Pedobacter terrae sp. nov., isolated from soil

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A Gram-negative, non-motile, rod-shaped bacterial strain, DS-57^T, was isolated from soil from Dokdo, Korea, and its taxonomic position was investigated using a polyphasic approach. It grew optimally at 25 °C and in trypticase soy broth without NaCl and trypticase soy broth with 0.5 % NaCl. Strain DS-57^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-57^T was most closely related to the genus *Pedobacter* of the family *Sphingobacteriaceae*, clustering coherently with *Pedobacter suwonensis*, *Pedobacter roseus* and *Pedobacter sandarakinus*. Strain DS-57^T exhibited 16S rRNA gene sequence similarity values of 99.2, 97.9 and 97.2 % with respect to the type strains of *P. suwonensis*, *P. roseus* and *P. sandarakinus*, respectively, and values less than 95.6 % with respect to the type strains of other *Pedobacter* species. Strain DS-57^T exhibited levels of DNA–DNA relatedness of 45, 17 and 15 % with respect to the type strains of *P. suwonensis*, *P. roseus* and *P. sandarakinus*, respectively. Differential phenotypic properties, together with the phylogenetic distinctiveness and the DNA–DNA relatedness data, were sufficient to allow the classification of strain DS-57^T as a species that is separate from recognized *Pedobacter* species. On the basis of phenotypic properties and phylogenetic distinctiveness, therefore, strain DS-57^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter terrae* sp. nov. is proposed. The type strain is DS-57^T (=KCTC 12762^T=DSM 17933^T).

The genus *Pedobacter* was proposed by Steyn *et al.* (1998) with the reclassification of two *Sphingobacterium* species and the description of two novel species. At the time of writing, the genus comprises 12 recognized species: *Pedobacter heparinus*, *Pedobacter piscium*, *Pedobacter africanus* and *Pedobacter saltans* (Steyn *et al.*, 1998), *Pedobacter cryoconitis* (Margesin *et al.*, 2003), *Pedobacter himalayensis* (Shivaji *et al.*, 2005), *Pedobacter caeni* (Vanparys *et al.*, 2005), *Pedobacter sandarakinus* (Yoon *et al.*, 2006), *Pedobacter roseus* (Hwang *et al.*, 2006), *Pedobacter aquatilis* (Gallego *et al.*, 2006), *Pedobacter ginsengisoli* (Ten *et al.*, 2006) and *Pedobacter suwonensis* (Kwon *et al.*, 2007). The genus *Pedobacter* is characterized chemotaxonomically by having MK-7 as the predominant menaquinone and iso-C_{15:0} and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c as the major fatty acids (Steyn *et al.*, 1998; Kwon *et al.*, 2007). Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Pedobacter* falls within the phylum *Bacteroidetes* (the *Cytophaga*–*Flavobacterium*–*Bacteroides* group) (Bernardet *et al.*, 2002; Kwon *et al.*,

2007). Here, we report on the taxonomic characterization of a *Pedobacter*-like bacterial strain, DS-57^T, which was isolated from a soil sample from Dokdo (37° 14' 12" N 131° 52' 07" E), an island of Korea.

Strain DS-57^T was isolated using the standard dilution plating technique on 2 × diluted nutrient agar (Difco) at 25 °C. The type strains of three *Pedobacter* species were used as reference strains for DNA–DNA hybridization and phenotypic characterization: *P. roseus* KACC 11594^T and *P. suwonensis* KACC 11317^T (obtained from the Korean Agricultural Culture Collection, Suwon, Korea) and *P. sandarakinus* DS-27^T (Yoon *et al.*, 2006). The morphological, physiological and biochemical characteristics of strain DS-57^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 25 °C. The cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy. The presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. For the transmission electron microscopic observations, the 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) using quarter-strength trypticase soy

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-57^T is DQ889723.

The cellular fatty acid content of strain DS-57^T and phylogenetically related *Pedobacter* species is presented in a supplementary table available with the online version of this paper.

broth (TSB; Difco) solidified with 1 % (w/v) agarose and R2A agar (Difco). 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 and 1.0–5.0 %, w/v, using increments of 1.0 %) was investigated in TSB prepared according to the formula of the Difco medium except that NaCl was excluded. The pH range for growth was determined in nutrient broth (Difco) that had been adjusted to various pH values (pH 4.5–10.5, using increments of 0.5 pH units), prior to sterilization, by the addition of HCl or Na₂CO₃. Growth at pH 4.5, 5.0 and 5.5 was investigated in nutrient broth adjusted to each value using a citrate/phosphate buffer (0.1 M citrate/0.2 M Na₂HPO₄) (Breznak & Costilow, 1994). 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). DNase activity was examined by using DNase test agar with methyl green (Difco). Hydrolysis of aesculin and nitrate reduction were studied as described previously (Lanyi, 1987). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Susceptibility to antibiotics was tested on TSA plates, using antibiotic discs containing the following concentrations (µg, unless indicated otherwise): polymyxin B, 100 U; streptomycin, 50; penicillin G, 20 U; chloramphenicol, 100; ampicillin, 10; cephalothin, 30; gentamicin, 30; novobiocin, 5; tetracycline, 30; kanamycin, 30; lincomycin, 15; oleandomycin, 15; neomycin, 30; carbenicillin, 100. Assimilation of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CHB and API ZYM systems (bioMérieux). The cells were suspended in AUX medium (bioMérieux), according to the manufacturer's instructions, for inoculation into the API 50CH system.

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), except that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by using a PCR with two universal primers, as described previously (Yoon *et al.*, 1998). The sequencing of the amplified 16S rRNA gene and the 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-57^T was harvested from

TSA plates after incubation for 3 days at 25 °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 using the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain DS-57^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-57^T determined in this study comprised 1477 nt (approx. 96 % of the *Escherichia coli* 16S rRNA gene sequence). The 16S rRNA gene sequence analyses showed that strain DS-57^T was phylogenetically most closely related to the genus *Pedobacter* of the family *Sphingobacteriaceae* (Fig. 1). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain DS-57^T fell within the cluster comprising the *Pedobacter* species, in particular forming a coherent cluster with *P. suwonensis*, *P. roseus*, *P. sandarakinus* and *P. aquatilis* (Fig. 1). Strain DS-57^T exhibited 16S rRNA gene sequence similarity values ranging from 93.8 % (*P. himalayensis*) to 99.2 % (*P. suwonensis*) with respect to the type strains of *Pedobacter* species (with the exception of *P. saltans*); the values were below 91.8 % for the other species used in the phylogenetic analysis.

Strain DS-57^T contained MK-7 (at a peak area ratio of approx. 87 %) as the predominant isoprenoid quinone; a significant amount of MK-6 (approx. 10 %) was also detected. The fatty acids constituting >0.5 % of the total fatty acid content for strain DS-57^T were as follows: branched fatty acids iso-C_{15:0} (28.3 %), iso-C_{17:1ω9c} (6.1 %) and anteiso-C_{15:0} (0.8 %); summed feature 3 (comprising C_{16:1ω7c} and/or iso-C_{15:0} 2-OH; 27.7 %) and summed feature 4 (comprising iso-C_{17:1} and/or anteiso-C_{17:1}; 1.2 %); hydroxyl fatty acids iso-C_{17:0} 3-OH (20.4 %), iso-C_{15:0} 3-OH (3.5 %), C_{16:0} 3-OH (1.6 %) and iso-C_{16:0} 3-OH (0.6 %); straight-chain fatty acid C_{16:0} (1.9 %); unsaturated fatty acids C_{16:1ω5c} (1.7 %) and C_{18:1ω5c} (0.9 %); and an unknown fatty acid (equivalent chain length, 16.582) (1.2 %). This fatty acid profile was similar to those of *Pedobacter* species, although there were differences in the proportions of some fatty acids, perhaps because of differences in cultivation conditions (Shivaji *et al.*, 2005; Vanparrys *et al.*, 2005; Yoon *et al.*, 2006; Hwang *et al.*, 2006; Kwon *et al.*, 2007) (see Supplementary Table S1, available with the online version of this paper). The DNA G+C content of strain DS-57^T was 39.7 mol%. These chemotaxonomic properties were in agreement with the monothetic phylogenetic classification of strain DS-57^T as a member of the genus *Pedobacter* (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparrys *et al.*, 2005; Yoon *et al.*, 2006; Hwang *et al.*, 2006).

The mean values for DNA–DNA relatedness between strain DS-57^T and the type strains of three phylogenetically

Table 1. Differential characteristics of strain DS-57^T and some phylogenetically related *Pedobacter* species

Taxa: 1, DS-57^T; 2, *P. suwonensis*, data from Kwon *et al.* (2007) and this study; 3, *P. roseus*, data from Hwang *et al.* (2006) and this study; 4, *P. sandarakinus*, data from Yoon *et al.* (2006); 5, *P. aquatilis*, data from Gallego *et al.* (2006). All species are positive for the following: catalase (data for *P. suwonensis* and *P. roseus* from this study), oxidase (data for *P. suwonensis* and *P. roseus* from this study), aesculin hydrolysis (data for *P. suwonensis* and *P. roseus* from this study), esterase (C4), esterase lipase (C8), acid phosphatase, alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase; assimilation of D-galactose, D-glucose, D-mannose, *N*-acetylglucosamine, amygdalin, salicin, D-cellobiose, maltose, lactose, melibiose, sucrose, trehalose, starch and gentiobiose; and susceptibility to chloramphenicol and tetracycline. All species are negative for the following: motility; Gram stain; sporulation; urease (data for *P. suwonensis* and *P. roseus* from this study), arginine dihydrolase (data for *P. suwonensis* and *P. roseus* from this study), indole and H₂S production (data for *P. suwonensis* and *P. roseus* from this study), nitrate reduction (data for *P. suwonensis* and *P. roseus* from this study), lipase (C14), α -chymotrypsin and β -glucuronidase; assimilation of glycerol, erythritol, D-arabinose, L-xylose, ribose, adonitol, sorbose, dulcitol, inositol, mannitol, sorbitol, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, caprate, adipate, malate, citrate and phenylacetate; and susceptibility to ampicillin, gentamicin, kanamycin and penicillin. +, Positive reaction; –, negative reaction; w, weakly positive reaction; ND, not determined.

Characteristic	1	2	3	4	5
Colony colour	Orange	Pinkish yellow	Pink	Orange	Pink
Maximum growth temperature (°C)	35	37	33	33	30
Growth on MacConkey medium	–	–	+	–	–
Cell shape	Rods	Rods	Short rods	Rods/long rods	Rods
Hydrolysis of*:					
Casein	+	+	+	–	–
Gelatin	+	+	+	–	–
Starch	+	+	+	–	+
Enzyme activities (API ZYM)*					
Valine arylamidase	+	–	–	–	+
Cystine arylamidase	–	–	–	w	+
Trypsin	–	–	w	–	+
α -Galactosidase	–	–	–	–	–
α -Mannosidase	–	–	–	–	+
α -Fucosidase	–	w	w	–	–
Assimilation of*:					
L-Arabinose	+	+	+	–	+
D-Xylose	+	+	+	–	–
Methyl β -D-xyloside	+	+	–	–	–
D-Fructose	+	+	+	–	+
L-Rhamnose	–	+	+	–	+
Methyl α -D-mannoside	+	+	+	w	+
Methyl α -D-glucoside	+	+	+	w	+
Arbutin	+	+	+	–	+
Inulin	+	–	–	–	–
D-Melezitose	–	+	–	–	+
D-Raffinose	+	+	+	–	+
Glycogen	–	+	+	+	–
Turanose	+	+	+	–	+
5-Ketogluconate	–	–	–	+	–
Susceptibility to*:					
Novobiocin	–	+	+	+	ND
Streptomycin	–	w	+	–	–
DNA G + C content (mol%)	39.7	44.2	41.3	39.7	38

*Data for *P. suwonensis* and *P. roseus* from this study.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain DS-57^T and some related taxa. Bootstrap percentages (based on 1000 replications) are shown at the nodes (if $\geq 50\%$). *Cytophaga hutchinsonii* ATCC 33406^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

related *Pedobacter* species, namely *P. suwonensis* 15-52^T, *P. roseus* CL-GP80^T and *P. sandarakinus* DS-27^T, which showed 16S rRNA gene sequence similarity values of $>97.0\%$ with respect to strain DS-57^T, were 15–45 % when their DNAs were used individually as labelled DNA probes. Strain DS-57^T was distinguishable from some phylogenetically related *Pedobacter* species through differences in several phenotypic characteristics, as listed in Table 1. The phylogenetic distinctiveness of DS-57^T, the differential phenotypic properties and the DNA–DNA relatedness data were sufficient to demonstrate that this strain is distinct from recognized *Pedobacter* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-57^T represents a novel member of the genus *Pedobacter*, for which the name *Pedobacter terrae* sp. nov. is proposed.

Description of *Pedobacter terrae* sp. nov.

Pedobacter terrae (ter'rae. L. gen. n. *terrae* of the earth).

Cells are Gram-negative, non-spore-forming, non-flagellated rods (0.3–0.5 $\times$ 0.8–3.5 μ m). Colonies on TSA are circular, convex, smooth, glistening, orange-coloured and 1.0–2.0 mm in diameter after incubation for 3 days at 25 °C. Optimal growth occurs at 25 °C; growth occurs at 4 and 35 °C, but not at 36 °C. Optimal pH for growth is 6.5–7.5; growth occurs at pH 5.5 and 9.0, but not at pH 5.0 or 9.5. Optimal growth occurs in TSB without NaCl and TSB with 0.5 % NaCl; growth does not occur in the presence of greater than 3 % (w/v) NaCl. Growth does not occur under anaerobic conditions on TSA or on TSA supplemented with nitrate. DNase-negative. Tweens 20, 40, 60 and 80 are hydrolysed, but hypoxanthine, xanthine and L-tyrosine are not. Lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Flexirubin-type pigments are absent. Susceptible to lincomycin and oleandomycin, but not to polymyxin B, cephalothin, carbenicillin or neomycin. The predominant menaquinone is MK-7. The major fatty acids (constituting

$>10\%$ of total fatty acids) are iso-C_{15:0}, C_{16:1} ω 7c 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-57^T (=KCTC 12762^T=DSM 17933^T), was isolated from soil.

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