Pedobacter panaciterrae sp. nov., isolated from soil in South Korea

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A Gram-negative, facultatively anaerobic, rod-shaped, non-motile, non-spore-forming bacterial strain, designated Gsoil 042^T, was isolated from a soil sample of a ginseng field in Pocheon province (South Korea) and was characterized taxonomically by using a polyphasic approach. The isolate was positive for catalase and oxidase, but negative for gelatinase and production of indole and H₂S. The predominant isoprenoid quinone was menaquinone 7 (MK-7). The major cellular fatty acids were summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 35.7 %), iso-C_{15:0} (24.3 %) and iso-C_{17:0} 3-OH (11.6 %). Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 042^T fell within the radiation of the cluster comprising *Pedobacter* species. On the basis of 16S rRNA gene sequence similarities, the phylogenetically closest recognized species of the genus *Pedobacter* to strain Gsoil 042^T included *Pedobacter heparinus* DSM 2366^T (98.1 %), *Pedobacter africanus* DSM 12126^T (97.8 %) and *Pedobacter caeni* LMG 22862^T (97.2 %). The 16S rRNA gene sequence similarity with other recognized species of the genus *Pedobacter* was less than 96.1 %. DNA–DNA hybridization experiments showed that the DNA–DNA relatedness between strain Gsoil 042^T and its phylogenetically closest neighbours was less than 43 %. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 042^T should be classified as representing a novel species in the genus *Pedobacter*, for which the name *Pedobacter panaciterrae* sp. nov. is proposed. The type strain is Gsoil 042^T (=KCTC 12594^T=LMG 23400^T).

The genus *Pedobacter* was first described by Steyn *et al.* (1998) and at present comprises species isolated from soil (*Pedobacter heparinus*, *Pedobacter africanus*, *Pedobacter saltans* and *Pedobacter sandarakinus*), commercial nitrifying inoculum (*Pedobacter caeni*), fish (*Pedobacter piscium*), a glacier in the Tyrolean Alps (*Pedobacter cryoconitis*) and glacial water in the Himalayan mountains, India (*Pedobacter himalayensis*) (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Yoon *et al.*, 2006). Three further species of the genus, *Pedobacter roseus*, *Pedobacter aquatilis* and *Pedobacter ginsengisoli* have been described recently (Hwang *et al.*, 2006; Gallego *et al.*, 2006; Ten *et al.*, 2006). Members of the genus *Pedobacter* have the

following characteristics: they are Gram-negative aerobic rods, are positive for catalase, oxidase and heparinase, may or may not show gliding motility, are negative for urease, lipase, gelatinase, indole production and nitrate reduction and contain iso-C_{15:0}, iso-C_{15:0} 2-OH, iso-C_{15:0} 3-OH, C_{16:0}, C_{16:1}ω5c, C_{16:1}ω7c, C_{16:0} 3-OH, iso-C_{17:0} 3-OH and iso-C_{17:1}ω9c fatty acids (Steyn *et al.*, 1998).

During the course of a study on the culturable aerobic and facultative anaerobic bacterial community living in the soil of a ginseng field in Pocheon province (South Korea), a large number of novel bacterial strains were isolated. In this study, we have characterized one of these isolates, strain Gsoil 042^T. The phenotypic, chemotaxonomic and phylogenetic analyses established the affiliation of the isolate to the genus *Pedobacter*. The data obtained suggest that the novel isolate represents a novel species of the genus *Pedobacter*.

Strain Gsoil 042^T was isolated originally from a soil sample from a ginseng field in Pocheon province. The soil sample

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 042^T is AB245368.

A table detailing the fatty acid compositions of *Pedobacter panaciterrae* sp. nov. and related *Pedobacter* species is available as supplementary material in IJSEM Online.

was suspended in 50 mM phosphate buffer (pH 7.0) and spread on plates of 1/5-strength modified R2A agar (0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acids, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4 , 0.05 g $MgSO_4$, 0.05 g $CaCl_2$, 15 g agar l^{-1}) after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 1 month. Single colonies on the plates were purified by transferring them onto fresh plates of modified R2A or half-strength modified R2A agar and incubating again. Purified colonies were tentatively identified from partial sequences of the 16S rRNA gene (Im *et al.*, 2005). One isolate, Gsoil 042^T, was cultured routinely on R2A agar (Difco) at 25 °C and maintained as a glycerol suspension (20 %, w/v) at -70 °C.

For phylogenetic analysis of strain Gsoil 042^T, genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Comparative 16S rRNA gene sequence analyses of strain Gsoil 042^T (1463 bp) showed that the strain is phylogenetically affiliated to *Pedobacter* species. The phylogenetic tree (Fig. 1) based on the neighbour-joining algorithm showed that strain Gsoil 042^T fell within the radiation of the cluster comprising *Pedobacter* species and was most closely related to *P. heparinus* DSM 2366^T. The almost-complete 16S rRNA gene sequence of strain Gsoil 042^T exhibited relatively high similarity levels with those of *P. heparinus* DSM 2366^T (98.1 %), *P. africanus* DSM 12126^T (97.8 %), *P. ginsengisoli* KCTC 12576^T (97.3 %) and *P. caeni* LMG 22862^T (97.2 %). The level of 16S rRNA gene sequence similarity with the type strains of other recognized species of the genus *Pedobacter*, including *P. piscium* DSM 11725^T, *P. cryoconitis* DSM 14825^T, *P. himalayensis* JCM 12171^T, *P. sandarakinus* KCTC 12559^T and *P. saltans* DSM 12145^T, was less than 96.1 %. In the phylogenetic tree constructed, strain Gsoil 042^T is clearly separated from *P. piscium*, *P. cryoconitis*, *P. himalayensis*, *P. sandarakinus* and *P. saltans* as well as from *P. roseus* CL-GP80^T and *P. aquatilis* AR107^T. The generally accepted criteria for delineating bacterial species state that strains with a DNA–DNA relatedness value of below 70 % (as measured by hybridization), or strains with 16S rRNA gene sequence dissimilarity above 3 %, are considered as belonging to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002). Taking into account this definition, our data indicate that strain Gsoil 042^T represents a novel species of the genus *Pedobacter*. To further verify the taxonomic position of strain Gsoil 042^T, DNA–DNA hybridization was performed with the three recognized members of the genus most closely related to strain Gsoil 042^T and *P. ginsengisoli* KCTC 12576^T.

Fig. 1. Phylogenetic dendrogram obtained by neighbour-joining clustering of 16S rRNA gene sequences showing the phylogenetic relationship between *Pedobacter panaciterrae* sp. nov. and other species of the genus *Pedobacter* and *Sphingobacterium spiritivorum*. Bootstrap values (expressed as percentages of 1000 replications) greater than 60 % are shown at branch points. Filled circles indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at $\times 1000$, with cells grown on R2A agar for 6 days at 25 °C. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). For studies on the assimilation of single carbon sources, a defined liquid medium containing basal salts was used, as follows (l^{-1}): K_2HPO_4 , 1.8 g; KH_2PO_4 , 1.08 g; $NaNO_3$, 0.5 g; NH_4Cl , 0.5 g; KCl , 0.1 g; $MgSO_4$, 0.1 g; and $CaCl_2$, 0.05 g. A vitamin solution (Widdel & Bak, 1992), a trace element solution (SL-10; Widdel *et al.*, 1983) and a selenite/tungstate solution (Tschech & Pfennig, 1984) were added to this medium and the pH was adjusted to 6.8. This liquid medium was dispensed into 96-well plates (0.25 ml per well) and filter-sterilized carbon sources were added to each well (at 0.1 %, w/v). The plates were incubated at 25 °C for up to 7 days, and were examined visually for growth. Negative-control wells did not contain any carbon sources. Positive-control wells comprised cultures grown in R2A broth. Some physiological characteristics were determined with API 20E galleries, according to the instructions of the manufacturer (bioMérieux). Anaerobic growth was determined in serum bottles containing R2A broth supplemented with thioglycolate ($1\text{ g }l^{-1}$), and in which the upper air layer had been replaced with nitrogen. Reduction of nitrate and nitrite was determined using serum bottles containing R2A broth supplemented with KNO_3 (10 mM) and $NaNO_2$ (10 mM), respectively. The reduction of nitrate and nitrite was monitored by ion chromatography on a model 790 personal IC (Metrohm) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). The degradation of DNA (using DNase agar from Scharlau, supplemented with 1 M HCl), casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan, cellulose and collagen (Ten *et al.*, 2004, 2005) was also investigated; reactions were read after 7 days. Growth at different temperatures and pH was assessed after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA; Difco) and MacConkey agar was also evaluated at 25 °C.

Cells of strain Gsoil 042^T were Gram-negative, facultatively anaerobic, non-spore-forming, non-motile and rod-shaped (0.5–1.0 μm wide and 1.8–2.2 μm long). After 6 days incubation at 25 °C on R2A, colonies were 1.8–2.0 mm in diameter, smooth, convex, round, glossy and white. On R2A agar, the optimal temperature for growth for strain Gsoil 042^T was 25 °C. The strain was able to grow at 30 °C, but not at 37 °C. The pH range for growth was pH 5.5–8.5, with an optimum between pH 6.5 and 7. Growth occurred in the absence of NaCl and in the presence of 1 % (w/v) NaCl, but not in the presence of 2 % (w/v) NaCl. The biochemical characteristics of strain Gsoil 042^T were similar to those reported for members of the genus *Pedobacter* (Steyn *et al.*, 1998), i.e. positive for oxidase and catalase, able to assimilate D-mannose, D-glucose, D-lactose, N-acetyl-D-glucosamine and amygdalin, negative for H_2S production and indole

production, urease, lipase and nitrate reduction and unable to produce acid from a large number of substrates. Phenotypic and chemotaxonomic characteristics that differentiate strain Gsoil 042^T from other members of the genus *Pedobacter* are listed in Table 1. In contrast to all other *Pedobacter* species, strain Gsoil 042^T utilizes inositol and does not hydrolyse aesculin.

For measurement of the G + C content of chromosomal DNA, the genomic DNA of strain Gsoil 042^T was extracted and purified as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides. The G + C content was then determined as described by Mesbah *et al.* (1989), using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under a vacuum and re-extracted in n-hexane/water (1 : 1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac Cartridges Silica (Waters) and analysed subsequently by HPLC, as described by Hiraishi *et al.* (1996). The cellular fatty acids were analysed after culture on TSA for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were then analysed by GC (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990).

The predominant menaquinone of strain Gsoil 042^T was MK-7. The cellular fatty acid profiles of strain Gsoil 042^T and related *Pedobacter* species are given in Supplementary Table S1 available in IJSEM Online. The major components were iso-C_{15:0}, iso-C_{17:0} 3-OH, C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH, which is a profile typical of members of the genus *Pedobacter* (Steyn *et al.*, 1998). However, some differences in the proportions of the fatty acids could be observed between strain Gsoil 042^T and its phylogenetically closest relatives, perhaps because of differences in cultivation conditions. The G + C content of the DNA of strain Gsoil 042^T was 44.2 mol%, which is in good agreement with the values determined for other members of the genus *Pedobacter* (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Yoon *et al.*, 2006).

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner), with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as the DNA–DNA hybridization values. Strain Gsoil 042^T exhibited relatively low levels of DNA–DNA relatedness with respect to *P. heparinus* DSM 2366^T (42 %), *P. africanus* KACC 11389^T (35 %), *P. caeni* DSM 16990^T (28 %) and *P. ginsengisoli* KCTC 12576^T (21 %), indicating that it is not related to them at the species level (Wayne *et al.*, 1987).

The phenotypic and phylogenetic characterizations indicated that strain Gsoil 042^T belongs to the genus *Pedobacter*. The phylogenetic distinctiveness, together with the DNA–DNA

Table 1. Physiological and biochemical characteristics of *Pedobacter panaciterrae* sp. nov. and related *Pedobacter* species

Taxa: 1, strain Gsoil 042^T; 2, *P. heparinus*; 3, *P. africanus*; 4, *P. ginsengisoli* Gsoil 104^T; 5, *P. caeni*; 6, *P. piscium*; 7, *P. cryoconitis*; 8, *P. himalayensis*; 9, *P. sandarakinus*; 10, *P. saltans*. Data are from Steyn *et al.* (1998), Margesin *et al.* (2003), Shivaji *et al.* (2005), Vanparys *et al.* (2005), Ten *et al.* (2006) and Yoon *et al.* (2006). All are positive for the following: catalase, oxidase and utilization of D-mannose, D-glucose, N-acetyl-D-glucosamine, amygdalin (not done for *P. himalayensis*), D-lactose, D-melibiose (variable for *P. saltans*), D-sucrose (variable for *P. saltans*) and D-trehalose (variable for *P. saltans*) as the sole carbon source. All are Gram-negative and negative for sporulation, production of indole and H₂S, urease, reduction of nitrate to nitrite, and utilization of L-sorbose, D-lyxose (not done for *P. himalayensis*), D-fucose (not done for *P. himalayensis*), dulcitol, citrate, L-lysine (not done for *P. sandarakinus*), gluconate (not done for *P. himalayensis*), adipate (not done for *P. himalayensis*), caprate (not done for *P. himalayensis*), phenylacetate (not done for *P. himalayensis*) and malate as the sole carbon source. +, Positive; −, negative; V, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10
Enzyme activities:										
Gelatinase	−	−	V	−	−	−	+	+	−	−
Lysine decarboxylase	−	+	+	−	ND	+	−	−	−	+
Tryptophan deaminase	−	−	−	−	ND	ND	−	+	−	−
Arginine dihydrolase	−	−	−	−	−	ND	−	+	−	−
Acid production from:										
D-Glucose	−	V	V	−	−	+	−	−	ND	V
D-Sucrose	+	−	−	−	ND	+	−	−	ND	−
D-Melibiose	+	−	−	−	ND	V	−	−	ND	−
D-Mannitol	−	−	−	−	ND	V	+	+	ND	−
Inositol	−	−	−	−	ND	−	−	+	ND	−
D-Sorbitol	−	+	−	−	ND	−	−	+	ND	−
Utilization of:										
D-Arabinose	+	−	−	−	−	−	−	+	−	V
L-Arabinose	+	+	V	−	+	+	+	+	−	+
D-Fructose	−	+	V	+	−	+	+	+	−	V
D-Ribose	−	−	−	−	−	−	−	+	−	−
D-Raffinose	+	−	−	+	+	+	+	+	−	V
L-Rhamnose	−	+	+	−	−	V	−	+	−	+
D-Xylose	+	+	V	+	−	+	+	+	−	+
L-Xylose	+	−	−	+	+	−	−	+	−	−
Starch	−	−	V	−	+	+	+	+	+	−
Inulin	+	−	−	+	−	−	+	+	−	−
Glycogen	−	−	−	−	−	−	+	−	+	−
Adonitol	−	+	−	−	−	−	−	+	−	+
Glycerol	+	−	−	+	−	−	−	+	−	+
D-Mannitol	−	+	−	−	−	−	−	+	−	−
D-Sorbitol	−	+	−	−	−	−	−	+	−	−
Xylitol	−	−	−	−	+	−	−	−	−	−
Pyruvate	−	+	V	−	ND	−	−	+	ND	−
L-Alanine	−	−	V	−	ND	−	−	+	ND	−
L-Aspartate	−	−	V	−	ND	−	+	+	ND	−
L-Serine	−	V	+	−	ND	V	−	+	ND	−
L-Glutamate	−	−	+	−	ND	+	−	+	ND	−
DNA G + C content (mol%)	44.2	43.0†	44.2†	43.6	42.7†	40.4†	43.4†	41.0†	39.7†	37.0†

*Data from Takeuchi & Yokota (1992).

†Data for type strain.

hybridization data, confirmed that strain Gsoil 042^T represents a species that is distinct from recognized *Pedobacter* species. There are some phenotypic differences between strain Gsoil 042^T and phylogenetically related

Pedobacter species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 042^T should be classified within the genus *Pedobacter* as representing a novel species, for which the name *Pedobacter panaciterrae* sp. nov. is proposed.

Description of *Pedobacter panaciterrae* sp. nov.

Pedobacter panaciterrae (pa.na.ci.ter'rae. N.L. n. *Panax* -acis scientific name of ginseng; L. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil from a ginseng field, the source of the type strain).

After 6 days incubation at 25 °C on R2A, colonies are 1.8–2.0 mm in diameter, smooth, convex, round, glossy and white. Cells are rod-shaped (0.5–1.0 µm wide and 1.8–2.2 µm long), facultatively anaerobic, Gram-negative, non-spore-forming and non-motile. Grows at 4–30 °C, with optimum growth occurring at 25 °C. Grows at pH 5.5–8.5, with optimum growth at pH 6.5–7.0. Tolerates 1% (w/v) NaCl, but not 2%. Growth occurs on TSA, but not on MacConkey agar. Positive for catalase and oxidase, but negative for lipase. Nitrate is not reduced to nitrite. Hydrolyses DNA, but not chitin, starch, carboxymethyl-cellulose, xylan, casein, collagen or aesculin. The following substrates are utilized for growth: D-glucose, D-mannose, D-arabinose, L-arabinose, D-xylose, L-xylose, N-acetyl-D-glucosamine, salicin, D-cellobiose, D-lactose, D-maltose, D-melibiose, D-sucrose, D-trehalose, D-raffinose, amygdalin, inositol, glycerol, inulin and dextran. The following substrates are not utilized for growth: D-galactose, D-fructose, D-fucose, ethanol, L-rhamnose, L-sorbose, D-lyxose, D-ribose, pyruvate, formate, acetate, propionate, DL-3-hydroxybutyrate, valerate, caprate, maleate, fumarate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, citrate, lactate, malate, malonate, succinate, glutarate, tartrate, itaconate, adipate, suberate, oxalate, gluconate, dulcitol, D-adonitol, D-mannitol, D-sorbitol, xylitol, methanol, glycogen, L-alanine, L-arginine, L-asparagine, L-aspartate, L-cysteine, L-glutamate, L-glutamine, L-histidine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. In API 20E tests, the Voges–Proskauer test and ONPG reaction are positive; tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease and gelatinase and production of H₂S and indole are negative. Acid is produced from D-melibiose and D-sucrose, but not from D-glucose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol or L-rhamnose. Major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. The predominant respiratory quinone is MK-7.

The G+C content of the DNA of the type strain is 44.2 mol%. The type strain, Gsoil 042^T (=KCTC 12594^T=LMG 23400^T), was isolated from soil from a ginseng field in Pocheon province, South Korea.

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