strains that were previously described as *Bacillus aquatilis* by Frankland and Frankland (1889) to accommodate rod-shaped, non-spore-forming, Gram staining-negative bacteria (Bergey et al. 1923; Frankland and Frankland 1889; Weeks 1955). This genus belongs to the *Cytophaga–Flavobacterium–Bacteroides* (CFB) group of the phylum *Bacteroidetes* (Ludwig and Klenk 2001; Bernardet

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S-42^T is KY117464.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10482-018-1105-x) contains supplementary material, which is available to authorized users.

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and Bowman 2010). At the time of writing, there are more than 180 species with validly published names (http://www.bacterio.net/flavobacterium.html). However, descriptions of the genus have been extensively emended; the key characteristics are yellow coloured colonies, inability to produce indole, inability to grow in anaerobic conditions, DNA G + C content in the range of 30–41 mol% (except *Flavobacterium caeni*; 52 mol%), most of the members contain iso-$C_{15:0}$ as a major fatty acid, menaquinone-6 as major respiratory quinone, and phosphatidylethanolamine as major polar lipid (Bernardet et al. 1996; Dong et al. 2013; Kang et al. 2013; Liu et al. 2010). Members of the genus *Flavobacterium* are cosmopolitan in distribution and have been frequently isolated from soil, water, sludge, sediments, plants, fish, and food products. Although most species are non-pathogenic, some species notably *Flavobacterium columnare*, *Flavobacterium branchiophila*, *Flavobacterium psychrophilum*, and *Flavobacterium spartansii* are recognized as fish pathogens (Pilarski et al. 2008; Wakabayashi et al. 1989; Duchaud et al. 2007; Starliper 2001; Louch and Faisal 2014). Some species have been isolated from wastewater polluted with heavy metal and sediment with very high arsenic content (Ao et al. 2014; Yoon et al. 2009). In addition, some urea hydrolyzing members of the genus *Flavobacterium* have also been reported (Pilarski et al. 2008; Nupur et al. 2013).

Strain S-42^T was isolated during a study of bacterial diversity of soil near Kyonggi University. This study describes a novel member of the genus *Flavobacterium*, isolated from stream bank soil near Kyonggi University, Suwon, South Korea and subjected to polyphasic taxonomic characterization.

Materials and methods

Isolation, cultivation and maintenance

Strain S-42^T was isolated using a modified culture technique with 6-well polycarbonate transwell plates (Corning, Inc.) from stream bank soil near Kyonggi University, Suwon, South Korea. Isolation, routine culture, and preservation were done as described previously (Dahal and Kim 2016; Dahal et al. 2017). Based on 16S rRNA gene sequence similarities and phylogenetic analyses, *Flavobacterium maotaiense* JCM 19927^T, *Flavobacterium hibernum* DSM 12611^T, and *Flavobacterium granuli* KACC 11820^T were selected for comparative analyses and were used as reference strains. In addition, the type species of the genus *Flavobacterium*, *Flavobacterium aquatile* KACC 11692^T was also selected for comparative physiological and chemotaxonomic analyses. All the reference strains were cultivated on R2A agar plate at 28 °C for 3–4 days.

Cell morphology

The morphologies of cells grown on R2A agar for 3–4 days at 28 °C were observed by light microscopy (BX50 microscope; Olympus, Japan), and transmission electron microscopy (Bio-TEM, Hitachi, H-7650, Japan). Colony morphology was observed on R2A agar after incubation at 28 °C for 4 days using a Zoom Stereo Microscope (SZ61; Olympus, Japan). Gram staining was performed according to the procedure described by Doetsch (1981).

Physiological and biochemical tests

Production of flexirubin-type pigment was investigated by flooding with 20% (w/v) KOH solution (Reichenbach 1992). Catalase activity was determined by production of bubbles with 3% (v/v) hydrogen peroxide (H_2O_2). Oxidase activity was determined using 1% (w/v) tetra-methyl-*p*-phenylenediamine dihydrochloride. Motility was assessed in SIM (Oxoid) medium and/or R2A medium containing 0.4% agar. Growth was assessed on various media, including R2A agar (MB Cell), nutrient agar (NA; Oxoid), tryptone soya agar (TSA; Oxoid), sorbitol MacConkey agar (MA; Oxoid), marine agar (Difco), brain heart infusion agar (BHI; Bacto), mueller–hinton agar (MHA; BBL), veal infusion agar (Difco), and Luria–Bertani agar (LBA; Oxoid). Growth at various temperatures, 4–37 °C (4, 10, 15, 20, 25, 28, 30, 32, 35, and 37 °C) was determined on R2A plates for 7 days. The pH range for growth was determined by cultivation at 28 °C in R2A broth adjusted to pH 4–12 (at 0.5 pH unit intervals) prior to sterilization using 5 M citrate/NaH_2PO_4 buffer (for pH 4.0–5.5), 5 M phosphate buffer (for pH 6–7.5), 5 M Tris buffer (for pH 8–10) (Breznak and Costilow 2007), and 5 M NaOH (for pH 10.5–12.0) each at a final concentration of 20 mM. Growth in NaCl was examined by cultivation

in R2A broth containing 0–5% NaCl (w/v, at 0.5% intervals). Anaerobic growth was assessed by cultivation on R2A agar at 28 °C for 10 days in a BBL (Becton–Dickinson) anaerobic jar with a GasPak™ EZ Gas Generating Container (Becton–Dickinson). Hydrolysis of Tween 80, Tween 60, and Tween 40 were assessed according to the method of Smibert and Krieg (1994). Hydrolysis of chitin, CM-cellulose, hypoxanthine, tyrosine, starch, and casein were evaluated as described previously (Dahal and Kim 2017). Production of hydrogen sulphide and indole was assessed using sulphide indole motility medium (SIM; Oxoid). A DNase assay was performed on DNase agar (Oxoid) by flooding 6 N HCl. Endospore formation was evaluated by staining with malachite green. Other physiological and biochemical tests were performed using API 20NE, and API ID 32GN test kits (bioMérieux). Enzyme activities were tested using an API ZYM kit (bioMérieux) according to the manufacturer's instructions.

Phylogenetic analysis

For 16S rRNA gene sequencing, genomic DNA was extracted using an InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA), and the 16S rRNA gene was amplified by PCR using universal bacterial primers 27F (forward) and 1492R (reverse) (Frank et al. 2008). PCR product was purified with multiscreen-filter plate (Millipore Corp., Bedford, MA, USA), and was sequenced with an Applied Biosystems 3770XL DNA analyser using a BigDye Terminator cycle sequencing kit v.3.1 (Applied Biosystems, USA). Almost complete sequence was assembled with SeqMan software (DNASTAR Inc.). The nearly complete length of 16S rRNA gene sequence for strain S-42^T was 1475 bp. The close phylogenetic neighbours were identified using the EzBioCloud (Yoon et al. 2017). All the 16S rRNA sequences of the close members were retrieved from NCBI GenBank and subjected to multiple sequence alignment using Clustal X 2.1 (Larkin et al. 2007). After multiple alignments, gaps at the 5′ and 3′ ends were deleted using the software package BioEdit (Hall 1999). Phylogenetic trees were constructed using MEGA6 (Tamura et al. 2013) by three different treeing methods: neighbour–joining method (Saitou and Nei 1987), maximum–parsimony algorithm (Fitch 1971), and maximum–likelihood algorithm (Felsenstein 1981). During phylogenetic analysis, evolutionary distances were calculated using Kimura two-parameter model (Kimura 1980), and bootstrap values were calculated based on 1000 replications (Felsenstein 1985).

Chemotaxonomic characterization

For the fatty acid analysis, cellular biomass of strain S-42^T and reference strains were harvested from R2A plate incubated at 28 °C for 3 days. Fatty acids were extracted using the standard MIDI protocol (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed with a gas chromatograph (HP 6890 Series GC System; Hewlett Packard) and identified using the TSBA6 database of the Microbial Identification System (Sasser 1990).

The respiratory quinone was extracted and analysed from freeze-dried cells as described by Minnikin et al. (1984). Isoprenoid quinone was extracted with methanol–water (10:1 v/v; water containing 0.3% sodium chloride) and petroleum ether at 80 °C, evaporated under a vacuum, re-extracted with acetone, and analysed by HPLC (Minnikin et al. 1984; Collins and Jones 1981). The polar lipids were extracted by the method described by Nguyen and Kim (2017). The polar lipids were analysed by two-dimensional TLC using chloroform/methanol/water (65:25:4; v/v/v) in the first dimension and chloroform/methanol/acetic acid/water (40:7.5:6:2, v/v/v/v) in the second. Appropriate detection reagents (Minnikin et al. 1984; Komagata and Suzuki 1987) were used to identify the spots; molybdophosphoric acid (phosphomolybdic acid reagent, 5% v/v solution in ethanol; Sigma-Aldrich, Germany) was used to detect total polar lipids, ninhydrin reagent (0.2% solution; Sigma Life Science, USA) was used to detect amino lipids, Zinzadze reagent (molybdenum blue spray reagent, 1.3%; Sigma Life Sciences) was used to detect phospholipids, and α-naphthol reagent was used to detect glycolipids.

Genotypic characterization

For DNA–DNA hybridization and DNA G + C mol%, genomic DNAs of strains were extracted according to the method presented by Cheng and Jiang (2006). DNA G + C content was determined according to the procedure described by Mesbah et al. (1989). DNA–DNA hybridization was measured

fluorometrically according to the method developed by Ezaki using photobiotin-labelled DNA probes and micro-dilution plates (Ezaki et al. 1989). All the assays were carried out in triplicate.

Results and discussion

Morphological and physiological characteristics

Cells (Fig. S1) appear as rod-shaped and are Gram stain-negative, non-motile, non-spore-forming, and aerobic. Colonies on R2A are yellow-pigmented, circular, entire, and convex. Cells are 1.8–2.2 μm long and 0.5–0.8 μm wide. After incubation on R2A agar for 4 days at 28 °C, the size of the colonies was 2–3 mm in diameter. The strain can grow at 15–35 °C, pH 6.0–10.0, and at 0.5% (w/v) NaCl concentration. Urea is hydrolysed. Flexirubin-type pigments are absent. The differential phenotypic features of strain S-42^T are presented in Table 1 with other closely related members of the genus *Flavobacterium*.

Phylogenetic analysis

The nucleotide sequence of the 16S rRNA gene of strain S-42^T has been deposited in GenBank/EMBL/DDBJ under the accession number KY117464. Preliminary comparisons with the 16S rRNA gene sequences in GenBank indicated that strain S-42^T belongs to the genus *Flavobacterium* and is closely related to *F. maotaiense* T9^T (97.4% sequence similarity), *F. hibernum* ATCC 51468^T (97.4%), and *F. granuli* Kw05^T (97.1%). The 16S rRNA gene sequence identity between strain S-42^T and other members of the genus *Flavobacterium* were <97.0%. A phylogenetic tree based on these sequences and ones with lower similarities is presented in Fig. 1.

Based on 16S rRNA gene sequence similarities and phylogenetic analysis, *F. maotaiense* JCM 19927^T, *F. hibernum* DSM 12611^T, *F. granuli* KACC 11820^T, and *F. aquatile* KACC 11692^T were selected for comparative analysis and were used as reference strains for biochemical tests, fatty acid analysis, menaquinone, and DNA–DNA hybridization.

Chemotaxonomic characteristics

The major cellular fatty acids were identified as iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ 3-OH, and iso-$C_{15:1}$ G (Table 2). Menaquinone-6 (MK-6) was detected as sole respiratory quinone. The major polar lipids of strain S-42^T were identified as phosphatidylethanolamine (PE) and an unidentified aminolipid (AL4). In addition, three unidentified aminolipids (AL1–AL3), and four unidentified polar lipids (L1–L4) were also detected in moderate amounts (Fig. S2).

DNA G + C content and DNA–DNA hybridization

The G + C content of type strain S-42^T was 35.8 mol%, which is in line with that (30–52 mol%) of members of the genus *Flavobacterium*. DNA–DNA relatedness of the strain S-42^T with reference strains showed DNA similarities of 45.6 ± 3.2% (reciprocal, 51.1 ± 2.7%)with *F. maotaiense* JCM 19927^T, 43.7 ± 2.9% (reciprocal, 45.3 ± 2.6%) with *F. hibernum* DSM 12611^T, and 29.7 ± 2.3% (reciprocal, 28.1 ± 3.3%) with *F. granuli* KACC 11820^T. DNA–DNA relatedness between the species demonstrated that, strain S-42^T differs genetically from closely related type strains of the genus *Flavobacterium* at the species level as the relatedness is below the recommended 70% cut off value (Wayne et al. 1987).

Maximum growth temperature (35° C); highest salt tolerance (0.5%); inability to assimilate L-proline and *N*-acetyl-glucosamine; and weak assimilation of D-mannose phenotypically differentiate strain S-42^T from its phylogenetically closely related type strains. Moreover, *F. maotaiense* JCM 19927^T, *F. hibernum* DSM 12611^T, *F. granuli* KACC 11820T, and *F. aquatile* KACC 11692^T are catalase positive but strain S-42^T showed a negative result for catalase activity (Table 1). Absence of fatty acids $C_{15:0}$, iso-$C_{15:1}\omega10c$; presence of minor fatty acids $C_{17:0}$, $C_{13:1}$, $C_{17:1}\omega5c$, $C_{18:1}\omega5c$, anteiso-$C_{17:0}$, iso-$C_{14:1}$ E, anteiso-$C_{15:1}$ A, anteiso-$C_{17:1}\omega9c$, $C_{17:0}$ 2-OH, and $C_{17:0}$ 3-OH and the differences in percentages of major and other minor fatty acids showed characteristic differences for strain S-42^T from the most closely related type strains (Table 2). The sole respiratory quinone was MK-6, as reported as the major respiratory quinone of all

Table 1 Phenotypic characteristics that differentiate strain S-42^T from phylogenetically closely related type strains of the genus *Flavobacterium*

Characteristic	1	2	3	4	5
Isolation source	Soil	(Freshwater)	(Antartic freshwater)	(Wastewater)	(Freshwater)
Colony colour	Yellow	Golden yellow	Yellow	Yellow	Yellow
Motility	−	−	+	−	−
Catalase	−	+	+	+	+
Maximum growth temperature (°C)	35	37	31	37	37
Highest salt tolerance (%, w/v)	0.5	1.0	2.0	2.0	0.5
Nitrate reduction	−	−	+	−	−
Flexirubin-type pigment	−	−	+	−	−
Hydrolysis of					
DNA	−	−	+	−	−
Casein	+	+	+	−	+
Starch	+	−	+	−	+
Gelatin	−	+	+	−	−
Tyrosine	−	−	+	−	+
Enzyme activities (API ZYM)					
Esterase (C4)	w	w	w	−	+
Esterase lipase (C8)	w	−	+	−	−
Cystine arylamidase	w	−	w	+	+
β-galactosidase	−	−	−	+	−
α-glucosidase	+	−	+	+	−
β-glucosidase	−	−	+	−	−
N-Acetyl-*β*-glucosaminidase	−	−	+	−	−
Assimilation of (API 20NE + ID 32GN)					
D-Glucose	+	−	+	+	−
D-Mannitol	−	+	+	−	−
D-Mannose	w	+	+	+	−
D-Melibiose	−	−	+	−	−
D-Ribose	−	+	−	−	−
D-Saccharose (Sucrose)	+	−	+	−	+
D-Sorbitol	−	−	+	−	−
Glycogen	+	+	+	−	−
Lactic acid	−	−	+	−	−
L-Arabinose	−	−	+	−	−
L-Proline	−	+	+	+	−
L-Rhamnose	−	−	+	−	−
L-Serine	−	−	+	−	−
Malic acid	−	+	−	−	−
N-acetyl-glucosamine	−	+	+	+	−
Potassium gluconate	−	+	−	−	−
Propionic acid	−	−	+	+	−

Table 1 continued

Characteristic	1	2	3	4	5
DNA G + C content (mol%)	35.8	(37.7)	(34.0)	(36.2)	(33.0)

Strains: 1, S-42^T; 2, *Flavobacterium maotaiense* JCM 19927^T (Feng et al. 2015); 3, *Flavobacterium hibernum* DSM 12611^T (McCammon et al. 1998); 4, *Flavobacterium granuli* KACC 11820^T (Aslam et al. 2005); 5, *Flavobacterium aquatile* KACC 11692^T (Bernardet et al. 1996). All data are from the present study except indicated in the parentheses. +, positive; w, weakly positive; −, negative

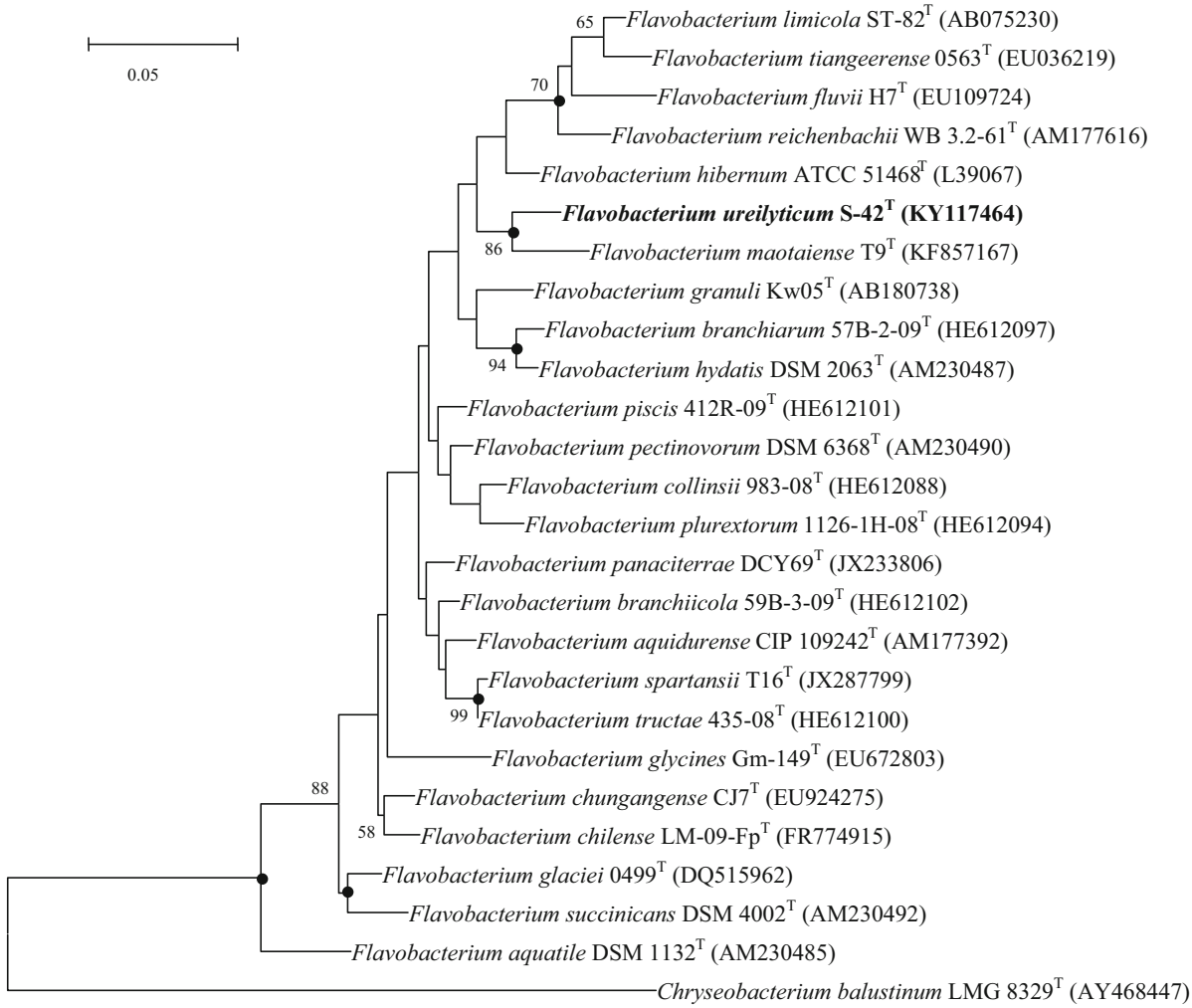

Fig. 1 Maximum–likelihood tree based on 16S rRNA gene sequences showing the phylogenetic position of strain S-42^T among closely related members of the genus *Flavobacterium*. Filled circles indicate nodes recovered by all three treeing methods (neighbour–joining, maximum–likelihood, and maximum–parsimony). The numbers at the nodes indicate the percentage of 1000 bootstrap replicates yielding this topology; only values > 50% are shown. *Chryseobacterium balustinum* LMG 8329^T was used as an out-group. GenBank accession numbers are given in parentheses. Bar, 0.05 substitutions per nucleotide position

Table 2 Cellular fatty acid profiles (% of totals) of strain S-42^T and phylogenetically closely related species of the genus *Flavobacterium*

Fatty acid	1	2	3	4	5
Saturated					
$C_{10:0}$	–	–	0.2	0.1	–
$C_{13:0}$	0.2	0.3	–	–	–
$C_{14:0}$	2.1	1.1	0.9	0.6	0.3
$C_{15:0}$	–	9.7	5.9	5.5	3.6
$C_{16:0}$	7.6	4.8	4.1	2.2	2.2
$C_{17:0}$	0.2	–	–	–	–
$C_{18:0}$	–	0.1	0.4	0.5	1.7
Unsaturated					
$C_{13:1}$ at 12-13	1.0	–	–	–	–
$C_{15:1}\omega6c$	2.3	3.1	3.9	6.1	2.5
$C_{17:1}\omega5c$	0.2	–	–	–	–
$C_{17:1}\omega6c$	1.0	1.6	–	3.6	–
$C_{17:1}\omega8c$	1.3	0.5	–	1.2	–
$C_{18:1}\omega5c$	0.7	–	–	–	–
Branched saturated					
iso-$C_{12:0}$	0.2	–	–	0.9	0.9
iso-$C_{13:0}$	0.5	0.2	0.2	1.9	1.9
iso-$C_{14:0}$	0.5	0.8	0.2	1.7	1.7
iso-$C_{15:0}$	29.8	18.6	19.9	27.7	32.3
iso-$C_{16:0}$	1.9	2.3	1.2	0.9	0.9
iso-$C_{17:0}$	0.7	0.1	0.3	0.4	0.4
anteiso-$C_{13:0}$	–	–	–	0.4	0.4
anteiso-$C_{15:0}$	7.4	11.2	3.7	4.4	4.4
anteiso-$C_{17:0}$	0.2	–	–	–	–
Branched unsaturated					
iso-$C_{14:1}$ E	0.4	–	–	–	–
iso-$C_{15:1}$ G	5.6	0.2	0.2	–	12.8
iso-$C_{15:1}\omega10c$	–	10.1	4.4	2.9	0.1
iso-$C_{16:1}$ H	0.7	0.4	–	0.8	1.2
anteiso-$C_{15:1}$ A	0.3	–	–	–	0.1
anteiso-$C_{17:1}\omega9c$	1.0	–	–	–	–
Hydroxy					
$C_{15:0}$ 2-OH	0.4	–	1.5	–	–
$C_{17:0}$ 2-OH	1.0	–	–	–	–
$C_{8:0}$ 3-OH	–	0.2	–	–	–
$C_{15:0}$ 3-OH	–	4.1	2.2	2.9	0.5
$C_{16:0}$ 3-OH	1.3	1.4	5.8	1.2	1.5
$C_{17:0}$ 3-OH	0.4	–	–	–	–
iso-$C_{15:0}$ 3-OH	6.7	4.6	8.9	5.1	2.3
iso-$C_{16:0}$ 3-OH	2.9	1.9	2.1	1.4	1.2
iso-$C_{17:0}$ 3-OH	6.8	4.2	11.1	15.1	16.2
anteiso-$C_{17:0}$ 3-OH	–	–	0.8	0.9	0.4
Summed features[a]					
3	10.5	16.1	19.6	7.1	5.5
9	4.4	1.6	1.7	2.7	4.2

Strains: 1, S-42^T; 2, *Flavobacterium maotaiense* JCM 19927^T; 3, *Flavobacterium hibernum* DSM 12611^T; 4, *Flavobacterium granuli* KACC 11820^T; 5, *Flavobacterium aquatile* KACC 11692^T. All data were obtained from this study. Fatty acids amounting to < 0.1% of the total fatty acids in all strains are not shown; –, not detected or < 0.1%

[a]Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 comprised $C_{16:1}\omega7c$ and/or $C_{16:\ 1}\omega6c$, summed feature 9 comprised iso-$C_{17:\ 1}\omega9c$ and/or $C_{16:0}$ 10-methyl

members of the genus *Flavobacterium*. In addition, the presence of four unidentified aminolipids and four unidentified polar lipids, their spot positions and amounts also differentiates strain S-42^T as a different species (Fig. S2) (Feng et al. 2015). Furthermore, strain S-42^T was genotypically distinguished by DNA–DNA relatedness values (29–46% of relatedness lower than 70% cut off value for species delineation). Based on this polyphasic characterization, strain S-42^T represents a novel member in the genus *Flavobacterium*, for which the name *Flavobacterium ureilyticum* sp. nov. is proposed.

Description of *Flavobacterium ureilyticum* sp. nov.

Flavobacterium ureilyticum (ur.e.i.ly′ti.cum. N.L. n. *urea* urea; N.L. neut. adj. *lyticum* from Gr. adj. *lytikos* dissolving; N.L. neut. adj. *ureilyticum* urea-dissolving).

Cells appear as rod-shaped and are Gram staining-negative, non-motile, non-spore-forming, and aerobic. Grows well on R2A but no growth is observed on NA, TSA, LBA, BHI, PDA, veal infusion agar, marine agar, and MacConkey agar. Colonies on R2A are yellow-pigmented, circular, entire, and convex. Cells are 1.8–2.2 μm long and 0.5–0.8 μm wide. After incubation on R2A agar for 4 days at 28 °C, the size of the colonies is 2–3 mm in diameter. Cells grow at 15–35 °C (optimum, 20–30 °C) and pH 6.0–10.0

(optimum pH, 7.0–9.0). No growth is observed in 1% NaCl. Oxidase test and urease activity are positive. Flexirubin pigments are not produced. Hydrogen sulphide is not produced from culture in SIM medium. Glucose is not fermented. Indole and catalase tests are negative. Gelatin is not liquefied. Casein, starch, and CM-cellulose are hydrolysed but chitin, tyrosine, DNA, hypoxanthine, Tween 40, Tween 60, and Tween 80 are not. Esculin ferric citrate is positive. Nitrate is not reduced to nitrite. The type strain shows the following enzyme activities: positive for, alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase; weakly positive for esterase (C4), esterase lipase (C8), and cystine arylamidase; and negative for, lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. The following substrates are assimilated: D-glucose, D-maltose, sucrose, glycogen, and D-mannose. The major fatty acids are iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ 3-OH, and iso-$C_{15:1}$ G. The sole respiratory quinone is MK-6. The major polar lipids are phosphatidylethanolamine and an unidentified aminolipid. The DNA G + C content of the type strain is 35.8 mol%.

The type strain, S-42^T (= KEMB 9005-537^T = KACC 19115^T = NBRC 112683^T), was isolated from stream bank soil near Kyonggi University, Suwon, Gyeonggi-Do, South Korea. The Digital Protologue database TaxonNumber for strain S-42^T is TA00529.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Human and animal rights This study does not describe any experimental work related to human.

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