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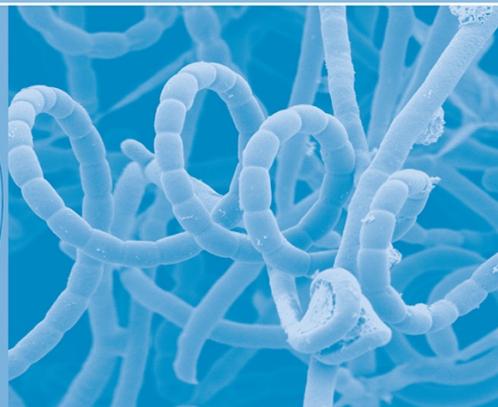
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Flavobacterium vireti sp. nov., isolated from soil

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Abstract A novel Gram-negative, aerobic, yellow-pigmented, non-motile and rod-shaped bacterium, designated as THG-SM1^T, was isolated from field soil collected from Suwon, South Korea. The strain was found to grow optimally at 28 °C, at pH 7.0 and in the absence of NaCl. Based on 16S rRNA gene sequence similarities, strain THG-SM1^T belongs to the genus *Flavobacterium* and is most closely related to *Flavobacterium terrae* KACC 11731^T, followed by *Flavobacterium columnare* KACC 11683^T and *Flavobacterium enshiense* KCTC 23775^T. The DNA G+C content of the novel isolate was determined to be 38.5 mol%. In DNA–DNA hybridization tests, the DNA relatedness between strain THG-SM1^T and its

closest phylogenetic neighbour *F. terrae* was below 50 %. Flexirubin-type pigments were found to be present. The major polar lipid and isoprenoid quinone were phosphatidylethanolamine and menaquinone 6 (MK-6), respectively. The main cellular fatty acids were identified as iso-C_{15:1}G, iso-C_{15:0} 3OH, iso-C_{16:0} and iso-C_{15:0}. The DNA–DNA hybridization result and differentiating chemotaxonomic and phenotypic characteristics showed that strain THG-SM1^T represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium vireti* sp. nov. is proposed. The type strain is THG-SM1^T (=KACC 18371^T = CCTCC AB2014312^T).

Keywords *Flavobacterium vireti* · 16S rRNA · Menaquinone (MK-6)

Hina Singh and Juan Du have contributed equally to this work.

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Introduction

The genus *Flavobacterium* was proposed by Bergey et al. (1923); the description of the genus has been revised by Bernardet et al. (1996), Dong et al. (2013) and Kang et al. (2013). The genus *Flavobacterium* belongs to the family *Flavobacteriaceae* within the phylum *Bacteroidetes*. The membership of the genus has been growing rapidly, as many novel species have been reported in recent years. The common characteristics of the members of the genus are Gram-negative, rod-shaped, aerobic, non-motile or motile by

gliding. Flagella have not been reported so far. Flexirubin-type pigments are produced. Their DNA G+C contents are in the range of 30–41 mol% (Bernardet and Bowman 2011; Xu et al. 2011), except for that of *Flavobacterium caeni* (52 mol%) (Liu et al. 2010). Members of the genus have menaquinone-6 (MK-6) as the predominant isoprenoid quinone and most of the members contain iso-C_{15:0} as a major fatty acid. The major polar lipid shown by most of the members of the genus is phosphatidylethanolamine (Park et al. 2006, 2007; Ryu et al. 2007, 2008; Sheu et al. 2011). Members of this genus have been reported from a wide range of environments such as soil (Kim et al. 2006; Yoon et al. 2006, 2007; Weon et al. 2007; Yang et al. 2011), sediments, freshwater, seawater, glaciers, microbial mats and deceased fish (Bernardet and Bowman 2011). *Flavobacterium* species have been reported to produce a variety of enzymes (Aslam et al. 2005; Park et al. 2006, 2007; Ryu et al. 2007; Liu et al. 2010). Some *Flavobacterium* species have also been reported play a key role in the uptake of organic matter from aquatic environments and degradation of dissolved organic material and biopolymers such as complex polysaccharides (Kirchman 2002; Bernardet and Bowman 2011). Some species of *Flavobacterium* are important fish pathogens (Frerichs and Roberts 1989; Noga 2000). In particular, *Flavobacterium columnare* is the etiological agent of columnaris disease, a common bacterial disease affecting the skin and gills of freshwater fish which may cause large mortalities.

In this study we determine the taxonomic position of strain THG-SM1^T by means of a polyphasic approach. The phenotypic and genotypic characterisation of the novel strain is described in this report.

Materials and methods

Isolation, morphological and physiological characterisation

A soil sample was collected in a clean zip lock cover from a field in Suwon, South Korea. Isolation of the novel isolate was carried out using a serial dilution method by plating on nutrient agar (NA; Difco, France). One gram of soil sample was suspended in 10 ml of 0.85 % (w/v) saline solution and was spread on to NA plates. The plates were incubated under

aerobic condition at 28 °C for one week. After incubation, several bacterial colonies were observed on the agar plates. Single colonies were purified by transferring them on to new NA plates and incubating in same condition. One bacterial strain forming yellow colonies, designated as THG-SM1^T, was selected for the further study. Strain THG-SM1^T was cultured routinely on NA plates and preserved as a suspension in nutrient broth (NB) with 25 % (v/v) glycerol and stored at –80 °C. Strain THG-SM1^T has been deposited in the Korean Agriculture Culture Collection (KACC 18371^T) and China Centre for Type Culture Collection (CCTCC AB 2014312^T). Three reference type strains (Table 1) were obtained from the Korean Collection for Type Cultures and Korean Agricultural Culture Collection.

Gram-reaction was tested by using a Gram stain kit (bioMérieux, France) according to the manufacturer's instructions. Cell morphology was observed at ×11,000

Table 1 Differential characteristics of strain THG-SM1^T and the type strains of closely related reference species

Characteristic	1	2	3	4
Colony color	Y	YO	Y	BY
Gliding motility	–	–	+	–
Catalase	–	–	+	+
Growth on				
NA	+	+	–	–
TSA	+	+	–	+
Hydrolysis of				
Tween 20	+	–	–	–
Starch	+	+	–	–
CM-cellulose	+	–	–	–
Enzyme activities (API ZYM)				
Lipase (C14)	+	–	+	–
Trypsin	–	–	+	+
Acid phosphatase	–	+	+	+
α-chymotrypsin	–	–	+	+
α-glucosidase	–	+	–	–
DNA G+C content (mol%)	38.5	34.4 ^a	32.0 ^b	34.0 ^c

Strain: 1. THG-SM1^T; 2. *Flavobacterium terrae* KACC 11731^T; 3. *Flavobacterium columnare* KACC 11683^T; 3. *Flavobacterium enshiense* KCTC 23775^T

Y yellow, BY bright yellow, YO yellowish orange, + positive, – negative

All data are from this study except the DNA G+C content of the reference strains: e^a and b^b data from Weon et al. (2007), c^c data from Dong et al. (2013)

magnification with a transmission electron microscope (Model JEM1010; JEOL) with cells grown on NA for 2 days at 28 °C. Cells were grown in NB for 2 days at 28 °C and then tested for gliding motility by the hanging-drop technique (Skerman 1967). Growth at different temperatures (4, 10, 15, 18, 25, 28, 30, 35, 37 and 42 °C) was assessed after 7 days of incubation using NA as basal medium. Different media were tested for growth at 28 °C for one week including Reasoner's 2A agar (R2A; Difco), NA, tryptone soya agar (TSA; Oxoid, England), Marine agar (MA; Difco), Luria Bertani agar (LB; Difco) and MacConkey Agar (Difco). pH conditions for growth (pH 4.0–10.0, at intervals of 0.5 pH units) was monitored in NB after 5 days of incubation at 28 °C. The following pH buffers were used (final concentration, 100 mM): acetate buffer was used for pH 4.0–6.5 and phosphate buffer was used for pH 7.0–10.0. The pH of NB was confirmed after autoclaving. The salinity test was performed by using 0–5 % (w/v) NaCl (at 0.5 % intervals) in NB. Growth was estimated by monitoring the optical density at 600 nm after 5 days of incubation at 28 °C. Anaerobic growth was tested in serum bottles containing NB supplemented with thioglycolate (0.1 %) and in which the air was substituted with nitrogen gas. The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992), Schmidt et al. (1994) and Bernardet et al. (2002). Catalase activity was determined by the production of bubbles from 3 % (v/v) H₂O₂ solution mixed with freshly grown cells. Oxidase activity was checked by using of 1 % (w/v) *N,N,N,N*-tetramethyl-*p*-phenylenediamine reagent (Sigma, USA) according to the manufacturer's instructions. Nitrate reduction was tested in nitrate broth containing 0.2 % KNO₃ (Skerman 1967). Indole production was analyzed using Kovács's reagent in 1 % tryptone broth (Skerman 1967). Urease activity was evaluated in Christensen's medium (Christensen 1946). Hydrolysis of the following substrates was tested using NA as basal medium: casein [2 % skim milk (Oxoid)], 1 % starch (Difco), aesculin [ferric citrate (0.02 %, 110 Fluka, Switzerland)], 12 % gelatin (Sigma), Tween 80 [0.01 % CaCl₂·2H₂O and 1 % Tween 80 (Sigma)], Tween 20 [0.01 % CaCl₂·2H₂O and 1 % Tween 20 (Sigma)], 1 % chitin (from crab shell, Sigma), 0.5 % *L*-tyrosine (Sigma), 0.1 % carboxymethyl-cellulose (CM-cellulose; Sigma) and DNA [DNase agar, Scharlau (Spain); DNase activity revealed by flooding the plates with 1 N HCl]. Plates were evaluated after 4 days of

incubation at 28 °C. Carbon utilization and enzyme production were examined using the API 20NE and API ZYM according to the manufacturer's instruction (bioMérieux, France). API 20NE was recorded after incubation for 48 h, under the optimal conditions for each strain while API ZYM was recorded after incubation for 10 h.

Phylogenetic analysis and DNA–DNA hybridization

The genomic DNA of strain THG-SM1^T was extracted and purified using a commercial Genomic DNA extraction kit (Solgent, Korea). The 16S rRNA gene was amplified with the universal bacterial primer pair 27F and 1492R (Weisburg et al. 1991) and the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, Korea). Seq-Man software version 4.1 (DNASTAR, Inc.) was used to compile the nearly complete (1,427 bp) 16S rRNA sequence of strain THG-SM1^T. The multiple alignments were performed using the CLUSTAL_X program (Thompson et al. 1997) and gaps were edited using the BioEdit program (Hall 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura 1983). The phylogenetic trees were constructed using the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) methods in the MEGA 6 program package (Tamura et al. 2013), with bootstrap values based on 1000 replications (Felsenstein 1985). Comparison of the 16S rRNA gene sequence of strain THG-SM1^T with validly named type strains was carried out using the EzTaxon-e server (Kim et al. 2012).

For determination of the DNA G+C content, genomic DNA was extracted, purified by the method of Moore and Dowhan (1995) and degraded enzymatically into nucleosides (nuclease P1 and alkaline phosphatase; Sigma). The nucleosides were analyzed using a reverse-phase HPLC system (Alliance 2690 system, Waters) as described previously (Mesbah et al. 1989) with reversed-phase column SunFire™ C18 (4.6 × 250 mm × 5 μm), flow rate 1.0 ml/min, solvent mixture of 200 mM (NH₄)H₂PO₄/acetonitrile (97:3, v/v) as mobile phase, and detector wavelength at 270 nm. The genomic DNA of *Escherichia coli* strain B (Sigma-Aldrich D4889) was used as a standard.

DNA–DNA hybridization was performed fluorometrically, according to the method developed by

Ezaki et al. (1989) with modifications (Stabili et al. 2008), using photobiotin-labelled DNA probes and micro-dilution wells. DNA–DNA hybridization was carried out to determine levels of relatedness of the novel strain THG-SM1^T with its closest relative *Flavobacterium terrae* KACC 11731^T. The optimum renaturation temperature (30.5 °C) was calculated as $[(0.51 \times G+C \text{ content}) + 47] - 36$ (Gillis et al. 1970), where 36 °C is the correction for the presence of 50 % formamide (McConaughy et al. 1969). Hybridization was performed with five replications 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.

Chemotaxonomy

For fatty acid analysis, cells were grown and harvested on NA. The cellular fatty acids were analyzed by capillary GLC (Hewlett Packard 6890) using the Microbial Identification software package with the Sherlock system MIDI 6.1 and the Sherlock Aerobic Bacterial Database (TSBA 6.1) (Sasser 1990). For quinone and polar lipid analyses, lyophilized cells of strain THG-SM1^T and *F. terrae* KACC 11731^T were used. The isoprenoid quinones were extracted using 100 mg freeze-dried cells and subsequently analyzed using a RP-HPLC system (Alliance 2690 system, Waters) [solvent: methanol/2-propanol (7:5, v/v); flow rate: 1.0 ml/min] as previously described (Hiraishi et al. 1996; Collins and Jones 1981; Tamaoky et al. 1983). The polar lipids of strain THG-SM1^T and the reference strain *F. terrae* KACC 11731^T were extracted and analyzed by two dimensional thin layer chromatography using Kiesel gel 60 F₂₅₄ plates (10 × 10 cm; Merck). Separately, each sample was spotted on the corner of a two-dimensional thin layer chromatography plate and developed in the first direction by using of chloroform : methanol : water (65:2:4, by vol) while in the second direction developed by using chloroform:acetic acid:methanol:water (80:15:12:4, by vol). TLC plates were sprayed with 5 % molybdophosphoric acid (total lipids; Sigma), 0.2 % ninhydrin reagent (aminolipids; Sigma) and 2.5 % α -naphthol reagent (glycolipids; Sigma). After spraying, it followed by heating at 120 °C for 10 min. TLC plates were also sprayed with Molybdenum blue reagent for detecting phospholipids. No heating step is needed for this reagent (Minnikin et al. 1977).

Results and discussion

Strain THG-SM1^T was isolated from field soil collected from Suwon, South Korea. The 16S rRNA gene sequence of strain THG-SM1^T was a continuous stretch of 1427 bp (NCBI GenBank accession number KM576853). Sequence similarities calculated using the EzTaxon-e server indicated that the closest relatives of strain THG-SM1^T were *F. terrae* KACC 11731^T, *F. columnare* KACC 11683^T and *Flavobacterium enshiense* KCTC 23775^T, with 16S rRNA gene sequence similarities of 98.7, 96.6 and 94.9 %, respectively. The relationship between strain THG-SM1^T and other members of the genus *Flavobacterium* was also evident in the phylogenetic trees (Fig. 1 and Supplementary Fig. S1). Strain THG-SM1^T was located in a clade with *F. terrae* and *F. columnare* in the genus *Flavobacterium*. These results clearly indicated that strain THG-SM1^T is a member of the genus *Flavobacterium*. The DNA–DNA relatedness value between strain THG-SM1^T and *F. terrae* KACC 11731^T was determined to be 48 ± 1.5 %, which is lower than the threshold value of 70 % recommended for the definition of bacterial species (Wayne et al. 1987; Stackebrandt and Goebel 1994). This low DNA–DNA relatedness suggests that strain THG-SM1^T represents a novel *Flavobacterium* species. The DNA G+C content of strain THG-SM1^T was determined to be 38.5 mol%, which lies within the range (30–41 mol%) for members of the genus *Flavobacterium* (Bernardet and Bowman 2011; Xu et al. 2011).

Strain THG-SM1^T was observed to be Gram-negative, aerobic, non-motile and rod-shaped (approximately 0.3–0.8 μm wide and 1.0–3.0 μm length; Supplementary Fig. S2). Colonies of strain THG-SM1^T on NA plates were observed to be smooth, circular, yellow coloured and convex, 2–3 mm in diameter. Strain THG-SM1^T was found to grow on NA and TSA, weakly on R2A but not on LB, MA and MacConkey agar. On NA, strain THG-SM1^T was found to be able to grow at 25–30 °C and at pH 7.0–8.0. The maximum growth of strain THG-SM1^T was observed at 28 °C, at pH 7.0 and in the absence of NaCl on NA. The test for oxidase was found to be positive. The isolate was found to be able to hydrolyze CM-cellulose, casein, starch, Tween-20 and gelatin but unable to hydrolyze aesculin, L-tyrosine, Tween 80, chitin, DNA and urea. Flexirubin-type pigments were found to be produced. Catalase test, nitrate reduction and indole production were found to be

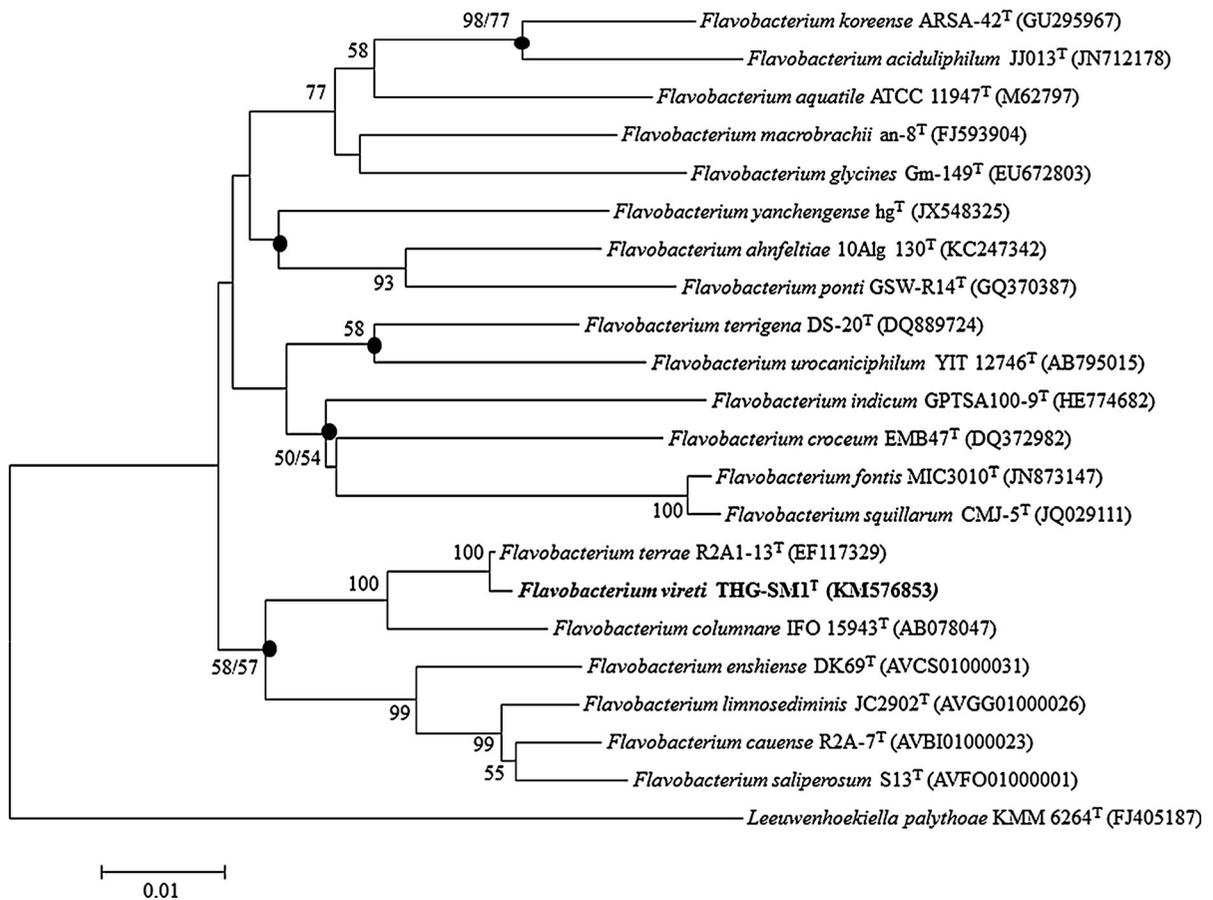


Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequence analysis showing the phylogenetic relationships of strain THG-SM1^T and members of the genus *Flavobacterium*. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony

algorithm. Bootstrap values more than 50 % based on 1000 replications are shown at branching points. *Leeuwenhoekiella palythoae* KCTC 22020^T was used as an out group. Scale bar, 0.02 substitutions per nucleotide position

negative. With regard to the reference strains used, all strains were found to be positive for the following activities: oxidase, flexirubin-type pigments, hydrolysis of casein and gelatin; and were negative for following activities: nitrate reduction, indole production, glucose acidification and arginine dihydrolase, hydrolysis of DNA, Tween 80, urea, aesculin, chitin and L-tyrosine. In API 20 NE kits, all strains showed negative results for assimilation of following substrates: D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate, phenylacetic acid. In API ZYM kits, all strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase

and Naphtol-AS-BI-phosphohydrolase but negative for α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The biochemical and physiological characteristics of strain THG-SM1^T and the most closely related *Flavobacterium* type strains are given in Table 1. The results suggest that the novel isolate represents a novel species of the genus *Flavobacterium*.

The cellular fatty acid profiles of strain THG-SM1^T and three reference strains are shown in Table 2. The major fatty acids (>7 % of the total fatty acids) was found to be iso-C_{15:1}G (8.0 %), iso-C_{15:0} 3OH (8.1 %), iso-C_{16:0} (9.2 %) and iso-C_{15:0} (22.3 %) as was also seen in the closely related reference type

strains. The fatty acid composition of strain THG-SM1^T was found to be very similar to those of the reference strains, with only limited variation in the proportions of the different components. Strain THG-SM1^T found to contain MK-6 as the respiratory quinone, in line with all other members of the genus *Flavobacterium*. The polar lipid profile of THG-SM1^T and the reference strain *F. terrae* KACC 11731^T are shown in supplementary Fig. S2. The major polar lipid in strain THG-SM1^T was identified as phosphatidylethanolamine which is similar to the polar lipid profile of the reference strain *F. terrae* KACC 11731^T. In addition, an unidentified aminolipid and an unidentified polar lipid (L1) were also detected in both strains. Additional unidentified polar lipids (L2 and

L3) were only observed in strain THG-SM1^T. Thus, the polar lipid profile of the novel isolate is in line with those of members of the genus *Flavobacterium* (Park et al. 2006, 2007; Ryu et al. 2007, 2008; Sheu et al. 2011).

On the basis of data obtained from this polyphasic taxonomy study including 16S rRNA, phylogenetic, phenotypic and chemotaxonomic properties, strain THG-SM1^T (=KACC 18371^T = CCTCC AB 2014312^T) is considered to represent a novel species of the genus *Flavobacterium*, for which name *Flavobacterium vireti* sp. nov. is proposed.

Description of *Flavobacterium vireti* sp. nov

Flavobacterium vireti (vi.re'ti. L. gen. n. *vireti* of the field)

Cells are Gram-negative, aerobic, non-motile and rod-shaped (0.3–0.8 μm × 1.0–3.0 μm). Growth occurs at 25–30 °C (optimum, 28 °C), at pH 7.0–8.0 (optimum, pH 7.0) and in the absence of NaCl. Colonies formed after 24 h of growth on NA at 28 °C are smooth, circular, yellow coloured and convex 2–3 mm in diameter. Growth occurs on NA and TSA, weakly on R2A but not on LA, MA and MacConkey agar. Oxidase positive and catalase negative. Flexirubin-type pigments are present. Nitrate reduction is not observed. Negative for indole production. Hydrolyses CM-cellulose, casein, starch, Tween-20 and gelatin but not aesculin, L-tyrosine, Tween 80, chitin, DNA and urea. Negative for glucose fermentation and arginine dihydrolase activity. Negative for assimilation of the following substrates in API 20 NE tests: D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. In the API ZYM tests, positive for following enzyme activities: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase and Naphtol-AS-BI-phosphohydrolase; and negative for following enzyme activities: trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. MK-6 menaquinone is the predominant isoprenoid quinone. The major polar lipid is

Table 2 Fatty acid profile of strain THG-SM1^T and the type strains of closely related species of the genus *Flavobacterium*

Fatty acid	1	2	3	4
Straight-chain				
C _{16:0}	4.8	5.3	2.4	4.6
C _{18:00}	2.1	1.6	–	–
Branched-chain				
iso-C _{13:0}	1.2	Tr	1.3	1.8
iso-C _{14:0}	4.8	4.2	3.9	2
iso-C _{15:0}	22.3	20.3	32.5	35.5
iso-C _{15:1}	8.0	6.2	7.2	3.6
iso-C _{16:0}	9.2	21.8	9.4	6.5
iso-C _{16:1}	3.37	3.2	Tr	Tr
anteiso-C _{15:0}	6.0	2.0	2.5	2.6
anteiso-C _{15:1} A	1.5	–	–	1.7
Unsaturated				
iso-C _{17:1ω9c}	4.7	8.8	4.2	15.1
Hydroxyl				
iso-C _{14:0} 3OH	1.6	1.1	1.2	Tr
C _{15:0} 3OH	1.2	5.2	Tr	4.3
iso-C _{15:0} 3OH	8.1	5.5	5.9	6.0
C _{16:0} 3OH	2.1	1.3	Tr	–
iso-C _{16:0} 3OH	2.9	2.6	6.3	Tr
iso-C _{17:0} 3OH	5.5	7.5	6.9	7.6
Summed feature 3*	2.9	1.8	Tr	2.0

Strain: 1. THG-SM1^T; 2. *Flavobacterium terrae* KACC 11731^T; 3. *Flavobacterium columnare* KACC 11683^T; 3. *Flavobacterium enshiense* KCTC 23775^T. All the data are from this study. Fatty acids amounting to less than 0.5 % in all strains are not listed

– Not detected, Tr traces (<0.5 %)

Summed feature 3* comprises C_{16:1ω6c} and/or C_{16:1ω7c}

phosphatidylethanolamine and the main cellular fatty acids are iso-C_{15:1}G, iso-C_{15:0} 3OH, iso-C_{16:0} and iso-C_{15:0}. The DNA G+C content of the type strain is 38.5 mol%.

The type strain, THG-SM1^T (=KACC 18371^T - = CCTCC AB 2014312^T), was isolated from field soil collected from Suwon, South Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain THG-SM1^T is KM576853.

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References

- Aslam Z, Im WT, Kim MK, Lee ST (2005) *Flavobacterium granulii* sp. nov., isolated from granules used in a wastewater treatment plant. *Int J Syst Evol Microbiol* 55:747–751
- Bergey DH, Harrison FC, Breed RS, Hammer BW, Huntoon FM (1923) Genus II. *Flavobacterium* gen. nov. In: Whitman W (ed) *Bergey's manual of determinative bacteriology*. Williams & Wilkins, Baltimore, pp 97–117
- Bernardet JF, Bowman JP (2011) Genus I. *Flavobacterium* Bergey et al. 1923. In: Whitman W (ed) *Bergey's manual of systematic bacteriology*, vol 4, 2nd edn. The Williams & Wilkins Co., Baltimore, pp 112–154
- Bernardet JF, Nakagawa Y, Holmes B, Subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes (2002) Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 52:1049–1070
- Bernardet JF, Segers P, Vancanneyt M, Berthe F, Kersters K, Vandamme P (1996) Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* 46:128–148
- Christensen WB (1946) Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J Bacteriol* 52:461–466
- Collins MD, Jones D (1981) Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol Rev* 45:316–354
- Dong K, Chen F, Du Y, Wang G (2013) *Flavobacterium ensiense* sp. nov., isolated from soil, and emended descriptions of the genus *Flavobacterium* and *Flavobacterium cauense*, *Flavobacterium saliperosum* and *Flavobacterium suncheonense*. *Int J Syst Evol Microbiol* 63:886–892
- Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39:224–229
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406–416
- Frerichs GN, Roberts RJ (1989) The bacteriology of teleosts. In: Roberts RJ (ed) *Fish pathology*. Bailliere Tindall, London, pp 289–291
- Gillis M, De Ley J, De Cleene M (1970) The determination of molecular weight of bacterial genome DNA from re-naturation rates. *Eur J Biochem* 12:143–153
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Hiraishi A, Ueda Y, Ishihara J, Mori T (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by high performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* 42:457–469
- Kang JY, Chun J, Jahng KY (2013) *Flavobacterium aciduliphilum* sp. nov., isolated from freshwater, and emended description of the genus *Flavobacterium*. *Int J Syst Evol Microbiol* 63:1633–1638
- Kim BY, Weon HY, Cousin S, Yoo SH, Kwon SW, Go SJ, Stackebrandt E (2006) *Flavobacterium daejeonense* sp. nov. and *Flavobacterium suncheonense* sp. nov., isolated from greenhouse soils in Korea. *Int J Syst Evol Microbiol* 56:1645–1649
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62:716–721
- Kimura M (1983) *The neutral theory of molecular evolution*. Cambridge University Press, Cambridge
- Kirchman DL (2002) The ecology of *Cytophaga*–*Flavobacteria* in aquatic environments. *FEMS Microbiol Ecol* 39(2):91–100
- Liu Y, Jin JH, Zhou YG, Liu HC, Liu ZP (2010) *Flavobacterium caeni* sp. nov., isolated from a sequencing batch reactor for the treatment of malachite green effluents. *Int J Syst Evol Microbiol* 60(2):417–421
- McConaughy BL, Laird CD, McCarthy BJ (1969) Nucleic acid reassociation in formamide. *Biochemistry* 8:3289–3295
- Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* 39:159–167
- Minnikin DE, Patel PV, Alshamaony L, Goodfellow M (1977) Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* 27:104–117
- Moore DD, Dowhan D (1995) Preparation and analysis of DNA. In: Ausubel FW, Brent R, Kingston RE, Moore DD,

- Seidman JG, Smith JA, Struhl K (eds) Current protocols in molecular biology. Wiley, New York, pp 2–11
- Noga EJ (2000) Fish disease diagnosis and treatment. Iowa State University Press, Ames
- Park M, Lu S, Ryu SH, Chung BS, Park W, Kim CJ, Jeon CO (2006) *Flavobacterium croceum* sp. nov., isolated from activated sludge. Int J Syst Evol Microbiol 56:2443–2447
- Park M, Ryu SH, Vu THT, Ro HS, Yun PY, Jeon CO (2007) *Flavobacterium defluvii* sp. nov., isolated from activated sludge. Int J Syst Evol Microbiol 57:233–237
- Reichenbach H (1992) The order *Cytophagales*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The Prokaryotes, a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, vol 4, 2nd edn. Springer, New York, pp 3631–3675
- Ryu SH, Park M, Jeon Y, Lee JR, Park W, Jeon CO (2007) *Flavobacterium filum* sp. nov., isolated from a wastewater treatment plant in Korea. Int J Syst Evol Microbiol 57:2026–2030
- Ryu SH, Park JH, Moon JC, Sung Y, Lee SS, Jeon CO (2008) *Flavobacterium resistens* sp. nov., isolated from stream sediment. Int J Syst Evol Microbiol 58:2266–2270
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI technical note 101. MIDI Inc, Newark
- Schmidt K, Connor A, Britton G (1994) Analysis of pigments: carotenoids and related polyenes. In: Goodfellow M, O'Donnell AG (eds) Chemical methods in prokaryotic systematics. Wiley, Chichester, pp 403–461
- Sheu SY, Chiu TF, Young CC, Arun AB, Chen WM (2011) *Flavobacterium macrobrachii* sp. nov., isolated from a freshwater shrimp culture pond. Int J Syst Evol Microbiol 61:1402–1407
- Skerman VBD (1967) A guide to the identification of the genera of bacteria, 2nd edn. Williams and Wilkins, Baltimore
- Stabili L, Gravili C, Tredici SM, Piraino S, Talà A, Boero F, Alifano P (2008) Epibiotic *Vibrio luminous* bacteria isolated from some hydrozoa and bryozoa species. Microb Ecol 56:625–636
- Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol 44:846–849
- Tamaoky J, Katayama-Fujiruma A, Kuraishi H (1983) Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. J Appl Bacteriol 54:31–36
- Tamura K, Stecher G, Peterson D, Filipitski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882
- Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore WEC, Murray RGE et al (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Evol Microbiol 37:463–464
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703
- Weon HY, Song MH, Son JA, Kim BY, Kwon SW, Go SJ, Stackebrandt E (2007) *Flavobacterium terrae* sp. nov. and *Flavobacterium cucumis* sp. nov., isolated from greenhouse soil. Int J Syst Evol Microbiol 57:1594–1598
- Xu M, Xin Y, Tian J, Dong K, Yu Y, Zhang J, Liu H, Zhou Y (2011) *Flavobacterium sinopsychrotolerans* sp. nov., isolated from a glacier. Int J Syst Evol Microbiol 61:20–24
- Yang JE, Kim SY, Im WT, Yi TH (2011) *Flavobacterium ginsenosidimutans* sp. nov., a bacterium with ginsenoside converting activity isolated from soil of a ginseng field. Int J Syst Evol Microbiol 61:1408–1412
- Yoon JH, Kang SJ, Oh TK (2006) *Flavobacterium soli* sp. nov., isolated from soil. Int J Syst Evol Microbiol 56:997–1000
- Yoon JH, Kang SJ, Lee JS, Oh TK (2007) *Flavobacterium terrigena* sp. nov., isolated from soil. Int J Syst Evol Microbiol 57:947–950