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Flavobacterium jejuensis sp. nov., isolated from marine brown alga *Ecklonia cava*[§]

So-Hyun Park[1], Ji-Young Kim[2,3], Young-Ju Kim[3]*, and Moon-Soo Heo[1]*

[1]*Department of Aquatic Life Medicine, College of Ocean Science, Jeju National University, Jeju 690-756, Republic of Korea*
[2]*Research Institute for Basic Science, Jeju National University, Jeju 690-756, Republic of Korea*
[3]*Jeju Biological Resource Co., Ltd, CTC Business Incubator, Jeju Tourism College, Jeju 690-756, Republic of Korea*

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A bacterial strain, designated EC11^T was isolated from brown alga *Ecklonia cava* collected from Jeju Island, Korea. EC11^T was identified as a Gram-negative, rod-shaped and yellow-pigmented bacterial strain. The strain EC11^T grew over a temperature range of 10°C to 30°C (optimally at 25°C), and a pH range of 6.0–10.5 (optimally at pH 7.5). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain EC11^T belongs to the genus *Flavobacterium*. Strain EC11^T shared close similarity with *Flavobacterium jumunjinense* HME7102^T (96.4%), *Flavobacterium dongtanense* LW30^T (95.8%), *Flavobacterium haoranii* LQY-7^T (95.3%), and *Flavobacterium urocaniciphilum* (95.1%). The major fatty acids (> 5%) were iso-$C_{17:0}$ 3-OH (22.4%), iso-$C_{15:0}$ 3-OH (19.0%), $C_{15:0}$ (12.4%), summed feature 3 (comprising $C_{16:1}$ *ω7c*/ $C_{16:1}$ *ω6c*; 9.78%), iso-$C_{15:1}$ G (9.6%), and iso-$C_{16:0}$ 3-OH (9.0%). The DNA G+C content was 28.1 mol% and the strain contained MK-6 as the predominant menaquinone. The major polar lipids were phosphatidylethanolamine, two unknown aminolipids and three unknown polar lipids. Based on phenotypic, chemotaxonomic and phylogenetic analysis, strain EC11^T represents a novel species of the *Flavobacterium* genus, for which the name *Flavobacterium jejuensis* sp. nov. is proposed. The type strain of *F. jejuensis* is EC11^T (=KCTC 42149^T = JCM 30735^T).

Keywords: brown alga, *Ecklonia cava*, *Flavobacterium*, 16S rRNA gene

Introduction

The genus *Flavobacterium*, a member of the family *Flavobacteriaceae* in the phylum *Bacteroidetes*, was first proposed by Bergey *et al.* (1923) and emended by Bernardet *et al.* (1996), Dong *et al.* (2013), and Kang *et al.* (2013). Members of this genus have been reported from wide range of habitats, such as sea water (Yoon *et al.*, 2011; Bhumika *et al.*, 2013; Song *et al.*, 2013), freshwater (Subhash *et al.*, 2013; Kim *et al.*, 2014), marine sediment (Fu *et al.*, 2011; Kaur *et al.*, 2012), marine alga (Miyashita *et al.*, 2010; Nedashkovskaya *et al.*, 2014), human clinical specimens (Holmes *et al.*, 1984) and soil (Weon *et al.*, 2007; Kim *et al.*, 2013; Ao *et al.*, 2014; Ngo *et al.*, 2015). *Flavobacterium* strains are Gram-negative, aerobic, non-motile or with gliding motility, non-spore-forming, yellow-pigmented, and rod shaped bacteria with menaquinone-6 (MK-6) as the major or sole respiratory quinone (Bergey *et al.*, 1923; Bernardet and Bowman, 2011). Species of the genus *Flavobacterium* have a DNA G+C content of 30–52 mol% (Bernardet and Bowman, 2006; Kuo *et al.*, 2013).

Materials and Methods

Bacterial strains

Strain EC11^T was isolated from the marine brown alga *Ecklonia cava* collected on Jeju Island (South Korea), using the standard dilution plating method. The alga sample was serially diluted (10-fold dilutions) using sterile 0.85% (w/v) NaCl solution and 0.1 ml aliquots of each dilution were spread onto R2A agar plates (Difco) followed by incubation for 7 days at 25°C. Isolated EC11^T was routinely cultivated on R2A agar at 25°C and preserved as a glycerol suspension (20%, v/v, glycerol in water) at -80°C. *Flavobacterium jumunjinense* KCTC 23618^T, *Flavobacterium dongtanense* KACC 15621^T and *Flavobacterium haoranii* KCTC 23008^T, were obtained from Korean Collection for Type Cultures (KCTC) and Korean Agricultural Culture Collection (KACC) and used as reference strains.

Morphology and physiological characteristics

The cell morphology of strain EC11^T was observed by light microscopy (Nikon) and scanning electron microscopy (SUPRA 55VP, ZEISS) with cells grown for 3 days at 25°C on R2A agar. Gram staining was performed using the Gram Stain Kit (BD Science), according to the manufacturer's instructions. Gliding motility was determined on R2A broth containing 0.5% agar, as described by Bowman (2000). Anaerobic growth was determined in an anaerobic jar with the AnaeroPack (Oxoid) on R2A agar at 25°C for 4 weeks. The temperature and pH range for growth of strain EC11^T were tested on R2A agar at 5–45°C (5, 10 15, 20, 25, 30, 35, 37, 40, and 45°C) and in R2A broth at pH 4.0–11.0 (intervals of 0.5 pH unit)

*For correspondence. (M.S. Heo) E-mail: msheo@jejunu.ac.kr; Tel.: +82-64-754-3473; Fax: +82-64-756-3493 / (Y.J. Kim) E-mail: jym1006@jejunu.ac.kr; Tel.: +82-64-746-0727; Fax: +82-64-746-0728
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for up to 2 weeks. The pH was adjusted prior to sterilization by the addition of sodium acetate/acetic acid and Na_2CO_3. Salt tolerance was investigated on R2A agar containing various concentrations of NaCl (0–7%, w/v) at 1% intervals and 25°C. Catalase activity was tested by observing bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined using 1% (w/v) tetramethyl *p*-phenylenediamine (bioMérieux). The hydrolysis of starch (3%), casein (1%), Tween 20, 40, 60, and 80 (1%), cellulose (1%) and DNA (1%) were tested after containing on R2A agar. These biochemical tests were performed using the API 20NE and API ZYM tests (bioMérieux), following the manufacturer's instructions.

Phylogenetic analysis

Genomic DNA from strain EC11^T was extracted and purified according to Wilson (1987). The 16S rRNA gene was amplified by PCR using universal 27F and 1522R primers (Weisburg *et al*., 1991). The PCR product was then cloned using the TOPO Cloning Kit (Invitrogen) and sequenced by Genotech. The complete 16S rRNA gene sequence (1,431 bp) was compiled with SeqMan software (DNAstar). Similarity searches were performed using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al*., 2012). Multiple sequence alignments were performed using the CLUSTAL X program, version 1.83 (Thompson *et al*., 1997) and gaps were edited in the BioEdit program (Hall, 1999). Phylogenetic analysis was performed using the software package MEGA version 6.0 (Tamura *et al*., 2013). Phylogenetic trees were reconstructed using the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Kluge and Farris, 1969), and maximum-likelihood (Felsenstein, 1981) methods. The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985), based on 1,000 replications.

Table 1. Biochemical characteristics of strain EC11^T and related type strains

Strains: 1, EC11^T; 2, *F. jumunjinense* KCTC 23618^T; 3, *F. dongtanense* KACC 15621^T; 4, *F. haoranii* KCTC23008^T. All data were obtained in this study. +, positive; -, negative. All strains are gram-negative, motile by gliding. All strain were positive for gelatin, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase and Naphthol-AS-BI-phosphohydrolase. All strains were negative for indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, D-arabinose, D-mannitol, caprate, citrate, phenylacetate, lipase (C14), α-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase.

Characteristic	1	2	3	4
Growth at 37°C	-	-	+	+
NaCl tolerance (%)	0–5	0–3	0–3	0–2
Nitrate reduction	+	-	-	-
Catalase	+	+	-	+
Oxidase	+	+	-	+
Hydrolysis of :				
Cellulose	+	-	-	-
DNA	+	-	-	+
STA (Starch)	-	-	-	+
CAS (casein)	+	+	+	-
Tween 20	-	-	-	+
Tween 80	-	-	+	+
Assimilation of:				
D-Glucose	+	+	-	+
D-Mannose	+	+	-	+
N-Acetyl-D-glucosamine	-	+	-	-
D-maltose	+	+	-	+
Gluconate	-	+	-	+
Adipate	+	+	-	+
Malate	-	+	-	-
Enzyme activities:				
α-Chymotrypsin	+	-	+	-
α-Glucosidase	+	-	-	+
β-Glucosidase	+	-	-	+
G+C mol%	28.1	36.5[a]	30[b]	34[c]

Data from [a] Joung *et al*. (2013); [b] Xiao *et al*. (2011); [c] Zhang *et al*. (2010).

Chemotaxonomy

For cellular fatty acid analysis, cells of strain EC11^T and three reference strains were grown at 25°C on R2A agar. After 3 days of growth, fatty acids were extracted, methylated according to the standard protocol of the Sherlock Microbial Identification System (MIDI; version 6.0), analyzed by gas chromatography (GC 7890A; Agilent), and then identified using the TSBA6 library (Sasser, 1990). The polar lipids of strain EC11^T were extracted according to the procedure described by Minnikin *et al*. (1984) and analyzed by 2-dimensional thin-layer chromatography (TLC; Minnikin *et al*., 1984; Komagata and Suzuki, 1987). The polar lipid pattern was determined by comparing results following staining with the molybdophosphoric acid, ninhydrin, Zinzadze and α-naphthol. To determine the G+C DNA content, genomic DNA was extracted as described (Marmur, 1961) from cells that had been cultured on R2A agar for 3 days at 25°C and analyzed by the thermal denaturation method (*Tm*), with *Escherichia coli* K-12 used as the control (Marmur and Doty, 1962).

Results and Discussion

Morphology and physiological characteristics

Strain EC11^T cells were Gram-negative, aerobic rods (0.2–0.3 μm × 1.3–3.4 μm), and exhibited gliding motility. The colonies were bright yellow, circular, smooth, translucent, and 1–2 mm in diameter after growth for 3 days at 25°C on R2A agar. Growth occurred at 10–30°C (optimum 25°C) and pH 6.0–10.5 (optimum 7.5). Growth occurred over a range of 0–5% NaCl (w/v), with optimal growth occurring in the absence of NaCl. The morphological, physiological and biochemical characteristics are noted below in the species description, and comparison with type strains of other *Flavobacterium* species is presented in Table 1.

Phylogenetic analysis

The 16S rRNA gene sequence of strain EC11^T showed the highest similarity to *F. jumunjinense* HME7102^T (96.4%), *F. dongtanense* LW30^T (95.8%), *F. haoranii* LQY-7^T (95.3%), and *F. urocaniciphilum* (95.1%). Phylogenetic analysis based

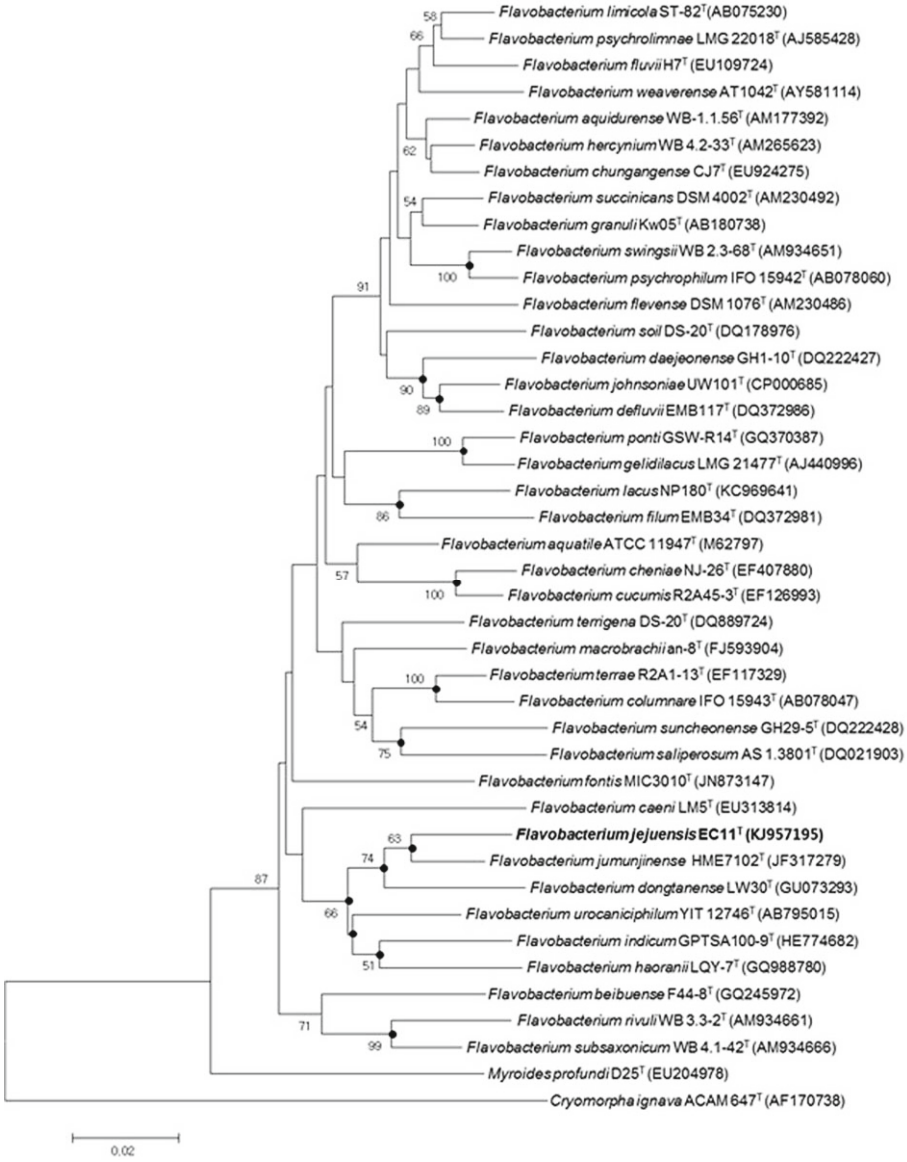

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of strain EC11^T and other related taxa. GenBank accession numbers are given in parentheses. Bootstrap value (>50%) based on 1,000 replications are shown. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Cryomorphaignava* ACAM 647^T was used as an out-group. Bar, 0.02 nucleotide substitutions per nucleotide position.

on 16S rRNA gene sequences using the neighbor-joining algorithm showed that strain EC11^T formed a phyletic lineage distinct from other members of the genus *Flavobacterium* (Fig. 1). The isolate formed a new phyletic line closely associated with *F. jumunjinense* HME7102^T, a result that is supported by all tree-making algorithms and by a 63% bootstrap value. The same relationship was also found in trees reconstructed using the maximum-parsimony and maximum-likelihood algorithms.

Chemotaxonomy

The major fatty acids (>5% of the total fatty acids) of strain EC11^T were iso-$C_{17:0}$ 3-OH (22.4%), iso-$C_{15:0}$ 3-OH (19.0%), $C_{15:0}$ (12.4%), Sum Feature 3 (comprising $C_{16:1}$ *ω7c*/ $C_{16:1}$ *ω6c*; 9.8%), iso-$C_{15:1}$ G (9.6%), and iso-$C_{16:0}$ 3-OH (9.0%) (Table 2). The major Fatty acid composition of strain EC11^T was highly similar to that of *F. jumunjinense* HME7102^T, with minor differences in their respective proportions. The fatty acid profile of strain EC11^T differs from those of other *Flavobacterium* species by having larger proportions of Summed Feature 3 (comprising $C_{16:1}$ *ω7c*/ $C_{16:1}$ *ω6c*; 9.8%).

The polar lipid profile consisted of phosphatidylethanolamine (PE), two unknown aminolipids (AL1 and AL2), and three unknown polar lipids (L1-L3; Fig. 2). They all contained phosphatidylethanolamine (PE), an unknown aminolipids (AL) and unknown polar lipids. PE is present in many species of the genus *Flavobacterium* (Ngo *et al.*, 2015). The polar lipid profile of strain EC11^T was very similar to those of the reference strains *F. jumunjinense* HME7102^T, *F. dongtanense* LW30^T, and *F. haoranii* LQY-7^T, but could be differentiated by the presence of absence of several other polar. The major respiratory quinone of the EC11^T strain was menaquinone-6 (MK6), which is also true of all other members of the genus *Flavobacterium*. The G+C content of the genomic DNA was found to be 28.1 mol%, which is lower than the corresponding values reported for the most closely

Table 2. Cellular fatty acid profiles of strain EC11^T and type strains of related species. Strains: 1, EC11^T; 2, *F. jumunjinense* KCTC 23618^T; 3, *F. dongtanense* KACC 15621^T; 4, *F. haoranii* KCTC 23008^T. All data were obtained in this study. -, not detected; TR, trace amount (<0.5%). All strains were incubated on R2A agar plate at 30°C for 3 days. Fatty acids that accounted for <0.5% of the total fatty acids in all strains were omitted.

Fatty acid	1	2	3	4
Saturated				
$C_{16:0}$	-	0.2	1.8	0.7
Hydroxylated				
$C_{15:0}$ 2-OH	2.2	TR	0.9	TR
$C_{15:0}$ 3-OH	2.2	0.9	0.6	2.2
$C_{16:0}$ 3-OH	TR	TR	1.0	0.96
$C_{17:0}$ 2-OH	1.2	TR	0.9	TR
$C_{17:0}$ 3-OH	1.2	TR	0.5	0.6
iso-$C_{14:0}$ 3-OH	1.0	TR	0.8	1.5
iso-$C_{15:0}$ 3-OH	19.0	22.5	7.0	3.9
iso-$C_{16:0}$ 3-OH	9.0	1.8	2.4	8.6
iso-$C_{17:0}$ 3-OH	22.4	21.9	10.1	5.1
Branched				
iso-$C_{13:0}$	-	2.1	TR	0.7
iso-$C_{14:0}$	-	1.5	0.9	7.8
iso-$C_{15:0}$	12.4	19.6	29.2	23.4
iso-$C_{15:1}$ G	9.6	10.7	16.2	16.8
iso-$C_{16:0}$	2.2	0.5	6.8	10.8
iso-$C_{16:1}$ G	-	-	-	2.5
iso-$C_{16:1}$ H	-	1.8	2.3	-
anteiso-$C_{15:0}$	1.4	1.3	4.4	6.2
anteiso-$C_{15:1}$ A	TR	-	1.3	1.2
Unsaturated				
$C_{15:1}$ *ω6c*	2.3	5.1	0.7	1.5
$C_{17:1}$ *ω6c*	1.0	TR	TR	TR
Summed features				
3	9.8	1.7	1.7	0.8
6	1.2	0.6	-	-
9	0.7	3.4	7.9	0.6

* Summed features were represent when two or three fatty acids that cannot be separated by MIDI system. Summed features 3, $C_{16:1}$ *ω7c*/ $C_{16:1}$ *ω6c*; Summed features 6, $C_{19:1}$ *ω11c*/ $C_{19:1}$ *ω9c*; Summed features 9, iso-$C_{17:1}$ *ω9c*.

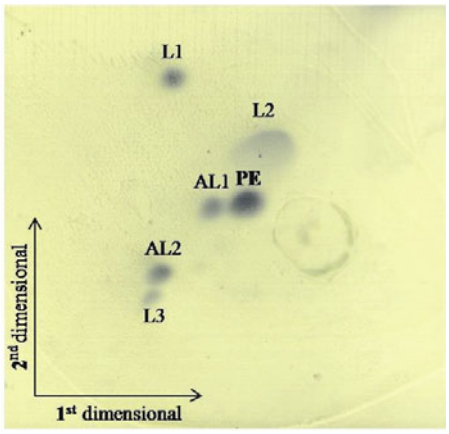

Fig. 2. Two dimensional thin-layer chromatogram of total polar lipids of strain EC11^T. Total polar lipids were spray with molybdophosphoric acid reagent. PE, phosphatidylethanolamine; AL1-2, unknown aminolipids; L 1–3, unknown lipids.

related genera (30–52 mol%) (Bernardet and Bowman, 2006; Kuo *et al.*, 2013), as shown in Table 1.

Taxonomic conclusion

Based on the phenotypic, phylogenetic, biochemical, and chemotaxonomic properties observed, strain EC11^T (=KCTC 42149^T =JCM 30735^T) represents a new species in the genus *Flavobacterium* for which the name *Flavobacterium jejuensis* sp. nov., is proposed.

Description of *Flavobacterium jejuensis* sp. nov.

Flavobacterium jejuensis (je.ju.en′sis.N.L. fem. adj. *jejuensis* referring to Jeju Island in the Republic of Korea, where the type strain was isolated).

Strain EC11^T are Gram-negative, motile by gliding, aerobic, and rod-shaped, approximately 0.19–0.29 μm wide and 1.31–3.39 μm long. Colonies were bright yellow, circular, smooth, and translucent after 3 day of incubation at 25°C on R2A agar. Growth occurs at 10–30°C (optimum temperature, 25°C), pH 6.0–10.5 (optimum pH, 7.5). Growth was observed at 0–5% NaCl (w/v). Oxidase and catalase activities are positive. Hydrolyses aesculin, gelatin, casein, cellulose, and DNA, but not hydrolyses starch, Tween 20, 40, 60, and 80. Nitrate reduction is positive, but negative for indole production, D-glucose fermentation, arginine dihydrolase, urease, and β-galactosidase activities. D-glucose, D-mannose, D-maltose and adipate is assimilated, but D-arabinose, D-mannitol, N-acetyl-D-glucosamine, gluconate, caprate, malate, citrate, and phenylacetate is not. In ZYM testing, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and β-glucosidase are present, but lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, D-acetyl-β-glucosamidase, α-mannosidase and α-ucosidase are absent. The main fatty acids (>5%) of strain EC11^T are iso-$C_{17:0}$ 3-OH (22.4%), iso-$C_{15:0}$ 3-OH (19.0%), $C_{15:0}$ (12.4%), ummed feature 3 (comprising $C_{16:1}$ *ω7c*/ $C_{16:1}$ *ω6c*; 9.8%), iso-$C_{15:1}$ G (9.6%), and iso-$C_{16:0}$ 3-OH (9.0%). The polar lipids profile consisted of were phosphatidylethanolamine, two unknown aminolipids and three unknown polar lipids. Menaquinone-6 is the predominant quinone. The G+C content is 28.1 mol%.

The type strain, EC11^T (=KCTC 42149^T =JCM 30735^T), was isolated from marine brown alga *Ecklonia cava* in the Jeju Island, Republic of Korea.

The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EC11^T is KJ957195.

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References

Ao, L., Zeng, X.C., Nie, Y., Mu, Y., Zhou, L., and Luo, X. 2014. *Flavobacterium arsenatis* sp. nov., a novel arsenic-resistant bacterium from high-arsenic sediment. *Int. J. Syst. Evol. Microbiol.* **64**, 3369–3374.

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. and Nakagawa, Y. 2006. An introduction to the family *Flavobacteriaceae*. pp. 445–480. The Prokaryotes. A Handbook on the Biology of Bacteria, 3rd edn. *In* Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., and Stackebrandt, E. (eds.) Springer, New York, N.Y., USA.

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. Microbiol.* **46**, 128–148.

Bhumika, V., Srinivas, T.N.R., and Kumar, P.A. 2013. *Flavobacterium nitratireducens* sp. nov., an amylolytic bacterium of the family *Flavobacteriacea*e isolated from coastal surface seawater. *Int. J. Syst. Evol. Microbiol.* **63**, 2490–2496.

Bowman, J.P. 2000. Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int. J. Syst. Evol. Microbiol.* **50**, 1861–1868.

Dong, K., Chen, F., Du, Y., and 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.

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.

Fu, Y., Tang, X., Lai, Q., Zhang, C., Zhong, H., Li, W., Liu, Y., Chen, L., Sun, F., and Shao, Z. 2011. *Flavobacterium beibuense* sp. nov., isolated from marine sediment. *Int. J. Syst. Evol. Microbiol.* **61**, 205–209.

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.

Holmes, B., Owen, R.J., Steigerwalt, A.G., and Brenner, D.J. 1984. *Flavobacterium gleum*, a new species found in human clinical specimens. *Int. J. Syst. Bacteriol.* **34**, 21–25.

Joung, Y.C., Kim, H.N., and Joh, K.S. 2013. *Flavobacterium jumunjinense* sp. nov., isolated from, a lagoon, and emended description of *Flavobacterium cheniae*, *Flavobacterium dongtanense*, and *Flavobacterium gelidilacus*. *Int. J. Syst. Evol. Microbiol.* **63**, 3937–3943.

Kang, J.Y., Chun, J., and Jahng, K.Y. 2013. *Flavobacterium aciduliphilum* sp. nov., isolated from freshwater, and emended description of the genus *Flavobacterium*. *Int. J. Syst. Evol. Microbiol.* **63**, 1633–1638.

Kaur, I., Kaur, C., Khan, F., and Mayilraj, S. 2012. *Flavobacterium rakeshii* sp. nov., isolated from marine sediment, and emended description of *Flavobacterium beibuense* Fu *et al.* 2011. *Int. J. Syst. Evol. Microbiol.* **62**, 2897–2902.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., *et al.* 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.

Kim, H., Kang, H., Joung, Y., and Joh, K. 2014. *Flavobacterium gyeonganense* sp. nov., isolated from freshwater, and emended descriptions of *Flavobacterium chungangense*, *Flavobacterium aquidurense*, *Flavobacterium tructae* and *Flavobacterium granuli*. *Int. J. Syst. Evol. Microbiol.* **64**, 4173–4178.

Kim, Y.J., Kim, S.R., Nguyen, N.L., and Yang, D.C. 2013. *Flavobacterium ginsengisoli* sp. nov., isolated from soil of a ginseng field. *Int. J. Syst. Evol. Microbiol.* **63**, 4289–4293.

Kluge, A.G. and Farris, F.S. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**, 1–32.

Komagata, K. and Suzuki, K. 1987. Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19**, 161–207.

Kuo, I., Saw, J., Kapan, D.D., Christensen, S., Kaneshiro, K.Y., and Donachie, S.P. 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.

Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* **3**, 208–218.

Marmur, J. and Doty, P. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**, 109–118.

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.

Miyashita, M., Fujimura, S., Nakagawa, Y., Nishizawa, M., Tomizuka, N., Nakagawa, T., and Nakagawa, J. 2010. *Flavobacterium algicola* sp. nov., isolated from marine algae. *Int. J. Syst. Evol. Microbiol.* **60**, 344–348.

Nedashkovskaya, O.I., Balabanova, L.A., Zhukova, N.V., Kim, S.J., Bakunina, I.Y., and Rhee, S.K. 2014. *Flavobacterium ahnfeltiae* sp. nov., a new marine polysaccharide-degrading bacterium isolated from a Pacific red alga. *Arch. Microbiol.* **196**, 745–752.

Ngo, H.T., Kook, M., and Yi, T.H. 2015. *Flavobacterium daemonensis* sp. nov., isolated from Daemo Mountain soil. *Int. J. Syst. Evol. Microbiol.* **65**, 983–989.

Saitou, N. and 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, DE, USA.

Song, L., Liu, H., Huang, Y., Dai, X., and Zhou, Y. 2013. *Flavobacterium marinum* sp. nov., isolated from seawater. *Int. J. Syst. Evol. Microbiol.* **63**, 3551–3555.

Subhash, Y., Sasikala, C., and Ramana, C.V. 2013. *Flavobacterium aquaticum* sp. nov., isolated from a water sample of a rice field. *Int. J. Syst. Evol. Microbiol.* **63**, 3463–3469.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703.

Weon, H.Y., Song, M.H., Son, J.A., Kim, B.Y., Kwon, S.W., Go, S.J., and Stackebrandt, E. 2007. *Flavobacterium terrae* sp. nov. and *Flavobacterium cucumis* sp. nov., isolated from greenhouse soil. *Int. J. Syst. Evol. Microbiol.* **57**, 1594–1598.

Wilson, K. 1987. Preparation of genomic DNA from bacteria. *In* Current Protocols in Molecular Biology, pp. 2.4.1–2.4.5. Green Publishing and Wiley-Interscience, New York, NY, USA.

Xiao, Y.P., Hui, W., Lee, J.S., Lee, K.C., and Quam, Z.X. 2011. *Flavobacterium dongtanense* sp. nov., isolated from the rhizosphere of a wetland reed. *Int. J. Syst. Evol. Microbiol.* **61**, 343–346.

Yoon, J.H., Park, S., Kang, S.J., Oh, S.J., Myung, S.C., and Kim, W. 2011. *Flavobacterium ponti* sp. nov., isolated from seawater. *Int. J. Syst. Evol. Microbiol.* **61**, 81–85.

Zhang, J., Jiang, R.B., Zang, X.X., Hang, B.J., He, J., and Li, S.P. 2010. *Flavoabacterium haoranii* sp. nov., a cypermethrin-degrading bacterium isolated from a wastewater treatment system. *Int. J. Syst. Evol. Microbiol.* **60**, 2882–2886.