Paenibacillus ginsengihumi sp. nov., a bacterium isolated from soil in a ginseng field

Myung Kyum Kim, Young-Ae Kim, Min-Ju Park and Deok-Chun Yang

Correspondence
Deok-Chun Yang
dcyang@khu.ac.kr

Korean Ginseng Center and Ginseng Genetic Resource Bank, Kyung Hee University,
1 Seocheon-dong, Giheung-gu, Yongin, Gyeonggi-do 446-701, Republic of Korea

Strain DCY16^T, a Gram-positive, spore-forming, rod-shaped, motile bacterium, was isolated from soil and characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain DCY16^T belonged to the genus *Paenibacillus*; highest sequence similarities were with *Paenibacillus validus* JCM 9077^T (94.4%), *P. chinjuensis* WN9^T (94.4%), *P. naphthalenovorans* DSM 14203^T (94.2%), *P. ehimensis* KCTC 3748^T (92.8%) and *P. elgii* KCTC 10016BP^T (92.4%). Chemotaxonomic data revealed that strain DCY16^T possessed menaquinone MK-7 and the predominant fatty acids were C_{15:0} anteiso, C_{17:0} anteiso, C_{16:0} and C_{16:0} iso. The DNA G+C content of strain DCY16^T was 50.9 mol%. Results of physiological and biochemical tests clearly demonstrated that strain DCY16^T represents a distinct *Paenibacillus* species. Based on these data, DCY16^T (=KCTC 13141^T =JCM 14928^T) should be classified as the type strain of a novel species, for which the name *Paenibacillus ginsengihumi* sp. nov. is proposed.

For many years, most aerobic, endospore-forming rods were assigned to the genus *Bacillus* (Claus & Berkeley, 1986). Recently, genomic analyses have shown that such classification has led to the genus *Bacillus* becoming a systematically heterogeneous taxon (Priest, 1981; Ash *et al.*, 1991; Slepecky & Hemphill, 1992; Stackebrandt & Liesack, 1993). 16S rRNA gene sequence analyses have revealed several phylogenetically distinct lineages within the genus *Bacillus* and some phylogenetic groups have been reclassified as new genera, e.g. *Alicyclobacillus* (Wisotzkey *et al.*, 1992), *Aneurinibacillus* (Shida *et al.*, 1996), *Brevibacillus* (Shida *et al.*, 1996), *Gracilibacillus* (Wainø *et al.*, 1999), *Halobacillus* (Spring *et al.*, 1996), *Paenibacillus* (Ash *et al.*, 1993), *Salibacillus* (Wainø *et al.*, 1999) and *Virgibacillus* (Heyndrickx *et al.*, 1998).

The genus *Paenibacillus* was proposed by Ash *et al.* (1993) to accommodate 11 species previously assigned to the genus *Bacillus* and its description was later emended by Shida *et al.* (1997). Members of the genus *Paenibacillus* are facultatively anaerobic or strictly aerobic, rod-shaped bacteria that produce ellipsoidal spores in swollen sporangia and have DNA G+C contents of 45–54 mol% (Ash *et al.*, 1993). *Paenibacillus* species have been isolated from various ecological habitats, including warm springs (Saha

et al., 2005), rice fields (Sánchez *et al.*, 2005), alkaline soils (Yoon *et al.*, 2005) and petroleum-hydrocarbon-contaminated sediment (Montes *et al.*, 2004). At the time of writing, there are more than 70 species of the genus *Paenibacillus* with validly published names; *Paenibacillus polymyxa* is the type species.

In a series of studies, attempts were made to isolate microorganisms from soils to investigate the community structure based on a culture-dependent method. In this study, one strain, DCY16^T, was isolated from soil of a ginseng field in South Korea and characterized using a polyphasic approach. The polyphasic approach, including phylogenetic analysis based on 16S rRNA gene sequences, genomic relatedness and determination of chemotaxonomic and phenotypic properties, was conducted to determine the precise taxonomic position of strain DCY16^T.

Strain DCY16^T was isolated from soil of a ginseng field via direct plating onto R2A agar (Difco). Single colonies on these plates were purified by transferring them onto new plates and subjecting them to an additional incubation for 5 days at 30 °C. Purified colonies were tentatively identified by using partial 16S rRNA gene sequences.

Cell morphology and motility were observed with a Nikon light microscope (×1000 magnification) and by transmission electron microscopy (JEM-1010) after negative staining with 1% (w/v) phosphotungstic acid. Gram reactions were determined according to the non-staining method, as described by Buck (1982). Oxidase activity was evaluated via the oxidation of 1% *p*-aminodimethylaniline

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY16^T is EF452662.

An extended phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain DCY16^T and members of the genus *Paenibacillus* is available as supplementary material with the online version of this paper.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain DCY16^T and closely related *Paenibacillus* species. Bar, 0.02 substitutions per nucleotide position. Bootstrap values, expressed as percentages of 1000 replications, are shown at branch points.

oxalate. Catalase activity was assessed by observing bubble production after the application of 3% (v/v) hydrogen peroxide solution. Acid production from carbohydrates was assessed by the procedures outlined by Cappuccino & Sherman (2002). Growth at various temperatures (4, 25, 30, 37 and 42 °C) was assessed on R2A agar and growth at different pH values was assessed in R2A broth. Growth on nutrient agar, trypticase soy agar (TSA), Luria–Bertani agar and MacConkey agar was also evaluated at 30 °C. The API 20NE and API ID32 GN microtest systems were used in these tests according to the recommendations of the manufacturer (bioMérieux).

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified via TLC and subsequently analysed by HPLC, as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). To perform fatty acid methyl ester analysis, the strain was allowed to grow on TSA for 48 h at 30 °C and then two loopfuls of the well-grown cells were harvested. Fatty acid methyl esters were prepared, separated and identified with the Sherlock Microbial Identification system (MIDI, Inc.) (Sasser, 1990).

For determination of G+C content, genomic DNA was extracted and purified with the Genomic-tip 100/G system (Qiagen) and then degraded enzymically into nucleosides. Nucleosides were analysed by HPLC as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989).

The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primer set 9F and 1512R (Weisburg *et al.*, 1991) and the purified PCR product was sequenced by Genotec, Daejeon, Korea (Kim *et al.*, 2005). The full sequence of the 16S rRNA gene was compiled with SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank and edited using the program BIOEDIT (Hall, 1999). Multiple alignments were performed with the program CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). The phylogenetic tree was constructed via the neighbour-joining

Table 1. Cellular fatty acid profiles of strain DCY16^T and type strains of closely related *Paenibacillus* species

Strains: 1, *P. ginsengihumi* sp. nov. DCY16^T (data from this study); 2, *P. validus* LMG 11161^T (data from Heyndrickx *et al.*, 1995); 3, *P. chinjuensis* WN9^T (Yoon *et al.*, 2002); 4, *P. naphthalenovorans* PR-N1^T (Daane *et al.*, 2002); 5, *P. ehimensis* IFO 15659^T (Lee *et al.*, 2004); 6, *P. elgii* SD17^T (Kim *et al.*, 2004); 7, *P. chitinolyticus* IFO 15660^T (Lee *et al.*, 2004); 8, *P. panacisoli* Gsoil 1411^T (Ten *et al.*, 2006). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. tr, Trace (<0.5%); ND, not detected/reported.

Fatty acid	1	2	3	4	5	6	7	8
Saturated								
C _{13:0} anteiso	ND	0.1	0.2	ND	ND	ND	ND	ND
C _{13:0} iso	ND	0.1	ND	ND	ND	ND	ND	ND
C _{14:0}	1.3	1.7	0.8	2.5	1.0	1.9	ND	ND
C _{14:0} iso	1.3	2.3	2.2	ND	1.8	2.1	ND	6.9
C _{15:0}	1.0	1.5	4.5	ND	1.2	1.2	ND	2.8
C _{15:0} anteiso	48.5	52.7	66.1	51.8	52.9	54.1	58.7	44.8
C _{15:0} iso	3.1	15.1	5.0	5.3	8.1	10.6	3.6	5.0
C _{16:0}	9.6	7.2	5.3	14.4	7.1	9.7	6.9	9.4
C _{16:0} iso	9.5	6.3	7.0	5.6	8.6	6.5	8.4	20.2
C _{17:0}	6.3	0.2	0.3	ND	ND	ND	ND	2.1
C _{17:0} anteiso	18.3	4.6	6.1	7.9	8.0	6.2	7.3	5.6
C _{17:0} iso	ND	3.3	1.6	3.2	3.3	3.2	2.0	ND
C _{18:0}	ND	0.3	ND	ND	ND	ND	ND	1.1
Unsaturated								
C _{16:1} ω 7c	ND	0.9	ND	ND	1.2	ND	ND	ND
C _{16:1} ω 11c	1.0	2.6	ND	9.3	5.2	4.5	tr	ND
C _{17:1} iso ω 10c	ND	0.5	ND	ND	ND	ND	ND	ND
C _{18:1} ω 9c	ND	0.3	ND	ND	ND	ND	ND	ND
C _{13:0} 2-OH	ND	ND	0.6	ND	ND	ND	ND	ND
C _{13:0} iso 3-OH	ND	ND	0.3	ND	ND	ND	ND	ND
Summed feature 5*	ND	0.3	ND	ND	ND	ND	ND	ND
ECL 14.966	ND	ND	ND	ND	ND	ND	ND	2.1

*Summed feature 5 contains C_{17:1} iso I and/or C_{17:1} anteiso B.

method (Saitou & Nei, 1987) in the program MEGA2 (Kumar *et al.*, 2001). Bootstrap analysis with 1000 replications was also carried out to obtain confidence levels for the branches (Felsenstein, 1985). The closest relatives of strain DCY16^T and the type strain of the type species of the genus *Paenibacillus* were included in the phylogenetic tree.

The 16S rRNA gene sequence of strain DCY16^T was a continuous stretch of 1512 nt. The phylogenetic tree (Fig. 1; an extended version of this tree is available as Supplementary Fig. S1 in IJSEM Online) shows that strain DCY16^T clustered within the genus *Paenibacillus* of the class 'Bacilli'. Highest sequence similarities were with *Paenibacillus validus* JCM 9077^T (94.4%), *P. chinjuensis* WN9^T (94.4%), *P. naphthalenovorans* DSM 14203^T (94.2%), *P. ehimensis* KCTC 3748^T (92.8%) and *P. elgii* KCTC 10016BP^T (92.4%). The phylogenetic position of strain DCY16^T was unique and distinct among members of the genus *Paenibacillus*.

Results obtained from chemotaxonomic analyses were in agreement with those of phylogenetic inference showing that strain DCY16^T belonged to the genus *Paenibacillus*. Strain DCY16^T contained menaquinone with seven isoprene units (MK-7). The majority of *Paenibacillus* species have MK-7 as the major quinone (Shida *et al.*, 1997). The cellular fatty acid profiles of strain DCY16^T and type strains

of closely related *Paenibacillus* species are shown in Table 1. The major cellular fatty acids in strain DCY16^T were C_{15:0} anteiso (48.5%), C_{17:0} anteiso (18.3%), C_{16:0} (9.6%), C_{16:0} iso (9.5%) and C_{17:0} (6.3%). Minor amounts of C_{15:0} iso (3.1%), C_{14:0} (1.3%), C_{14:0} iso (1.3%), C_{15:0} (1.0%) and C_{16:1 ω 11c} (2.3%) were also present. According to published data, the cellular fatty acid pattern of strain DCY16^T is similar to those of phylogenetically closely related *Paenibacillus* species. C_{15:0} anteiso, the main fatty acid in strain DCY16^T, is indicative of members of the genus *Paenibacillus* (Ash *et al.*, 1993; Shida *et al.*, 1997). However, some qualitative and quantitative differences in fatty acid content were observed between strain DCY16^T and its closest relatives. The second most abundant fatty acid in strain DCY16^T was C_{17:0} anteiso (18.3%), which was present at much higher levels in strain DCY16^T than in its closest relatives (Table 1). Slight differences in the proportions of the other fatty acids were also found.

The G + C content of the genomic DNA of strain DCY16^T was 50.9 mol%.

Phenotypic characteristics of strain DCY16^T were compared with those of *Paenibacillus* species that formed a coherent phylogenetic cluster. As shown in Table 2, strain DCY16^T showed similar G + C content and growth temperature to closely related *Paenibacillus* species.

Table 2. Differential phenotypic characteristics of strain DCY16^T and closely related *Paenibacillus* species

Strains: 1, *P. ginsengihumi* sp. nov. DCY16^T (data from this study); 2, *P. validus* LMG 11161^T (data from Heyndrickx *et al.*, 1995); 3, *P. chinjuensis* WN9^T (Yoon *et al.*, 2002); 4, *P. naphthalenovorans* PR-N1^T (Daane *et al.*, 2002); 5, *P. ehimensis* IFO 15659^T (Lee *et al.*, 2004); 6, *P. elgii* SD17^T (Kim *et al.*, 2004); 7, *P. chitinolyticus* IFO 15660^T (Lee *et al.*, 2004); 8, *P. panacisoli* Gsoil 1411^T (Ten *et al.*, 2006). +, Positive; –, negative; v, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Optimum growth temperature (°C)	35–40	28–35	30–37	30–37	ND	20–45	ND	37
Colony colour	Pale yellow	Greyish white	Pink	White	ND	White	ND	Slightly yellow
Nitrate reduction	+	–	–	v	+	+	+	+
Production of indole	–	–	ND	–	ND	+	ND	–
Production of acid from glucose	–	+	+	+	+	+	+	–
Oxidase	–	–	+	+	+	–	v	+
Enzyme activity								
α -Glucosidase (starch hydrolysis)	–	+	+	v	+	+	–	–
Protease (gelatin hydrolysis)	–	–	+	–	+	ND	ND	+
Urease	–	–	–	+	ND	ND	ND	–
Assimilation of:								
Arabinose	–	–	–	–	+	–	–	–
Fucose	–	–	–	–	ND	ND	ND	–
Glucose	–	+	+	+	+	+	+	–
Maltose	–	+	+	+	ND	+	ND	–
Mannose	–	+	ND	ND	ND	+	ND	–
Rhamnose	–	–	–	–	ND	ND	ND	–
Mannitol	–	+	–	+	+	+	–	–
DNA G + C content (mol%)	50.9	50–52 ^a	53	49	52–55 ^b	51.7	51–53 ^c	53.9

*Ranges of values for: a, the type strains of *P. validus* and *P. gordonae*; b, three strains, including the type strain; c, two strains, including the type strain.

However, enzyme activities and assimilation of sugars by strain DCY16^T differed from those of the other *Paenibacillus* species.

Therefore, on the basis of the data presented, strain DCY16^T represents a novel species of the genus *Paenibacillus* for which the name *Paenibacillus ginsengihumi* sp. nov. is proposed.

Description of *Paenibacillus ginsengihumi* sp. nov.

Paenibacillus ginsengihumi (gin.seng.i.hu'mi. N.L. n. *ginsengum* ginseng; L. n. *humus* soil; N.L. gen. n. *ginsengihumi* of the soil of a ginseng field, the source of the type strain).

Cells are Gram-positive, aerobic, motile and rod-shaped, about 0.5–0.8 µm wide and 2.0–3.5 µm long. Colonies grown on R2A agar (Difco) for 5 days are circular and pale yellow. Growth occurs at 25–42 °C, but not at 4 °C. The optimal temperature for growth is 37 °C and the pH growth range is between pH 7.0 and 9.0. Catalase-positive and oxidase-negative. Aerobic nitrate reduction is positive and indole production is negative. Aesculin is hydrolysed, but not starch or gelatin. Assimilates maltose, melibiose, sucrose and D-mannitol. Does not assimilate 2-ketoglucuronate, 3-hydroxybenzoate, DL-3-hydroxybutyrate, 4-hydroxybenzoate, 5-ketoglucuronate, acetate, adipate, caprate, citrate, gluconate, itaconate, DL-lactate, malate, malonate, phenylacetate, propionate, suberate, n-valerate, arabinose, fucose, glucose, maltose, mannose, rhamnose, ribose, inositol, mannitol, sorbitol, L-alanine, L-histidine, L-proline, L-serine, N-acetyl-D-glucosamine, salicin or glycogen. Produces esterase (C4), esterase (C8), β-galactosidase, β-glucosidase and naphthol-AS-BI-phosphohydrolase, but not N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, α-glucosidase, leucine arylamidase, lipase (C14), α-mannosidase, protease, trypsin, urease or valine arylamidase. The predominant menaquinone is MK-7. The major fatty acid is C_{15:0} anteiso.

The type strain is DCY16^T (=KCTC 13141^T =JCM 14928^T), isolated from soil of a ginseng field in South Korea. The DNA G+C content of the type strain is 50.9 mol%.

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