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Flavobacterium cheonhonense sp. nov., Isolated from a Freshwater Reservoir[§]

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A novel bacterium, designated strain ARSA-15^T, was isolated from a freshwater sample collected from the Cheonho reservoir, Cheonan, Republic of Korea. The isolate was deep-yellow pigment, Gram-negative, rod-shaped, non-motile, and catalase- and oxidase-positive. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate belongs to the genus *Flavobacterium*, and shared less than 97% sequence similarity with recognized *Flavobacterium* species. The novel species was able to grow at 10–37°C, pH 6.5–10.0, and in 0–0.5% (w/v) NaCl concentrations. Chemotaxonomically, iso-$C_{15:1}$, iso-$C_{15:0}$, and iso-$C_{16:0}$ were observed to be the predominant cellular fatty acid, and menaquinone-6 (MK-6) was the predominant respiratory quinone. The major polar lipid patterns of strain ARSA-19^T was phosphatidylethanolamine, unknown aminolipid (AL1 and AL2), and unidentified polar lipids (L1, L2, and L3). The genomic DNA G+C content of the isolate was 39.2 mol%. On the basis of polyphasic approach, strain ARSA-15^T represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium cheonhonense* sp. nov. is proposed. The type strain is ARSA-15^T (=KACC 14967^T =KCTC 23180^T =JCM 17064^T).

Keywords: freshwater, phylogenetic analysis, *Flavobacterium*

Introduction

The name *Flavobacterium* was proposed in 1923 for a genus of the family Bacteriaceae, encompassing the rod-shaped, nonendospore-forming chemoorganotrophic bacteria (Bergey *et al.*, 1923). Description of the genus *Flavobacterium* (family *Flavobacteriaceae*, phylum *Bacteroidetes*) was emended by Bernardet *et al.* (1996). Members of the genus *Flavobacterium* have been isolated worldwide from a variety of habitats such as freshwater sediments, marine environments, glacier ice, soil, Antarctic habitats, earthworm digestive tract, and bacterial aggregates of a wastewater treatment plant (Bernardet *et al.*, 1996). Recently, several novel species have been isolated from various freshwater environments and subsequently added to the genus (Wang *et al.*, 2006; Cousin *et al.*, 2007; Kim *et al.*, 2009; Qu *et al.*, 2009; Lee *et al.*, 2011). In this study, a *Flavobacterium*-like strain was recovered from a freshwater reservoir of Cheonan, Korea and taxonomically characterized.

Materials and Methods

Bacterial strain

Freshwater samples were collected from Cheonho reservoir (36°49′N 127°10′E), Cheonan, Republic of Korea. Water samples were inoculated on R2A agar (Difco, USA) using standard dilution plating techniques and incubated at 25°C for 4 days. After primary isolation and purification, the strain was cultivated at 25°C on the same medium. Stock cultures were preserved at -80°C in Criobilles tubes (AES Laboratory). We then deposited this organism into the Korean Agricultural Culture Collection, Korean Collection for Type Cultures, and Japan Collection of Microorganisms (=KACC 14967^T =KCTC 23180^T =JCM 17064^T). Reference strains, *Flavobacterium dankookense* KACC 14968^T, *Flavobacterium koreense* KACC 14969^T, and *Flavobacterium aquatile* JCM 20475^T, were obtained from the KACC and JCM.

Phylogenetic analysis

Genomic DNA was extracted using the InstaGene^{TM} Matrix (Bio-Rad, USA). PCR amplification of 16S rRNA gene was accomplished using the 8-27F (5′-AGAGTTTGATCMTGG CTCAG-3′) and 1492-1510R (5′-TACGGYTACCTTGTTA CGACTT-3′) universal primer set with the GeneAmp PCR system 9700 (Applied Biosystems, USA). The PCR product was purified using the PCRquick-spin^{TM} PCR product purification kit (iNtRON, Korea) and direct sequence determination of the purified 16S rRNA gene was performed by using sequencing primers (27F, 518F, 800R, and 1492R) (Lane, 1991) with an ABI3730XL automated sequencer (Applied Biosystems) at Macrogen (Korea). The identification of phylogenetic neighbors was achieved using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007) and PHYDIT program version 3.1 (Chun and Bae, 2000). Sequences of

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related species were downloaded, together with that of *Escherichia coli* KCTC 2441^{T} (GenBank accession no. EU014689). We aligned the most closely related sequences with the sequences of reference strains, using the software BioEdit version 7.0.9.0 (Hall, 1999). The phylogenetic tree was constructed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4.1 (Tamura *et al.*, 2007). Distance calculation (distance options according to the Kimura 2-parameter) and clustering with the Neighbor-joining was performed, and bootstrap values were estimated based on 1,000 replications. In addition, to examine the phylogenetic relationships of the strain ARSA-15^{T} and closely related strains, we constructed a median-joining network (Bandelt *et al.*, 1999) using the software NETWORK version 4.6.0.0 available at http://www.fluxus-engineering.com/sharenet.htm. *Capnocytophaga granulosa* LMG 16022^{T} (GenBank accession no. U41347) was used as an out-group. The network was generated using partial 16S rRNA gene sequences (920 bp).

Phenotypic and biochemical characteristics

Cell morphology was observed by phase-contrast microscopy (AXIO; Zeiss, Germany) with cells grown on R2A agar. Gram staining was performed using a Sigma-Aldrich Gram-stain kit, according to the manufacturer's instructions. Motility was tested by the hanging-drop method (Skerman, 1967) using a CHT light microscope (Olympus, Japan) at 1,000× magnification. Catalase and oxidase activities were determined using 3% hydrogen peroxide and oxidase reagent (bioMérieux, France), respectively. Growth was tested on nutrient agar (NA; Difco), trypticase soy agar (TSA; Difco), MacConkey agar (Difco), and Anacker & Ordal's agar (Anacker and Ordal, 1955). The temperature range for growth was tested on R2A agar at 4, 10, 15, 20, 25, 30, 37, and 45°C. The pH range for growth was determined on R2A agar with the pH adjusted to pH 5.0–11.0 at 0.5 pH unit intervals. The influence of salinity was tested using modified R2A supplemented with 0, 0.5, 1.0, 1.5, and 2.0 (%, w/v) NaCl. To adjust the pH of the R2A medium, phosphate buffer was added and 1 M HCl or 1 M NaOH was used to achieve the desired pH. The optimum temperature and pH were tested by R2A broth for 14 days. Anaerobic growth was evaluated on R2A agar in a GasPak anaerobic system (BBL, USA). Gliding motility was tested by observing the spread of colony edges on R2A plates (Perry, 1973) and by microscopic observation of hanging drops of a R2A broth culture under a CHT microscope (Olympus, Japan). Production of flexirubin-type pigments

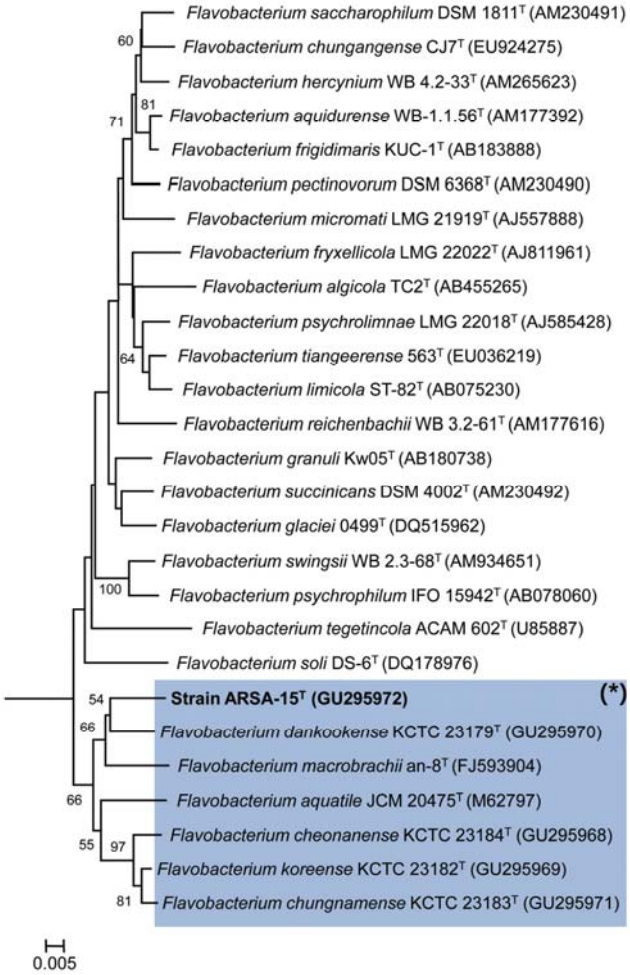

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain ARSA-15^{T}. *E. coli* KCTC 2441^{T} (EU014689) was used as an out-group. Bootstrap values (>50%) based on 1,000 replications are shown at branch nodes. Scale bar represents 0.005 substitutions per nucleotide position.

Table 1. Phenotypic characteristics that differentiate strain ARSA-15^T from other related species of the genus *Flavobacterium*. Strains: 1, ARSA-15^T; 2, *F. dankookense* KACC 14968^T; 3, *F. macrobrachii* an-8^T; 4, *F. koreense* KACC 14969^T; 5, *F. aquatile* JCM 20745^T. All data from this study. DY, deep yellow; Y, yellow; CY, cream yellow; PY, pale yellow; +, positive; -, negative; w, weak positive.
All strains are positive for hydrolysis of starch, alkaline phosphatase, leucine arylamidase, valine arylamidase, catalase, and oxidase. All strains are negative for CM-cellulose, chitin, tyrosine, xanthine, hypoxanthine, nitrate reduction, nitrite reduction, L-tryptophane, D-glucose, L-arginine, urea, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, phenylacetic acid, lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, Gram staining, and motility.

Characteristic	1	2	3	4	5
Colony pigmentation	DY	Y	Y	CY	PY
Flexirubin-type pigments	-	+	-	+	-
Ranges for growth					
Temperature (°C)	10–37	4–37	15–30	4–37	10–30
pH (pH unit)	6.5–10.0	6.5–9.5	7.0–8.0	6.0–9.5	6.5–9.5
Growth on 1% NaCl (w/v)	-	w	+	-	+
Hydrolysis of:					
Gelatine	-	-	+	+	-
Capric acid	-	w	-	w	w
Casein	-	-	w	-	+
Esculin	-	-	-	+	+
Enzymatic activities:					
Ala-Phe-Pro-arylamidase	-	+	+	+	+
Glutamyl arylamidase	-	-	+	-	-
L-proline arylamidase	-	+	+	+	+
Glu-Gly-Arg-arylamidase	-	-	-	+	-
Esterase (C4)	+	+	-	-	+
Esterase lipase (C8)	-	+	+	-	+
Cystine arylamidase	-	-	-	+	+
Acid phospatase	+	+	-	+	-
Naphtol-AS-BI-phosphohydrolase	+	+	+	+	-
α-Glucosidase	-	-	-	-	+
DNA G+C content (mol%)	39.2	33.3	35.5	31.5	34.7

and Congo red adsorption were investigated using 20% KOH test and 0.01% aqueous solution, according to the minimal standards for the description of new taxa in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). Substrate utilization and enzyme activity tests were conducted by using the API 20NE, Vitek, and API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Hydrolysis of starch, casein, Tween 80, CM-cellulose, chitin, xanthine, and hypoxanthine, were performed using the methods of Smibert and Krieg (1994).

Determination of DNA G+C content

For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain ARSA-15^T and references strains were extracted and purified as described by Moore and Dowhan (1995) and enzymatically degraded into nucleosides. The DNA G+C content was determined as described by Mesbah *et al.* (1989), using reversed-phase high-performance liquid chromatography (HPLC; Supelco, USA).

Chemotaxonomy

Cell biomass for the analysis of chemotaxonomic characterization was obtained from cells grown in R2A agar at 25°C for 4 days. Fatty acid profiles of strain ARSA-15^T and reference strains were determined on the identical conditions. Cells were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification (MIDI) System. The fatty acids were analyzed by gas chromatography (model 6890; Hewlett Packard, USA) using the Microbial Identification software package. Isoprenoid quinones and polar lipids were obtained using the methods of Minnikin *et al.* (1984).

Results and Discussion

Phylogenetic analysis

We found that the 16S rRNA gene sequence of strain ARSA-15^T is a continuous stretch of 1403 nucleotides. The 16S rRNA gene sequence of strain ARSA-15^T exhibits similarity ranging from 91.2–96.8% to members of the genus *Flavobacterium*, with the highest to *F. dankookense* KACC 14968^T (96.8% sequence similarity), *F. macrobrachii* an-8^T (96.4%), *F. koreense* KACC 14969^T (96.3%), *F. chungnamense* KACC 14971^T (96.1%), *F. cheonanense* KACC 14972^T (95.9%), and *F. aquatile* JCM 20745^T (94.1%). A neighbor-joining tree placed strain ARSA-15^T in the genus *Flavobacterium*, most closely related to *F. dankookense* KACC 14968^T, and *F. macrobrachii* an-8^T. In addition, we applied the median-joining network method (Bandelt *et al.*, 1999) using partial 16S rRNA gene sequences of seven strains which belong to a single clade (Fig. 1). This network shows the comprehensive

Table 2. Fatty acid compositions of novel species and the type strains of closely related species. Strains: 1, ARSA-15^T; 2, *F. dankookense* KACC 14968^T; 3, *F. macrobrachii* an-8^T; 4, *F. koreense* KACC 14969^T; 5, *F. aquatile* JCM 20745^T. All data from this study. -, not detected; Tr, trace (fatty acids that amount to <0.5% of the total fatty acid content in all strains are not shown).

Fatty acid	1	2	3	4	5
Saturated					
$C_{13:0}$	-	Tr	-	-	-
$C_{14:0}$	0.5	0.92	0.6	0.52	-
$C_{16:0}$	1.3	1.8	1.7	1.3	-
Branched					
iso-$C_{13:0}$	0.5	Tr	-	Tr	-
iso-$C_{14:0}$	1.3	2.5	1.4	2.8	1.1
iso-$C_{15:0}$	17.7	21.8	9.0	12.0	17.7
anteiso-$C_{15:0}$	4.9	4.9	2.0	5.2	10.2
iso-$C_{15:1}$	18.6	9.3	12.7	12.7	9.9
anteiso-$C_{15:1}$	1.6	0.7	2.2	2.1	1.8
iso-$C_{16:0}$	11.9	7.3	5.3	4.9	1.5
iso-$C_{16:1}$	3.4	2.9	3.0	3.3	1.1
Unsaturated					
$C_{15:1}\omega 6c$	2.2	4.0	3.4	3.8	20.4
$C_{15:1}\omega 8c$	-	-	-	-	1.2
$C_{17:1}\omega 6c$	1.0	1.0	4.0	-	5.4
$C_{17:1}\omega 8c$	0.8	0.6	-	Tr	-
iso-$C_{17:1}\omega 9c$	2.3	1.8	-	2.2	1.7
Hydroxy					
iso-$C_{14:0}$ 3-OH	0.8	0.7	1.7	1.1	-
$C_{15:0}$ 2-OH	0.9	0.9	3.3	1.4	-
$C_{15:0}$ 3-OH	1.3	1.7	4.9	2.1	5.6
iso-$C_{15:0}$ 3-OH	8.4	7.7	11.2	11.5	3.7
$C_{16:0}$ 3-OH	-	1.4	2.3	2.2	Tr
iso-$C_{16:0}$ 3-OH	7.6	10.4	10.8	13.8	3.1
$C_{17:0}$ 2-OH	1.5	1.5	1.2	1.7	1.7
$C_{17:0}$ 3-OH	1.0	0.4	2.0	1.0	1.1
iso-$C_{17:0}$ 3-OH	8.8	5.9	7.9	10.4	4.8
Summed feature 3[a]	1.8	8.2	7.0	3.4	3.1
Summed feature 9[b]	-	-	1.2	-	-

[a] Summed features represent groups two or three fatty acids that could not be separated by GLC with MIDI system. Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7c$.
[b] Summed feature 9 comprises $C_{16:0}$ 10-methyl and/or iso-$C_{17:0}\omega 9c$.

sequential order in which the species diversified according to the number of substitutions. As shown in Fig. 2, the differences between 16S rRNA gene sequences of the strain ARSA-15^T, *F. dankookense* KACC 14968^T, and *F. macrobrachii* an-8^T are found in 45–52 nucleotides (Supplementary data Fig. S1) The result of the network study was congruent with the phylogenetic inference based on the NJ study.

Phenotypic characteristics and DNA G+C content

Cells of strain ARSA-15^T were aerobic, Gram-negative, non-motile, non-spore-forming, short rods, 0.5–0.6 μm in width and 0.8–1.2 μm in length. Colonies grown on R2A agar for 4 days were circular deep-yellow pigmented. Strain ARSA-15^T grew well on R2A, and NA; no growth was observed on TSA, and MacConkey agar. Phenotypic characteristics of strain ARSA-15^T and closely related species are shown in Table 1. The DNA G+C content of the isolate was 39.2 mol%, a value consistent with those of other *Flavobacterium* species (30-41 mol%; Bernardet and Bowman, 2011) (Table 1).

Chemotaxonomy

The dominant fatty acids of strain ARSA-15^T are iso-$C_{15:1}$ (18.6%), iso-$C_{15:0}$ (17.7%), and iso-$C_{16:0}$ (11.9%). The proportion of iso-$C_{13:0}$, iso-$C_{15:1}$, iso-$C_{16:0}$, iso-$C_{16:1}$, $C_{17:1}\omega 8c$, and iso-$C_{17:1}\omega 9c$ are larger than those recorded for other types of species and smaller than $C_{15:1}\omega 6c$, $C_{15:0}$ 3-OH, $C_{16:0}$ 3-OH, and Summed feature 3. The detailed fatty acids profile of strain ARSA-15^T is compared with those of closely related members in Table 2. Cells of isolates contained menaquinone 6 (MK-6) as the major respiratory quinone. The polar lipids consist of phosphatidylethanolamine, unknown aminolipid (AL1 and AL2), and unidentified polar lipids (L1, L2, and L3) as major components (Supplementary data Fig. S2).

Description of *Flavobacterium cheonhonense* sp. nov.

Flavobacterium cheonhonense (che.on.ho.nen'se. N.L. neut. adj. cheonhonense, of or belonging to Cheonho reservoir).

Colonies are circular, deep-yellow pigmented and translucent on R2A agar. The organism has properties of adherence.

Cells are Gram-negative, rod-shaped (0.5–0.6 μm wide and 0.8–1.2 μm long), strictly aerobic, non-motile, non-gliding, catalase- and oxidase-positive. Congo red is not absorbed by the colonies. Flexirubin-type pigments are not produced. Grows on R2A, NA, and Anacker and Ordal's agar but not on MacConkey, DNase test agar, and TSA. The isolate grows on R2A at 10–37°C (optimum, 25°C) but not at 4 or 45°C. The pH range for growth is 6.5–10.0 (optimum, 7.5–8.0). No growth is observed in the presence of 1% or higher NaCl concentration. The type strain shows hydrolysis of starch but not Tween 80, casein, CM-cellulose, chitin, tyrosine, xanthine, and hypoxanthine. The novel strain is positive for tyrosine arylamidase and phosphatase (Vitek system). In the API ZYM system, alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase are present. The major fatty acids (>10% of the total fatty acids) are iso-$C_{15:1}$, iso-$C_{15:0}$, and iso-$C_{16:0}$. The polar lipids consist of phosphatidylethanolamine, unknown aminolipid (AL1 and AL2), and unidentified polar lipids (L1, L2, and L3) as major components. The predominant menaquinone is MK-6 and DNA G+C content of the type strain is 39.2 mol%. The GenBank accession number for 16S rRNA gene sequence is GU295972. The type strain, ARSA-15^T (=KACC 14967^T =KCTC 23180^T =JCM 17064^T), was isolated from Cheonho reservoir in Cheonan, Republic of Korea.

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References

Anacker, R.L. and Ordal, E.J. 1955. Study of a bacteriophage infecting the *myxobacterium Chondrococcus columnaris*. *J. Bacteriol.* **70**, 738–741.

Bandelt, H.J., Forster, P., and Rohl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48.

Bergey, D.H., Harrison, F.C., Breed, R.S., Hammer, B.W., and Huntoon, F.M. 1923. Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore, USA.

Bernardet, J.F. and Bowman, J.P. 2011. Genus I. Flavobacterium Bergey *et al.* 1923. *In* Whitman, W. (ed.), Bergey's Manual of Systematic Bacteriology, pp. 112–154, 2nd ed., Vol. 4, The Williams & Wilkins Co., Baltimore, USA.

Bernardet, J.F., Nakagawa, Y., and 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.

Bernardet, J.F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K., and 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. Evol. Microbiol.* **46**, 128–148.

Chun, J. and Bae, K.S. 2000. Phylogenetic analysis of *Bacillus subtilis* and related taxa based on partial *gyrA* gene sequences. *Antonie van Leeuwenhoek* **78**, 123–127.

Chun, J.S., Lee, J.H., Jung, Y.Y., Kim, M., Kim, S., Kim, B.K., and Lim, Y.W. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **57**, 2259–2261.

Cousin, S., Päuker, O., and Stackebrandt, E. 2007. *Flavobacterium aquidurense* sp. nov. and *Flavobacterium hercynium* sp. nov., from a hard-water creek. *Int. J. Syst. Evol. Microbiol.* **57**, 243–249.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95–98.

Kim, J.H., Kim, K.Y., and Cha, C.J. 2009. *Flavobacterium chungangense* sp. nov., isolated from a freshwater lake. *Int. J. Syst. Evol. Microbiol.* **59**, 1754–1758.

Lane, D.J. 1991. 16S/23S rRNA sequencing. Nucleic acid techniques in bacterial systematics, pp. 115–175. *In* Stackebrandt, E. and Goodfellow, M. (eds.). Wiley, Chichester, UK.

Lee, S., Weon, H.Y., Kim, S.J., and Ahn, T.Y. 2011. *Flavobacterium koreense* sp. nov., *Flavobacterium chungnamense* sp. nov. and *Flavobacterium cheonanense* sp. nov., isolated from a freshwater reservoir. *J. Microbiol.* **49**, 387–392.

Mesbah, M., Premachandran, U., and Whitman, W.B. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**, 159–167.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Moore, D.D. and Dowhan, D. 1995. Preparation and analysis of DNA. Current Protocols in Molecular Biology, pp. 2–11. *In* Ausubel, F.W., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (eds.). Wiley, New York, N.Y., USA.

Perry, L.B. 1973. Gliding motility in some non-spreading *Flexibacteria*. *J. Appl. Bacteriol.* **36**, 227–232.

Qu, J.H., Yuan, H.L., Li, H.F., and Deng, C.P. 2009. *Flavobacterium cauense* sp. nov., isolated from sediment of a eutrophic lake. *Int. J. Syst. Evol. Microbiol.* **59**, 2666–2669.

Skerman, V.B.D. 1967. A guide to the identification of the Genera of Bacteria, 2nd edn. Williams & Wilkins, Baltimore, USA.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization. Methods for General and Molecular Bacteriology, pp. 607–654. *In* Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.). American Society for Microbiology, Washington, D.C., USA.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596–1599.

Wang, Z.W., Liu, Y.H., Dai, X., Wang, B.J., Jiang, C.Y., and Liu, S.J. 2006. *Flavobacterium saliperosum* sp. nov., isolated from freshwater lake sediment. *Int. J. Syst. Evol. Microbiol.* **56**, 439–442.