Pedobacter sandarakinus sp. nov., isolated from soil

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A Gram-negative, non-motile, rod-shaped bacterial strain, designated DS-27^T, was isolated from a soil sample, and its taxonomic position was investigated by using a polyphasic approach. The organism grew optimally at 30 °C and in the presence of 0–0.5 % (w/v) NaCl. Strain DS-27^T contained MK-7 as the predominant menaquinone and iso-C_{15:0}, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and iso-C_{17:0} 3-OH as the major fatty acids. The DNA G + C content was 39.7 mol%. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain DS-27^T is most closely related to the genus *Pedobacter* of the family *Sphingobacteriaceae*. Similarity values between the 16S rRNA gene sequences of strain DS-27^T and the type strains of recognized *Pedobacter* species ranged from 90.6 to 95.5 %. Differential phenotypic properties, together with the phylogenetic distinctiveness, were sufficient to categorize strain DS-27^T as representing a species that is separate from recognized *Pedobacter* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain DS-27^T (=KCTC 12559^T = CIP 108922^T) was classified in the genus *Pedobacter* as a member of a novel species, for which the name *Pedobacter sandarakinus* sp. nov. is proposed.

The genus *Pedobacter* was proposed by Steyn *et al.* (1998) with the reclassification of two *Sphingobacterium* species as *Pedobacter heparinus* and *Pedobacter piscium* and the description of two novel species, *Pedobacter africanus* and *Pedobacter saltans*. Subsequently, three further species, *Pedobacter cryoconitis* (Margesin *et al.*, 2003), *Pedobacter himalayensis* (Shivaji *et al.*, 2005) and *Pedobacter caeni* (Vanparrys *et al.*, 2005), have been described. In this study, we report on the taxonomic characterization of a *Pedobacter*-like bacterial strain, DS-27^T, which was isolated from a soil sample from the island of Dokdo (37° 14' 12" N, 131° 52' 07" E), Korea.

Strain DS-27^T was isolated by the standard dilution plating technique on twofold diluted nutrient agar (Difco) at 25 °C. The morphological, physiological and biochemical characteristics of strain DS-27^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 30 °C. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. The presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. For transmission electron microscopic observation, cells were negatively stained with 1 % (w/v) phosphotungstic acid and, following air-drying, the grids were examined

with a Philips CM-20 transmission electron microscope. Gliding motility was determined as described by Bowman (2000) using quarter-strength trypticase soy broth (TSB; Difco) solidified with 1 % (w/v) agarose. 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. Growth in the absence of NaCl and at various NaCl concentrations [0.5 % (w/v) and 1.0–5.0 % (w/v) at intervals of 1.0 %] was investigated in TSB prepared according to the formula of the Difco medium except that no NaCl was used. The pH range for growth was determined in nutrient broth (Difco) that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization to various levels 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 using nitrogen. 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). Hydrolysis of aesculin and nitrate reduction were studied as described by Lanyi (1987). The presence of flexirubin pigment was investigated according to the method of Reichenbach (1992). Susceptibility to antibiotics was tested on TSA plates using discs containing the following antibiotics: polymyxin B

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-27^T is DQ235228.

Table 1. Differential phenotypic characteristics of *Pedobacter* species

Species: 1, *P. sandarakinus* sp. nov.; 2, *P. heparinus*; 3, *P. africanus*; 4, *P. piscium*; 5, *P. saltans*; 6, *P. cryoconitis*; 7, *P. himalayensis*; 8, *P. caeni*. Data are from Steyn *et al.* (1998), Margesin *et al.* (2003), Shivaji *et al.* (2005) and Vanparys *et al.* (2005). +, Positive reaction; –, negative reaction; w, weakly positive reaction; v, variable reaction; ND, not determined. Data in parentheses are for the type strain. All species are positive for catalase, oxidase, hydrolysis of aesculin, activities (not done for *P. cryoconitis* and *P. himalayensis*) of esterase lipase (C8), acid phosphatase, alkaline phosphatase, leucine arylamidase and β -galactosidase, and assimilation of D-glucose, D-mannose, N-acetylglucosamine, amygdalin (not done for *P. himalayensis*), lactose, melibiose (variable for *P. saltans*), sucrose (variable for *P. saltans*) and D-trehalose (variable for *P. saltans*). All species are negative for Gram stain, sporulation, urease, production of indole and H₂S, nitrate reduction, and assimilation of erythritol, methyl β -D-xyloside, L-sorbose, dulcitol, inositol, D-melezitose, D-lyxose (not done for *P. himalayensis*), D-tagatose (not done for *P. himalayensis*), D-fucose (not done for *P. himalayensis*), D-arabitol (not done for *P. himalayensis*), L-arabitol (not done for *P. himalayensis*), gluconate (not done for *P. himalayensis*), adipate (not done for *P. himalayensis*), caprate (not done for *P. himalayensis*), phenylacetate (not done for *P. himalayensis*), citrate and malate.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Orange	Translucent yellow	Translucent yellow	Yellow or creamish white	Light yellow	Creamish white	Pale white	Creamish white to yellow
Maximum growth temperature (°C)	33	37	37	25–30	25–30	25	25	37
Growth on MacConkey's medium	–	–	–	–	–	–	+	ND
Motility	–	Gliding	–	–	Gliding	Gliding	–	–
Cell shape	Rods/long rods	Short rods	ND	Long rods	ND	Long rods	Long rods	Rods
Arginine dihydrolase	–	–	–	–	–	–	+	–
Lysine decarboxylase	–	+	+	+	+	–	–	ND
Tryptophan deaminase	–	–	–	–	–	–	+	ND
Enzyme activity (API ZYM)								
Esterase (C4)	+	+	–	+	–	ND	ND	–
Lipase (C14)	–	–	–	V*	–	ND	ND	–
Valine arylamidase	–	–	V (–)	+	V	ND	ND	–
Cystine arylamidase	W	+	–	+	–	ND	ND	–
Trypsin	–	–	–	+	–	ND	ND	–
α -Chymotrypsin	–	+	–	+	–	ND	ND	–
α -Galactosidase	–	+	–	+	–	ND	ND	–
β -Glucuronidase	–	–	–	V*	V	ND	ND	–
α -Glucosidase	+	+	+	+	+	ND	ND	–
β -Glucosidase	+	+	V (–)	+	+	+	+	–
N-Acetyl- β -glucosaminidase	+	+	+	+	+	ND	ND	–
α -Mannosidase	–	+	V (w)	+	–	ND	ND	–
α -Fucosidase	–	–	–	–	–	ND	ND	–
Assimilation of:								
Glycerol	–	–	–	–	+	–	+	–
D-Arabinose	–	–	V (–)	–	V	–	+	–
L-Arabinose	–	+	V (+)	+	+	+	+	+
Ribose	–	–	V (–)	–	–	–	+	–
D-Xylose	–	+	V (+)	+	+	+	+	–
L-Xylose	–	–	–	–	–	–	+	+
Adonitol	–	+	–	–	+	–	+	–
D-Galactose	+	+	+	V	+	+	+	–
D-Fructose	–	+	V (–)	+	V	+	+	–
L-Rhamnose	–	+	+	V	+	–	+	–
Mannitol	–	+	–	–	–	–	+	–
D-Sorbitol	–	+	–	–	–	–	+	–
Methyl α -D-mannoside	W	+	+	–	–	–	+	+
Methyl α -D-glucoside	W	+	+	+	V	W	+	+
Arbutin	–	V (–)	V (–)	+	+	+	+	+
Salicin	+	+	V (+)	+	+	+	+	+
D-Cellobiose	+	+	+	+	–	+	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
Maltose	+	+	V (+)	+	+	+	+	+
Inulin	—	—	—	—	—	W	+	—
D-Raffinose	—	—	—	+	V	+	+	+
Starch	+	—	V (—)	+	—	+	+	+
Glycogen	+	—	—	—	—	+	—	—
Xylitol	—	—	—	—	—	—	—	+
Gentiobiose	+	+	V (+)	+	+	+	ND	+
D-Turanose	—	+	+	+	V	+	ND	—
L-Fucose	—	+	V (—)	—	—	—	+	—
2-Ketogluconate	—	—	—	—	—	+	ND	—
5-Ketogluconate	+	—	—	—	—	—	ND	—
Susceptibility to antibiotics								
Ampicillin	—	—	—	ND	V	—	—	—
Chloramphenicol	+	+	V	ND	+	—	—	—
Kanamycin	—	—	ND	ND	ND	—	—	—
Penicillin	—	+	V	ND	V	—	—	ND
Tetracycline	+	+	—	ND	+	—	—	+
DNA G + C content (mol%)	39.7	42.3–43.0	43.7–44.2	40.4–41.0	36.9–37.1	43.4	41.0	42.7

*Data from Takeuchi & Yokota (1992).

(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 activity and other physiological and biochemical properties were tested by using the API 20E, API 50CHB and API ZYM systems (bioMérieux).

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in TSB at 30 °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 analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain DS-27^T was harvested from TSA plates after incubation for 5 days at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by 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-27^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-27^T, comprising 1475 nt (approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence), was determined in this study. The 16S rRNA gene sequence analyses showed that strain DS-27^T was phylogenetically most closely affiliated to the genus *Pedobacter* of the family *Sphingobacteriaceae* (Fig. 1). In the neighbour-joining tree based on 16S rRNA gene sequences, strain DS-27^T joined the cluster comprising *Pedobacter* species, except *P. saltans*, with a bootstrap resampling value of 99.8 % (Fig. 1). *P. saltans* exhibited low 16S rRNA gene sequence similarity values to other *Pedobacter* species (89.9–91.4 %) as well as to strain DS-27^T, and joined the cluster comprising *Sphingobacterium* species with a high bootstrap resampling value (Fig. 1). Accordingly, *P. saltans* may have to be reclassified as a member of the genus *Sphingobacterium* or within a new genus. The same tree topology was found in the trees generated with the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain DS-27^T exhibited 16S rRNA gene sequence similarity values of 90.6 % to *P. saltans* DSM 12145^T, 93.2–95.5 % to the type strains of the other *Pedobacter* species and <90.8 % to the other species used in the phylogenetic analysis.

Strain DS-27^T contained menaquinone-7 (MK-7), at a peak area ratio of approximately 92 %, as the predominant isoprenoid quinone. The fatty acid profile of strain DS-27^T included (>1.0 % of the total fatty acids): branched fatty acids iso-C_{15:0} (35.8 %), iso-C_{17:1}ω₉c (3.8 %) and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain DS-27^T and some other related taxa. Bootstrap values of >50% (1000 replications) are shown at each node. *Cytophaga hutchinsonii* ATCC 33406^T was used as outgroup. Dots indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.02 substitutions per nucleotide position.

iso-C_{17:0} (1.8%); summed feature 3, comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (21.7%); hydroxy fatty acids iso-C_{17:0} 3-OH (17.7%), iso-C_{15:0} 3-OH (3.0%) and C_{15:0} 2-OH (1.2%); straight-chain fatty acid C_{15:0} (4.2%); and unsaturated fatty acids C_{15:1}ω6c (1.6%) and C_{17:1}ω8c (1.1%). This fatty acid profile was similar to those of *Pedobacter* species analysed previously, although there were differences in the proportions of some fatty acids, perhaps because of differences in cultivation conditions (Steyn *et al.*, 1998; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005). The DNA G+C content of strain DS-27^T was 39.7 mol%. There were no distinct phenotypic, particularly chemotaxonomic, properties to differentiate strain DS-27^T from the genus *Pedobacter* (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005). Accordingly, it was concluded that strain DS-27^T is more closely affiliated to the genus *Pedobacter* than it is to the genus *Sphingobacterium*, based on phylogenetic and chemotaxonomic relatedness, the absence of urease activity and the inability to assimilate D-melezitose (Steyn *et al.*, 1998; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005). Strain DS-27^T was distinguishable from recognized *Pedobacter* species based on several phenotypic characteristics as listed in Table 1. The 16S rRNA gene sequence similarity values were low enough to allocate strain DS-27^T to a species that is separate from the recognized *Pedobacter* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-27^T should be placed in the genus *Pedobacter* as a member of a novel species, for which the name *Pedobacter sandarakinus* sp. nov. is proposed.

Description of *Pedobacter sandarakinus* sp. nov.

Pedobacter sandarakinus [san.da.ra.ki'nus. N.L. masc. adj. *sandarakinus* (from Gr. adj. *sandarakinus*) orange-coloured].

Cells are Gram-negative, non-spore-forming, non-flagellated rods (0.3–0.5 × 0.7–4.0 μm); occasionally cells >50 μm in length may occur. Colonies on TSA are circular, convex, smooth, glistening, orange-coloured and 1.5–2.5 mm in diameter after incubation for 5 days at 30 °C. Optimal growth occurs at 30 °C; growth occurs at

4 and 33 °C, but not at 34 °C. Optimal pH for growth is 7.0–7.5; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 0–0.5% (w/v) NaCl; growth does not occur in the presence of >3% (w/v) NaCl. Growth does not occur under anaerobic conditions on TSA or on TSA supplemented with nitrate. Tweens 20, 40, 60 and 80 are hydrolysed, but casein, gelatin, starch, hypoxanthine, xanthine and L-tyrosine are not. Flexirubin-type pigments are absent. In assays with the API ZYM system, naphthol-AS-BI-phosphohydrolase is present. Susceptible to novobiocin, lincomycin and oleandomycin, but not to polymyxin B, streptomycin, cephalothin, gentamicin, carbenicillin or neomycin. The predominant menaquinone is MK-7. The major fatty acids (>10% of total fatty acids) are iso-C_{15:0}, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and iso-C_{17:0} 3-OH. The DNA G+C content is 39.7 mol% (HPLC). Other phenotypic properties are shown in Table 1.

The type strain, DS-27^T (= KCTC 12559^T = CIP 108922^T), was isolated from soil.

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