Paenibacillus ginsengarvi sp. nov., isolated from soil from ginseng cultivation

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A Gram-positive, aerobic, non-motile, rod-shaped, spore-forming bacterium, designated Gsoil 139^T, was isolated from soil from 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 139^T belongs to the family *Paenibacillaceae*. The greatest sequence similarity was found with respect to the type strains of *Paenibacillus hodogayensis* (95.6 %) and *Paenibacillus koleovorans* (93.8 %). The strain showed less than 93.8 % sequence similarity with respect to other species of the genus *Paenibacillus*. The G+C content of the genomic DNA was 48.1 mol%. In addition, the presence of MK-7 as the major menaquinone and C_{15:0} anteiso as a major fatty acid (27.9 %) justifies its affiliation to the genus *Paenibacillus*. On the basis of its phenotypic characteristics and phylogenetic distinctiveness, strain Gsoil 139^T represents a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus ginsengarvi* sp. nov. is proposed. The type strain is Gsoil 139^T (=KCTC 13059^T =DSM 18677^T).

The genus *Paenibacillus* was defined in 1993 after an extensive comparative analysis of the 16S rRNA gene sequences of 51 species of the genus *Bacillus* (Ash *et al.*, 1991, 1993). When the genus was first described, it comprised 11 species, including *Paenibacillus polymyxa* as the type species. Currently, the genus *Paenibacillus* encompasses over 70 species (Euzéby, 1997; updated January 2007). Members of the genus *Paenibacillus* are aerobic or facultatively anaerobic organisms with typically Gram-positive cell walls and produce ellipsoidal endospores in swollen sporangia. The DNA G+C contents range from 39 to 54 mol% and anteiso-C_{15:0} is the major cellular fatty acid (Shida *et al.*, 1997).

During the course of an investigation of 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 139^T. Phenotypic, chemotaxonomic and phylogenetic analyses have established the affiliation of this isolate to the genus *Paenibacillus*, and the data obtained in this work

suggest that the isolate represents a novel species of this genus.

Strain Gsoil 139^T was originally isolated from soil from a ginseng field in Pocheon Province. The soil sample was suspended in 50 mM phosphate buffer (l⁻¹: 5.355 g K₂HPO₄, 2.62 g KH₂PO₄; pH 7.0) and serial decimal dilutions of the suspension were spread-plated onto modified R2A agar plates containing the following (l⁻¹): 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 K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂ and 15 g agar. The plates were then incubated at 30 °C for 1 month. On the 10⁷-diluted plate, 40–50 colonies appeared, of which strain Gsoil 139^T was one. Single colonies were purified by transferring them onto new plates (modified R2A medium) and repeated incubation. Strain Gsoil 139^T was one of the isolates that appeared on the modified R2A agar plates under aerobic conditions. It was routinely cultured on R2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

For phylogenetic analysis of strain Gsoil 139^T, DNA was extracted using a genomic DNA extraction kit (Core Biosystems); the 16S rRNA gene was amplified by a PCR and then sequencing of the purified PCR product was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 139^T is AB271057.

An extended 16S rRNA gene sequence-based neighbour-joining tree is available as supplementary material with the online version of this paper.

carried out according to Kim *et al.* (2005). The complete 16S rRNA gene sequence was 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 the CLUSTAL_X program (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) and 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 analysis of the 16S rRNA gene sequence of strain Gsoil 139^T (1469 bp) showed that this strain is phylogenetically affiliated to *Paenibacillus* species. The phylogenetic tree (Fig. 1) based on the neighbour-joining algorithm showed that strain Gsoil 139^T fell within the radiation of the cluster comprising *Paenibacillus* species (see the expanded phylogenetic tree; Supplementary Fig. S1 available in IJSEM Online), joining *Paenibacillus hodogayensis* JCM 12520^T with a bootstrap resampling value of 99.0%. The closest phylogenetic neighbours of strain Gsoil 139^T are *P. hodogayensis* SG^T (95.6%) and *Paenibacillus koleovorans* TB^T (93.8%). The 16S rRNA gene sequence similarity to type strains of all other *Paenibacillus* species with validly published names was below 93.8%; according to Wayne *et al.* (1987), the phylogenetic definition of a species requires strains to show approximately ≥70% DNA–DNA relatedness. The available data indicate that organisms having less than 97.0% 16S rRNA gene sequence similarity will not exhibit more than 60% reassociation, irrespective of the hybridization method applied (Stackebrandt & Goebel, 1994; Keswani & Whitman, 2001). This phylogenetic result demonstrated that strain Gsoil 139^T was not related to any previously described *Paenibacillus* taxa at the species level.

The Gram reaction was performed using the non-staining method, as described by Buck (1982). Cell morphology and

motility were observed with a Nikon light microscope (×1000 magnification) using the hanging drop technique with cells allowed to grow on R2A agar for 3 days at 30 °C. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Acid production from 49 carbon sources was tested at 30 °C with API 50 CH in combination with API 50 CHB/E medium (bioMérieux), which was used for inoculum preparation, and evaluated after 2 days. Growth at a variety of temperatures (4, 15, 18, 25, 30, 37, 42 and 45 °C) was assessed on R2A agar and at pH 5.0–10.0 (in increments of 0.5 pH units) was assessed in R2A broth. Some physiological characteristics and the utilization of various substrates as sole carbon sources were determined using API 32GN, API 20NE and API 20E galleries according to the instructions of the manufacturer (bioMérieux). Tests for the degradation of DNA [in which DNase agar (Scharlau) plates were flooded with 1 M HCl], casein, chitin, starch (Atlas, 1993), olive oil (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. 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 30 °C.

Cells of strain Gsoil 139^T were Gram-positive, non-motile rods, 0.3–0.5 µm in diameter and 1.5–3.0 µm long after 2 days culture on R2A agar. They formed oval spores positioned centrally in swollen sporangia. Colonies grown on R2A agar plates for 2 days were 0.5–1.5 mm in diameter, circular, convex, non-glossy and white. On R2A agar, the optimum temperature for growth was 37 °C. Strain Gsoil 139^T showed oxidase and catalase activities and hydrolysed DNA weakly. Physiological characteristics that can be used to differentiate strain Gsoil 139^T from its closest phylogenetic relatives (*P. hodogayensis* and *P. koleovorans*) are listed in Table 1.

To determine the G+C content of the chromosomal DNA, the genomic DNA of the strain was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides; the DNA G+C content was

Fig. 1. Phylogenetic tree based on comparative analysis of the 16S rRNA gene sequences of strain Gsoil 139^T and phylogenetically related species of the genus *Paenibacillus* constructed using the neighbour-joining method. Bootstrap percentages (from 1000 replications) greater than 70% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position. An extended version of this tree is available as Supplementary Fig. S1 in IJSEM Online.

Table 1. Phenotypic characteristics of strain Gsoil 139^T and type strains of phylogenetically related *Paenibacillus* species

Strains: 1, Gsoil 139^T; 2, *P. hodogayensis* JCM 12520^T (data from Takeda *et al.*, 2005); 3, *P. koleovorans* JCM 11186^T (Takeda *et al.*, 2002). All of the strains are negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, gelatin hydrolysis, indole production and citrate utilization. None of the strains produces acid from D-fructose, D-fucose, L-fucose, D-galactose, D-lyxose, D-mannose, L-rhamnose, L-sorbose, D-tagatose, L-xylose, adonitol, D-arabitol, L-arabitol, dulcitol, erythritol, inositol, D-sorbitol, xylitol, inulin, β -gentiobiose, D-melezitose, gluconate, starch or glycogen. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Anaerobic growth	–	–	+
Optimum growth temperature (°C)	37	30	30
Catalase activity	+	+	–
Oxidase activity	+	+	–
Voges–Proskauer test	+	–	–
Acid production from:			
Amygdalin	+	w	–
L-Arabinose	+	–	–
Arbutin	+	–	–
D-Glucose	+	+	–
Glycerol	+	+	–
D-Lactose	–	w	–
D-Mannitol	–	+	–
D-Melibiose	+	+	–
Methyl α -D-glucoside	+	w	–
D-Raffinose	+	–	–
D-Ribose	+	w	–
Salicin	–	w	–
Sucrose	+	+	–
D-Xylose	+	–	–
DNA G + C content (mol%)	48.1	55.0	54.0–55.8

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 vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude quinone in n-hexane was purified using silica Sep-Pak Vac cartridges (Waters) and subsequently analysed by HPLC, as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on TSA for 3 days at 30 °C. 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 gas chromatography (6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Duplicate experiments were performed.

The DNA G + C content of strain Gsoil 139^T was 48.1 mol%, which lies within the range observed for members of the genus *Paenibacillus*. The respiratory quinone system present in Gsoil 139^T supports its

affiliation with the genus *Paenibacillus*, in which most species have MK-7 as the major quinone (Shida *et al.*, 1997). The fatty acids found in isolate Gsoil 139^T are shown in Table 2 and are compared with values available for phylogenetically related *Paenibacillus* strains. Anteiso-branched C_{15:0}, the major fatty acid present in members of the genus *Paenibacillus* (Shida *et al.*, 1997), was also the major fatty acid component of strain Gsoil 139^T, comprising 27.9% of the total. However, some qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 139^T and the phylogenetically closest relatives. The next most predominant fatty acid found in strain Gsoil 139^T was iso-C_{16:0}, comprising 26.8%. This value is much higher than those reported for the reference species (Table 2).

All of the characteristics determined for strain Gsoil 139^T are in accordance with those for the genus *Paenibacillus*. On the basis of the phylogenetic distance from established *Paenibacillus* species, indicated by the relatively low 16S rRNA gene sequence similarities (<96%), and the specific combination of phenotypic characteristics, it is demonstrable that Gsoil 139^T is not affiliated to any species of this genus. Therefore, on the basis of the data presented, strain Gsoil 139^T should be placed in the genus *Paenibacillus* within a novel species, for which the name *Paenibacillus ginsengarvi* sp. nov. is proposed.

Description of *Paenibacillus ginsengarvi* sp. nov.

Paenibacillus ginsengarvi (gin.seng.ar'vi. N.L. n. *ginsengum* ginseng; L. n. *arvum* a field; N.L. gen. n. *ginsengarvi* of a ginseng field, the source of the type strain).

Table 2. Cellular fatty acid profile of strain Gsoil 139^T and type strains of phylogenetically related species of the genus *Paenibacillus*

Strains: 1, Gsoil 139^T; 2, *P. hodogayensis* JCM 12520^T (data from Takeda *et al.*, 2005); 3, *P. koleovorans* JCM 11186^T (Takeda *et al.*, 2002). NR, not reported.

Fatty acid	1	2	3
Straight-chain saturated			
C _{14:0}	0.7	NR	NR
C _{15:0}	3.4	19.2	13.3
C _{16:0}	3.2	7.6	22.8
C _{17:0}	0.3	NR	NR
Branched saturated			
iso-C _{14:0}	10.0	NR	NR
iso-C _{15:0}	21.9	NR	NR
iso-C _{16:0}	26.8	9.6	NR
iso-C _{17:0}	4.0	NR	NR
anteiso-C _{15:0}	27.9	39.7	37.4
anteiso-C _{17:0}	1.8	NR	NR

Cells are Gram-positive, non-motile rods that form oval spores positioned centrally in swollen sporangia. Oxidase and catalase reactions are positive. Nitrate is not reduced to nitrite. Anaerobic growth does not occur. Grows between 18 and 45 °C (optimum 37 °C) and at pH 5.0–8.5 (optimum pH 6.5–7.0). Tolerates NaCl at 2 % (w/v) but not 3 %. Growth occurs on TSA and nutrient agar but not on MacConkey agar. Hydrolyses DNA weakly, but does not hydrolyse chitin, starch, cellulose, xylan, lipids or casein. Positive for β -glucosidase and β -galactosidase. The following substrates are utilized for growth: D-glucose, maltose, D-melibiose, D-ribose, sucrose, salicin and gluconate. The following substrates are not utilized for growth: D-mannose, L-fucose, L-rhamnose, L-arabinose, propionate, caprate, phenylacetate, 3-hydroxybenzoate, 4-hydroxybenzoate, malonate, acetate, 3-hydroxybutylate, valerate, citrate, lactate, malate, 5-ketogluconate, 2-ketoglutarate, itaconate, adipate, suberate, D-sorbitol, D-mannitol, inositol, glycogen, N-acetyl-D-glucosamine, gelatin, urea, nitrate, L-serine, L-tryptophan, L-alanine, L-arginine, L-histidine and L-proline. Positive for β -galactosidase and the Voges–Proskauer test in API 20E tests. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, gelatin hydrolysis, indole and hydrogen sulphide production are negative. Acids are produced from glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, methyl β -D-xyloside, D-glucose, methyl α -D-glucoside, amygdalin, arbutin, D-cellobiose, maltose, D-melibiose, sucrose, trehalose, D-raffinose and D-turanose. Acids are not produced from erythritol, L-xylose, adonitol, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α -D-mannoside, N-acetyl-D-glucosamine, salicin, D-lactose, inulin, D-melezitose, starch, glycogen, xylitol, β -gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. MK-7 is the predominant menaquinone. The major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{15:0}. The G + C content of the genomic DNA of the type strain is 48.1 mol%.

The type strain, Gsoil 139^T (=KCTC 13059^T =DSM 18677^T), was isolated from soil from a ginseng field in Pocheon Province, South Korea.

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