**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 1411T, 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 1411T 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 1411T 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-C15:0 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 1411T 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 1411T (KCTC 13020T = LMG 23405T).

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 1411T. 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 1411T 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, 0.25 g Casamino acids, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g CaH2NaO6, 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 1411T was one of the isolates which appeared on the modified R2A agar plates under aerobic conditions. It 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-C15:0 is the major cellular fatty acid (Shida et al., 1997a).

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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 1411T, 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 neighbor-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 1411T was a continuous stretch of 1487 bp. Comparative 16S rRNA gene sequence analyses showed that strain Gsoil 1411T is phylogenetically affiliated to species of the genus Paenibacillus. The phylogenetic tree based on the neighbor-joining algorithm (Fig. 1) showed that strain Gsoil 1411T 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 1411T were Paenibacillus xylanilyticus XIL14T, Paenibacillus illinoisensis JCM 9907T, Paenibacillus pabuli NCIMB 12781T and Paenibacillus amylolyticus NRRL NRS-290T 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 KH2PO4, 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 thioglycollate (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 thioglycollate (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 1411T 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 1411T 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 yellow-
ish. On R2A agar, the optimum temperature for growth was
37 °C. Strain Gsoil 1411T showed oxidase and catalase
activity and reduced nitrate to nitrite. Physiological
characteristics that differentiate strain Gsoil 1411T from
its closest phylogenetic relatives, P. xylanilyticus, P.
ilinoisensis, 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
hexane/water (1:1, v/v). The crude quinone in
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

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

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>In 5 % (w/v) NaCl</td>
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<td>ND</td>
<td>V</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>At 30 °C</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Oxidase</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Nitrate reduction</td>
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<td>+</td>
<td>-</td>
<td>+</td>
</tr>
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<td>V</td>
<td>W</td>
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<td></td>
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</tr>
<tr>
<td>d-Rhamnose</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>53-9</td>
<td>50-5</td>
<td>48-0</td>
<td>45-0</td>
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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 1411T was
53-9 mol%, which lies within the range of values reported
for members of the genus Paenibacillus. Affiliation of Gsoil
1411T 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
1411T is given in Table 2 and is compared with those of the
type strains of phylogenetically related Paenibacillus species.
Anteiso-branched C15:0, 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 1411T,
comprising 44-8 % of the total. However, some qualitative
and quantitative differences in fatty acid content were
observed between strain Gsoil 1411T and its phylogenetically
closest relatives. The second most abundant fatty acid in
strain Gsoil 1411T was iso-C16:0 comprising 20-2 % of the

Fig. 2. Negatively stained transmission electron micrograph of
a single cell of strain Gsoil 1411T, showing peritrichous flagella.
Bar, 1 μm.
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. amylyticus* NRRL NRS-290^T* (Shida et al., 1997b). ND, Not detected; ECL, equivalent chain-length.

<table>
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<th>Fatty acid</th>
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<td>C14:0</td>
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<td>2-0</td>
<td>0-7</td>
<td>2-9</td>
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<tr>
<td>C15:0</td>
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<td>ND</td>
<td>1-0</td>
<td>0-1</td>
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<td>C16:10:011</td>
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<td>7-1</td>
<td>1-5</td>
<td>2-2</td>
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<tr>
<td>iso-C16:0</td>
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<td>4-7</td>
<td>6-3</td>
<td>4-8</td>
<td>9-0</td>
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<tr>
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<tr>
<td>anteiso-C15:0</td>
<td>44-8</td>
<td>47-9</td>
<td>56-6</td>
<td>73-7</td>
<td>46-2</td>
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<tr>
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<td>5-6</td>
<td>4-9</td>
<td>4-9</td>
<td>4-1</td>
<td>2-2</td>
</tr>
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</table>

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

Description of *Paenibacillus panacisoli* sp. nov.

*Paenibacillus panacisoli* (pa.na.ci.so’i.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-xylene, L-xylene, 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, phenylacetae, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, malonate, pyruvate, acetate, 3-hydroxybutyrate, valerate, fumarate, salicin, citrate, lactate, malate, succinate, tartrate, glutarate, itaconate, adipate, suberate, oxalate, D-cellulbiose, 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-C15:0, iso-C16:0 and C16:0. 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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**References**


