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Flavobacterium sharifuzzamanii sp. nov., Isolated from the Sediments of the East China Sea

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Abstract

A novel bacterial strain A7.6^T was isolated from the sediments collected near the Zhairuo Island located in the East China Sea and characterized using a polyphasic approach. Cells were Gram-stain-negative, rod-shaped, non-spore forming, non-flagellated but motile by gliding. The strain was aerobic, positive for oxidase and catalase activities. The strain can grow at 4–35 °C, pH 5.5–9.0, and 0–3% (w/v) NaCl concentration. The major polar lipid was phosphatidylethanolamine, the predominant fatty acids (> 10%) were iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c). The genomic G+C content was 33.6 mol% and the major respiratory quinone was menaquinone 6. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain A7.6^T belonged to the genus *Flavobacterium* and was closely related to *Flavobacterium tistrianum* GB 56.1^T (98.4% similarity), *F. nitrogenifigens* NXU-44^T (98.4%), *F. ginsenosidimutans* THG 01^T (98.0%) and *F. anhuiense* D3^T (97.7%). Average nucleotide identities and digital DNA–DNA hybridizations values for genomes ranged from 75.9 to 91.4% and 21.4 to 43.9% between strain A7.6^T and its closest phylogenetic neighbors. The polyphasic characterization indicated that strain A7.6^T represented a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium sharifuzzamanii* is proposed. The type strain is A7.6^T (= KCTC 62405^T = MCCC 1K03485^T). The NCBI GenBank accession number for the 16S rRNA gene of A7.6^T is MH396692, and for the genome sequence is QJGZ00000000. The digital protologue database (DPD) Taxon Number is TA00643.

Abbreviations

PE	Phosphatidylethanolamine
ANI	Average nucleotide identity
dDDH	Digital DNA–DNA hybridization

Introduction

The genus *Flavobacterium* belonging to the family *Flavobacteriaceae* was first proposed in 1923 by Bergey et al. [4] and since then it has been emended several times [6, 10, 17, 22]. At the time of writing, more than 200 species comprise in the genus *Flavobacterium* with validly published names (http://www.bacterio.net/flavobacterium.html), among which *F. aquatile* is the type species [6]. Members of this genus have been isolated from various environmental sources, such as soil, aquatic environments, fresh and marine water sediments, glaciers, microbial mat, Antarctic and Arctic habitats, animal, plants, marine organisms including fish, mammals, oysters, algae, and also from cyanobacterial aggregates, activated sludge, oil contaminated soils, and waste treatment plants [8, 15]. This present study focuses on

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A7.6^T is MH396692. The GenBank/EMBL/DDBJ accession numbers for the genome sequence of strain A7.6^T, *F. tistrianum* KCTC 42679^T, *F. nitrogenifigens* DSM 29982^T, *F. ginsenosidimutans* KCTC 42980^T are QJGZ00000000, QJRH00000000, QJRI00000000, and QJRJ00000000, respectively. The Digital protologue database (DPD) Taxon Number for strain A7.6^T is TA00643.

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the description of a novel strain of the genus *Flavobacterium*, designated A7.6^T using polyphasic approach.

Materials and Methods

Bacterial Strain Isolation and Culture Conditions

In July 2017, sediment samples were collected near Zhairuo Island (29°56′53″N, 122°04′55″E) located in the East China Sea. Approximately 1 g of sediments was suspended in seawater and spread onto the mineral salt medium (MM) agar plates [27]. After incubation at 28 °C for 7 days, strain A7.6^T was picked and purified by repeated streaking on fresh MM agar plates. After purification, strain A7.6^T was routinely cultured on Luria Bertani (LB; Difco) medium at 28 °C and preserved as a glycerol suspension (25% v/v) at -80 °C. The isolate A7.6^T has deposited at the KCTC (Korean Collection for Type Cultures) and the MCCC (Marine Culture Collection of China). For the comparative investigation, the reference strains *F. tistrianum* KCTC 42679^T, *F. ginsenosidimutans* KCTC 42980^T, and *F. anhuiense* KCTC 22128^T were obtained from the Korean Collection for Type Cultures (KCTC) and *F. nitrogenifigens* DSM 29982^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). All strains were cultured and tested under the same laboratory conditions.

Cell Growth, Physiology, Morphology, and Biochemical Characteristics

Strain A7.6^T was examined for growth on LB, nutrient agar (NA; Oxoid), tryptone soya agar (TSA; Oxoid), R2A agar (R2A; Oxoid), marine agar 2216 (BD), and MM agar at 28 °C for 3 days under aerobic conditions. Anaerobic growth on LB agar was examined using an anaerobic box (AnaeroPack-Anaero; Mitsubishi Gas Chemical Co., INC. Japan) at 28 °C for 7 days according to the manufacturer's instruction. Growth at different temperatures (4, 15, 20, 25, 28, 30, 35, 37, 40, 45, and 50 °C), different pH (pH 5.0–10.0, at 0.5 intervals) and different NaCl concentrations (0–5%, w/v, at 0.5 intervals) were investigated in LB broth at 28 °C by reading OD_{600} values over 5 days. For adjusting pH of growth, four different types of buffering agents were used at the final concentration of 30 mM [7, 23]. Cellular morphology was examined by light (E100; Nikon) and transmission electron (JEM1230; Hitachi) microscopy, using cells from exponentially growing on the LB agar plates. Gram staining was performed with a commercial Gram stain kit (BD) following the manufacturer's protocol. Motility was investigated by motility test medium [3] and gliding motility was examined by the hanging-drop method following the protocol of Bernardet et al. [5]. Congo-red absorption and flexirubin-type pigments production were determined following the protocol described by Bernardet et al. [5]. Carotenoid pigments were extracted, purified and analyzed following the protocol described by Asker et al. [2]. Tetramethyl *p*-phenylenediamine (1% w/v) was used to determine the oxidase activity and catalase activity was determined in a 3% H_2O_2 solution. Hydrolysis of casein, starch, CM-cellulose, filter paper, hypoxanthine, xanthine, tyrosine, gelatin, Tweens (20, 40, 60, and 80), nitrate reduction, urease activity, methyl red and Voges–Proskauer (MR-VP) reactions were determined according to previously described methods [7]. The biochemical properties, enzyme activities, acid productions, and carbon source utilization of strain A7.6^T and reference strains were evaluated using API ZYM, API 20NE, and API 50CH (bioMérieux), and GEN III MicroPlates (Biolog) kit systems following the manufacturer's instruction.

Phylogenetic Analysis

Bacterial genomic DNA was extracted and purified using a genomic DNA extraction kit (Takara). The 16S rRNA gene of strain A7.6^T was amplified with the universal primers 27F and 1492R [26]. The amplified DNA fragment was purified using gel extraction kits (Cwbio; China), ligated into the pMD19T vector (Takara) for sequencing. The almost full-length 16S rRNA gene sequence was used for pairwise sequence alignment performed by the BLASTN program (http://www.ncbi.nlm.nih.gov) [1] as well as the EzTaxon-e server (https://www.ezbiocloud.net) [36]. To construct the phylogenetic trees, 16S rRNA gene sequences of related validly published species were obtained from EzTaxon server. Multiple sequences alignments of all sequences were performed by using ClustalX2 software [32]. Phylogenetic trees were constructed by using MEGA 7 program [21] following the neighbor-joining [29], maximum-likelihood [12], and maximum-parsimony [13] methods. Kimura's two-parameter model was used to calculate the evolutionary distance [19] while 1000 random replicates were used to estimate the bootstraps values [11].

DNA G+C Content and DNA–DNA Relatedness

The genome of strain A7.6^T and type strains *F. tistrianum* KCTC 42679^T, *F. nitrogenifigens* DSM 29982^T, and *F. ginsenosidimutans* KCTC 42980^T were sequenced for investigating the DNA–DNA relatedness. The sequencing was performed at the Beijing Genomics Institute with HiSeq platform by Solexa PE150 sequencing technology. The Velvet algorithms were used for *de novo* assembly of the short reads [38]. The genome sequence of *F. anhuiense* KCTC 22128^T (FMVC00000000), and those from the top 20 closest neighbors available in GenBank were also retrieved for the comparison. The DNA G+C content of strain A7.6^T and

the four reference strains were calculated from their genome sequence. The digital DNA–DNA hybridization (dDDH) values between the strain A7.6^T and type strains were determined by Genome-to-Genome Distance Calculator (GGDC) 2.1 web server (http://ggdc.dsmz.de/distcalc2.php). Formula 2 was used for the analysis of dDDH, as suggested previously by GGDC [25]. The average nucleotide identity (ANI) calculations were determined using the ANI calculator with the EzTaxon-e server (https://www.ezbiocloud.net/tools/ani) [37].

Chemotaxonomic Characterization

For analysis of the whole cell fatty acid profile, strain A7.6^T and the four reference strains were grown on LB broth under the same conditions (at 28 °C, 180 rpm) and the cells were harvested at the exponential growth phase (optical density = 1.0 at 600 nm). The cellular fatty acids were extracted and analyzed according to the guidelines of the Microbial Identification System (MIDI) [30] using the RTSBA6 database. For the extraction and identification of polar lipids of A7.6^T, the isolate was grown on LB broth at 28 °C, 180 rpm for 2 days, and harvested at the exponential growth phase. Then polar lipids were extracted with chloroform/methanol (1:2, v/v) and identified by two-dimensional TLC on silica gel 60 F_{254} (Merck) plates (10 cm × 10 cm) as described by Tindall [33]. To determine respiratory quinones, strain A7.6^T was cultured on LB broth for 2 days at 28 °C, harvested at the exponential growth phase and freeze-dried. Then respiratory quinones were extracted and analyzed following previously described method [20].

Results and Discussion

Cell Growth, Physiology, Morphology, and Biochemical Characteristics

Detailed phenotypic features of strain A7.6^T that differentiate it from its most closely related members are summarized in species description, Table 1 and Table S1. Additionally, the pigments extracted from strain A7.6^T exhibited peaks with an absorption maximum at approximately 450 nm. The mass spectrum of the main peak gave $[M+H]^+$ at *m/z* 569.3, which was identical to the standard zeaxanthin indicating that the main carotenoid identified from strain A7.6^T was zeaxanthin, a carotenoid already reported in several members of the family *Flavobacteriaceae* [2, 9, 18, 22, 34].

Phylogenetic Analysis

The nearly complete 16S rRNA gene sequence of strain A7.6^T contains 1475 bp. On the basis of 16S rRNA gene sequence similarity, the close relatives of strain A7.6^T were identified to be *F. tistrianum* GB 56.1^T (98.4%), *F. nitrogenifigens* NXU-44^T (98.4%), *F. ginsenosidimutans* THG 01^T (98.0%), and *F. anhuiense* D3^T (97.7%). The sequence similarity between strain A7.6^T and other *Flavobacterium* species was less than 97.5%. As *Flavobacterium* species are the closest neighbors of strain A7.6^T, it is obvious that it branches in the *Flavobacterium* clade which is revealed by the neighbor-joining tree (Fig. 1) and also by the maximum-likelihood, maximum-parsimony trees (Fig. S2, S3).

DNA G+C Content and DNA–DNA Relatedness

The DNA G+C content of strain A7.6^T is 33.6 mol% (Table 1), which is within the range of 30–52 mol% reported for the members of the genus *Flavobacterium* [22]. The genome sequences of strain A7.6^T and *F. tistrianum* KCTC 42679^T, *F. nitrogenifigens* DSM 29982^T, and *F. ginsenosidimutans* KCTC 42980^T have registered into the GenBank and the GenBank/EMBL/DDBJ accession number for the genome sequences of these strains are presented in Table 2. The genome size of A7.6^T was determined to be 5.3 Mbp, which is similar to the ones of the reference strains (Table 2). As shown in Table 2, the genome-based dDDH value and ANI score between the strain A7.6^T and the reference strains were significantly lower than the 70% cut-off value for dDDH and 95% for ANI, proposed for the delineation of bacterial species [14, 28]. According to dDDH and ANI results, it is clearly indicated that the strain A7.6^T should represent a novel species of the genus *Flavobacterium*.

Chemotaxonomic Characterization

The major fatty acids (> 10%) of A7.6^T were iso-$C_{15:0}$ and summed feature 3 (comprising $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c). Strain A7.6^T showed a similar fatty acid profile to the closest related reference strains, but there were remarkable quantitative differences under the same growth condition (Table 3). Total polar lipids of strain A7.6^T were composed of phosphatidylethanolamine (PE), phosphatidylinositol mannoside (PIM), three unidentified polar lipids (L1-3), and two unidentified aminophospholipids (APL1-2) (Fig. S4). The presence of a large amount of PE in polar lipids profile of A7.6^T was common in other *Flavobacterium* species [10]. The predominant respiratory quinone of strain A7.6^T was menaquinone-6 (MK-6), which is the only one or major quinone found in all members of *Flavobacterium* [5]. These chemotaxonomic results reveal that strain A7.6^T is a novel species in the genus *Flavobacterium*.

Based on the polyphasic characterization, strain A7.6^T represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium sharifuzzamanii* sp. nov. is proposed.

Table 1 Differential characteristics of A7.6^T and closely related species of the genus *Flavobacterium*

Characteristic	1	2	3	4	5
Isolation source	Marine sediments	Biospere reserve soil[a]	Rhizosphere of switchgrass[b]	Ginseng field soil[c]	Field-soil[d]
Colony pigmentation	LY	BY	PY	BY	Y
Temperature range for growth, °C (optimum)	4–35 (28)	15–30 (25–30)[a]	15–30 (28)[b]	10–37(25–30)[c]	5–37 (25–30)[d]
pH range for growth (optimum)	5.5-9.0 (6.0–8.0)	6.0–8.0 (6.0–7.0)[a]	5.5–10.5[b]	5.0–10.0 (7.0)[c]	4.0–10.0 (6.0–8.0)[d]
NaCl range for growth, % w/v (optimum)	0–3 (0-0.5)	0–2 (0–1)[a]	1[b]	0–1 (0)[c]	0–2 (0–1)[d]
Gliding motility	Motile by gliding	Motile by gliding	Non-motile	Non-motile	Motile by gliding
Oxidase activity	+	−	+	+	+
Methyl red test	+	−	+	−	−
Nitrite reduction	+	−	+	+	+
Hydrolysis of					
Gelatin	+	−	−	−	−
Enzyme activity (API ZYM)					
Esterase (C4), esterase lipase (C8), lipase (C14), α-chymotrypsin, β-galactosidase, β-glucuronidase, β-glucosidase	+	−	+	+	+
α-galactosidase	+	+	−	−	+
α-fucosidase	−	−	+	−	+
API 20NE test					
Arginine dihydrolase	+	−	+	+	+
Urease activity	+	−	+	−	−
Assimilation of malate	+	−	−	−	−
Acid production from (API 50CH)					
D-arabinose	+	−	−	−	−
Salicin	−	−	−	+	+
D-melibiose	−	+	−	+	+
D-saccharose	+	−	−	+	−
Amidon	+	+	+	−	−
Glycogen	+	+	+	+	−
Carbon source utilization (GEN III)					
D-melibiose	−	+	+	+	+
D-salicin	−	+	−	+	+
DNA G+C content (mol%)	33.6	34.0	34.1	33.5	34.4

All data were obtained in this study unless otherwise stated

Strain: *1* A7.6^T, *2 F. tistrianum* KCTC 42679^T, *3 F. nitrogenifigens* DSM 29982^T, *4 F. ginsenosidimutans* KCTC 42980^T, *5 F. anhuiense* KCTC 22128^T. *LY* Light yellow, *BY* Bright yellow, *PY* Pale yellow, *Y* Yellow; + positive reaction, − negative reaction

[a]Suwannachart et al. [31]

[b]Kämpfer et al. [16]

[c]Yang et al. [35]

[d]Liu et al. [24]

Description of *Flavobacterium sharifuzzamanii* sp. nov.

Flavobacterium sharifuzzamanii (sha.ri.fuz.za.ma´ni.i N.L. gen. n. *sharifuzzamanii* in honor of the first author's teacher, Professor Dr SM Sharifuzzaman).

Cells are Gram-stain-negative, non-flagellated, non-spore forming, aerobic, rod-shaped with size approximately 0.5–0.7 μm in width and 1.3–2.1 μm in length, and motile by gliding. Colonies are light yellow in color, smooth, circular and 2–4 mm in diameter after 2 days of incubation at 28 °C on LB agar medium. Flexirubin-type

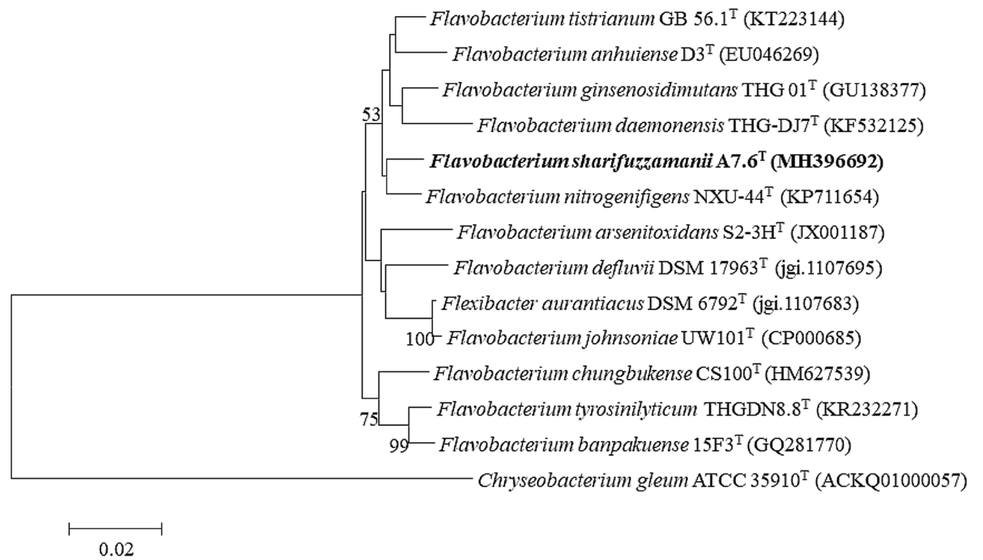

Fig. 1 Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences, showing the phylogenetic position of strain A7.6^{T} with the most closely related members. Bootstrap values were expressed as a percentage of 1000 replicates at the nodes and only those higher than 50% were given at the branch points. *Chryseobacterium gleum* ATCC 35910^{T} (ACKQ01000057) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position

Table 2 The digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) values between A7.6^{T} and closely related species of the genus *Flavobacterium*

Member of genus *Flavobacterium*	GenBank accession No.	Genome size (Mbp)	DDH (%)	ANI (%)
A7.6	QJGZ00000000	5.3	–	–
F. tistrianum KCTC 42679^{T}	QJRH00000000	5.1	41.3	90.6
F. nitrogenifigens DSM 29982^{T}	QJRI00000000	5.5	43.9	91.4
F. ginsenosidimutans KCTC 42980^{T}	QJRJ00000000	5.5	32.7	86.8
F. anhuiense KCTC 22128^{T}	FMVC00000000[a]	5.3	37.1	89.0
F. johnsoniae UW101^{T}	CP000685[a]	6.1	26.8	82.9
F. defluvii DSM 17963^{T}	FQWC00000000[a]	4.9	26.1	82.3
F. cutihirudinis DSM 25795^{T}	QRDQ00000000[a]	4.9	25.6	81.6
F. phragmitis BLN2 ^{T}	FOMH00000000[a]	6.0	28.8	84.4
F. aquidurense DSM 18293^{T}	MUGR00000000[a]	5.4	23.4	80.2
F. seoulense EM1321^{T}	JNCA00000000[a]	3.8	21.4	75.9

All data were obtained in this study unless otherwise stated

[a]Retrieved from NCBI GenBank

pigment and carotenoid (zeaxanthin) pigments are produced. Congo red is not absorbed by the colonies. Cells grow on LB, TSA, NA, and marine agar 2216, but weak on R2A agar and MM agar. Growth occurs at 4–35 °C (optimum at 28 °C), at pH 5.5-9.0 (optimum at 6.0–8.0), and in 0–3% (w/v) NaCl (optimum in 0-0.5%). Oxidase and catalase activities are present. Cells are able to hydrolyze starch, gelatin, Tween 20, but unable to hydrolyze CM-Cellulose, hypoxanthine, xanthine, tyrosine, filter paper (Crystalline cellulose), casein, Tweens 40, 60, and 80, and are positive for nitrate reduction, nitrite reduction, methyl red and Voges–Proskauer tests. According to API ZYM kits, all enzyme activities except α-mannosidase and α-fucosidase are positive. The API 20NE results show that cells are positive for glucose fermentation, arginine dihydrolase, hydrolysis of β-glucosidase, urease activity, PNPG (β-galactosidase) activity, assimilation of glucose, arabinose, mannose, *N*-acetyl-glucosamine, maltose and malate. Acid production occurs from D-arabinose, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetyl-glucosamine, amygdalin, esculin, D-cellobiose, D-maltose, D-lactose, D-saccharose, amidon, glycogen, and gentiobiose. From the GEN III microplate system, cells utilize dextrin, D-maltose, D-cellobiose, gentiobiose, sucrose, stachyose, D-raffinose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, α-D-glucose, D-mannose, D-fructose, D-galactose, L-rhamnose, L-serine, pectin, D-galacturonic acid. The dominant fatty acids (> 10%) are iso-$C_{15:0}$ and summed feature 3 (comprising $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c). The polar lipid profile

Table 3 Cellular fatty acid profiles (%) of A7.6^T and closely related species of the genus *Flavobacterium*

Fatty acids	1	2	3	4	5
Saturated					
$C_{10:0}$	2.3	tr	1.8	2.3	–
$C_{14:0}$	2.7	2.9	tr	1.1	tr
$C_{15:0}$	3.4	-	2.4	tr	3.7
$C_{16:0}$	7.9	15.2	6.2	8.7	10.4
Unsaturated					
$C_{15:1}$ ω6c	2.2	tr	1.5	2.4	tr
$C_{17:1}$ ω6c	tr	2.7	2.7	1.5	tr
$C_{17:1}$ ω8c	tr	tr	tr	1.2	tr
Hydroxy					
$C_{15:0}$ 3-OH	tr	tr	tr	1.6	–
$C_{16:0}$ 3-OH	6.1	5.9	4.6	4.6	6.0
$C_{17:0}$ 3-OH	tr	-	1.2	–	1.1
Branched chain					
iso-$C_{15:0}$	24.9	22.5	21.2	23.7	26.6
iso-$C_{15:0}$ 3-OH	5.7	6.8	6.4	4.1	5.2
iso-$C_{15:1}$ G	4.1	3.1	5.7	4.7	4.4
iso-$C_{16:0}$	tr	tr	2.1	2.6	1.3
iso-$C_{16:0}$ 3-OH	tr	tr	1.6	1.2	1.5
iso-$C_{17:0}$ 3-OH	7.8	8.6	9.7	8.1	8.4
antesiso-$C_{15:0}$	2.5	tr	1.4	1.2	2.6
antesiso-$C_{19:0}$	1.2	–	tr	2.1	-
Summed feature[a]					
2	tr	1.4	1.2	tr	–
3	20.7	19.8	19.1	20.6	18.9
9	1.3	1.8	-	1.3	tr

All data were obtained during this study

Strain: *1* A7.6^T, *2 F. tistrianum* KCTC 42679^T, *3 F. nitrogenifigens* DSM 29982^T, *4 F. ginsenosidimutans* KCTC 42980^T, *5 F. anhuiense* KCTC 22128^T, – not found, *tr* trace amount (< 1%)

[a]Summed features are groups of two or three fatty acids that could not be separated by the MIDI system. Summed feature 2 is composed of iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH; Summed feature 3 is composed of $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c; Summed feature 9 is composed of iso-$C_{17:1}$ ω9c and/or $C_{16:0}$ 10-methyl

contains PE, one PIM, two APLs and three Ls. The major respiratory quinone is MK-6 and the DNA G+C content is 33.6 mol%.

The type strain is A7.6^T (= KCTC 62405^T = MCCC 1K03485^T), isolated from marine sediments collected near Zhairuo Island (29°56′53″N, 122°04′55″E) located in the East China Sea.

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Compliance with Ethical Standards

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

References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
2. Asker D, Beppu T, Ueda K (2007) *Mesoflavibacter zeaxanthinifaciens* gen. nov., sp. nov., a novel zeaxanthin-producing marine bacterium of the family *Flavobacteriaceae*. Syst Appl Microbiol 30:291–296
3. Barrow GI, Feltham RKA (1993) Cowan and steel's manual for the identification of medical bacteria, 3rd edn. Cambridge University Press, Cambridge
4. 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
5. Bernardet JF, Nakagawa Y, Holmes B (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
6. 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
7. Chen C, Su Y, Tao TY, Fu GY, Zhang CY, Sun C, Zhang XQ, Wu M (2017) *Maripseudobacter aurantiacus* gen. nov., sp nov., a novel member of the family *Flavobacteriaceae* isolated from a sedimentation basin. Int J Syst Evol Microbiol 67:778–783
8. Chen WM, Su CL, Kwon SW, Sheu SY (2018) *Flavobacterium effusum* sp. nov., isolated from a freshwater river. Int J Syst Evol Microbiol 68:3111–3117
9. Choi JY, Kim JH, Lee PC (2018) *Flavobacterium kingsejongi* sp. nov., a carotenoid-producing species isolated from Antarctic penguin faeces. Int J Syst Evol Microbiol 68:911–916
10. Dong K, Chen F, Du Y, Wang G (2013) *Flavobacterium enshiense* 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
11. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
12. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376
13. Fitch WM (1971) Toward defining course of evolution-minimum change for a specific tree topology. Syst Zool 20:406–416
14. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91
15. Huq MA, Akter S, Lee SY (2018) *Flavobacterium chungangensis* sp nov., a bacterium isolated from soil of Chinese cabbage garden. Curr Microbiol 75:842–848
16. Kämpfer P, Busse HJ, McInroy JA, Xu J, Glaeser SP (2015) *Flavobacterium nitrogenifigens* sp. nov., isolated from switchgrass (*Panicum virgatum*). Int J Syst Evol Microbiol 65:2803–2809

17. 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
18. Kim JH, Choi BH, Jo M, Kim SC, Lee PC (2014) *Flavobacterium faecale* sp. nov., an agarase-producing species isolated from stools of Antarctic penguins. Int J Syst Evol Microbiol 64:2884–2890
19. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. J Mol Evol 16:111–120
20. Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial systematics. Method Microbiol 19:161–207
21. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
22. Kuo I, Saw J, Kapan DD, Christensen S, Kaneshiro KY, Donachie SP (2013) *Flavobacterium akiainvivens* sp. nov., from decaying wood of *Wikstroemia oahuensis*, Hawai'i, and emended description of the genus *Flavobacterium*. Int J Syst Evol Microbiol 63:3280–3286
23. Li DD, Liu C, Zhang YQ, Wang XJ, Wang N, Peng M, Song XY, Su HN, Zhang XY, Zhang YZ, Shi M (2017) *Flavobacterium arcticum* sp. nov., isolated from Arctic seawater. Int J Syst Evol Microbiol 67:1070–1074
24. Liu H, Liu R, Yang SY, Gao WK, Zhang CX, Zhang KY, Lai R (2008) *Flavobacterium anhuiense* sp. nov., isolated from field soil. Int J Syst Evol Microbiol 58:756–760
25. Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60
26. Okai M, Kihara I, Yokoyama Y, Ishida M, Urano N (2015) Isolation and characterization of benzo[a]pyrene-degrading bacteria from the Tokyo Bay area and Tama River in Japan. FEMS Microbiol Lett 362:fnv143
27. Qi YB, Wang CY, Lv CY, Lun ZM, Zheng CG (2017) Removal capacities of polycyclic aromatic hydrocarbons (PAHs) by a newly isolated strain from oilfield produced water. Int J Environ Res Public Health 14
28. Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131
29. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
30. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. In: vol 1502. MIDI Technical Note 101 MIDI Inc., Newark
31. Suwannachart C, Rueangyotchanthana K, Srichuay S, Pheng S, Fungsin B, Phoonsiri C, Kim SG (2016) *Flavobacterium tistrianum* sp. nov., a gliding bacterium isolated from soil. Int J Syst Evol Microbiol 66:2241–2246
32. 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
33. Tindall BJ (1990) Lipid Composition of *Halobacterium lacusprofundi*. FEMS Microbiol Lett 66:199–202
34. Wu YH, Yu PS, Zhou YD, Xu L, Wang CS, Wu M, Oren A, Xu XW (2013) *Muricauda antarctica* sp. nov., a marine member of the *Flavobacteriaceae* isolated from Antarctic seawater. Int J Syst Evol Microbiol 63:3451–3456
35. 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
36. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617
37. Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286
38. Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829

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