Pedobacter ginsengisoli sp. nov., a DNase-producing bacterium isolated from soil of a ginseng field in South Korea

Leonid N. Ten,^{1,2†} Qing-Mei Liu,^{1†} Wan-Taek Im,¹ Myungjin Lee,¹ Deok-Chun Yang³ and Sung-Taik Lee¹

Correspondence
Wan-Taek Im
wandra@kaist.ac.kr

¹Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1, Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea

²National University of Uzbekistan, Students town, Tashkent, 700-174, Uzbekistan

³Department of Oriental Medicinal Material and Processing College of Life Science, Kyung Hee University, 1 Seocheon, Kihung Yongin, Kyunggi 449-701, Republic of Korea

A Gram-negative, strictly aerobic, rod-shaped, non-motile, non-spore-forming bacterial strain, designated Gsoil 104^T, was isolated from a soil sample from a ginseng field in Pocheon Province (South Korea) and was characterized taxonomically by using a polyphasic approach. On the basis of 16S rRNA gene sequence similarities, strain Gsoil 104^T was shown to belong to the family *Sphingobacteriaceae*, being related to *Pedobacter africanus* DSM 12126^T (97.0%), *Pedobacter caeni* LMG 22862^T (96.9%), *Pedobacter cryoconitis* DSM 14825^T (96.8%) and *Pedobacter heparinus* DSM 2366^T (96.6%). The phylogenetic distance from any other *Pedobacter* species with a validly published name was greater than 3.4% (i.e. <96.6% 16S rRNA gene sequence similarity). DNA–DNA hybridization experiments showed that values for DNA–DNA relatedness between strain Gsoil 104^T and its phylogenetically closest neighbours were below 37%. The G + C content of the genomic DNA was 43.6 mol%. The predominant respiratory quinone was MK-7. The major fatty acids were C_{16:1}ω7c, iso-C_{15:0}, C_{16:0}, iso-C_{17:0} 3-OH and iso-C_{15:0} 2-OH. These chemotaxonomic data support the affiliation of strain Gsoil 104^T to the genus *Pedobacter*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 104^T represents a novel species in the genus *Pedobacter*, for which the name *Pedobacter ginsengisoli* sp. nov. is proposed. The type strain is Gsoil 104^T (=KCTC 12576^T =LMG 23399^T).

The genus *Pedobacter* was first described by Steyn *et al.* (1998) and currently comprises species isolated from soil (*Pedobacter heparinus*, *P. africanus*, *P. saltans* and *P. sandarakinus*), from a commercial nitrifying inoculum (*Pedobacter caeni*), from fish (*Pedobacter piscium*), from a glacier in the Tyrolean Alps (*Pedobacter cryoconitis*) and from glacial water in the Himalayan mountains of India (*Pedobacter himalayensis*) (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Yoon *et al.*, 2006). Two further species of the genus, *Pedobacter roseus* and *Pedobacter aquatilis*, have been described recently (Gallego *et al.*, 2006; Hwang *et al.*, 2006). The members of the genus *Pedobacter* have the following characteristics: they are Gram-negative rods, they are obligately aerobic, positive for catalase, oxidase and heparinase, they may or may not show gliding motility, they are negative for urease, lipase,

gelatinase, indole production and nitrate reduction and they 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 104^T. Our phenotypic, genotypic, chemotaxonomic and phylogenetic analyses have established the affiliation of the isolate to the genus *Pedobacter*. The data obtained also suggest that the isolate represents a novel species of the genus *Pedobacter*.

Strain Gsoil 104^T was originally isolated from a soil sample from a ginseng field in Pocheon Province (South Korea). The soil sample 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,

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 104^T is AB245371.

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 $CaCl_2$ and 15 g agar l^{-1}) after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 25 °C for 1 month. Single colonies on the plates were purified by transferring them onto new plates and were incubated once again on modified R2A or on half-strength modified R2A. Purified colonies were tentatively identified from partial sequences of the 16S rRNA gene (Im *et al.*, 2005). Gsoil 104^T was one of the dominant isolates on the modified R2A agar plates under aerobic conditions. It was routinely cultured on R2A agar at 30 °C and was maintained as a glycerol suspension (20 %, w/v) at -70 °C.

A nearly-complete 16S rRNA gene sequence of strain Gsoil 104^T was determined as described below. DNA was extracted using a commercial genomic DNA-extraction kit (Core Biosystem) 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 the GenBank database. Multiple alignments were performed with 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 method (Saitou & Nei, 1987) and the maximum-parsimony method (Fitch, 1971) using 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 104^T (1430 bp) showed that the strain is phylogenetically affiliated to *Pedobacter* species. The phylogenetic tree (Fig. 1) based on the neighbour-joining algorithm showed strain Gsoil 104^T to have a distinct phylogenetic position within the genus *Pedobacter*. The almost-complete 16S rRNA gene sequence of strain Gsoil 104^T exhibits similarity levels of $\leq 97\%$ with respect to the type strains of six *Pedobacter* species with validly published names, namely *P. africanus* DSM 12126^T (97.0 %), *P. caeni* LMG 22862^T

(96.9 %), *P. cryoconitis* DSM 14825^T (96.8 %), *P. heparinus* DSM 2366^T (96.6 %), *P. piscium* DSM 11725^T (96.5 %) and *P. himalayensis* JCM 12171^T (96.4 %). The levels of 16S rRNA gene sequence similarity with *P. sandarakinus* KCTC 12559^T and *P. saltans* DSM 12145^T are only 93.7 and 90.1 %, respectively. In the phylogenetic tree constructed, strain Gsoil 104^T is clearly separated from *P. sandarakinus* and *P. saltans* as well as from *P. roseus* CL-GP80^T and *P. aquatilis* AR107^T. Strain Gsoil 104^T and *Pedobacter* species (except *P. saltans*) form a monophyletic clade with a high bootstrap value (100 %) that was supported by the neighbour-joining and maximum-parsimony methods employed in this study for phylogenetic tree construction. The generally accepted criteria for delineating bacterial species state that strains with a DNA-DNA relatedness value 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). On this basis, our data indicate that strain Gsoil 104^T is highly likely to represent a novel species of the genus *Pedobacter*.

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 $\times 1000$, using cells grown for 6 days at 25 °C on R2A agar. Catalase activity was determined from bubble production in 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. To determine the assimilation of single carbon sources, a defined liquid medium containing basal salts was used, as follows (g 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 poured into 96 wells and 25 $\times$ stock filter-sterilized carbon sources were added to each well. The wells 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 and

Fig. 1. Phylogenetic dendrogram, obtained by neighbour-joining clustering of 16S rRNA gene sequences, showing phylogenetic relationships between strain Gsoil 104^T, species of the genus *Pedobacter* and *Spingobacterium spirittivorum*. Bootstrap values (expressed as percentages of 1000 replications) greater than 60 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated using the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position.

anaerobic reduction of nitrate as the final electron acceptor were determined in serum bottles containing R2A broth to which thioglycolate (1 g l^{-1}) had been added and in which the upper air layer had been replaced with N_2 ; for determination of nitrate reduction, nitrate was added as KNO_3 at a concentration of 10 mM. Aerobic reduction of nitrate was later confirmed by inoculation into serum bottles (25 ml) containing 12 ml R2A media, with nitrate being added as KNO_3 at 10 mM. The reduction of nitrate 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 and cellulose (Ten *et al.*, 2004) was also investigated; reactions were read after 7 days. Growth at different temperatures (4, 15, 25, 30, 37 and 42°C) and various pH values (pH 4.5–10.0, using increments of 0.5 pH units) 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 (Difco) and MacConkey agar was also evaluated, at 25°C .

The cells of strain Gsoil 104^T were Gram-negative, aerobic, non-spore-forming, non-motile and rod-shaped. After 6 days incubation at 25°C on R2A, colonies were 2–5 mm in diameter, smooth, convex, round, glossy and white. On R2A agar, the optimal growth temperature for Gsoil 104^T was 25°C ; the strain was able to grow at 4– 30°C , but not at 37°C . The strain grew between pH 5.0 and 8.5, with an optimum between pH 6.5 and 7.5. 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 104^T were found to be 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, N-acetyl-D-glucosamine and amygdalin, negative for H_2S production, indole production, urease, lipase and nitrate reduction and unable to produce acid from a large number of substrates. The phenotypic and chemotaxonomic characteristics that differentiate strain Gsoil 104^T from related *Pedobacter* species are listed in Table 1. In contrast to all other *Pedobacter* species, Gsoil 104^T does not hydrolyse aesculin.

For measurement of the G+C content of chromosomal DNA, the genomic DNA of the strain was extracted and purified as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides. The G+C content of the DNA 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 quinone in n-hexane was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analysed by HPLC, as described previously (Hiraishi *et al.*, 1996).

Cellular fatty acids were analysed after culture on trypticase soy agar 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). Summed features, which are mixtures of two or three fatty acids, were analysed further using GC/MS.

The cellular fatty acid profiles of strain Gsoil 104^T and related *Pedobacter* species are presented in Table 2. The major components were $\text{C}_{16:1\omega7c}$, iso- $\text{C}_{15:0}$, iso- $\text{C}_{17:0}$ 3-OH and iso- $\text{C}_{15:0}$ 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 104^T and the phylogenetically closest relatives. One of the major fatty acids found in strain Gsoil 104^T was $\text{C}_{16:0}$ (15.6% of total). This value is generally much higher than that reported for other *Pedobacter* species; only *P. cryoconitis* DSM 14825^T (9.1%) and *P. himalayensis* HHS 22^T (7.3%) have comparable levels of $\text{C}_{16:0}$. The DNA G+C content for the genus *Pedobacter* ranges from 36.9 mol% (reported for *P. saltans*) to 44.2 mol% (reported for *P. africanus*) (Steyn *et al.*, 1998). The G+C content of the DNA of strain Gsoil 104^T was within this range (43.6 mol%). The predominant respiratory quinone of strain Gsoil 104^T was MK-7.

To clarify the taxonomic position of strain Gsoil 104^T, DNA–DNA hybridization was performed with the four members of the genus most closely related to strain Gsoil 104^T. 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 hybridization values. Strain Gsoil 104^T exhibited relatively low levels of DNA–DNA relatedness with respect to *P. africanus* KACC 11389^T (36%), *P. cryoconitis* KACC 11394^T (30%), *P. caeni* DSM 16990^T (25%) and *P. heparinus* DSM 2366^T (19%), indicating that it is not related to them at the species level (Wayne *et al.*, 1987).

The results obtained from the phenotypic and phylogenetic characterizations indicated that strain Gsoil 104^T belongs to the genus *Pedobacter*. The phylogenetic distinctiveness of strain Gsoil 104^T, together with the DNA–DNA hybridization data, confirmed that this isolate represents a species that is distinct from recognized *Pedobacter* species. There are some phenotypic differences between strain Gsoil 104^T and phylogenetically related *Pedobacter* species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 104^T should be classified within the genus *Pedobacter* as the type strain of a novel species, for which the name *Pedobacter ginsengisoli* sp. nov. is proposed.

Table 1. Physiological and biochemical characteristics of strain Gsoil 104^T and related *Pedobacter* species

Taxa: 1, strain Gsoil 104^T; 2, *P. africanus*; 3, *P. caeni*; 4, *P. cryoconitis*; 5, *P. heparinus*; 6, *P. piscium*; 7, *P. himalayensis*; 8, *P. sandarakinus*; 9, *P. saltans*. Data for reference species are from Steyn *et al.* (1998), Margesin *et al.* (2003), Shivaji *et al.* (2005), Vanparys *et al.* (2005) and Yoon *et al.* (2006). All taxa are positive for the following: aerobic growth, 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*), sucrose (variable for *P. saltans*) and D-trehalose (variable for *P. saltans*) as sole carbon sources. All strains are negative for the following: Gram stain, 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, inositol, 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 sole carbon sources. Symbols: +, positive; −, negative; V, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Enzyme activities									
Gelatinase	−	V	−	+	−	−	+	−	−
Lysine decarboxylase	−	+	ND	−	+	+	−	−	+
Tryptophan deaminase	−	−	ND	−	−	ND	+	−	−
Arginine dihydrolase	−	−	−	−	−	ND	+	−	−
Acid production from:									
D-Glucose	−	V	−	−	V	+	−	ND	V
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	−	+	ND	−	V	V	+	ND	−
L-Glutamate	−	+	ND	−	−	+	+	ND	−
DNA G+C content (mol%)	43·6	43·7–44·2	42·7	43·4	42·3–43·0	40·4–41·0	41·0	39·7	36·9–37·1

*Data from Takeuchi & Yokota (1992).

Description of *Pedobacter ginsengisoli* sp. nov.

Pedobacter ginsengisoli (gin.sen.gi.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil from a ginseng field, the source of the type strain).

Cells are Gram-negative, aerobic, non-spore-forming, non-motile and rod-shaped (0·5–1·0 × 2·5–4·5 µm). After 6 days incubation at 25 °C on R2A, colonies are 2–5 mm in diameter, smooth, convex, round, glossy and white. Grows at 4–30 °C; the optimum temperature for growth is 25 °C. The minimum pH for growth lies between 4·5 and

Table 2. Fatty acid composition (%) of strain Gsoil 104^T and related *Pedobacter* species

Strains: 1, strain Gsoil 104^T; 2, *P. africanus* DSM 12126^T (data from Steyn *et al.*, 1998); 3, *P. caeni* LMG 22862^T (Vanparrys *et al.*, 2005); 4, *P. cryoconitis* DSM 14825^T (Shivaji *et al.*, 2005); 5, *P. heparinus* DSM 2366^T (Steyn *et al.*, 1998); 6, *P. piscium* DSM 11725^T (Steyn *et al.*, 1998); 7, *P. himalayensis* HHS 22^T (Shivaji *et al.*, 2005); 8, *P. sandarakinus* KCTC 12559^T (Yoon *et al.*, 2006); 9, *P. saltans* DSM 12145^T (Steyn *et al.*, 1998). tr, Trace amount (<1%); –, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
Straight-chain									
saturated									
C _{14:0}	–	1.5	2.5	1.0	1.1	1.3	1.0	–	tr
C _{15:0}	–	tr	–	–	1.1	–	–	4.2	1.4
C _{16:0}	15.5	3.8	3.0	9.1	3.0	3.3	7.3	–	4.0
C _{15:0} 2-OH	1.0	–	–	–	–	–	–	1.2	–
C _{16:0} 3-OH	2.1	3.1	2.8	–	1.5	4.5	–	–	tr
C _{17:0} 2-OH	2.9	–	1.3	–	–	–	–	–	–
Branched									
saturated									
iso-C _{15:0}	21.5	26.6	21.3	15.0	28.2	26.2	33.0	35.8	31.4
iso-C _{17:0}	–	–	–	–	–	–	–	1.8	–
iso-C _{15:0} 2-OH	10.9	10.4	NA*	2.0	10.4	10.7	2.0	NA*	8.9
iso-C _{15:0} 3-OH	3.6	2.1	3.3	1.9	2.5	2.5	1.5	3.0	2.8
iso-C _{16:0} 3-OH	1.1	–	–	–	–	–	–	–	–
iso-C _{17:0} 3-OH	13.1	14.7	12.1	4.2	15.2	9.2	6.0	17.7	12.7
anteiso-C _{15:0}	1.2	tr	tr	–	tr	2.6	–	–	2.9
Summed	–	–	39.9	–	–	–	–	21.7	–
feature 3†									
Monounsaturated									
C _{15:1} ω6c	–	tr	–	–	–	–	–	1.6	1.4
C _{16:1} ω5c	–	2.1	2.5	–	1.4	3.5	–	–	tr
C _{16:1} ω7c	23.3	23.7	NA*	27.0	20.2	31.4	20.0	NA*	19.6
C _{17:1} ω8c	–	–	–	–	–	–	–	1.1	–
iso-C _{17:1} ω9c	2.4	4.4	2.2	8.6	6.3	1.6	4.0	3.8	6.6
anteiso-	–	–	tr	–	–	1.2	–	–	–
C _{17:1} ω9c	–	–	–	–	–	–	–	–	–
Unknown‡									
ECL 13.566	–	2.6	–	–	5.0	tr	–	–	1.1
ECL 13.961	1.4	–	–	–	–	–	–	–	–
ECL 16.580	–	1.0	–	–	1.0	tr	–	–	1.2

*Not applicable (fatty acids are components of unresolved summed feature 3).

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

‡Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. ECL, Equivalent chain length.

5.0, the optimum pH is 6.5–7.5 and the maximum pH lies between 8.5 and 9.0. Tolerates 1 % (w/v) NaCl, but not 2 %. Growth occurs on trypticase soy agar, but not on

MacConkey agar. Positive for catalase and oxidase, but negative for lipase. Negative for hydrolysis of chitin, starch, cellulose, xylan, casein and aesculin and positive for hydrolysis of DNA. The following substrates are utilized for growth: D-glucose, D-galactose, D-mannose, D-fructose, D-xylose, L-xylose, N-acetyl-D-glucosamine, salicin, D-cellobiose, D-lactose, D-maltose, D-melibiose, sucrose, D-trehalose, D-raffinose, amygdalin, glycerol, inulin and dextran. The following substrates are not utilized for growth: D-arabinose, L-arabinose, 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, inositol, D-adonitol, D-mannitol, D-sorbitol, xylitol, methanol, glycogen, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, 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 is positive and tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, gelatinase, β-galactosidase and production of hydrogen sulphide and indole are all negative. Acid is not produced from D-glucose, D-melibiose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose or sucrose. The major fatty acids are C_{16:1}ω7c, iso-C_{15:0}, C_{16:0}, iso-C_{17:0} 3-OH and iso-C_{15:0} 2-OH. The G + C content of the genomic DNA is 43.6 mol%. The predominant respiratory quinone is MK-7.

The type strain, Gsoil 104^T (=KCTC 12576^T=LMG 23399^T), was isolated from a soil sample from a ginseng field in Pocheon Province (South Korea).

Acknowledgements

This work was supported by the Brain Pool Program (grant 031-4-17) funded by the Ministry of Science and Technology and by the 2006 research program of the Rural Development Administration, Republic of Korea.

References

- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric DNA–DNA hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

- Fitch, W. M. (1971).** Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gallego, V., García, M. T. & Ventosa, A. (2006).** *Pedobacter aquatilis* sp. nov., isolated from drinking water, and emended description of the genus *Pedobacter*. *Int J Syst Evol Microbiol* **56**, 1853–1858.
- Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996).** Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.
- Hwang, C. Y., Choi, D. H. & Cho, B. C. (2006).** *Pedobacter roseus* sp. nov., isolated from a hypertrophic pond, and emended description of the genus *Pedobacter*. *Int J Syst Evol Microbiol* **56**, 1831–1836.
- Im, W.-T., Jung, H.-M., Cui, Y.-S., Liu, Q.-M., Zhang, S.-L. & Lee, S.-T. (2005).** Cultivation of three hundred bacterial species from the soil of the ginseng field and mining the novel lineage bacteria. In *Proceedings of the International Meeting of the Federation of Korean Microbiological Societies*, abstract A035, p. 169. Seoul: Federation of Korean Microbiological Societies.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005).** *Sphingopyxis granulii* sp. nov., a β -glucosidase-producing bacterium in the family *Sphingomonadaceae* in α -4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kimura, M. (1983).** *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kouker, G. & Jaeger, K.-E. (1987).** Specific and sensitive plate assay for bacterial lipases. *Appl Environ Microbiol* **53**, 211–213.
- Kumar, S., Tamura, K. & Nei, M. (2004).** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Margesin, R., Spröer, C., Schumann, P. & Schinner, F. (2003).** *Pedobacter cryoconitis* sp. nov., a facultative psychrophile from alpine glacier cryoconite. *Int J Syst Evol Microbiol* **53**, 1291–1296.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Moore, D. D. & Dowhan, D. (1995).** Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, chapter 2. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI Technical Note 101. Newark, DE: MIDI.
- Shivaji, S., Chaturvedi, P., Reddy, G. S. N. & Suresh, K. (2005).** *Pedobacter himalayensis* sp. nov., from the Hamta glacier located in the Himalayan mountain ranges of India. *Int J Syst Evol Microbiol* **55**, 1083–1088.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M. & 10 other authors (2002).** Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Steyn, P. L., Segers, P., Vancanneyt, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998).** Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. *Int J Syst Bacteriol* **48**, 165–177.
- Takeuchi, M. & Yokota, A. (1992).** Proposals of *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov., *Sphingobacterium heparinum* comb. nov., *Sphingobacterium thalpophilum* comb. nov., and two genospecies of the genus *Sphingobacterium*, and synonymy of *Flavobacterium yabuuchiae* and *Sphingobacterium spiritivorum*. *J Gen Appl Microbiol* **38**, 465–482.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004).** Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tschech, A. & Pfennig, N. (1984).** Growth yield increase linked to caffeine reduction in *Acetobacterium woodii*. *Arch Microbiol* **137**, 163–167.
- Vanparys, B., Heylen, K., Lebbe, L. & De Vos, P. (2005).** *Pedobacter caeni* sp. nov., a novel species isolated from a nitrifying inoculum. *Int J Syst Evol Microbiol* **55**, 1315–1318.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987).** International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Widdel, F. & Bak, F. (1992).** Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, 2nd edn, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Widdel, F., Kohring, G. & Mayer, F. (1983).** Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov. *Arch Microbiol* **134**, 286–294.
- Yoon, J.-H., Lee, M.-H., Kang, S.-J., Park, S.-Y. & Oh, T.-K. (2006).** *Pedobacter sandarakinus* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1273–1277.