Flavobacterium daejeonense sp. nov. and *Flavobacterium suncheonense* sp. nov., isolated from greenhouse soils in Korea

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Two yellow-pigmented, Gram-negative, rod-shaped bacterial strains, GH1-10^T and GH29-5^T, were isolated from greenhouse soils in Korea. 16S rRNA gene sequence analysis indicated that these strains were related to members of the genus *Flavobacterium*. Strain GH1-10^T was most closely related to *Flavobacterium psychrolimnae* and *Flavobacterium denitrificans*, with sequence similarities of 95.9 and 95.2 %, respectively. Strain GH29-5^T was most closely related to '*Flavobacterium salidiodium*', *F. denitrificans* and *Flavobacterium frigoris*, with sequence similarities of 94.3, 92.5 and 92.5 %, respectively. The major cellular fatty acids of GH1-10^T were iso-C_{15:0}, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) and iso-C_{17:0} 3-OH, and those of GH29-5^T were iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1} G and iso-C_{15:0} 3-OH. Both strains contained menaquinone with six isoprene units (MK-6) as the sole quinone. The DNA G + C contents of GH1-10^T and GH29-5^T were 35 and 39 mol%, respectively. Based on the phylogenetic and phenotypic data presented, it is concluded that the two bacteria represent two separate novel species of the genus *Flavobacterium*. The names proposed to accommodate these organisms are *Flavobacterium daejeonense* sp. nov., with type strain GH1-10^T (= KACC 11422^T = DSM 17708^T), and *Flavobacterium suncheonense* sp. nov., with type strain GH29-5^T (= KACC 11423^T = DSM 17707^T).

We isolated two yellow-pigmented bacterial strains, GH1-10^T and GH29-5^T, from greenhouse soils in Korea. 16S rRNA gene sequence analysis indicated that these strains represented two novel species of the genus *Flavobacterium*.

The genus *Flavobacterium* was proposed by Frankland in 1889. The genus belongs within the *Cytophaga–Flavobacterium–Bacteroides* (CFB) group, which is also known as the phylum *Bacteroidetes* (Ludwig & Klenk, 2001). Members of the CFB group are widely distributed in nature, particularly in marine environments (Glöckner *et al.*, 1999; Kirchman, 2002). Recently, *Flavobacterium* species have been isolated from seawater and freshwater, but relatively few have been isolated from soil environments.

Abbreviation: CFB, *Cytophaga–Flavobacterium–Bacteroides*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains GH1-10^T and GH29-5^T are DQ222427 and DQ222428, respectively.

A table giving the cellular fatty acid content of strains GH1-10^T and GH29-5^T is available as supplementary material in IJSEM Online.

R2A medium (Difco; Reasoner & Geldreich, 1985) was used for strain isolation, and the two novel strains were routinely grown at 28 °C. Growth was assessed at 4, 10, 20, 25, 30, 33, 37 and 40 °C, at pH 4, 5, 6, 7, 8, 9 and 10, and at 0, 1, 3, 5 and 7 % NaCl. The isolates were tested for a number of key characteristics by using standard procedures (Smibert & Krieg, 1994), such as Gram staining, production of catalase, oxidase and indole, Voges–Proskauer reaction, and hydrolysis of agar, casein, DNA, gelatin and starch. Hugh–Leifson medium (Hugh & Leifson, 1953) was used to test for glucose oxidation/fermentation. CM-cellulose (Sigma) (0.1 %) and Whatman powder CF11 (0.1 %) were used to test for hydrolysis of cellulose; degradation of alginic acid (0.5 %, w/v), chitin from crab shells (1 %, w/v), egg yolk (5 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was also tested. The urease test, absorption of Congo red and production of flexirubin-type pigments were determined by using the methods of MacFaddin (2000), Bernardet *et al.* (2002) and Reichenbach (1989), respectively. Cell motility was examined by using 1/10-strength R2A medium, and gliding motility was observed by using

oil-immersion phase-contrast microscopy of the edge of colonies in exponential growth phase. Strains were additionally characterized by using the whole test spectrum of the API 20NE, API 50CH and API ZYM (bioMérieux) systems according to the manufacturer's instructions.

For analysis of fatty acids, the strains were grown at 28 °C on R2A agar. After 48 h growth, cells were harvested, and identification of fatty acids was performed by using the standard protocol of the Microbial Identification System (MIDI; Microbial ID). Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). DNA G+C contents were determined by using an HPLC-based method as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18-S; Supelco).

The 16S rRNA gene sequence was determined by PCR amplification (Kwon *et al.*, 2003) and direct sequencing (Hiraishi, 1992). A phylogenetic tree was generated by using the neighbour-joining algorithm (Saitou & Nei, 1987).

Colonies of strains GH1-10^T and GH29-5^T were yellow, convex and round with clear margins. Both strains grew on R2A medium, trypticase soy agar (TSA; Difco) and nutrient agar (NA; Difco), but did not grow on MacConkey agar (Difco). GH1-10^T grew well on TSA, but GH29-5^T showed only weak growth when subcultured on TSA. Strain GH29-5^T showed no colour changes in all tubes of the API 50CH system as well as for the glucose fermentation reaction in the API 20NE system. Phenotypic characteristics of both strains are given in the species descriptions below and in Table 1.

Cellular fatty acid analysis showed that strains GH1-10^T and GH29-5^T contained iso-C_{15:0} as the most abundant component, present at 23.0 and 29.9% of the total, respectively. Strain GH1-10^T also contained considerable amounts of summed feature 3 (20.5%; iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) and iso-C_{17:0} 3-OH (10.7%). Strain GH29-5^T contained high levels of iso-C_{17:0} 3-OH (17.7%), iso-C_{15:1} G (12.0%) and iso-C_{15:0} 3-OH (11.1%) (see Supplementary Table S1 in IJSEM Online). The fatty acid

Table 1. Phenotypic characteristics of *Flavobacterium daejeonense* sp. nov., *Flavobacterium suncheonense* sp. nov. and some related *Flavobacterium* species

Taxa: 1, *F. daejeonense* GH1-10^T; 2, *F. suncheonense* GH29-5^T; 3, *F. aquatile*; 4, *F. denitrificans*; 5, *F. johnsoniae*; 6, *F. flevense*; 7, *F. psychrolimnae*; 8, *F. xanthum*; 9, *F. micromati*; 10, *F. columnare*; 11, *F. gelidilacus*. Data are taken from Bernardet *et al.* (1996), Holmes *et al.* (1988), McCammon & Bowman (2000), Van Trappen *et al.* (2004, 2005), Yabuuchi *et al.* (1983) and this study. Symbols: +, positive test; (+), positive test, weak or delayed response; –, negative; v, test results vary between strains; (v), variable among references; ND, no available data.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Growth on:											
Trypticase soy agar	+	(+)	(+)	+	+	+	+	+	(+)	–	+
Nutrient agar	+	+	–	ND	+	+	+	+	+	–	+
Growth at 25 °C	+	+	+	+	+	+	+	(+)	(+)	+	+
Flexirubin-type pigment	–	–	–	+	+	–	–	–	–	+	–
Congo red absorption	–	–	–	ND	(v)	–	–	–	–	+	–
Glucose utilization	+	–	ND	+	+	+	+	+	–	–	–
Acid from carbohydrate	+	–	+	–	+	+	–	+	–	–	–
Degradation of:											
Gelatin	–	+	(v)	ND	+	–	–	+	–	+	v
Casein	–	+	+	ND	+	–	+	+	–	+	+
Starch	+	–	(v)	ND	+	ND	+	+	–	–	+
CM-cellulose	–	–	–	ND	+	–	–	–	–	–	–
Agar	–	–	–	ND	–	+	–	–	–	–	–
Alginate	–	–	ND	ND	+	–	–	–	–	ND	–
Pectin	–	–	ND	ND	+	+	–	–	–	ND	–
Chitin	–	–	–	ND	+	–	–	–	–	–	–
Aesculin	+	–	(v)	ND	+	+	+	+	+	–	–
DNA	–	–	–	ND	+	–	–	–	–	+	–
Tyrosine	–	+	(v)	ND	+	–	–	–	–	–	–
Precipitation on egg-yolk agar	–	+	+	ND	–	–	–	–	–	+	–
β-Galactosidase activity	+	–	ND	ND	+	+	–	–	–	–	–
Nitrate reduction	+	–	ND	+	+	ND	–	+	–	ND	–
DNA G+C content (mol%)	35	39	33	35	34	35	34	36	33	32	30

profiles of the two strains showed only minor variations. Both strains contained menaquinone with six isoprene units (MK-6) as the sole quinone. The DNA G + C contents of strains GH1-10^T and GH29-5^T were 35 and 39 mol%, respectively.

In the phylogenetic tree constructed based on the neighbour-joining algorithm (Saitou & Nei, 1987), strains GH1-10^T and GH29-5^T fell within the radiation of the cluster comprising members of the genus *Flavobacterium*, but occupied quite different phylogenetic positions within the genus (Fig. 1). The nearly complete 16S rRNA gene sequences (about 1410 nucleotide positions) of strains GH1-10^T and GH29-5^T were compared with those of the type strains of a wide range of related recognized species and of '*Flavobacterium saliodium*'. None of the reference taxa had a sequence similarity to the two new isolates above the 97 % recommended cut-off value for the delineation of genomic species (Stackebrandt & Goebel, 1994). The 16S rRNA gene sequence of strain GH1-10^T showed highest similarities to *Flavobacterium psychrolimnae* LMG 22018^T (95.9 %) and *Flavobacterium denitrificans* ED5^T (95.2 %). Strain GH29-5^T showed highest 16S rRNA gene sequence similarities to '*F. saliodium*' AS 1.3801 (94.3 %), *F. denitrificans* ED5^T (92.5 %) and *Flavobacterium frigoris* LMG 21922^T (92.5 %).

These gene sequence comparisons clearly demonstrated that strains GH1-10^T and GH29-5^T represent members of the genus *Flavobacterium*. The fatty acid profiles and other phenotypic characteristics of the two strains further support this conclusion. We therefore suggest that GH1-10^T and GH29-5^T are the type strains of two novel species, for which the names *Flavobacterium daejeonense* sp. nov. and *Flavobacterium suncheonense* sp. nov. are proposed, respectively.

Description of *Flavobacterium daejeonense* sp. nov.

Flavobacterium daejeonense (dae.jeon.en'se. N.L. neut. adj. *daejeonense* pertaining to Daejeon, a city in Korea, from where the type strain was isolated).

Cells are Gram-negative rods (0.5 × 2–3 µm). Growth occurs at 5–35 °C, pH 6–8 and 0–3 % NaCl. Gliding motility is absent. Colonies are yellow, convex and round with clear margins. Catalase and oxidase tests are positive. Starch and aesculin are degraded, but gelatin, casein, cellulose, agar, alginate, pectin, chitin, DNA, tyrosine and egg yolk are not. Congo red is not absorbed. Flexirubin-type pigments are not produced. Voges–Proskauer reaction is negative. Positive reactions for nitrate reduction, aesculin hydrolysis and

Fig. 1. Phylogenetic relatedness of strains GH1-10^T, GH29-5^T and the type strains of *Flavobacterium* species on the basis of 16S rRNA gene sequence comparison. The dendrogram was generated by using neighbour-joining analysis (Saitou & Nei, 1987). Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values > 60 % are shown. Bar, two substitutions per 100 nt.

β -galactosidase, but negative reactions for indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis (API 20NE). Assimilates D-glucose, L-arabinose, D-mannose and D-maltose, but not D-mannitol, N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate or phenylacetic acid. Enzymic activity is detected for alkaline phosphatase, esterase C4, esterase lipase C8 (weak), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase and N-acetyl- β -glucosaminidase (API ZYM). No activity is detected for lipase C14, cystine arylamidase, trypsin, α -chymotrypsin, β -glucuronidase, β -glucosidase, α -mannosidase or α -fucosidase (API ZYM). Produces acid from L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, aesculin, D-maltose, D-lactose, D-melibiose, sucrose, inulin and D-raffinose (API 50CH). Major cellular fatty acids are iso-C_{15:0} (23%), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c; 20.5%) and iso-C_{17:0} 3-OH (10.7%). The sole quinone is MK-6, and the DNA G+C content is 35 mol%.

The type strain, GH1-10^T (= KACC 11422^T = DSM 17708^T), was isolated from greenhouse soil, Daejeon city, Republic of Korea.

Description of *Flavobacterium suncheonense* sp. nov.

Flavobacterium suncheonense (sun.cheon.en'se. N.L. neut. adj. *suncheonense* pertaining to Suncheon, a city in Korea, from where the type strain was isolated).

Cells are Gram-negative rods (0.3 $\times$ 1.5–2.5 μ m). Growth occurs at 15–37 °C, pH 6–8 and 0–1% NaCl. Gliding motility is absent. Colonies are yellow, convex and round with clear margins. Catalase and oxidase tests are positive. Gelatin, casein, tyrosine and egg yolk are degraded, but starch, cellulose, agar, alginate, pectin, chitin, aesculin and DNA are not. Congo red is not absorbed. Flexirubin-type pigment is not present. Voges–Proskauer reaction is negative. Positive reaction for gelatin hydrolysis, but negative reactions for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis and β -galactosidase (API 20NE). Does not assimilate all carbohydrates tested (API 20NE). Enzymic activity is detected for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase (API ZYM). No activity is detected for lipase C14, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase (API ZYM). No acids are produced from all the substrates tested (API 50CH). Major cellular fatty acids are iso-C_{15:0} (29.9%), iso-C_{17:0} 3-OH (17.7%), iso-C_{15:1} G (12.0%) and iso-C_{15:0} 3-OH (11.1%). The sole quinone is MK-6, and the G+C content is 39 mol%.

The type strain, GH29-5^T (= KACC 11423^T = DSM 17707^T), was isolated from greenhouse soil, Suncheon city, Republic of Korea.

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