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Cohnella panacarvi sp. nov., a Xylanolytic Bacterium Isolated from Ginseng Cultivating Soil

YOON, MIN-HO[1]*, LEONID N. TEN[2], AND WAN-TAEK IM[3]

[1]*Department of BioEnvironmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Korea*
[2]*Department of Biology & Medicinal Sciences, Pai Chai University, Daejeon 302-735, Korea*
[3]*Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea*

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Abstract A Gram-positive, aerobic, rod-shaped, nonmotile, endospore-forming bacterium, designated Gsoil 349^T, was isolated from soil of a ginseng field and characterized using a polyphasic approach. Comparative analysis of 16S rRNA gene sequences revealed that the strain Gsoil 349^T belongs to the family *Paenibacillaceae*, and the sequence showed closest similarity with *Cohnella thermotolerans* DSM 17683^T (94.1%) and *Cohnella hongkongensis* DSM 17642^T (93.6%). The strain showed less than 91.3% 16S rRNA gene sequence similarity with *Paenibacillus* species. In addition, the presence of MK-7 as the major menaquinone and anteiso-$C_{15:0}$, iso-$C_{16:0}$, and $C_{16:0}$ as major fatty acids suggested its affiliation to the genus *Cohnella*. The G+C content of the genomic DNA was 53.4 mol%. On the basis of its phenotypic characteristics and phylogenetic distinctiveness, strain Gsoil 349^T should be treated as a novel species within the genus *Cohnella* for which the name *Cohnella panacarvi* sp. nov. is proposed. The type strain is Gsoil 349^T (=KCTC 13060^T= DSM 18696^T).

Keywords: *Cohnella panacarvi* sp. nov., ginseng soil, polyphasic taxonomy

The genus *Paenibacillus* was defined in 1993 after an extensive comparative analysis of 16S rRNA gene sequences of 51 species of the genus *Bacillus* [1, 2]. Currently, the genus *Paenibacillus* encompasses over 70 species [7], which have been isolated from various ecological habitats, including soils [26] and the phyllospheres and rhizospheres of trees [6, 20]. As was noticed by Kämpfer *et al*. [12], although some progress has been made in the reclassification of the bacilli, some questions are still remaining, and extensive comparative work would lead to the creation of novel taxa that may be closely associated with the genera *Bacillus* or *Paenibacillus*. In particular, "*Paenibacillus hongkongensis*" was recently reclassified as *Cohnella hongkongensis* into the novel genus *Cohnella* that currently also includes *Cohnella thermotolerans* as the type species. Members of the genus *Cohnella* are spore-forming, aerobic, nonmotile, and thermotolerant organisms that contain MK-7 as the main menaquinone and iso-$C_{16:0}$, anteiso-$C_{15:0}$, and $C_{16:0}$ as major fatty acids.

During the course of study of the culturable aerobic and facultative 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 349^T. Phenotypic, chemotaxonomic, and phylogenetic analyses have established the affiliation of this isolate to the genus *Cohnella*. The data obtained in this study suggest that the isolate represents a novel species of this genus, and the name *Cohnella panacarvi* sp. nov. is proposed.

MATERIALS AND METHODS

Isolation of Bacterial Strain and Culture Condition

Strain Gsoil 349^T was originally isolated from soil of a ginseng field in Pocheon Province (South Korea). The soil sample was suspended in 50 mM phosphate buffer (pH 7.0), and serial decimal dilutions of the suspension were spread on modified-R2A agar plates (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,

*Corresponding author
Phone: 82-42-821-6733; Fax: 82-42-823-9241;
E-mail: mhyoon@cnu.ac.kr

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 $CaCl_2$, 15 g agar per liter). The plates were incubated at 30°C for one month. Single colonies on the plates were purified by transferring them onto new plates and were incubated once again on the modified R2A medium. Strain Gsoil 349^T was one of the isolates that appeared on the modified R2A agar plates under aerobic condition. It was routinely cultured on R2A agar at 30°C and maintained as a glycerol suspension (20%, w/v) at −70°C.

Phenotypic and Biochemical Characteristics

Gram reaction was performed by the nonstaining method as described by Buck [4]. Cell morphology and motility were observed with a Nikon light microscope at ×1,000 using the hanging drop technique, with the cells allowed to grow on R2A agar for 3 days at 30°C. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman [5]. Acid production from 49 carbon sources was tested at 30°C with API 50 CH in combination with API 50CHB/E medium (bioMérieux) 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 growth at a variety of pH values was assessed in R2A broth. Substrate utilization as sole carbon source and some physiological characteristics were determined with API 32GN, API 20NE, and API 20E galleries according to the instructions of the manufacturer (bioMérieux). Tests for degradation of DNA (DNASE agar, Scharlau, with DNAse activity by flooding plates with 1 M HCl), casein, chitin, starch [3], lipid [16], xylan, and cellulose [25] were performed and evaluated after 10 days. Salt tolerance was tested on R2A medium supplemented with 1–10% (w/v) NaCl after 5 days of incubation. Growth on nutrient agar, trypticase soy agar (TSA), and MacConkey agar was also valuated at 30°C.

PCR Amplification, 16S rRNA Gene Sequencing, and Phylogenetic Analysis

For phylogenetic analysis of strain Gsoil 349^T, DNA was extracted using a genomic DNA extraction kit (Core Biosystem, Korea), the 16S rRNA gene was amplified by PCR, and sequencing of the purified PCR product was carried out according to Kim *et al*. [14]. The 16S rRNA gene full sequences were compiled using the SeqMan software (DNASTAR, Madison, WI, U.S.A.). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by the Clustal_X program [28]. Gaps were edited in the BioEdit program [10]. Evolutionary distances were calculated using the Kimura two-parameter model [15]. The phylogenetic trees were constructed by using the neighbor-joining method [21], and the maximum-parsimony method [9] using the MEGA3 Program [17] with bootstrap values based on 1,000 replications [8].

Determination of DNA G+C Content

For the measurement of G+C content of the chromosomal DNA, the genomic DNA of the strain was extracted and purified as described by Moore [19], enzymatically degraded into nucleosides, and then the G+C content of DNA was determined as described by Mesbah *et al.* [18] using a reverse-phase HPLC.

Cellular Fatty Acids and Isoprenoid Quinones

Cellular fatty acid profiles were determined for strains grown on TSA agar (Difco) for three 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 analyzed by gas chromatography (Hewlett Packard 6890) using the Microbial Identification software package [22]. The value range was obtained by duplicate experiments. 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 analyzed by HPLC, as previously described by Shin *et al.* [23].

Nucleotide Sequence Accession Numbers

The 16S rRNA gene sequence of strain Gsoil 349^T determined in this study was deposited in NCBI/EMBL/DDBJ under the accession number AB271056. The accession numbers of the reference strains that are closely related to strain Gsoil 349^T are indicated in Fig. 1.

RESULTS AND DISCUSSION

Morphological and Phenotypic Characteristics

Cells of strain Gsoil 349^T were Gram-positive, nonmotile, thin-rods that formed oval spores that lay central in swollen sporangia. After two day on R2A, colonies were 0.5–1.0 mm in diameter, circular, convex, non-glossy, and white colored. On R2A agar, the optimum temperature for growth was 30°C and growth occurred within the range 18–45°C. Growth occurred in the absence of NaCl and in the presence of 1.0% (w/v) NaCl, but not in the presence of 2% (w/v) NaCl. Strain Gsoil 349^T showed oxidase and catalase activities and actively hydrolyzed xylan. Physiological characteristics that differentiate strain Gsoil 349^T from its closest phylogenetic relatives are listed in Table 1. It should be noted that in contrast to *Cohnella thermotolerans* DSM 17683^T and *Cohnella hongkongensis* DSM 17642^T, strain Gsoil 349^T was able to produce acid from many carbohydrates. At the same time, it was found that strain Gsoil 349^T as well as two other *Cohnella* species utilized D-glucose, L-arabinose, D-mannose, and D-maltose, but these three strains did not assimilate propionate, phenylacetate,

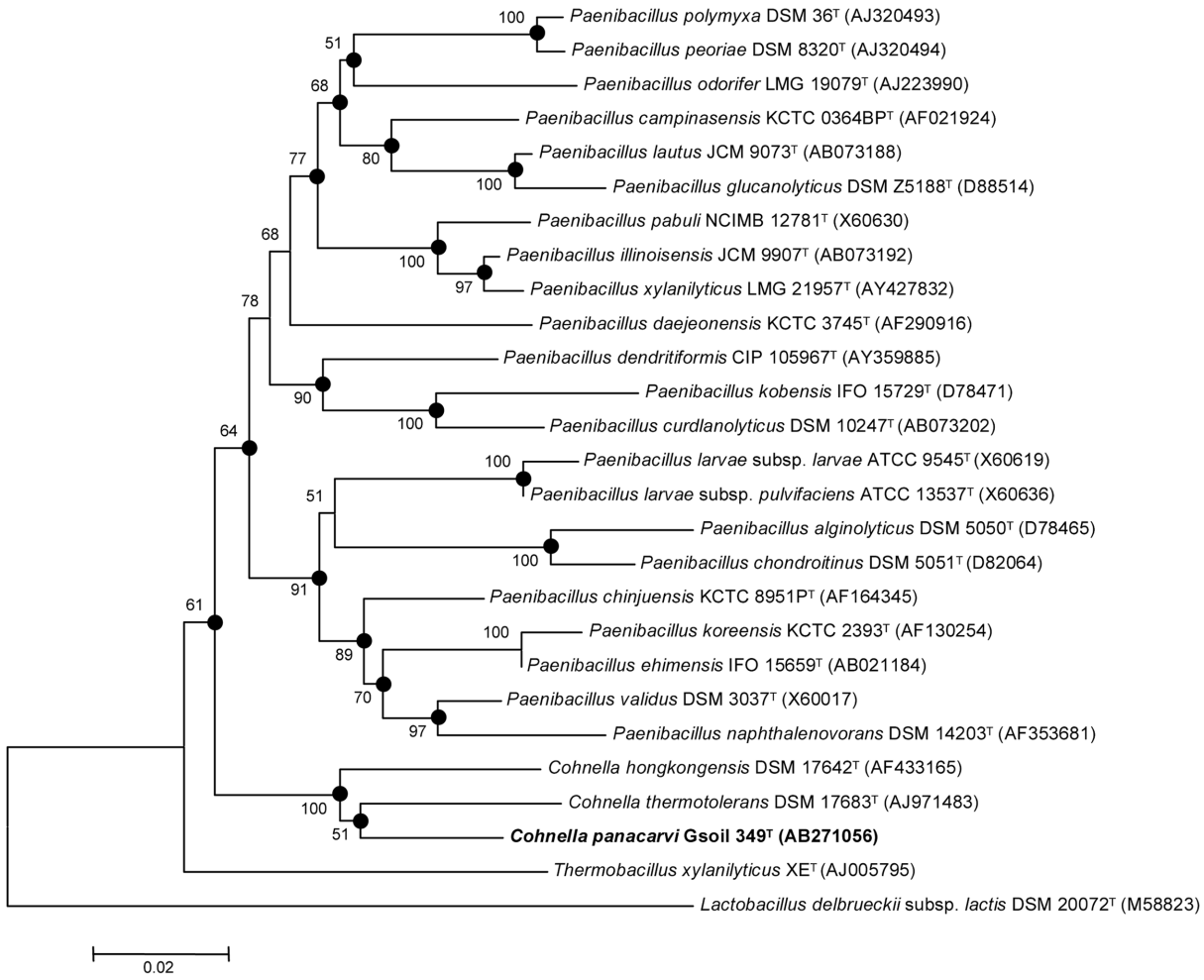

Fig. 1. Neighbor-joining tree (based on 16S rRNA gene sequences) showing the phylogenetic positions of strain Gsoil 349^T among neighboring species of the genera *Cohnella* and *Paenibacillus*.

Numbers on branch nodes are bootstrap values (1,000 resamplings; only values over 50% are given). Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. The tree was rooted by using *Lactobacillus delbrueckii* subsp. *lactis* as an outgroup. Bar, 2 % sequence divergence.

3-hydroxybenzoate, 4-hydroxybenzoate, acetate, citrate, lactate, malate, adipate, suberate, D-sorbitol, D-mannitol, inositol, L-serine, L-tryptophane, L-alanine, L-histidine, and L-proline as sole carbon source.

Cellular Fatty Acid and Menaquinone Compositions

The predominant menaquinone was MK-7. The respiratory quinone system supports affiliation of Gsoil 349^T to the genus *Cohnella*, where all species have MK-7 as the major quinone [12]. The fatty acids found in isolate Gsoil 349^T are shown in Table 2 and are compared with values available for other *Cohnella* species. Anteiso-$C_{15:0}$, iso-$C_{16:0}$, and $C_{16:0}$, the major fatty acids in the genus *Cohnella* [12], were also the predominant fatty acid components of strain Gsoil 349^T, comprising 79.9% of the total. At the same time, some qualitative and quantitative differences in fatty acid content could be observed between the strain Gsoil 349^T and the phylogenetically closest relatives. The second major fatty acid found in the strain Gsoil 349^T was iso-$C_{16:0}$ comprising 16.8%. This value is higher than that reported for *Cohnella hongkongensis* DSM 17642^T but lower than that found for *Cohnella thermotolerans* DSM 17683^T [12]. A similar situation is observed with the third major fatty acid $C_{16:0}$ (Table 2). It should be noticed that the fatty acid profile of Gsoil 349^T was similar to that of some *Paenibacillus* species; but according to fatty acid data compiled for paenibacilli by Kämpfer [11], strain Gsoil 349^T could be differentiated from *Paenibacillus* species by significantly larger amounts of iso-$C_{16:0}$ and $C_{16:0}$.

DNA G+C Content

The DNA G+C content of strain Gsoil 349^T was 53.4 mol%, which is close to the values observed for other members of the genus *Cohnella*.

Table 1. Comparison of the phenotypic characteristics of *Cohnella panacarvi* sp. nov. and phylogenetically related *Cohnella* species.

Characteristic	1	2	3
Nitrate reduction	+	ND	+
Oxidase	+	+	ND
Catalase	(+)	ND	(+)
Assimilation of			
N-Acetyl-D-glucosamine	–	–	+
Gluconate	–	+	+
D-Melibiose	–	+	+
L-Rhamnose	–	(+)	+
D-Ribose	–	+	+
Salicin	+	–	–
Acid production from			
L-Arabinose	+	–	–
D-Cellobiose	+	–	–
Dulcitol	+	–	–
D-Glucose	+	–	–
D-Maltose	+	–	–
D-Mannose	+	–	–
D-Melibiose	+	–	–
Methyl α-D-glucoside	+	–	–
D-Lactose	+	–	–
D-Raffinose	+	–	–
Sucrose	+	–	–
D-Trehalose	+	–	–
D-Xylose	+	–	–
DNA G+C content (mol%)	53.4	59.0	47.6

Strains: 1, *Cohnella panacarvi* Gsoil 349^T (present study); 2, *Cohnella thermotolerans* DSM 17683^T [12]; 3, *Cohnella hongkongensis* DSM 17642^T [12, 27].
Results are scored as +, positive; –, negative; (+), weakly positive; ND, not determined.
[a]Data in parenthesis are from Kämpfer *et al.* [12].

Table 2. Cellular fatty acid profiles of *Cohnella panacarvi* Gsoil 349^T and other species of the genus *Cohnella*[a].

Fatty acids	1	2	3
Straight-chain saturated			
$C_{14:0}$	2.5	1.0	5.0
$C_{15:0}$	2.4	1.4	8.0
$C_{16:0}$	9.4	6.6	25.3
$C_{17:0}$	ND[b]	ND[b]	1.2
Branched saturated			
iso-$C_{14:0}$	3.6	2.1	2.3
iso-$C_{15:0}$	6.0	3.2	8.1
iso-$C_{16:0}$	16.8	45.5	11.9
iso-$C_{17:0}$	0.9	ND[b]	ND[b]
anteiso-$C_{13:0}$	0.8	ND[b]	0.8
anteiso-$C_{15:0}$	53.7	28.4	31.2
anteiso-$C_{17:0}$	2.6	6.7	2.6
Monounsaturated			
$C_{16:1}\omega7c$ alcohol	0.5	ND[b]	ND[b]
$C_{16:1}\omega11c$	0.8	ND[b]	0.9
$C_{17:1}\omega6c$	ND[b]	1.0	ND[b]
iso-$C_{17:1}$	ND[b]	1.1	1.9
$C_{18:1}\omega7c$	ND[b]	4.0	ND[b]

Strains: 1, *Cohnella panacarvi* Gsoil 349^T (present study); 2, *Cohnella thermotolerans* DSM 17683^T [12]; 3, *Cohnella hongkongensis* DSM 17642^T [12].
[a]Values are shown as a percentage of the total fatty acid content for each strain.
[b]ND, not detected.

Phylogenetic Analysis Based on 16S rRNA Gene Sequences

Comparative 16S rRNA gene sequence analyses of strain Gsoil 349^T (1,478 bp) showed that the strain belongs to the family *Paenibacillaceae*. The phylogenetic tree (Fig. 1) based on the neighbor-joining and maximum-parsimony algorithms showed that strain Gsoil 349^T was not affiliated to the *Paenibacillus* species but that it fell within the radiation of the cluster comprising the *Cohnella* species. The levels of 16S rRNA gene sequence similarity to *Cohnella thermotolerans* DSM 17683^T and *Cohnella hongkongensis* DSM 17642^T were 94.1 and 93.6%, respectively. Significantly lower sequence similarities (<91.3%) were found with all recognized species of the genus *Paenibacillus.* The phylogenetic definition of a species generally includes "strains with approximately 70% or greater DNA-DNA relatedness" [29]. According to the available compilation of data, organisms that have less than 97.0% sequence similarity will not reassociate to more than 60%, irrespective of the hybridization method applied [13, 24]. This phylogenetic result demonstrated that strain Gsoil 349^T was not related to any of the previously described *Cohnella* taxa at the species level.

Taxonomic Conclusions

All characteristics determined for strain Gsoil 349^T are in accordance with those of the genus *Cohnella.* On the basis of phylogenetic distance from established *Cohnella* species, indicated by relatively low 16S rRNA gene sequence similarities (<94.2%) and a specific combination of phenotypic characteristics, it is demonstrable that Gsoil 349^T is not affiliated to any species of this genus. Therefore, on the basis of the data presented, strain Gsoil 349^T should be placed in the genus *Cohnella* as a novel species, for which the name *Cohnella panacarvi* sp. nov. is proposed.

Description of *Cohnella panacarvi* sp. nov.

Cohnella panacarvi (pa.na.car.vi. N.L. n. Panax-acis, scientific name of ginseng; arvum, a field; N.L. gen. n. *panacarvi*, of a ginseng field).

Cells are Gram-positive, aerobic, nonmotile, spore-forming, and thin rod-shaped with length of approximately 1.5–3.5 μm and width of 0.2–0.4 μm. Spores are oval, central, occurring in swollen sporangia. After two day on R2A, colonies are 0.5–1.0 mm in diameter, circular, convex,

nonglossy, and white colored. Oxidase reaction is positive and activity for catalase is weakly positive. Nitrate is reduced to nitrite in aerobic conditions. Grows between 18°C and 45°C; the optimum temperature for growth is 30°C. The bacterium grows within pH values of between 5.5 and 8.0; the optimum pH is 6.5–7.0. The strain tolerates 1% (w/v) NaCl, but not 2%. Growth occurs on TSA and nutrient agar but not on MacConkey agar. Able to hydrolyze xylan and starch (weakly), but not DNA, chitin, cellulose, and casein. Activities for urease and gelatinase are negative. Activities for β-galactosidase and β-glucosidase are positive. The following substrates are utilized for growth: D-glucose, L-arabinose, D-mannose, D-maltose, and salicin. The following substrates are not utilized for growth: L-fucose, L-rhamnose, D-ribose, gluconate, propionate, caprate, phenylacetate, 3-hydroxybenzoate, 4-hydroxybenzoate, malonate, acetate, 3-hydroxybutylate, valerate, citrate, lactate, malate, 5-ketogluconate, 2-ketoglutarate, itaconate, adipate, suberate, D-sucrose, D-sorbitol, D-mannitol, inositol, D-melibiose, glycogen, *N*-acetyl-D-glucosamine, gelatin, urea, nitrate, L-serine, L-tryptophane, L-alanine, L-arginine, L-histidine, and L-proline. In API 20E tests, Voges-Proskauer test, activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophane deaminase, gelatin hydrolysis, and indole and hydrogen sulfide production are negative. Acids are produced from L-arabinose, D-galactose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, methyl α-D-glucoside, amygdalin, D-cellobiose, D-maltose, D-melibiose, D-sucrose, D-lactose, D-trehalose, D-raffinose, and D-turanose. Acids are not produced from D-arabinose, erythritol, L-xylose, adonitol, glycerol, L-sorbose, inositol, D-mannitol, D-sorbitol, methyl α-D-mannoside, methyl β-D-xyloside, *N*-acetyl-D-glucosamine, arbutin, salicin, inulin, D-melezitose, starch, glycogen, xylitol, β-gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, and 5-ketogluconate. MK-7 is the predominant menaquinone. The predominant fatty acid is anteiso-$C_{15:0}$ and iso-$C_{16:0}$. The G+C content of the genomic DNA is 53.4 mol%.

The type strain, Gsoil 349^T (= KCTC 13060^T=DSM 18696^T), was isolated from soil of a ginseng field of Pocheon Province, South Korea.

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