Flavobacterium terrigena sp. nov., isolated from soil

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A Gram-negative, rod-shaped, *Flavobacterium*-like bacterial strain, DS-20^T, was isolated from soil from the island of Dokdo, Korea, and subjected to a polyphasic taxonomic study. Strain DS-20^T grew optimally at pH 6.5–7.0 and 25 °C. It contained MK-6 as the predominant menaquinone and iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{17:1}ω9c as the major fatty acids. The DNA G + C content was 38.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-20^T belonged to the genus *Flavobacterium*. Levels of 16S rRNA gene sequence similarity between strain DS-20^T and the type strains of recognized *Flavobacterium* species were below 94.9 %. Strain DS-20^T differed from phylogenetically related *Flavobacterium* species in several phenotypic characteristics. On the basis of its phenotypic and phylogenetic distinctiveness, strain DS-20^T was classified in the genus *Flavobacterium* as representing a novel species, for which the name *Flavobacterium terrigena* sp. nov. is proposed. The type strain is DS-20^T (=KCTC 12761^T=DSM 17934^T).

The genus *Flavobacterium* was proposed by Bergey *et al.* (1923). Recently, descriptions of many novel *Flavobacterium* species, including *Flavobacterium saliperosum* (Wang *et al.*, 2006), *Flavobacterium soli* (Yoon *et al.*, 2006), *Flavobacterium weaverense* and *Flavobacterium segetis* (Yi & Chun, 2006), *Flavobacterium daejeonense* and *Flavobacterium suncheonense* (Kim *et al.*, 2006), *Flavobacterium croceum* (Park *et al.*, 2006), *Flavobacterium indicum* (Saha & Chakrabarti, 2006), *Flavobacterium glaciei* (Zhang *et al.*, 2006), and *Flavobacterium aquidurensense* and *Flavobacterium hercynium* (Cousin *et al.*, 2007), have increased considerably the number of species belonging to the genus. *Flavobacterium ferrugineum* was reclassified recently in a new genus, *Terrimonas*, as *Terrimonas ferruginea* (Xie & Yokota, 2006). Accordingly, at the time of writing, the genus *Flavobacterium* comprises 45 recognized species. Here we report on the taxonomic characterization of a *Flavobacterium*-like bacterial strain, DS-20^T.

Strain DS-20^T was isolated from a soil sample collected on the island of Dokdo (37° 14' 12" N 131° 52' 07" E), Korea, using the standard dilution plating technique at 25 °C on nutrient agar (NA; Difco). The morphological, physiological and biochemical characteristics of strain DS-20^T were investigated by using routine cultivation on NA at 25 °C. Cell morphology was examined by light microscopy (Nikon

E600) and transmission electron microscopy with cells from exponentially growing cultures. Cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after air-drying with a Philips CM-20 transmission electron microscope. Gliding motility was determined as described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was measured on NA. The pH range for growth was determined in nutrient broth (NB; Difco) that, prior to sterilization, was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units) by the addition of HCl or Na₂CO₃. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on NA and on NA supplemented with nitrate, both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). DNase activity was examined by using DNase test agar with methyl green (Difco). Hydrolysis of aesculin and reduction of nitrate were studied as described by Lanyi (1987). Hydrolysis of carboxymethylcellulose (CM-cellulose) was tested as described by Bowman (2000) using NA as the basal medium. The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Congo red adsorption was determined according to the method of Bernardet *et al.* (2002). Assimilation of various substrates was determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturer. Sensitivity to antibiotics was tested on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-20^T is DQ889724.

A table giving Biolog assimilation data for strain DS-20^T is available as supplementary material in IJSEM Online.

NA plates by using antibiotic discs containing the following: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) or carbenicillin (100 µg). Enzyme activities and other physiological and biochemical properties were tested by using the API 20E and API ZYM systems (bioMérieux) according to the manufacturer's instructions.

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in NB at 25 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR by using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the

method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid analysis, cell mass of strain DS-20^T was harvested from NA plates after incubation for 3 days at 25 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined according to the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain DS-20^T are given in the species description or are shown in Table 1. The almost complete 16S rRNA gene sequence of strain DS-20^T determined in this study comprised 1470 nt, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-20^T falls within the radiation of the cluster comprising *Flavobacterium* species (Fig. 1). In

Table 1. Differential phenotypic characteristics of strain DS-20^T and phylogenetically related *Flavobacterium* species

Taxa: 1, strain DS-20^T (*F. terrigena* sp. nov.); 2, *F. columnae* (data from Bernardet *et al.*, 1996); 3, *F. croceum* (Park *et al.*, 2006); 4, *F. saliperosum* (Wang *et al.*, 2006); 5, *F. suncheonense* (Kim *et al.*, 2006). +, Positive reaction; –, negative reaction; w, weakly positive reaction; ND, not determined. All taxa are positive for hydrolysis of casein and gelatin. All are negative for hydrolysis of aesculin, agar (not determined for *F. croceum*), CM-cellulose and starch, and β-galactosidase activity (not determined for *F. saliperosum*).

Characteristic	1	2	3	4	5
Catalase	+	+	–	+	+
Oxidase	+	+	+	–	+
Gliding motility	–	+	–	–	–
Congo red absorption	–	+	–	–	–
Flexirubin-type pigments	+	+	–	+	–
Growth on:					
Seawater media	–	–	ND	–	ND
Nutrient agar	+	–	–	+	+
Trypticase soy agar	–	–	W	W	W
Optimum growth temperature (°C)	25	20–25	25–35	28	28
Maximum growth temperature (°C)	30	33*	ND	34	37
Acid produced aerobically from carbohydrates	–	–	+	–	–
Degradation of:					
DNA	–	+	ND	ND	–
Tyrosine	+	–	–	+	+
Nitrate reduction	–	+	–	–	–
H ₂ S production	–	+	–	–	ND
API ZYM results:					
Esterase (C4)	+	ND	–	ND	+
Esterase lipase (C8)	+	ND	–	ND	+
Acid phosphatase	–	ND	W	ND	+
Naphthol-AS-BI-phosphohydrolase	–	ND	W	ND	+
N-Acetyl-β-glucosaminidase	–	ND	–	ND	+
DNA G+C content (mol%)	38.2	32–33	40.8	41	39

*Some strains are able to grow at 37 °C.

the neighbour-joining phylogenetic tree, strain DS-20^T joined the clade comprising *F. saliperosum*, *F. suncheonense*, *Flavobacterium columnare*, *F. indicum* and *F. croceum* with a bootstrap resampling value of 83.8 %; this tree topology was also found in trees generated with the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain DS-20^T exhibited 16S rRNA gene sequence similarity values of 92.3–94.9 % to the type strains of recognized *Flavobacterium* species.

The results obtained from chemotaxonomic analyses were consistent with the phylogenetic affiliation of strain DS-20^T to the genus *Flavobacterium*. The predominant isoprenoid quinone detected in strain DS-20^T was menaquinone-6 (MK-6) at a peak area ratio of approximately 95 %; this is consistent with other *Flavobacterium* species (Bernardet *et al.*, 1996, 2002; Kim *et al.*, 2006; Yi *et al.*, 2005; Yi & Chun, 2006; Yoon *et al.*, 2006; Wang *et al.*, 2006). The fatty acid profile of strain DS-20^T comprised (> 1 % of the total fatty acids): branched fatty acids iso-C_{15:0} (30.1 %), iso-C_{17:1}ω9*c* (11.7 %), iso-C_{15:1} (9.0 %), iso-C_{16:0} (5.2 %), iso-C_{16:1} (2.2 %); hydroxy fatty acids iso-C_{17:0} 3-OH (16.0 %), iso-C_{15:0} 3-OH (6.6 %) and iso-C_{16:0} 3-OH (3.6 %); straight-chain fatty acid C_{15:0} (4.3 %); summed feature 3 (C_{16:1}ω7*c* and/or iso-C_{15:0} 2-OH, 2.4 %); and unsaturated fatty acid C_{15:1}ω6*c* (1.3 %). This fatty acid profile was similar to those of other *Flavobacterium* species, although there were differences in the proportions of some components, probably because of differences in cultivation conditions and extraction procedures (Aslam *et al.*, 2005; Bernardet *et al.*, 1996, 2002; Wang *et al.*, 2006). The DNA G + C content of strain DS-20^T was 38.2 mol%. Strain DS-20^T differed from phylogenetically related *Flavobacterium* species in several phenotypic characteristics, as shown in Table 1. The phylogenetic distinctiveness, together with differential phenotypic properties, was sufficient to classify strain DS-20^T as representing a species that is distinct from recognized *Flavobacterium* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-20^T should be classified in the genus *Flavobacterium* as the type strain of a novel species, for which the name *Flavobacterium terrigena* sp. nov. is proposed.

Description of *Flavobacterium terrigena* sp. nov.

Flavobacterium terrigena (ter.ri.ge'na. L. masc. or fem. n. *terrigena* child of the earth, referring to the isolation of the type strain from soil).

Cells are Gram-negative, aerobic short rods or rods (0.3–0.6 µm × 0.7–2.5 µm). No flagella are detected. Non-motile. Colonies on NA are circular, flat to raised, smooth, glistening, dark yellow in colour and 1.0–2.0 mm in diameter after 3 days incubation at 25 °C. Optimal temperature for growth is 25 °C; growth occurs at 10 and 30 °C, but not at 4 or 31 °C. Optimal pH for growth is 6.5–7.0; growth occurs at pH 6.0 and 8.0, but not at pH 5.5 or 8.5. No anaerobic growth occurs on NA or on NA supplemented with nitrate. Tweens 20, 40, 60 and 80 are hydrolysed, but hypoxanthine,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain DS-20^T and other *Flavobacterium* species. Bootstrap values (expressed as percentages of 1000 replications) of > 50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated using the maximum-likelihood and maximum-parsimony algorithms. *Cellulophaga lytica* ATCC 23178^T (GenBank accession no. M62796) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

xanthine and urea are not. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase. In assays with the API ZYM system, positive for alkaline phosphatase, leucine arylamidase and valine arylamidase, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. Sensitive to polymyxin B, streptomycin, penicillin G, chloramphenicol, ampicillin, cephalothin, gentamicin, novobiocin, tetracycline, carbenicillin, kanamycin, lincomycin and neomycin, but not to oleandomycin.

Pyruvic acid methyl ester, α -ketovaleric acid, succinic acid, L-glutamic acid, glycyl L-glutamic acid, L-ornithine, L-proline and L-threonine are assimilated, and α -cyclodextrin, α -ketobutyric acid, α -ketoglutaric acid and L-serine are weakly assimilated. The predominant menaquinone is MK-6. Major fatty acids ($>10\%$ of the total fatty acids) are iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{17:1} ω 9c. The DNA G+C content of the type strain is 38.2 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1 or in Supplementary Table S1 available in IJSEM Online.

The type strain, DS-20^T (= KCTC 12761^T = DSM 17934^T), was isolated from soil.

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