Flavobacterium terrae sp. nov. and *Flavobacterium cucumis* sp. nov., isolated from greenhouse soil

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Two bacterial strains, R2A1-13^T and R2A45-3^T, were isolated from greenhouse soils in Korea. The cells of both strains were Gram-negative, aerobic and rod-shaped. 16S rRNA gene sequence analysis placed the isolates in the genus *Flavobacterium* within the family *Flavobacteriaceae*. Strain R2A1-13^T was found to be related to *Flavobacterium columnare* IAM 14301^T, *Flavobacterium saliperosum* CGMCC1.3801^T and *Flavobacterium croceum* EMB47^T, with sequence similarities of 96.8, 95.0 and 94.6 %, respectively. Strain R2A45-3^T was found to be related to *F. croceum* EMB47^T and *Flavobacterium aquatile* ATCC 11947^T, with sequence similarities of 94.7 and 94.6 %, respectively. Both strains contained iso-C_{15:0} and iso-C_{16:0} as the main fatty acids and contained a menaquinone with six isoprene units (MK-6) as the major isoprenoid quinone. The G+C contents of the DNA from strains R2A1-13^T and R2A45-3^T were 34 and 38 mol%, respectively. A polyphasic taxonomic study revealed that these strains belong to two novel species within the genus *Flavobacterium*, for which the names *Flavobacterium terrae* sp. nov. and *Flavobacterium cucumis* sp. nov. are proposed. The type strains of *F. terrae* sp. nov. and *F. cucumis* sp. nov. are R2A1-13^T (=KACC 11731^T=DSM 18829^T) and R2A45-3^T (=KACC 11732^T=DSM 18830^T), respectively.

Since the genus *Flavobacterium* was proposed by Bergey *et al.* (1923), the description of this genus has been revised several times (Bernardet *et al.*, 1996). Since 2005, the following novel species of the genus *Flavobacterium* have been described: *Flavobacterium antarcticum*, *F. croceum*, *F. daejeonense*, *F. denitrificans*, *F. frigidimaris*, *F. fryxellicola*, *F. granuli*, *F. indicum*, *F. psychrolimnae*, *F. saliperosum*, *F. segetis*, *F. soli*, *F. suncheonense* and *F. weaverense* (Aslam *et al.*, 2005; Horn *et al.*, 2005; Nogi *et al.*, 2005; Van Trappen *et al.*, 2005; Yi *et al.*, 2005; Kim *et al.*, 2006; Park *et al.*, 2006; Saha & Chakrabarti, 2006; Wang *et al.*, 2006; Yi & Chun, 2006; Yoon *et al.*, 2006). *Flavobacterium ferrugineum* has also been reclassified as *Terrimonas ferruginea* (Xie & Yokota, 2006).

In the present study, soil samples were collected from greenhouses planted with lettuce (*Lactuca sativa* L.) and cucumber (*Cucumis sativus* L.) in the Daejeon and Sangju

regions of Korea. Soil samples were suspended in sterilized water and then diluted solutions were spread on R2A agar (Difco) and incubated at 30 °C. Two bacterial isolates, R2A1-13^T and R2A45-3^T, were obtained; they were grown routinely on R2A medium at 30 °C.

The 16S rRNA gene was amplified by using a PCR with two universal primers as described previously (Kim *et al.*, 2006). BLAST searches in GenBank were used to identify species that were related to the novel isolates and the sequences of the two isolates (about 1430 bp) were aligned by using CLUSTAL W software (Thompson *et al.*, 1994). The phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and maximum parsimony (Fitch, 1971) in the MEGA3 program (Kumar *et al.*, 2004). The stability of the relationships was assessed by means of bootstrapping (based on 1000 replicates).

In the neighbour-joining phylogenetic tree (Fig. 1), the two isolates were clearly grouped within the genus *Flavobacterium*. Strain R2A1-13^T shared 96.8 % 16S rRNA gene sequence similarity with *Flavobacterium columnare* IAM

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains R2A1-13^T and R2A45-3^T are EF117329 and EF126993, respectively.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, for R2A1-13^T, R2A45-3^T and *Flavobacterium* species. Numbers at branch nodes are bootstrap percentages based on 1000 resamplings; only values greater than 50 % are shown. Bar, 0.02 substitutions per nucleotide position. *Sphingobacterium mizutaii* DSM 11724^T was used as an outgroup. The maximum-parsimony tree showed essentially the same topology (not shown).

14301^T (100 % bootstrap support). It also shared relatively high levels of sequence similarity with *F. saliperosum* CGMCC 1.3801^T (95.0 %) and *F. croceum* EMB47^T (94.6 %). Strain R2A45-3^T was related to *F. croceum* EMB47^T and *Flavobacterium aquatile* ATCC 11947^T, with sequence similarities of 94.7 and 94.6 %, respectively. The maximum-parsimony tree showed essentially the same topology.

Biochemical and physiological tests were performed as described by Kim *et al.* (2006). The cells of both strains were Gram-negative, aerobic and rod-shaped, growing well on R2A and nutrient agar (Difco), weakly on tryptic soy agar (Difco) and not at all on MacConkey agar (Difco). Strain R2A1-13^T could be differentiated from its closest relative, *F. columnare*, by the absence of gliding motility, by the ability to grow on nutrient agar and tryptic soy agar and by the ability to hydrolyse starch. Strain R2A45-3^T could be distinguished from *F. croceum* by the ability of the former to grow on nutrient agar and to hydrolyse starch. Additional phenotypic characteristics that served to differentiate the

two strains and other related *Flavobacterium* species are shown in Table 1.

Chemotaxonomic characteristics were determined from cells grown at 30 °C for 1 day on R2A medium. Analysis of the fatty acid methyl esters was performed by using GLC according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were analysed by using HPLC as described previously (Groth *et al.*, 1996). The DNA G + C contents of the isolates were determined by using the HPLC method described by Mesbah *et al.* (1989), with a reversed-phase column (Supelcosil LC-18 S; Supelco).

The major fatty acids in both strains were iso-C_{15:0} and iso-C_{16:0}. The detailed fatty acid compositions are shown in Table 2. Both strains contained a menaquinone with six isoprene units (MK-6) as the major isoprenoid quinone. The G + C contents of the DNA of strains R2A1-13^T and R2A45-3^T were 34 and 38 mol%, respectively.

On the basis of the data obtained in this polyphasic taxonomic study, strains R2A1-13^T and R2A45-3^T represent

Table 1. Physiological and biochemical characteristics of strains R2A1-13^T and R2A45-3^T and related *Flavobacterium* species

Strains: 1, R2A1-13^T; 2, R2A45-3^T; 3, *F. aquatile* (data from Bernardet *et al.*, 1996); 4, *F. columnare* (Bernardet *et al.*, 1996); 5, *F. croceum* EMB47^T (Park *et al.*, 2006); 6, *F. indicum* GPTSA100-9^T (Saha & Chakrabarti, 2006); 7, *F. saliperosum* JCM 13331^T (Wang *et al.*, 2006); 8, *F. suncheonense* KACC 11423^T (Kim *et al.*, 2006). +, Positive; –, negative; (+), weakly positive; v, variable among references according to Bernardet *et al.* (1996).

Characteristic	1	2	3	4	5	6	7	8
Colony pigmentation*	YO	Y	CY	GY	Y	YO	Y	Y
Growth on:								
Nutrient agar	+	+	–	–	–	(+)	+	+
Tryptic soy agar	(+)	(+)	+	–	(+)	–	+	(+)
Oxidase/catalase	+/-	+/+	+/+	+/+	+/-	+/(+)	-/+	+/+
Gliding motility	–	+	+	+	–	–	–	–
Flexirubin-type pigments	+	–	–	+	–	–	+	–
Nitrate reduction	–	–	v	v	–	–	–	–
Hydrolysis of:								
Aesculin	–	+	v	–	–	–	–	–
Gelatin	+	+	v	+	+	+	+	+
Starch	+	+	v	–	–	+	–	–
Tyrosine	–	+	v	–	–	–	+	+
DNA G+C content (mol%)	34	38	33	32	41	31	41	39

*CY, Cream–yellow; GY, greenish yellow; Y, yellow; YO, yellowish orange.

two novel *Flavobacterium* species, for which the names *Flavobacterium terrae* sp. nov. and *Flavobacterium cucumis* sp. nov. are proposed.

Description of *Flavobacterium terrae* sp. nov.

Flavobacterium terrae (ter'rae. L. gen. n. *terrae* of the soil).

Cells are Gram-negative, non-spore-forming rods, 0.5 × 2.5–4.5 µm. Colonies on R2A medium are yellowish-orange with irregular edges. Growth occurs at 5–37 °C (optimum, 30 °C), at pH 6–8 (optimum, pH 7) and with 0–2 % NaCl (optimum, 0–2 %). Oxidase-positive. Catalase-negative. Gliding motility is not observed. Flexirubin-type pigments are present. Negative for nitrate reduction, indole and arginine dihydrolase production, glucose fermentation and urease and aesculin hydrolysis (API 20NE test strip). Casein, gelatin and starch are hydrolysed, but alginic acid, chitin, CM-cellulose, DNA, pectin, tyrosine and urea are not. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated (API 20NE test strip). Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present; lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent (API ZYM

test strip). MK-6 is the major respiratory lipoquinone. The major fatty acids are iso-C_{15:0} and iso-C_{16:0}. The G+C content of the genomic DNA is 34 mol%.

The type strain, R2A1-13^T (=KACC 11731^T=DSM 18829^T), was isolated from greenhouse soil cultivated with lettuce in the Daejeon region of Korea.

Description of *Flavobacterium cucumis* sp. nov.

Flavobacterium cucumis (cu'cu.mis. L. gen. n. *cucumis* of the cucumber, referring to the isolation source of the type strain, a soil cultivated with cucumber plants).

Cells are Gram-negative, non-spore-forming rods, 0.5 × 2.0–3.0 µm. Colonies on R2A medium are yellow with irregular edges. Growth occurs at 5–37 °C (optimum, 30 °C), at pH 6–8 (optimum, pH 7) and with 0–2 % NaCl (optimum, 0–2 %). Catalase- and oxidase-positive. Gliding motility is observed. Flexirubin-type pigments are not present. Negative for nitrate reduction, indole and arginine dihydrolase production and glucose fermentation (API 20NE test strip). Aesculin, casein, gelatin, starch and tyrosine are hydrolysed, but alginic acid, chitin, CM-cellulose, DNA, pectin and urea are not. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated (API 20NE test strip). Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase,

Table 2. Cellular fatty acid compositions of strains R2A1-13^T and R2A45-3^T and related *Flavobacterium* type strains

Strains: 1, R2A1-13^T; 2, R2A45-3^T; 3, *F. aquatile* DSM 1132^T; 4, *F. columnare* LMG 13035^T; 5, *F. croceum* EMB47^T (data from Park *et al.*, 2006); 6, *F. indicum* GPTSA100-9^T (Saha & Chakrabarti, 2006); 7, *F. saliperosum* JCM 13331^T (data in columns 1–4 and 7 are from this study); 8, *F. suncheonense* KACC 11423^T (Kim *et al.*, 2006). –, <1% or not detected; NR, not reported.

Fatty acid	1	2	3	4	5	6	7	8
iso-C _{13:0}	–	–	–	1.3	1.0	NR	–	1.9
iso-C _{14:0}	4.7	5.3	3.1	3.8	8.7	NR	–	–
iso-C _{14:0} 3-OH	1.1	1.8	–	1.5	2.6	NR	–	–
C _{15:0}	–	–	–	–	10.8	NR	–	–
C _{15:0} 2-OH	–	–	–	–	2.0	NR	–	–
C _{15:0} 3-OH	2.2	1.5	2.1	1.9	1.3	NR	–	–
anteiso-C _{15:0}	2.8	4.4	3.5	2.2	5.1	NR	4.4	2.1
anteiso-C _{15:1} A	–	–	–	–	1.6	NR	–	–
iso-C _{15:0}	18.4	17.5	17.0	30.1	9.3	18.5	25.6	29.9
iso-C _{15:0} 3-OH	6.7	5.1	6.4	5.2	5.9	5.0	4.8	11.1
iso-C _{15:1} G	10.7	9.9	7.4	14.6	11.5	18.0	6.6	12.0
C _{15:1} ω6c	1.1	2.0	10.8	–	–	NR	1.3	–
C _{16:0}	–	1.2	2.6	1.1	1.0	NR	1.1	1.0
C _{16:0} 3-OH	1.3	1.7	2.3	–	–	NR	–	–
iso-C _{16:0}	16.6	18.8	7.2	8.7	8.5	5.1	11.5	1.0
iso-C _{16:0} 3-OH	5.0	12.6	10.4	7.4	16.5	4.5	2.4	–
iso-C _{16:1} G	3.0	–	–	–	2.9	NR	–	–
iso-C _{16:1} H	–	4.0	2.9	4.1	–	NR	2.1	–
C _{17:0} 2-OH	–	–	–	–	–	NR	1.8	–
C _{17:0} 3-OH	1.5	–	1.1	–	–	NR	–	–
iso-C _{17:0} 3-OH	10.3	5.3	6.5	6.6	2.7	9.0	10.3	17.7
C _{17:1} ω6c	–	–	2.5	–	–	NR	–	–
C _{17:1} ω8c	–	–	1.3	–	–	NR	–	–
iso-C _{17:1} ω9c	6.2	1.5	1.5	3.7	–	NR	17.9	7.5
Summed feature 3*	1.3	2.0	7.0	–	3.6	16.6	2.3	9.8

*Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present; lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent (API ZYM test strip). MK-6 is the major respiratory lipoquinone. The major fatty acids are iso-C_{15:0} and iso-C_{16:0}. The G+C content of the genomic DNA is 38 mol%.

The type strain, R2A45-3^T (=KACC 11732^T=DSM 18830^T), was isolated from greenhouse soil cultivated with cucumber in the Sangju region of Korea.

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