Paenibacillus panacisoli sp. nov., a xylanolytic bacterium isolated from soil in a ginseng field in South Korea

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A Gram-positive, facultatively anaerobic, motile, spore-forming bacterium, designated Gsoil 1411^T, was isolated from soil of a ginseng field in Pocheon Province (South Korea) and was characterized using a polyphasic approach. Comparative analysis of 16S rRNA gene sequences revealed that strain Gsoil 1411^T belongs to the family *Paenibacillaceae*, with closest sequence similarity to the type strains of *Paenibacillus xylanilyticus* (95.7%), *Paenibacillus illinoisensis* (95.2%) and *Paenibacillus pabuli* (94.8%). Strain Gsoil 1411^T showed less than 94% sequence similarity to the type strains of other recognized members of the genus *Paenibacillus*. In addition, the presence of MK-7 as the major menaquinone, anteiso-C_{15:0} as a major fatty acid (44.8%) and the presence of PAEN513F and PAEN862F signature sequences suggest that it is affiliated to the genus *Paenibacillus*. The G + C content of the genomic DNA was 53.9 mol%. On the basis of its phenotypic characteristics and phylogenetic distinctiveness, strain Gsoil 1411^T is suggested to represent a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus panacisoli* sp. nov. is proposed. The type strain is Gsoil 1411^T (=KCTC 13020^T =LMG 23405^T).

The genus *Paenibacillus* was defined in 1993 after an extensive comparative analysis of 16S rRNA gene sequences of 51 species of the genus *Bacillus* (Ash *et al.*, 1991, 1993). At that time, the genus comprised 11 recognized species, with *Paenibacillus polymyxa* as the type species. At the time of writing, there are 69 recognized species within the genus *Paenibacillus* (<http://www.bacterio.cict.fr>). Members of the genus are aerobic, or facultatively anaerobic, organisms that produce ellipsoidal endospores in swollen sporangia and whose cell walls show structures typical of Gram-positive bacteria. The DNA G + C content ranges from 39 to 54 mol% and anteiso-C_{15:0} is the major cellular fatty acid (Shida *et al.*, 1997a).

During the course of study on the culturable aerobic and facultatively anaerobic bacterial community in soil from a

ginseng field in Pocheon Province (South Korea), a large number of bacteria were isolated. In this study, we have characterized one of these isolates, strain Gsoil 1411^T. Phenotypic, chemotaxonomic and phylogenetic analyses establish the affiliation of this isolate to the genus *Paenibacillus*. The data obtained also suggest that the isolate represents a novel species of this genus.

Strain Gsoil 1411^T was originally isolated from soil from a ginseng field. The soil sample was suspended in 50 mM phosphate buffer (pH 7.0) and serial ten-fold dilutions of the suspension were spread on modified R2A agar plates (per litre: 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 C₃H₃NaO₃, 0.3 g K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂, 15 g agar). The plates were incubated at 30 °C for 1 month. Single colonies on the plates were purified by transferring them onto new plates and they were incubated again on modified R2A or half-strength modified R2A. The purified colonies were tentatively identified by partial sequencing of the 16S rRNA gene (Im *et al.*, 2005). Strain Gsoil 1411^T was one of the isolates which appeared on the modified R2A agar plates under aerobic conditions. It

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Gsoil 1411^T is AB245384.

An expanded phylogenetic tree based on 16S rRNA gene sequences of strain Gsoil 1411^T and the type strains of representative members of the genus *Paenibacillus* is available as a supplementary figure in IJSEM Online.

was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at -70 °C.

For phylogenetic analysis of strain Gsoil 1411^T, DNA was extracted using a genomic DNA extraction kit (Core Biosystem), the 16S rRNA gene was amplified by PCR and sequencing of the purified PCR product was carried out according to Kim *et al.* (2005). The full 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using CLUSTAL_X (Thompson *et al.*, 1997). 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).

The 16S rRNA gene sequence of strain Gsoil 1411^T was a continuous stretch of 1487 bp. Comparative 16S rRNA gene sequence analyses showed that strain Gsoil 1411^T is phylogenetically affiliated to species of the genus *Paenibacillus*. The phylogenetic tree based on the neighbour-joining algorithm (Fig. 1) showed that strain Gsoil 1411^T appeared within the genus *Paenibacillus*, but occupied a distinct phylogenetic position (an expanded phylogenetic tree is available as Supplementary Fig. S1 in IJSEM Online). Species found to be closely related to strain Gsoil 1411^T were *Paenibacillus xylanilyticus* XIL14^T, *Paenibacillus illinoisensis* JCM 9907^T, *Paenibacillus pabuli* NCIMB 12781^T and *Paenibacillus amylolyticus* NRRL NRS-290^T with 16S rRNA gene sequence similarity levels of 95.7, 95.2, 94.8 and 94.2 %, respectively.

Gram reaction testing was performed by the non-staining method as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000 magnification, with cells grown for 3 days at 30 °C on

R2A agar. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). For single carbon source assimilation studies, we used a defined liquid medium plus basal salt medium containing (per litre): 1.8 g K₂HPO₄, 1.08 g KH₂PO₄, 0.5 g NaNO₃, 0.5 g NH₄Cl, 0.1 g KCl, 0.1 g MgSO₄ and 0.05 g CaCl₂. To this medium, a vitamin solution (Widdel & Bak, 1992), trace element solution SL-10 (Widdel *et al.*, 1983) and selenite/tungstate solution (Tschech & Pfennig, 1984) were added and the pH of the medium was adjusted to 6.8. This liquid medium was aliquoted (0.24 ml) into 96-well trays and filter-sterilized carbon sources (0.1 ml) were added to each well (individually at 0.1 %, w/v). Growth in the 96-well plates was examined visually after incubation at 30 °C for 7 days. Negative control wells did not contain any added carbon source. A positive control culture was grown in a well containing R2A medium. Fermentative acid production and oxidative acid production from carbohydrates were tested by growth in O-F basal medium with bromothymol blue (Atlas, 1993) supplemented with 1 % carbohydrate [soft-agar stabs with (fermentative) and without (oxidative) sterile mineral oil overlay]. The O-F medium tubes were incubated at 30 °C for 5 days. Tests for acid production from carbohydrates and some other phenotypic characteristics, as given in the species description below, were determined with API 20E galleries according to the manufacturer's instructions (bioMérieux). Anaerobic growth was tested in serum bottles with the addition of thioglycolate (1 g l⁻¹) to R2A broth and substituting the upper air layer with nitrogen gas. A test for anaerobic nitrate reduction, as the final electron acceptor, was performed in serum bottles with the addition of thioglycolate (1 g l⁻¹) to R2A broth and substituting the upper air layer with nitrogen gas, while nitrate was added as KNO₃ at concentrations of 10 mM. Aerobic nitrate reduction was later confirmed by inoculations, in each case, into three serum bottles (25 ml) containing 12 ml R2A media, while nitrate was added as KNO₃ at concentrations of 10 mM. Reduction of nitrate and nitrite was monitored by ion chromatography (model 790 personal IC; Metrohm) equipped with a conductivity detector and anion exchange

Fig. 1. Comparative analysis of the 16S rRNA gene sequences of strain Gsoil 1411^T and the type strains of phylogenetically related representatives of the genus *Paenibacillus* based on the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) greater than 80 % are shown at branch points. Dots indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.005 substitutions per nucleotide position.

column (Metrosep Anion Supp 4; Metrohm). Tests for degradation of DNA (DNase agar Scharlau by flooding plates with 1 M HCl), casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. Growth at different temperatures (4, 15, 25, 30, 37, 42 and 45 °C) and various pH values (pH 4.0–10.0 at intervals 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 (TSA) and MacConkey agar was also evaluated at 30 °C.

Cells of strain Gsoil 1411^T were Gram-positive, motile rods that formed oval spores that lay subterminally in swollen sporangia. Peritrichous flagella were observed (Fig. 2). After 1 day on R2A, colonies were 0.5–1.0 mm in diameter, convex, irregular, undulate, non-glossy and slightly yellowish. On R2A agar, the optimum temperature for growth was 37 °C. Strain Gsoil 1411^T showed oxidase and catalase activity and reduced nitrate to nitrite. Physiological characteristics that differentiate strain Gsoil 1411^T from its closest phylogenetic relatives, *P. xylanilyticus*, *P. illinoisensis*, *P. amylolyticus* and *P. pabuli*, are listed in Table 1.

For the measurement of G + C content of the chromosomal DNA, genomic DNA was extracted and purified as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides. The G + C content of DNA was then determined as described by Mesbah *et al.* (1989) using reverse-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and reextracted 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 by Hiraishi *et al.* (1996). Cellular fatty acids were analysed following growth on R2A 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

Fig. 2. Negatively stained transmission electron micrograph of a single cell of strain Gsoil 1411^T, showing peritrichous flagella. Bar, 1 µm.

Table 1. Differential phenotypic characteristics of strain Gsoil 1411^T and the type strains of phylogenetically related *Paenibacillus* species

Strains: 1, Gsoil 1411^T; 2, *P. xylanilyticus* XIL14^T (data from Rivas *et al.*, 2005); 3, *P. illinoisensis* NRRL NRS-1356^T (Shida *et al.*, 1997b); 4, *P. pabuli* NRRL NRS-924^T (Shida *et al.*, 1997b); 5, *P. amylolyticus* NRRL NRS-290^T (Shida *et al.*, 1997a). All strains are positive for catalase activity and acid production from L-arabinose and D-mannitol, but are negative for citrate and L-malate utilization. All produce ellipsoidal or oval spores in swollen sporangia. +, Positive; –, negative; V, variable; ND, no data; W, weakly positive.

Characteristic	1	2	3	4	5
Growth:					
At pH 5.6	+	–	+	+	+
In 5 % (w/v) NaCl	+	ND	–	V	–
At 50 °C	–	–	+	–	–
Oxidase	+	–	–	–	–
Nitrate reduction	+	–	–	–	+
Indole production	–	–	ND	–	–
Voges–Proskauer reaction	–	–	–	ND	–
Hydrolysis of:					
Casein	W	–	W	V	W
Gelatin	+	+	+	ND	+
Starch	–	+	+	+	+
Assimilation of:					
Acetate	–	ND	+	+	–
Succinate	–	ND	–	–	–
Propionate	–	–	–	ND	–
Acid production from:					
D-Rhamnose	–	+	–	ND	–
D-Melibiose	+	+	+	ND	+
Sucrose	–	+	–	ND	+
DNA G + C content (mol%)	53.9	50.5	48.0	45.0	53.0

acids were then analysed by GC (Hewlett Packard 6890) using the Microbial Identification software package (Sasser, 1990).

The DNA G + C content of strain Gsoil 1411^T was 53.9 mol%, which lies within the range of values reported for members of the genus *Paenibacillus*. Affiliation of Gsoil 1411^T to the genus *Paenibacillus* was also supported based on analysis of the respiratory quinone system; the majority of species in the genus have MK-7 as the major quinone (Shida *et al.*, 1997a). The fatty acid profile of isolate Gsoil 1411^T is given in Table 2 and is compared with those of the type strains of phylogenetically related *Paenibacillus* species. Anteiso-branched C_{15:0}, the major fatty acid found in members of the genus *Paenibacillus* (Shida *et al.*, 1997a), was also the major fatty acid component of strain Gsoil 1411^T, comprising 44.8 % of the total. However, some qualitative and quantitative differences in fatty acid content were observed between strain Gsoil 1411^T and its phylogenetically closest relatives. The second most abundant fatty acid in strain Gsoil 1411^T was iso-C_{16:0}, comprising 20.2 % of the

Table 2. Cellular fatty acid profile (%) of strain Gsoil 1411^T and the type strains of phylogenetically related species of the genus *Paenibacillus*

Strains: 1, Gsoil 1411^T; 2, *P. xylanilyticus* XIL14^T (data from Rivas *et al.*, 2005); 3, *P. illinoisensis* NRRL NRS-1356^T (Shida *et al.*, 1997b); 3, *P. pabuli* NRRL NRS-924^T (Shida *et al.*, 1997a); 4, *P. amylolyticus* NRRL NRS-290^T (Shida *et al.*, 1997b). ND, Not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4	5
Saturated:					
C _{14:0}	ND	3.9	2.0	0.7	2.9
C _{15:0}	2.8	ND	1.0	0.1	0.5
C _{16:0}	9.4	16.6	24.7	10.1	12.8
C _{18:0}	1.1	ND	ND	ND	ND
Unsaturated:					
C _{16:1ω11}	ND	8.1	ND	ND	0.1
Branched-chain:					
iso-C _{14:0}	6.9	2.3	1.8	0.7	2.3
iso-C _{15:0}	5.0	7.1	1.5	2.2	2.0
iso-C _{16:0}	20.2	4.7	6.3	4.8	9.0
iso-C _{17:0}	2.1	4.5	1.4	1.3	3.5
anteiso-C _{15:0}	44.8	47.9	56.6	73.7	46.2
anteiso-C _{17:0}	5.6	4.9	4.9	4.1	2.2
Unknown:*					
ECL 14.966	2.1	ND	ND	ND	ND

*The unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

total. This value is much higher than that reported for the reference species tested (Table 2). We also found slight differences in the proportions of the other fatty acids.

All characteristics determined for strain Gsoil 1411^T are in accordance with those of the genus *Paenibacillus*. On the basis of its phylogenetic distance from recognized *Paenibacillus* species, as indicated by relatively low 16S rRNA gene sequence similarities (<96%), and specific combination of phenotypic characteristics, it is clear that Gsoil 1411^T is not affiliated to any recognized species of this genus. Therefore, on the basis of the data presented, strain Gsoil 1411^T is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus panacisoli* sp. nov. is proposed.

Description of *Paenibacillus panacisoli* sp. nov.

Paenibacillus panacisoli (pa.na.ci.so'li. N.L. n. *Panax* -*acis* scientific name of ginseng; L. n. *solum* soil; N.L. gen. n. *panacisoli* of soil of a ginseng field).

Cells are Gram-positive, facultatively anaerobic, motile, spore-forming and rod-shaped with a length of approximately 2.0–5.0 µm and a width of 0.4–0.6 µm. Spores are oval, subterminal and occur in swollen sporangia. After 1 day on R2A, colonies are 0.5–1.0 mm in diameter,

convex, irregular, undulate, non-glossy and slightly yellowish. Oxidase and catalase reactions are positive. Nitrate is reduced to nitrite. The optimum temperature for growth is 37 °C, the maximum growth temperature is between 42 and 45 °C, and the minimum growth temperature is between 15 and 20 °C. The minimum pH for growth is between 4.5 and 5.0, the optimum pH is 6.5 and the maximum pH is between 8.5 and 9.0. Tolerates 5% (w/v) NaCl, but not 7%. No growth occurs on TSA, MacConkey or nutrient agar. Hydrolyses xylan and casein (weakly), but not chitin, starch, cellulose, DNA or aesculin. Utilizes D-mannose, D-ribose, D-xylose, L-xylose, D-lactose, D-raffinose, gluconate (weakly), glycerol and inulin for growth, but not D-glucose, D-fructose, D-galactose, D-mannose, D-fucose, sucrose, D-trehalose, ethanol, L-rhamnose, L-sorbose, D-arabinose, L-arabinose, D-lyxose, formate, propionate, caprate, maleate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, malonate, pyruvate, acetate, 3-hydroxybutyrate, valerate, fumarate, salicin, citrate, lactate, malate, succinate, tartrate, glutarate, itaconate, adipate, suberate, oxalate, D-cellobiose, D-maltose, D-melibiose, D-raffinose, D-adonitol, dulcitol, D-sorbitol, D-mannitol, xylitol, inositol, amygdalin, methanol, glycogen, dextran, *N*-acetyl-D-glucosamine, L-cysteine, glycine, L-iso-leucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-tryptophan, L-tyrosine, L-valine, L-alanine, L-arginine, L-asparagine, L-aspartate, L-glutamate, L-glutamine, L-histidine, L-proline or L-threonine. In API 20E tests, positive for gelatin hydrolysis and tryptophan deaminase. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, β-galactosidase, urease, indole and hydrogen sulfide production, citrate utilization and the Voges–Proskauer reaction. Acid is produced from D-mannitol and weakly from L-arabinose, D-melibiose and amygdalin, but not from inositol, D-sorbitol, L-rhamnose, sucrose or D-glucose. MK-7 is the predominant menaquinone. The major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and C_{16:0}. The G + C content of the genomic DNA is 53.9 mol%.

The type strain, Gsoil 1411^T (=KCTC 13020^T=LMG 23405^T), was isolated from soil of a ginseng field of Pocheon Province, South Korea.

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