Flavobacterium soli sp. nov., isolated from soil

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A Gram-negative, rod-shaped *Flavobacterium*-like bacterial strain, DS-6^T, was isolated from soil from the island of Dokdo, Korea, and subjected to a polyphasic taxonomic study. Strain DS-6^T grew optimally at pH 7.0 and 25 °C in the presence of 0–0.5 % (w/v) NaCl. It contained MK-6 as the predominant menaquinone and iso-C_{15:0} and iso-C_{17:0} 3-OH as the major fatty acids. The DNA G + C content was 36.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain DS-6^T belonged to the genus *Flavobacterium*. Levels of 16S rRNA gene sequence similarity between strain DS-6^T and the type strains of *Flavobacterium* species were below 95.4 %. Strain DS-6^T differed from some phylogenetically related *Flavobacterium* species in several phenotypic characteristics. On the basis of phenotypic and phylogenetic distinctiveness, DS-6^T (=KCTC 12542^T = CIP 108840^T) was classified in the genus *Flavobacterium* as the type strain of a novel species, for which the name *Flavobacterium soli* sp. nov. is proposed.

The genus *Flavobacterium*, which belongs to the *Cytophaga–Flavobacterium–Bacteroides* group, was proposed by Bergey *et al.* (1923). At the time of writing, the genus comprises 34 recognized species, including the recently described species *Flavobacterium antarcticum* (Yi *et al.*, 2005), *Flavobacterium granuli* (Aslam *et al.*, 2005), *Flavobacterium fryxellicola* and *Flavobacterium psychrolimnae* (Van Trappen *et al.*, 2005), and *Flavobacterium denitrificans* (Horn *et al.*, 2005). In this study, we report on the taxonomic characterization of a *Flavobacterium*-like bacterial strain, DS-6^T.

Strain DS-6^T was isolated from soil samples 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 (Difco). The morphological, physiological and biochemical characteristics of strain DS-6^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 25 °C. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy using 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 using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was measured on TSA. To investigate tolerance to NaCl, trypticase soy broth (TSB)

was prepared according to the formula of the Difco medium and NaCl concentrations were varied [0, 0.5 and 1.0–5.0 % (w/v) at intervals of 1.0 %]. The pH range for growth was determined in nutrient broth (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 TSA and on TSA 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 investigated as described by Cowan & Steel (1965) using DNase test agar (Difco). Hydrolysis of aesculin and nitrate reduction were studied as described by Lanyi (1987). Hydrolysis of carboxymethylcellulose was tested as described by Bowman (2000) using TSA as the basal medium. The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Congo red adsorption was determined as described by Bernardet *et al.* (2002). Sensitivity to antibiotics was tested on TSA plates using discs containing the following antibiotics: 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) and carbenicillin (100 µg). Assimilation of various substrates, enzyme activities, and other physiological and biochemical properties were tested by using the API 50CHB and API ZYM systems (bioMérieux) according to the manufacturer's instructions.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-6^T is DQ178976.

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in TSB 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 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 then analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid analysis, cell mass of strain DS-6^T was harvested from TSA 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-6^T are given in the species description (see later) or are shown in Table 1. The almost complete 16S rRNA gene sequence of strain DS-6^T determined in this study comprised 1471 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analysis revealed that strain DS-6^T was most closely phylogenetically affiliated to the genus *Flavobacterium* (Fig. 1). However, the 16S rRNA gene sequence of strain DS-6^T showed less than 95.4 % similarity to those of the type strains of other *Flavobacterium* species (Fig. 1).

The results obtained from chemotaxonomic analyses were consistent with the phylogenetic affiliation of strain DS-6^T to the genus *Flavobacterium*. The predominant isoprenoid quinone detected in strain DS-6^T was menaquinone-6 (MK-6) at a peak area ratio of approximately 93 %; this is consistent with other *Flavobacterium* species (Bernardet *et al.*, 1996, 2002; Yi *et al.*, 2005). The fatty acid profile of strain DS-6^T comprised (of those making up >1 % of the total fatty acids): branched fatty acids iso-C_{15:0} (26.6 %), iso-C_{17:1ω9c} (9.6 %), iso-C_{15:1} (6.3 %), iso-C_{16:0} (4.1 %),

Table 1. Differential phenotypic characteristics of *Flavobacterium soli* sp. nov. and phylogenetically related *Flavobacterium* species

Species: 1, *F. soli* sp. nov.; 2, *F. johnsoniae* (data from Reichenbach, 1989; Bernardet *et al.*, 1996); 3, *F. denitrificans* (data from Horn *et al.*, 2005); 4, *F. tegetincola* (data from McCammon & Bowman, 2000); 5, *F. antarcticum* (data from Yi *et al.*, 2005); 6, *F. flevense* (data from Reichenbach, 1989; Bernardet *et al.*, 1996). +, Positive reaction; –, negative reaction; ND, not determined; V, variable among references; v, variable reaction. All species are positive for growth on trypticase soy agar and nutrient agar (not determined for *F. denitrificans*). All species are negative for urease, H₂S production (not determined for *F. denitrificans*) and indole production.

Characteristic	1	2	3	4	5	6
Catalase activity	+	+	+	+	+	–
Oxidase activity	+	+	–	–	+	+
Gliding motility	–	+	ND	+	–	+
Flexirubin-type pigments	–	+	+	–	–	–
Growth on sea water media	+	–	ND	+	+	+
Optimum growth temperature (°C)	25	25–30	25	20	21.2	25
Maximum growth temperature (°C)	33	<37	30	27	25.1	<35
Highest NaCl concentration (%) tolerated	2	1	<5	5	4	ND
Acid produced aerobically from carbohydrates	–	+	–	+	+	–
Degradation of:						
Gelatin	+	+	ND	–	+	–
Casein	–	+	ND	–	+	–
Starch	–	+	ND	–	–	V
Carboxymethylcellulose	–	+	ND	–	–	–
Agar	–	–	ND	–	–	+
Aesculin	+	+	ND	–	–	+
DNA	–	+	ND	–	+	–
Tyrosine	–	+	ND	–	–	–
Nitrate reduction	–	V	+	–	–	V
β-Galactosidase	–	+	ND	–	–	+
DNA G+C content (mol%)	36.9	33	34.6	34	38	33 or 35

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *Flavobacterium soli* sp. nov. DS-6^T and of other *Flavobacterium* species. Bootstrap values (expressed as percentages of 1000 replications) of > 50% are shown at branching points. *Cellulophaga lytica* ATCC 23178^T was used as an out-group (not shown). Bar, 0.01 substitutions per nucleotide position.

anteiso-C_{15:0} (2.7%) and iso-C_{16:1} (1.9%); hydroxy fatty acids iso-C_{17:0} 3-OH (11.5%), iso-C_{15:0} 3-OH (6.0%) and iso-C_{16:0} 3-OH (2.5%); straight-chain fatty acids C_{15:0} (8.2%) and C_{16:0} (2.0%); unsaturated fatty acids C_{15:1}ω6c (1.9%), C_{17:1}ω6c (1.4%) and C_{17:1}ω8c (1.1%); and summed feature 3 comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (6.0%). This fatty acid profile was similar to those of other *Flavobacterium* species, although there were differences in the proportions of some fatty acids, probably because of differences in cultivation conditions and extraction procedures (Bernardet *et al.*, 1996, 2002; Aslam *et al.*, 2005). The DNA G+C content of strain DS-6^T was 36.9 mol%. Strain DS-6^T differed from phylogenetically related *Flavobacterium* species by several phenotypic characteristics, as shown in Table 1. Therefore, on the basis of the data presented, DS-6^T should be classified in the genus *Flavobacterium* as the type strain of a novel species, for which the name *Flavobacterium soli* sp. nov. is proposed.

Description of *Flavobacterium soli* sp. nov.

Flavobacterium soli (so'li. L. gen. n. *soli* of soil).

Cells are Gram-negative, aerobic rods (0.3–0.6 μm × 1.0–3.0 μm). No flagella are detected. Non-motile. Colonies on TSA are circular, slightly convex, smooth, glistening, cream-yellow in colour and 2.0–3.0 mm in diameter after 3 days incubation at 25 °C. Optimal temperature for growth is 25 °C; growth occurs at 4 and 33 °C, but not at 34 °C. Optimal pH for growth is 7.0; growth occurs at pH 6.5 and 8.0, but not at pH 6.0 or 8.5. Growth occurs in the presence

of 0–2% (w/v) NaCl; optimal growth occurs in the presence of 0–0.5% (w/v) NaCl. No anaerobic growth occurs on plain TSA and on TSA supplemented with nitrate. Tweens 20, 40, 60 and 80 are hydrolysed, but hypoxanthine and xanthine are not. Congo red absorption is negative. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are positive, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are negative. Sensitive to penicillin G, chloramphenicol, cephalothin, novobiocin, tetracycline, lincomycin and oleandomycin, but not to polymyxin B, streptomycin, ampicillin, gentamicin, carbenicillin, kanamycin or neomycin. The following substrates are utilized: L-arabinose, glucose, mannose, aesculin, cellobiose, maltose, starch, gentiobiose and 5-ketogluconate. The following substrates are not utilized: glycerol, erythritol, D-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-D-xyloside, galactose, fructose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, salicin, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate and 2-ketogluconate. The predominant menaquinone is MK-6. The major fatty acids (> 10% of the total fatty acids) are iso-C_{15:0} and iso-C_{17:0}.

3-OH. The DNA G + C content is 36.9 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DS-6^T (=KCTC 12542^T = CIP 108840^T), was isolated from soil.

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