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ORIGINAL PAPER

Paenibacillus ginsengisoli sp. nov., a novel bacterium isolated from soil of a ginseng field in Pocheon province, South Korea

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Abstract A Gram-positive, aerobic or facultative anaerobic, motile, spore-forming bacterial strain, designated Gsoil 1638^{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. It grew well on nutrient agar medium, utilized a fairly narrow spectrum of carbon sources and tolerated 10% NaCl. The isolate was positive for catalase and oxidase tests but negative for the degradation of macromolecules such as casein, collagen, starch, chitin, CM-cellulose, xylan and DNA. The G + C content of the genomic DNA was 50.7 mol%. The predominant isoprenoid quinone was menaquinone 7 (MK-7). The major fatty acids were anteiso-$C_{15:0}$ (44%) and $C_{16:0}$ (25%). Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 1638^{T} fell within the radiation of the cluster comprising *Paenibacillus* species and joined *Paenibacillus anaericanus* DSM 15890^{T} with a bootstrap value of 100%. These two strains shared 99.5% 16S rRNA gene sequence similarity with each other. The phylogenetic distance from any other validly described species within the genus *Paenibacillus* was less than 96.2%. DNA–DNA relatedness value between strain Gsoil 1638^{T} and its closest phylogenetic neighbor, *Paenibacillus anaericanus*, was 62%. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 1638^{T} (= KCTC 13931^{T} = LMG 23406^{T} = CCUG 52472^{T}) was classified in the genus *Paenibacillus* as the type strain of a novel species, for which the name *Paenibacillus ginsengisoli* sp. nov. is proposed.

Keywords Facultative anaerobic · *Paenibacillus ginsengisoli* sp. nov. · Polyphasic taxonomy

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Introduction

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

was formed by 11 species, including *Paenibacillus polymyxa* as the type species. Currently, the genus *Paenibacillus* encompasses over 60 species and two subspecies (Euzéby 2006). Members of the genus *Paenibacillus* are aerobic or facultatively anaerobic organisms that produce ellipsoidal endospores in swollen sporangia and the cell wall shows structures typical of Gram-positive bacteria, but sometimes stains are Gram-negative. 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 a course of study on the culturable aerobic and facultative anaerobic bacterial community in 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 1638^T. Phenotypic, chemotaxonomic and phylogenetic analyses establish the affiliation of the isolate to the genus *Paenibacillus*. The data obtained in this study suggest that the isolate represents a novel species of this genus and the name *Paenibacillus ginsengisoli* sp. nov. is proposed.

Materials and methods

Isolation of bacterial strain and culture condition

Strain Gsoil 1638^T was isolated from a soil sample of a ginseng field in Pocheon province (South Korea). The soil sample was thoroughly suspended with 50 mM phosphate buffer (pH 7.0) and subsequently diluted serially in same buffer. Aliquots were plated on one fifth strength modified R2A media containing (per liter): 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 acid, 0.25 g soytone, 0.5 g dextrose, 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. 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 the modified R2A or one half strength modified R2A media. Strain Gsoil 1638^T was one of isolates that appeared on the modified-R2A agar plates in aerobic condition. The strain was routinely cultured on R2A agar (Difco) at 25°C and maintained as a glycerol suspension (20%, w/v) at –70°C.

Paenibacillus anaericanus KACC 11533^T (= DSM 15890^T) was received from the Korean Agricultural Culture Collection (Suwon, Republic of Korea) and used as a reference strain for DNA–DNA hybridization and other experiments.

Phenotypic and biochemical characteristics

Gram reaction was performed by the non-staining method as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at × 1000 with cells grown for 3 days at 25°C on R2A agar. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman (2002). For single-carbon-source assimilation studies, a defined liquid medium containing basal salts was used (per liter): 1.8 g K_2HPO_4, 1.08 g KH_2PO_4, 0.5 g $NaNO_3$, 0.5 g NH_4Cl, 0.1 g KCl, 0.1g $MgSO_4$, 0.05 g $CaCl_2$. To this medium, a vitamin solution (Widdel and Bak 1992), trace elements solution SL-10 (Widdel et al. 1983) and selenite/tungstate solution (Tschech and Pfennig 1984) were added and the pH of the medium was adjusted to 6.8. This liquid medium was aliquoted into 96 well trays each and filter-sterilized carbon sources were added into each well [individually at 0.1% (w/v)]. Growth in the 96 well plates was examined visually after incubation at 25°C for up to 7 days. A negative control well did not contain an added carbon source. A positive control included a well containing R2A broth. Fermentative acid production and oxidative acid production from carbohydrates were tested by growth in OF basal medium with bromothymol blue (Atlas 1993) supplemented with 1% carbohydrate [soft-agar stabs with (fermentative) and without (oxidative) sterile mineral oil overlay]. The OF medium tubes were incubated at 25°C for 5 days. Some physiological characteristics were determined with API 20E galleries according to the instructions of the manufacturer (bioMérieux, France). Tests for anaerobic growth was performed in serum bottles containing R2A broth supplemented with thioglycolate (1 g/l) under a nitrogen atmosphere. Tests for degradation of

DNA [DNASE agar, Scharlau (Spain), with DNAse activity by flooding plates with 1 M HCl], casein, chitin, starch (Atlas 1993), lipid (Kouker and Jaeger 1987), xylan, cellulose and collagen (Ten et al. 2004; 2005) were performed and evaluated after 7 days. Growth at different temperatures (4, 15, 20, 25, 30, 37, 42 and 45°C) and various pH values (pH 4.5–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A medium, containing (per liter): 0.5 g of proteose peptone, 0.5 g yeast extract, 0.5 g casamino acid, 0.5 g glucose, 0.5 g soluble starch, 0.3 g K_2HPO_4, 0.3 g sodium pyruvate, 0.05 g magnesium sulfate heptahydrate and 15 g agar, supplemented with 1–15% (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA), and MacConkey agar was also evaluated at 25°C.

PCR amplification, 16S rRNA gene sequencing and phylogenetic analysis

DNA was extracted using a commercial genomic DNA extraction kit (Core Biosystem, Korea) and PCR-mediated amplification of the 16S rRNA gene and sequencing of purified PCR product were carried out according to Kim et al. (2005). The 16S rRNA full gene sequences were compiled using SeqMan software (DNASTAR, Madison, WI, USA). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. The multiple alignments were performed 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). The phylogenetic trees were constructed by using the neighbor-joining method (Saitou and Nei 1987) and the maximum-parsimony method (Fitch 1972) using the MEGA3 Program (Kumar et al. 2004) with bootstrap values based on 1000 replications (Felsenstein 1985).

DNA extraction and determination of DNA G + C content

Chromosomal DNA for determination of G + C content was extracted from cells and purified as described by Moore (1995). DNA base composition was determined using the HPLC method. DNA was enzymatically degraded into nucleotides as described by Mesbah et al. (1989). The nucleotide mixture obtained was then separated by HPLC using a Waters Nova-Pak® C_{18} column (3.9×300 mm) and eluted by a mixture of 0.2 M $(NH_4)H_2PO_4$ and acetonitrile (20:1, v/v) at a flow rate of 1.0 ml/min and detected by UV absorbance at 270 nm. DNA of *Escherichia coli* (Sigma) was used as the calibration reference.

DNA–DNA hybridization

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). Hybridization was performed 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 DNA hybridization values.

Cellular fatty acids and isoprenoid quinones

Cellular fatty acids were analyzed in organisms grown on TSA agar for 3 days. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The fatty acids analyzed by a gas chromatograph (Hewlett Packard 6890) were identified by the Microbial Identification software package (Sasser 1990). 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). Then, the crude quinone in *n*-hexane was purified using Sep-Pak® Vac Cartridges Silica (Waters) and subsequently analyzed by HPLC, as previously described by Hiraishi et al. (1996).

Nucleotide sequence accession numbers

The 16S rRNA gene sequence of strain Gsoil 1638^T determined in this study has been deposited in NCBI GenBank under the accession number AB245382. The accession numbers of the

reference strains which are closely related to strain Gsoil 1638^T are indicated in Fig. 1.

Results and discussion

Strain Gsoil 1638^T was Gram-positive, aerobic or facultatively anaerobic and rod-shaped organism that was about 1.5–4.5 μm in length and 0.3–0.8 μm in width. Colonies of Gsoil 1638^T were circular, convex, smooth, non-glossy, slightly yellowish and 0.3–0.6 mm in diameter after incubation for 2 days on R2A at 25°C. The optimal temperature for growth was 25°C and growth occurred within the narrow range 20–30°C in contrast to closest phylogenetic relatives presented in Table 1. The pH range for growth was 5.0–8.5 and the optimum growth pH was 6.5–7.0. The strain Gsoil 1638^T was isolated from a non-saline environment and did not require NaCl to grow but, in contrast to phylogenetic neighbors, it can tolerate 10% (w/v) NaCl. Oxidase and catalase reactions were positive. Other physiological characteristics of strain Gsoil 1638^T are summarized in the species description. Physiological characteristics that differentiate strain Gsoil 1638^T from the closest members of the genus *Paenibacillus*, which were chosen according to 16S rDNA gene sequence similarities (see below) and constructed phylogenetic tree (Fig. 1), are

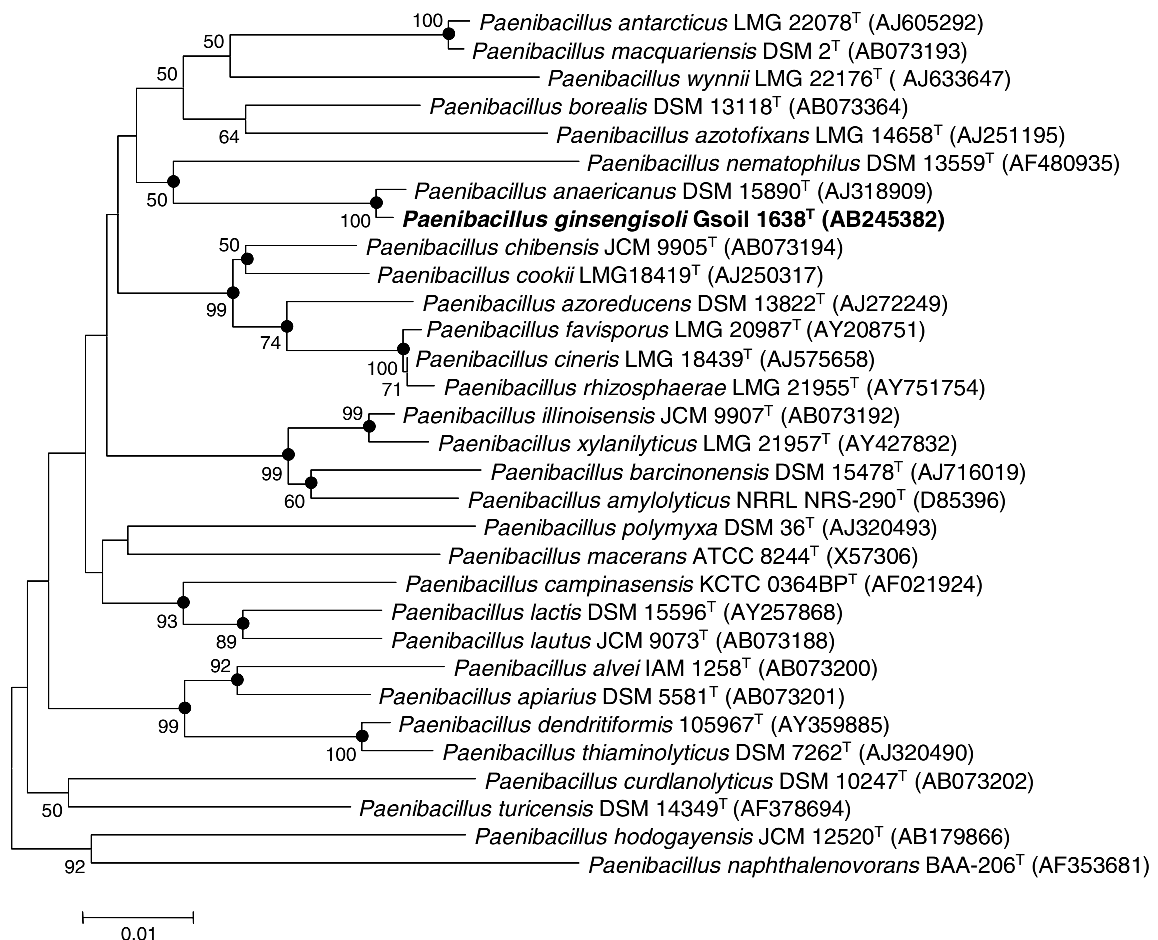

Fig. 1 Neighbor-joining tree (Saitou and Nei 1987) based on 16S rRNA gene sequences showing the phylogenetic position of strain Gsoil 1638^T among phylogenetic neighbors. Numbers on branch nodes are bootstrap values (1000 resamplings, only values over 49% are given). Dots indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm (Fitch 1972). Bar, 1% sequence divergence

Table 1 Comparison of the phenotypic characteristics of *Paenibacillus ginsengisoli* sp. nov. and those reported for phylogenetically related *Paenibacillus* species

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gram staining	+	−	−	+	−	−	−	+	+		−	−	+	+
Oxidase	+	+	−	−	−	+	+	+	+	−	−	−	+	−
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ONPG test	(+)	+	−		−	+	+	+	+	ND	−	+	−	+
Voges Proskauer test	+	−	+	+	−	+	−	−	+	+	−	−	−	+
Growth at/in														
3% (w/v) NaCl	+	−	−	ND	(+)	+	+	+	+	ND	+	−	+	+
10% (w/v) NaCl	(+)	−	−	−	−	−	−	−	−	−	−	−	−	−
5°C	−	+	−	−	+	−	−	ND	−	−	+	+	+	+
10°C	−	+	+	+	+	−	−	ND	+	−	+	+	+	+
37°C	−	+	+	+	+	+	+	+	+	+	−	−	−	−
pH 5.6	+	+	−	+	+	+	+	+	(+)	+	−	−	ND	+
Hydrolysis of														
Casein	−	−	−	−	+	(+)	(+)	−	−	−	−	−	−	+
Starch	−	+	+	+	−	ND	ND	+	ND	−	+	+	+	+
Gelatin	−	−	−	−	−	−	−	+	−	−	−	−	−	+
Esculin	−	+	+	ND	+	+	+	ND	ND	ND	+	+	−	+
Utilization of														
N-acetyl-D-glucosamine	−	+	ND	ND	+	ND	ND	ND	+	−	ND	ND	ND	−
L-arabinose	+	−	−	ND	+	ND	ND	−	ND	−	ND	ND	ND	+
D-melibiose	+	+	−	ND	v	ND	ND	ND	ND	+	ND	ND	ND	+
D-galactose	+	+	−	ND	+	ND	ND	ND	ND	+	ND	ND	ND	+
D-xylose	−	+	−	ND	+	ND	ND	+	ND	−	ND	ND	ND	+
Acetate	−	+	ND	−	−	ND	ND	ND	ND	+	ND	ND	ND	−
Acid production from														
D-mannitol	−	−	−	+	+	−	+	+	+	+	+	+	−	+
D-sorbitol	−	−		−	+	−	(+)	ND	ND	+	−	+	−	+
D-sucrose	−	−	+	+	+	+	+	+	+	+	+	+	+	+
D-melibiose	−	−	−	+	+	+	+	+	+	+	+	+	+	+
Amygdalin	−	−	+	ND	+	+	+	ND	+	+	+	+	+	+
L-arabinose	(+)	−	−	+	+	+	+	ND	+	−	−	v	+	+
DNA G + C mol%)	50.7	42.6[a]	44.0	52.8	53.6	51.6	51.5	53.0	50.9	48-53	39.0	44.6	40.7	43-46

Strains and data sources: 1, *Paenibacillus ginsengisoli* Gsoil 1638^T (present study); 2, *Paenibacillus anaericanus* KACC 11533^T (present study); 3, *Paenibacillus nematophilus* DSM 13559^T (Enright et al. 2003); 4, *Paenibacillus chibensis* JCM 9905^T (Shida et al. 1997b); 5 *Paenibacillus borealis* DSM 13118^T (Elo et al. 2001); 6, *Paenibacillus* cooki LMG 18419^T (Logan et al. 2004); 7, *Paenibacillus cineris* LMG 18439^T (Logan et al. 2004); 8, *Paenibacillus favisporus* LMG 20987^T (Velazquez et al. 2004); 9, *Paenibacillus rhizosphaerae* LMG 21955^T (Rivas et al. 2005); 10, *Paenibacillus azotofixans* DSM 5976^T (data from Seldin and Penido 1986; Pires and Seldin 1997; Shida et al. 1997b; Elo et al. 2001); 11, *Paenibacillus macquariensis* CIP 103269^T (data from Shida et al. 1997a; Elo et al. 2001; Rodríguez-Díaz et al. 2005); 12, *Paenibacillus wynnii* LMG 22176^T (Rodríguez-Díaz et al. 2005); 13, *Paenibacillus antarcticus* LMG 22078^T (Montes et al. 2004); 14, *Paenibacillus polymyxa* DSM 36^T (data from Shida et al. 1997a; Elo et al. 2001). *Symbols:* +, positive; (+), weakly positive; -, negative; ND, not determined

[a]Data from Horn et al. (2005)

listed by Table 1. According to comparative phenotypic analysis from our study, strain Gsoil 1638^T and *Paenibacillus anaericanus* KACC 11533^T had markedly different phenotypic profiles: only *P. anaericanus* uses D-raffinose, D-xylose, D-mannitol, glycerol, acetate, fumarate, L-alanine, L-arginine and *N*-acetyl-D-glucosamine as sole carbon sources for growth, can hydrolyse starch and esculin, and does not grow in 3–10% (w/v) NaCl. In contrast, strain Gsoil 1638^T utilized inulin, L-arabinose, L-xylose and oxalate as sole carbon sources for growth, produced acid from L-arabinose and did not grow at 5 and 40°C, with a narrow growth temperature range (20–30°C).

The predominant menaquinone was MK-7. The major fatty acids found in isolate Gsoil 1638^T are shown in Table 2 and are compared with

Table 2 Fatty acid composition of *Paenibacillus ginsengisoli* sp. nov. and phylogenetically related *Paenibacillus* species

Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Straight-chain saturated														
$C_{12:0}$	ND	ND	1.8	ND	ND[a]	1.2	ND	ND	ND	ND	ND	ND	ND	ND
$C_{14:0}$	8.5[b]	ND	15.5	0.3	18.6	4.3	3.0	3.0	3.0	5.0	0.7	5.7	1.6	0.7
$C_{15:0}$	2.9	ND	0.9	0.6	0.2	1.3	1.9	1.9	1.4	2.2	1.2	ND	4.2	0.5
$C_{16:0}$	25.4	11.0	22.7	5.3	10.0	11.6	18.7	18.7	16.5	15.5	2.9	30.9	4.0	9.1
$C_{18:0}$	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3	ND	ND	ND	1.1
$C_{17:0}$ 2-OH	ND	ND	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Branched saturated														
iso-$C_{14:0}$	2.5	ND	1.8	0.8	4.9	1.3	1.2	0.9	1.4	4.7	0.8	6.3	3.0	0.6
iso-$C_{15:0}$	5.9	37.5	1.5	4.0	11.5	6.3	6.2	6.5	8.0	8.7	5.2	6.9	15.0	5.5
iso-$C_{16:0}$	5.8	5.0	13.2	12.2	10.5	12.9	9.1	11.1	10.1	5.3	2.6	4.4	3.2	7.7
iso-$C_{17:0}$	2.0	7.4	1.4	3.2	4.9	4.3	3.0	3.3	4.1	1.1	0.4	1.7	2.1	7.0
iso-$C_{16:0}$3-OH	ND	ND	1.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
iso-$C_{17:0}$3-OH	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.9	ND	ND	ND	ND
anteiso-$C_{13:0}$	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.8	ND	ND	ND	ND
anteiso-$C_{15:0}$	43.7	12.6	27.7	57.8	35.2	37.3	43.2	48.8	45.5	45.4	81.0	34.0	55.3	49.9
anteiso-$C_{17:0}$	3.3	5.4	8.3	14.3	2.1	19.5	10.7	3.3	9.8	2.1	0.7	ND	1.9	16.7
Mono-unsaturated														
$C_{16:1}\omega$ 7c alcohol	ND	3.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.0	ND
$C_{16:1}\omega$ 11c	ND	8.1	ND	ND	1.3	ND	ND	ND	ND	ND	0.6	10.1	7.7	ND
iso-$C_{17:1}\omega$ 9c	ND	2.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
$C_{18:1}\omega$ 5c	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.8	ND	ND	ND	ND
Summed feature 4[c]	ND	2.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Others	ND	3.7	2.5	1.5	0.8	ND	3.0	2.5	0.2	3.2	3.9	ND	ND	1.2

Strains and data sources: 1, *Paenibacillus ginsengisoli* Gsoil 1638^T (present study); 2, *Paenibacillus anaericanus* KACC 11533^T (present study); 3, *Paenibacillus nematophilus* DSM 13559^T (Enright et al. 2003); 4, *Paenibacillus chibensis* JCM 9905^T (Shida et al. 1997b); 5, *Paenibacillus borealis* DSM 13118^T (Elo et al. 2001); 6, *Paenibacillus* cooki LMG 18419^T (Logan et al. 2004); 7, *Paenibacillus cineris* LMG 18439^T (Logan et al. 2004); 8, *Paenibacillus favisporus* LMG 20987^T (Rivas et al. 2005); 9, *Paenibacillus rhizosphaerae* LMG 21955^T (Rivas et al. 2005); 10, *Paenibacillus azotofixans* DSM 36^T (Yoon et al. 2003); 11, *Paenibacillus macquariensis* CIP 103269^T (Shida et al. 1997a); 12, *Paenibacillus wynnii* LMG 22176^T (Rodríguez-Díaz et al. 2005); 13, *Paenibacillus antarcticus* LMG 22078^T (Montes et al. 2004); 14, *Paenibacillus polymyxa* DSM 36^T (Yoon et al. 2003)

[a] Not detected

[b] Values are shown as a percentage of the total fatty acid content for each strain

[c] Summed feature represent group of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained $C_{16:1}\omega$ 7c and/or iso-$C_{15:0}$2-OH

values available for phylogenetically related *Paenibacillus* strains. Strain Gsoil 1638^T contained a large amount of anteiso-branched and straight-chain saturated fatty acids, namely 12-methyl tetradecanoic acid (anteiso-$C_{15:0}$) which is typical for members of the genus *Paenibacillus* (Shida et al. 1997a), and *n*-hexadecanoic acid ($C_{16:0}$). The content of $C_{16:0}$ (25%) is much higher than that reported for other members of the genus *Paenibacillus*: only a few *Paenibacillus* spp. such as *Paenibacillus wynnii* LMG 22176^T (31%) (Rodríguez-Díaz et al. 2005), *Paenibacillus nematophilus* DSM 13559^T (23%) (Enright et al. 2003) and *Paenibacillus azoreducens* DSM 13822^T (22%) (Meehan et al. 2001) have comparable levels of this fatty acid. Gsoil 1638^T could be differentiated from its closest phylogenetic neighbor *P. anaericanus* by its much higher content of anteiso-$C_{15:0}$ and $C_{16:0}$ and by the absence of any mono-unsaturated fatty acids. The G + C content for strain Gsoil 1638^T was 50.7 mol%, which lies within the range of 39–54% reported for the genus *Paenibacillus* (Shida et al. 1997a). This characteristic clearly distinguishes our isolate from *P. anaericanus* which had a G + C content of 42.6% (Horn et al. 2005).

The length of the almost complete 16S rRNA gene sequence of strain Gsoil 1638^T was 1495 bp.

Preliminary comparison of the sequence against the GenBank database indicated that members of the genus *Paenibacillus* were the closest phylogenetic neighbors. The phylogenetic tree (Fig. 1) based on the neighbor-joining algorithm showed that strain Gsoil 1638^T apparently clustered with *P. anaericanus* DSM 15890^T and that they shared 99.5% 16S rRNA gene sequence similarity with each other. According to the BLAST search, the next phylogenetically related species were *Paenibacillus borealis* DSM 13118^T (96.1%), *P. wynnii* LMG 22176^T (95.8%), *Paenibacillus macquariensis* DSM 2^T (95.3%) and *Paenibacillus chibensis* JCM 9905^T (95.2%). The phylogenetic distance from any other validly described species within the genus *Paenibacillus*, including *Paenibacillus cooki* LMG 18419^T, *Paenibacillus cineris* LMG 18439^T, *Paenibacillus favisporus* LMG 20987^T, *Paenibacillus rhizosphaerae* LMG 21955^T, *Paenibacillus antarcticus* LMG 22078^T and *Paenibacillus azotofixans* DSM 5976^T was less than 95%. Generally recommended and accepted criteria for delineating bacterial species state that strains with a DNA–DNA relatedness below 70%, as measured by hybridization, or with 16S rDNA gene sequence dissimilarity above 3% are considered as belonging to separate species (Wayne et al. 1987; Stackebrandt and Goebel 1994; Stackebrandt et al. 2002). Taking into account this definition, the above mentioned data indicate that strain Gsoil 1638^T is species that is clearly separated from other *Paenibacillus* spp., with the exception of *P. anaericanus*. DNA–DNA hybridization experiments were performed to establish whether our strain and *P. anaericanus* are related at species level or not. The DNA–DNA relatedness value of strain Gsoil 1638^T to the closest phylogenetic relative, *P. anaericanus* KACC 11533^T, was 62%. Although this value is just below the threshold for species delineation, the two strains were found to be phenotypically and chemotaxonomically distinct from each other (see above), which supports separate species status for these two members of the genus *Paenibacillus*.

Taxonomic conclusions

The results obtained from the phenotypic and phylogenetic characterizations indicated that strain Gsoil 1638^T belongs to the genus *Paenibacillus*. The phylogenetic distinctiveness and DNA–DNA hybridization experiments confirmed that strain Gsoil 1638^T represents a species distinct from recognized *Paenibacillus* species. Differential taxonomic properties of our isolate and its closest neighbors are detailed in Table 1. Comparative phenotypic and chemotaxonomic analysis from this study confirmed that strain Gsoil 1638^T differs from closest relative, *P. anaericanus*, in terms of several phenotypic characteristics and cellular fatty acid composition. Therefore, on the basis of the data presented, strain Gsoil 1638^T should be classified in the genus *Paenibacillus* as a novel species, for which the name *Paenibacillus ginsengisoli* sp. nov. is proposed.

Description of *Paenibacillus ginsengisoli* sp. nov.

Paenibacillus ginsengisoli (gin.sen.gi.so′li. N.L. n. ginsengum, ginseng; L. n. solum, soil; N.L. gen. n. ginsengisoli, of soil of a ginseng field, the source of the organism).

The description is based on phenotypic and chemotaxonomic data taken from this investigation of the strain Gsoil 1638^T. Cells are Gram-positive, aerobic or facultative anaerobic, motile, and rod-shaped, 1.5–4.5 μm in length and 0.3–0.8 μm in width. Oval terminal spores are observed in swollen sporangia. Colonies on R2A medium after 2 days are 0.3–0.6 mm in diameter, circular, convex, smooth, non-glossy and slightly yellowish. Oxidase and catalase reactions are positive, lipase is negative. Growth occurs at 20 and 30°C, but not at 15 and 37°C. The bacterium grows within pH values of between 5.0 and 8.5; the optimum pH is 6.5–7.0. The bacterium tolerates 10% (w/v) NaCl. Growth occurs on TSA and R2A media, but not on MacConkey agar. Strain does not hydrolyse casein, collagen, esculin, starch, chitin, CM-cellulose, DNA, and xylan. The following substrates are utilized for growth as sole carbon sources: D-glucose, D-galactose, D-mannose, L-arabinose, L-xylose, D-cellobiose, D-lactose, D-maltose, D-melibiose, D-sucrose, D-trehalose, salicin, amygdalin, glycogen, dextran, inulin and oxalate. The following substrates are

not utilized for growth: D-fructose, D-xylose, D-fucose, L-rhamnose, L-sorbose, D-arabinose, D-lyxose, D-raffinose, D-ribose, inositol, D-mannitol, xylitol, glycerol, ethanol, D-adonitol, dulcitol, D-sorbitol, methanol, *N*-acetyl-glucosamine, pyruvate, acetate, 3-hydroxybutylate, valerate, fumarate, citrate, lactate, malate, succinate, tartrate, gluconate, formate, propionate, caprate, maleic acid, phenyl-acetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, malonate, glutarate, itaconate, adipate, suberate, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamate, L-glutamine, L-histidine, L-proline, L-threonine, L-cysteine, glycine, L-iso-leucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-tryptophane, L-tyrosine and L-valine. In API 20E tests, the ONPG reaction is weakly positive, the Voges–Proskauer test is positive; gelatin hydrolysis, lysine decarboxylase, arginine dihydrolaseornithine decarboxylase, tryptophane deaminase, hydrogen sulphide production, urease production, and indole production are all negative. Acid is weakly produced from D-glucose and L-arabinose but not from D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose and amygdalin. The major fatty acids are anteiso-$C_{15:0}$ and $C_{16:0}$. DNA G + C content is 50.7 mol%.

The type strain Gsoil 1638^T (= KCTC 13931^T = LMG 23406^T = CCUG 52472^T) was isolated from a soil sample of a ginseng field in Pocheon province (South Korea). The DDBJ/EMBL/NCBI GenBank accession number for 16S rRNA gene sequence of strain Gsoil 1638^T is AB245382.

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