

Paenibacillus swuensis sp. nov., a Bacterium Isolated from Soil[§]

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Strain DY6^T, a Gram-positive endospore-forming motile rod-shaped bacterium, was isolated from soil in South Korea and characterized to determine its taxonomic position. Phylogenetic analyses based on the 16S rRNA gene sequence of strain DY6^T revealed that strain DY6^T belongs to the genus *Paenibacillus* in the family *Paenibacillaceae* in the class *Bacilli*. The highest degree of sequence similarities of strain DY6^T were found with *Paenibacillus gansuensis* B518^T (97.9%), *P. chitinolyticus* IFO 15660^T (95.3%), *P. chinjuensis* WN9^T (94.7%), and *P. rigui* WPCB173^T (94.7%). Chemotaxonomic data revealed that the predominant fatty acids were anteiso-C_{15:0} (38.7%) and C_{16:0} (18.0%). A complex polar lipid profile consisted of major amounts of diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol. The predominant respiratory quinone was MK-7. Based on these phylogenetic, chemotaxonomic, and phenotypic data, strain DY6^T (=KCTC 33026^T =JCM 18491^T) should be classified as a type strain of a novel species, for which the name *Paenibacillus swuensis* sp. nov. is proposed.

Keywords: *Bacilli*, *Paenibacillaceae*, *Paenibacillus*, taxonomy

Introduction

The genus *Paenibacillus*, in the family *Paenibacillaceae*, was originally proposed with *Paenibacillus polymyxa* as the type species (Ash *et al.*, 1993, 1994). At the time of writing, the genus *Paenibacillus* comprises 148 species with validly published names (<http://www.bacterio.cict.fr/paenibacillus.html>). Members of the genus *Paenibacillus* are aerobic or facultative aerobic, Gram-staining-positive, but few strains are Gram-staining-negative. *Paenibacillus* spp. have L-ornithine

in the cell wall without teichoic acid.

We isolated a strain (designated DY6^T) from a gamma ray-irradiated soil sample collected from Mt. Deogyusan (GPS; N35°51'38" E127°44'47"; altitude 1500 m), Jeonbuk Province, South Korea while isolating radiation-resistant soil bacteria. Strain DY6^T was a Gram-positive bacterium with pale yellow color pigmented colonies on 1/2 R2A agar (Difco, USA). Based on the 16S rRNA gene sequence analysis, strain DY6^T belonged to the genus *Paenibacillus*, and the results of polyphasic taxonomic investigation indicated that strain DY6^T represents a novel *Paenibacillus* sp.

Materials and Methods

Isolation of the bacterial strain and culture conditions

Strain DY6^T was isolated from a gamma ray-irradiated soil sample (pH 6.6) collected from Mt. Deogyusan, Jeonbuk Province, South Korea. The collected soil was exposed to 5 kGy gamma radiation (cobalt-60 gamma irradiator, AECL, IR-79). One g of the gamma ray-irradiated soil sample in 10 ml saline [0.85% (w/v) NaCl] was serially diluted and 100 µl of each dilution was spread on a 1/2 R2A agar and incubated at 30°C. Single colonies were transferred to a new R2A agar plate and incubated for 3 days at 30°C. This purified colony was tentatively identified by 16S rRNA gene sequence using EzTaxon-e (<http://eztaxon-e.ezbiocloud.net>) (Kim *et al.*, 2012).

Strain DY6^T was deposited at the Japan Collection of Microorganisms (JCM 18491^T) and Korean Collection for Type Cultures (KCTC 33026^T). The type strain, *Paenibacillus gansuensis* B518^T, was obtained from the Korean Agricultural Culture Collection (KACC 12291^T). All strains were maintained on R2A agar unless otherwise stated.

16S rRNA sequencing and phylogenetic analysis

The 16S rRNA gene of strain DY6^T was amplified using the 9F and 1492R universal bacterial primer set (Weisburg *et al.*, 1991) and sequenced by Genotech (Korea) using the 9F, 518F, 785F, and 800R universal bacterial primer set. The partial sequences of the 16S rRNA gene were compiled with SeqMan software (DNASTAR Inc., USA) and then compared using EzTaxon-e server. The 16S rRNA sequences of related taxa obtained from GenBank were edited with the BioEdit program (Hall, 1999). Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al.*, 1997) and a phylogenetic tree was constructed using the MEGA5 program (Tamura *et al.*, 2011). Pairwise distances for the neighbor-joining algorithm (Saitou and Nei, 1987) were calculated according to the Kimura two-parameter model (Kimura, 1983). A bootstrap analysis with 1,000 replicates

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was conducted (Felsenstein, 1985). The min-mini heuristic method (Fitch, 1971) with a search factor of one was applied for the maximum-parsimony analysis (MEGA 5 Program).

Phenotypic and biochemical characteristics

Cell morphology and motility were examined by a light microscopy (Nikon E600) and transmission electron microscopy (Carl Zeiss LEO912AB). Gram-staining was performed as described by Gerhardt *et al.* (1994). The hanging drop technique was used to examine motility, after the cells had grown for 2 days at 30°C on R2A agar. Sporulation was induced in modified Schaeffer's medium [0.1% KCl, 0.01% MgCl₂, 1.0 mM Ca(NO₃)₂, 0.01 mM MnCl₂, 0.001 mM FeSO₄, and 8 g/L nutrient broth] according to Kempf *et al.* (2005). Spore morphology was determined using a BX50 (Olympus) microscope. Growth under anaerobic conditions was tested by culturing the organisms on R2A agar, nutrient agar (NA, Difco) and trypticase soy agar (TSA, Difco) plates in GasPak jars (BBL) at 30°C. Oxidase activity was evaluated with 1% (w/v) tetramethyl-p-phenylene diamine, and catalase activity was determined by applying a 3% (v/v) hydrogen peroxide solution. Gamma radiation resistance was tested as described by Im *et al.* (2008), Lim *et al.* (2006b, 2012), and Srinivasan *et al.* (2012a, 2012b). Aerobic growth on different media was also assessed on TSA, NA, and TGY (1% tryptone, 0.1% glucose, 0.5% yeast extract) agar. The API 20NE, API 20E, API ID32GN, API 50CH, and API ZYM microtest systems were employed according to the manufacturer's recommendations (bioMérieux, France) to study carbon source utilization, enzyme activities, and H₂S production of the strains (DY6^T and *P. gansuensis* KACC 12291^T). Hydrolysis of casein, starch, Tween 20, Tween 40, Tween 80, and tyrosine were tested on R2A agar according to methods described previously (Lanyi, 1987; Smibert and Krieg, 1994). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, and 42°C) was assessed on R2A agar for 2 days. Growth at various pH levels (4, 5, 6, 7, 8, 9, 10, and 11) was assessed in R2A broth (MBcell) at 30°C. The pH of the medium was maintained using three buffers (final concentration, 50 mM): acetate buffer (pH 4.0–5.0); phosphate buffer (pH 6.0–8.0) and Tris buffer (pH 9.0–11.0). NaCl tolerance was tested on R2A broth at 30°C that had been supplemented with 0–10% (w/v) NaCl (1% intervals).

Chemotaxonomic and genomic analyses

Cells were grown on TSA for 2 days at 30°C to perform the fatty acid analysis. Two loops of well-grown cells were harvested, and fatty acid methyl esters were prepared, separated, and identified (Sherlock ver. 6.01; database TSBA6; MIDI, Inc., USA) (Sasser, 1990). Isoprenoid quinones were extracted, purified via thin-layer chromatography (TLC), and subsequently analyzed by high performance liquid chromatography (HPLC), as described previously (Collins and Jones, 1981; Shin *et al.*, 1996). Polar lipids were extracted as described by Minnikin *et al.* (1984) and identified using two-dimensional TLC as described previously (Minnikin *et al.*, 1984; Komagata and Suzuki, 1987). The first mobile phase for TLC development was chloroform/methanol/water (65:25:4, v/v/v), and the second mobile phase was chloroform/me-

thanol/acetic acid/water (80:12:15:4, v/v/v/v). The appropriate detection reagents for each type of polar lipids were used as described previously (Lee *et al.*, 2013).

DNA-DNA hybridization was performed with five replications according to Ezaki *et al.* (1989). The highest and lowest values were excluded, and the remaining three values were utilized. Genomic DNA was extracted, purified with

Table 1. Differential characteristics between strain DY6^T and *P. gansuensis* KACC 12291^T

Strains: 1, DY6^T; 2, *P. gansuensis* KACC 12291^T

All data were obtained in this study. Both strains are Gram-positive, motile.

Characteristic	1	2
Size (µm)		
Length	1.5–2.5	1.7–2.4
Width	1.0–1.2	0.7–0.9
Production of acid from glucose	-	+
Growth at		
15°C	+	-
20°C	+	-
37°C	-	+
Enzyme production (API ZYM)		
Catalase	+	-
α-Chymotrypsin	w	+
β-Glucosidase (esculin hydrolysis)	w	+
Valine arylamidase	w	-
Hydrolysis		
Starch	+	-
Tyrosine	+	-
Tween 80	-	+
Acid production (API 50CH)		
Amygdalin	-	+
D-Arabinose	-	+
L-Arabinose	-	+
Arbutin	-	+
D-Fructose	-	+
D-Lactose	-	+
Maltose	-	+
D-Mannose	-	+
Melezitose	-	+
L-Rhamnose	-	+
Starch	-	+
D-Sucrose	-	+
D-Tagatose	-	+
D-Trehalose	w	+
N-Acetylglucosamine	-	+
Glycerol	-	+
Xylitol	w	+
Utilization of carbon source (API ID 32GN)		
L-Arabinose	+	-
D-Glucose	+	-
D-Ribose	+	-
D-Sucrose	+	-
Caprate	-	+
Gluconate	-	w
2-Ketogluconate (α)	+	-
L-Alanine	+	-
L-Proline	+	-
L-Serine	+	-
D-Mannitol	+	-
G+C content	47.7	49.0

+, positive; -, negative; w, weak positive.

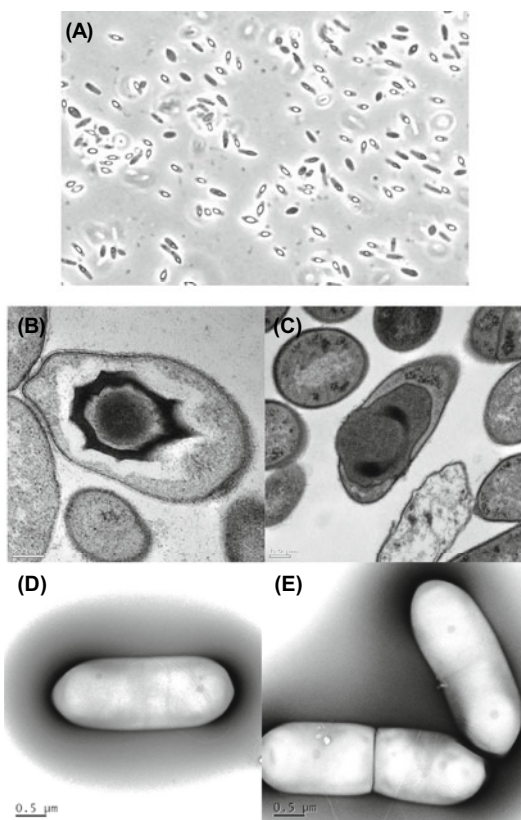


Fig. 1. Microscopic images of strain DY6^T (A), (B) and (C) the endospores on Schaeffer's medium after 4 days; (D) and (E) morphology of cells with flagella by transmission electron microscopy on R2A agar after 2 days at 30°C. Bar, 0.5 μm.

the Genomic-tip system 100/G (QIAGEN, Japan), enzymatically degraded into nucleosides, and analyzed using reverse-phase HPLC to determine G + C content (Tamaoka and Komagata, 1984; Mesbah *et al.*, 1989).

Results and Discussion

Morphological and phenotypic characteristics

Strain DY6^T was pale yellow colored when routinely cultured on R2A agar at 30°C. Cells were Gram-positive, strictly aerobic, motile, endospore forming, and rod-shaped (Fig. 1). Strain DY6^T did not show gamma radiation resistance in comparison with the control strains *Deinococcus radiodurans* R1^T and *Escherichia coli* K-12 (data not shown). They grew at temperatures of 10–30°C with optimal growth at 30°C. Strain DY6^T grew at pH values of 6–10. The physiological characteristics of strain DY6^T are in the species description and differential characteristics between strain DY6^T and closely related type strains are shown in Table 1.

Phylogenetic analysis

The 16S rRNA gene sequence of strain DY6^T was a continuous stretch of 1,492 nucleotides. Strain DY6^T belongs to the class *Bacilli*, order *Bacillales*, family *Paenibacillaceae*. The highest degree of sequence similarity of strain DY6^T was found with *Paenibacillus* species, *P. gansuensis* B518^T (97.9%) (Lim *et al.*, 2009), *P. chitinolyticus* IFO 15660^T (95.3%) (Lee *et al.*, 2004), *P. chinjuensis* WN9^T (94.7%) (Yoon *et al.*, 2002), and *P. rigui* WPCB173^T (94.7%) (Baik *et al.*, 2011). Strain DY6^T clearly belonged to the genus *Paenibacillus* lineage in the phylogenetic trees (Fig. 2).

Chemotaxonomic and genomic analyses

The predominant cellular fatty acids of strain DY6^T were anteiso-C_{15:0} (38.7%) and C_{16:0} (18.0%). Minor fatty acids were anteiso-C_{17:0} (9.0%), iso-C_{15:0} (8.2%), iso-C_{17:0} (7.9%), and C_{16:1} ω11c (6.1%), iso-C_{16:0} (5.4%), C_{14:0} (1.6%), iso-C_{17:1} ω10c (1.6%), iso-C_{14:0} (1.1%), summed feature 4 (iso I-C_{17:1}/anteiso B) (0.7%), C_{17:0} (0.5%), C_{18:0} (0.4%), C_{16:1} alcohol ω7c (0.4%), C_{10:0} (0.2%), C_{12:0} (0.2%), and iso-C_{13:0} (0.1%). Strain DY6^T had larger amounts of C_{16:0} (18.0%), and C_{16:1} ω11c (6.1%), whereas another closely related type strain *P. gan-*

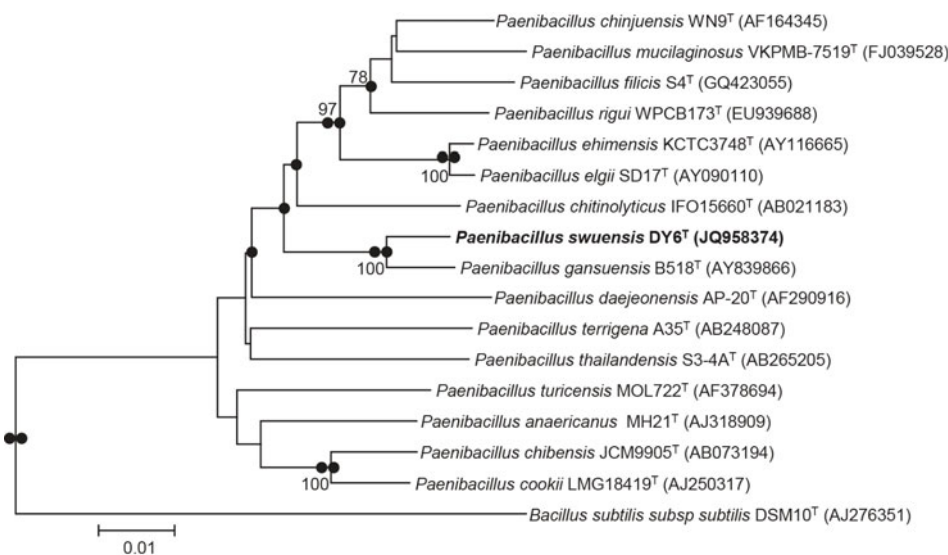


Fig. 2. Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences of strain DY6^T and representatives of related taxa. Numbers at the nodes indicate the bootstrap values (>70%) expressed as percentage of 1000 replicates. Single closed circles indicate the corresponding nodes recovered by the maximum-parsimony algorithm and double closed circles indicate the corresponding nodes recovered by both maximum-parsimony and maximum-likelihood algorithms. Bar represents 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid profiles of strain DY6^T and *P. gansuensis* KACC 12291^T.Strains: 1, DY6^T; 2, *P. gansuensis* KACC 12291^T
Both strains were grown on TSA agar at 30°C for 2 days. tr, trace (<1.0%)

Fatty acids	1	2
Saturated		
14:0	1.6	tr
14:0 iso	1.1	tr
15:0 iso	8.2	9.7
15:0 anteiso	38.7	46.2
16:0	18.0	5.9
16:0 iso	5.4	6.4
17:0 iso	7.9	10.2
17:0 anteiso	9.0	13.1
Unsaturated		
16:1 ω11c	6.1	1.8
17:1 iso ω10c	1.6	2.2
Summed feature 4 (17:1 iso I / anteiso B)	tr	1.8

suensis had smaller amounts of corresponding fatty acids. Strain DY6^T could be differentiated from *P. gansuensis* KACC 12291^T based on differences in the compositions of the fatty acids (Table 2). MK-7 is the predominant quinone of strain DY6^T, similar to other *Paenibacillus* species (Baik *et al.*, 2011; Kong *et al.*, 2013).

Strain DY6^T contained a major amount of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG); various unknown polar lipids (L₁₋₂), unknown glycolipid (GL), unknown phosphoglycolipids (PGL₁₋₂), unknown phospholipids (PL₁₋₃), and unknown aminophospholipids (APL₁₋₃) (Supplementary data Fig. S1). The polar lipid profile of strain DY6^T was dominated by DPG, PE, and PG, which is common in members of the genus *Paenibacillus* (Baik *et al.*, 2011; Kong *et al.*, 2013). The G + C content of genomic DNA from strain DY6^T was 47.7 mol%. Strain DY6^T exhibited <70% DNA-DNA relatedness with *P. gansuensis* KACC 12291^T (19.7±3.6%; reciprocal analysis, 6.3±4.9%) and was delineated as a different genomic species (Wayne *et al.*, 1987; Stackebrandt and Goebel, 1994).

Taxonomic conclusion

Strain DY6^T clearly showed typical features of the genus *Paenibacillus*, with the presence of the predominant respiratory quinone MK-7; major fatty acids such as anteiso-C_{15:0} and C_{16:0}; the polar lipid profile consisting of major amounts of DPG, PE, and PG. Strain DY6^T can be distinguished from closely related species *Paenibacillus gansuensis* by its ability to grow at 15°C; assimilation of L-arabinose, D-glucose, D-ribose, D-sucrose, caprate, gluconate, 2-ketogluconate (α), L-alanine, L-proline, L-serine, D-mannitol, and production of catalase. Based on the phylogenetic, chemotaxonomic, and phenotypic data, we conclude that strain DY6^T is a representative novel species, for which the name *Paenibacillus swuensis* sp. nov. is proposed.

Description of *Paenibacillus swuensis* sp. nov.

Paenibacillus swuensis (*swu.en'sis*. N.L. masc. adj. *swuensis* of or belonging to Seoul Women's University, where the taxonomic study was performed).

Cells are Gram-positive, strictly aerobic, motile, endospore-forming, and rod-shaped (1.5–2.5 × 1.0–1.2 mm) when grown on R2A agar at 30°C for 2 days. Growth occurs on TSA, LB, NA, and R2A. Growth occurred at temperatures of 10–30°C (optimum 30°C). Strain DY6^T grew well at pH 6–10 (optimum pH 7) and tolerates up to 3% NaCl (w/v) with optimum growth at 0–1% NaCl (w/v). Hydrolysis of casein, starch, Tween 20, and tyrosine was positive, but Tween 40 and Tween 80 were negative. Gelatin is not liquefied; Voges-Proskauer (acetoin) and H₂S production are negative (API 20E). Nitrate was reduced to nitrite (API 20NE). Oxidase-positive and catalase-positive. Acid production from glucose and indole production was negative (API 20NE).

Growth is observed (API ID 32GN) with acetate, *N*-acetyl-D-glucosamine, L-alanine, L-arabinose, D-glucose, glycogen, 4-hydroxybenzoate, D,L-3-hydroxybutyrate, 2-ketogluconate (α), D,L-lactate, malonate, D-maltose, D-mannitol, D-mannose, D-melibiose, L-proline, propionate, D-ribose, L-serine, D-sucrose, and *n*-valerate. Growth is not observed with adipate, caprate, citrate, L-fucose, gluconate, L-histidine, 3-hydroxybenzoate, itaconate, 5-ketogluconate, L-malate, myo-inositol, phenyl acetate, L-rhamnose, salicin, D-sorbitol, and suberate. Acid is produced (API 50CH) with D-adonitol (ribitol), D-cellobiose, gentiobiose, glycogen, inulin, D-lyxose, D-melibiose, D-trehalose, D-ribose, xylitol, D-xylose, and L-xylose. Acid is not produced with *N*-acetyl-glucosamine, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol (galactitol), erythritol, esculin, D-fructose, D-fucose, L-fucose, D-galactose, gluconate, glucose, glycerol, inositol, D-lactose, maltose, mannitol, D-mannose, melezitose, α-methyl-D-glucoside, α-methyl-D-mannoside, β-methyl-D-xyloside, D-raffinose, L-rhamnose, salicin, sorbitol, L-sorbose, starch, D-sucrose, D-tagatose, turanose, 2-ketogluconate, or 5-ketogluconate. In tests with the API Zym system, enzyme production was positive for acid phosphatase, alkaline phosphatase, α-chymotrypsin, esterase (C4), esterase (C8), α-galactosidase, β-galactosidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase. Enzyme production was negative for *N*-acetyl-β-glucosaminidase, cysteine arylamidase, α-fucosidase, α-glucosidase, leucine arylamidase, lipase (C14), α-mannosidase, β-glucosidase, β-glucuronidase, or trypsin.

The predominant cellular fatty acids of strain DY6^T are anteiso-C_{15:0} (38.7%) and C_{16:0} (18.0%). MK-7 is the predominant quinone. The polar lipid profile consisted of major amounts of DPG, PE, and PG. The DNA G + C content of the type strain is 47.7 mol%.

The type strain, DY6^T (=KCTC 33026^T =JCM 18491^T) was isolated from a soil sample collected from Mt. Deogyusan (GPS; N 35°51'38" E 127°44'47"; altitude 1500 m), Jeonbuk Province, South Korea.

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