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Paenibacillus cucumis sp. nov. Isolated from Greenhouse Soil[§]

Jae-Hyung Ahn[1], Byoung-chan Kim[2], Byung-Yong Kim[1], Soo-Jin Kim[1], Jaekyeong Song[1], Soon-Wo Kwon[1], and Hang-Yeon Weon[1]*

[1]*Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea*
[2]*Korean Collection for Type Cultures (KCTC), Biological Resource Center (BRC), Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Republic of Korea*

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Strain CO 4-7^T was isolated from greenhouse soil used for cultivation of cucumbers in Korea. The 16S rRNA gene sequence of strain CO 4-7^T showed the highest sequence similarity with *Paenibacillus contaminans* CKOBP-6^T (94.2%) among the type strains. Strain CO 4-7^T was a strictly aerobic, Gram-staining-positive, endospore-forming, and motile rod-shaped bacterium. Strain CO 4-7^T grew at 10–45°C (optimum, 30°C), at pH 6.0–7.5 (optimum, pH 6.5) and in the presence of 0–5% NaCl (optimum, 0.5%). The DNA G+C content of strain CO 4-7^T was 48.5 mol%. It contained MK-7 as the major isoprenoid quinone and anteiso-$C_{15:0}$ (51.8%), $C_{16:0}$ (12.7%), and iso-$C_{16:0}$ (8.6%) as the major fatty acids. The cell wall contained *meso*-diaminopimelic acid. Based on evidence from our polyphasic taxonomic study, it was concluded that strain CO 4-7^T should be classified as a novel species of the genus *Paenibacillus*, for which, the name *Paenibacillus cucumis* sp. nov. is proposed. The type strain is CO 4-7^T (=KACC 17444^T=JCM 19515^T).

Keywords: *Paenibacillus cucumis*, polyphasic taxonomy, novel species, soil

Introduction

The genus *Paenibacillus*, previously classified as the genus *Bacillus*, was proposed as a new genus based on 16S rRNA gene sequence by Ash *et al.* (1993). The genus was characterized as rod-shaped, Gram-positive or variable, endospore-forming, motile by peritrichous flagella, and facultatively anaerobic or strictly aerobic bacterium (Priest, 2009). The members of the genus *Paenibacillus* have anteiso-$C_{15:0}$ as the major fatty acid, *meso*-diaminopimelic acid (DAP) as the cell wall peptidoglycan, and DNA G+C contents ranging from 40–59%. There is no phenotypic characteristic that allows for the differentiation of the genus *Paenibacillus* from other aerobic and endospore-forming genera although the presence of an endospore that distinctly swells the sporangium is indicative (Priest, 2009). The normal habitat of the genus is the soil and several species have been associated with the rhizosphere of plants, enhancing the growth of the plants by the production of phytohormones or by providing nutrients including nitrogen. Currently, the genus comprises more than 150 recognized species with *P. polymyxa* as the type species (http://www.bacterio.net). We describe one isolate, discovered during the course of an investigation of bacterial communities in agricultural soils, which was shown to represent a novel species of the genus *Paenibacillus* based on phenotypic data and phylogenetic inference.

Materials and Methods

Bacterial strains

Strain CO 4-7^T was isolated from a greenhouse soil cultivated with cucumber located at a height of 216 m in Cheongsong, Korea (36°24.61′N, 129°3.84′E). The soil sample had the following chemical properties: pH 7.0; total organic carbon, 39 g/kg; total nitrogen, 2.2 g/kg; available P_2O_5, 642 mg/kg; exchangeable K, 0.42 $cmol_c$/kg; exchangeable Ca, 14.6 $cmol_c$/kg, exchangeable Na, 0.45 $cmol_c$/kg, and exchangeable Mg, 4.7 $cmol_c$/kg. The soil texture was sandy loam. The soil sample was diluted serially in saline solution (0.85%, w/v), spread on R2A agar (Difco, USA) and incubated for 5 days at 28°C. A selected colony was subcultured repeatedly to isolate a pure culture. Selected type strains of the genus *Paenibacillus* were obtained from the Korean Agricultural Culture Collection (KACC, Suwon, Korea) for comparative taxonomic analysis.

Phylogenetic analysis

The 16S rRNA gene was amplified by PCR using two universal primers, as described previously (Kwon *et al.*, 2003) and sequenced using an Applied Biosystems ABI 3100 DNA sequencer with the primers 800R (5′-TACCAGGGTATCTAATCC-3′), 518F (5′-CCAGCAGCCGCGGTAATACG-3′), and 984F (5′-AACGCGAAGAACCTTAC-3′). The nearly complete 16S rRNA gene sequence of strain CO 4-7^T (1,471 nucleotides) was obtained from the sequence fragments using the SeqMan software (DNASTAR) and imported along with the 16S rRNA gene sequences of the selected type strains belonging to the genus *Paenibacillus* into the ARB software package (version 5.5) (Ludwig *et al.*, 2004). These sequences were aligned by the SINA aligner, (version 1.1) (Pruesse *et al.*, 2012) using the SILVA 16S rRNA database (SSURef-111)

*For correspondence. E-mail: why@korea.kr; Tel.: +82-31-290-8478; Fax: +82-31-290-8488
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(Pruesse *et al.*, 2007) for the reference sequences, and exported to the MEGA program (version 5.1) (Tamura *et al.*, 2011). The maximum-likelihood, neighbor-joining, and maximum-parsimony trees were constructed using the positional variability filter for bacteria provided by the ARB package. Nucleotide similarity values were calculated using EzTaxon-e server (Kim *et al.*, 2012).

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CO 4-7^T is KF289905.

Determination of DNA G+C content

The DNA G+C content was determined as described by Gonzalez and Saiz-Jimenez (2002), using the CFX96 system (Bio-Rad, USA). The genomic DNA of strain CO 4-7^T was extracted using PureHelix Genomic DNA Prep Kit (NanoHelix, Korea). A 50-µl reaction consisted of 5 µg of genomic DNA, SYBR Green I (Molecular Probes, USA; 1:100,000 final dilution), and 0.1× standard saline citrate (SSC). Thermal conditions consisted in a ramp from 25–100°C at a rate of 1°C/min. The DNA G+C content of strain CO 4-7^T was calculated using the melting temperature of the genomic DNA of strain CO 4-7^T and formula proposed by Gonzalez and Saiz-Jimenez (2002).

Morphological, physiological, and biochemical characterization

The cell morphology and motility of strain CO 4-7^T was examined using oil-immersion phase-contrast microscopy (Axioplan 2; Zeiss, Germany) with cells grown for 3 days on R2A agar at 28°C. In addition to R2A agar, growth of strain CO 4-7^T was tested on trypticase soy agar (TSA), nutrient agar (NA), Luria-Bertani agar (LB), and marine agar 2216 (MA). Growth at 0%, 0.5%, 1.0%, 2.0%, 3.0%, and 4.0% NaCl (w/v), and at various temperatures (5–50°C at intervals of 5°C) was investigated after 7 days of incubation on R2A agar. Growth was monitored for 20 days of incubation in R2A broth at pH 5.0–10.0 in increments of 0.5 units. pH was adjusted by using following buffer systems: citric acid/Na_2HPO_4 (pH 5.0–6.0), NaH_2PO_4/Na_2HPO_4 (pH 6.5–8.0), Tris/HCl (pH 8.5 and 9.0), and Na_2CO_3/$NaHCO_3$ (pH 9.5 and 10.0) (Gomori, 1955). Gram staining behavior was determined by means of the KOH test (Smibert and Krieg, 1994) and L-alanine aminopeptidase activity (Bactident Aminopeptidase test kit; Merck, Germany). Activities of catalase and oxidase, and hydrolyses of starch (1.0%, w/v), casein (10% skimmed milk, w/v), lipid (1.0% tributyrin), chitin (0.5%, w/v), and carboxy-methylcellulose (CM-cellulose, 0.1%, w/v) were conducted as described by Smibert and Krieg (1994). Growth under anaerobic condition was tested by incubating R2A agar plates in AnaeroGen sachet pouches (Oxoid, UK) at 28°C for 2 weeks. Other biochemical characteristics were determined using the API 20E, 20NE, and 50CH systems, following the instructions of the manufacturer (bioMérieux, France). The API test strips were examined after 7 days at 28°C.

Chemotaxonomy

Cells were grown on R2A agar for 3 days at 28°C and fatty acid methyl esters (FAMEs) were extracted and prepared according to the standard protocol of the Microbial Identification System (MIDI; Microbial ID, USA). Briefly, the method entailed: (i) saponification of whole-cell preparations (40 mg of cells from plate culture) at 100°C with 1 ml of methanolic NaOH (15% [w/v] NaOH in 50% [v/v] methanol); (ii) esterification of the fatty acids at 80°C with 2 ml of 3.25 N HCl in 46% (v/v) methanol; (iii) extraction of the FAMEs into 1.25 ml of 1:1 (v/v) methyl-tert-butyl ether-hexane; and (iv) aqueous washing of the organic extract with 3 ml of 1.2% (w/v) NaOH. The washed extracts were then analyzed on a gas chromatograph (Agilent Ultra 2 column; carrier gas, hydrogen; temperature ramping from 120 to 260°C at a rate of 5°C/min) with a flame ionization detector. Individual fatty acids were identified using MIDI standards and the TSBA6 database (version 6.10) (Microbial ID, USA).

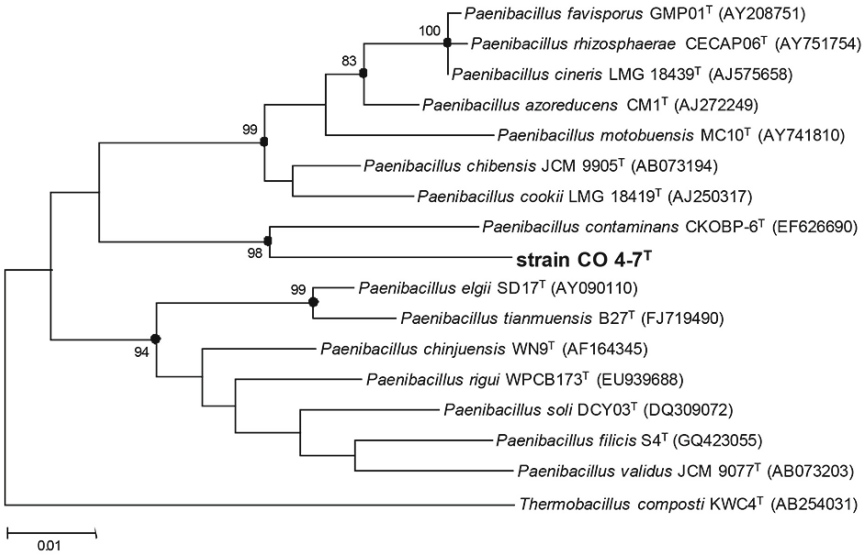

Fig. 1. Maximum-likelihood tree based on a comparative analysis of 16S rRNA gene sequences showing the relationships of strain CO 4-7^T with selected type strains belonging to the genus *Paenibacillus*. Bootstrap values (>70%) based on 500 resamplings are shown at branching points. The dots indicate that the corresponding branches were also recovered in the neighbor-joining and maximum-parsimony trees. The scale bar indicates 0.01 estimated change per nucleotide. *Thermobacillus composti* KWC4^T was used as an outgroup.

The presence of isoprenoid quinones was investigated using high-performance liquid chromatography (HPLC), as described previously (Groth *et al.*, 1996). Isoprenoid quinone-containing extracts were dissolved in acetone and were applied as 4-cm bands to 20 × 20-cm TLC plates of aluminum-backed silica gel 60 F254 sheets. After development with hexane-ethyl ether (9:1, v/v), separated components were revealed using short wave (254 nm) ultraviolet light. Bands were marked with a pencil, cut from the plates, and extracted with 1-ml diethyl ether; the solvent was removed by evaporation under a stream of nitrogen. The extracts were resuspended in 200 µl isopropanol. The filtered samples were analyzed by reverse-phase HPLC. Extraction and analysis of polar lipids by two-dimensional thin layer chromatography (TLC), were performed according to Minnikin *et al.* (1984).

The cell-wall diamino acid was determined using whole-cell hydrolysates (6 N HCl, 100°C, 18 h) subjected to thin-layer chromatography on cellulose plates as previously described (Rhuland *et al.*, 1955).

Results and Discussion

Phylogenetic analysis

Analyses of the 16S rRNA gene sequences showed that strain CO 4-7^T shared the highest sequence similarity with *Paenibacillus contaminans* CKOBP-6^T (94.2%) among the previously identified type strains. Phylogenetic analysis based on the maximum-likelihood tree showed that strain CO 4-7^T formed a clade with *Paenibacillus contaminans* CKOBP-6^T with a 98% bootstrap value (Fig. 1), which was also supported by the neighbor-joining and maximum-parsimony trees.

Table 1. Differential characterization of strain CO 4-7^T and selected type strains of *Paenibacillus* speices

Strains: 1, CO 4-7^T; 2, *P. contaminans* KACC 17159^T; 3, *P. tianmuensis* KACC 16677^T; 4, *P. elgii* KACC 15271^T; 5, *P. chinjuensis* KACC 12279^T; 6, *P. filicis* KACC 14197^T. +, Positive; -, negative; v, variable depending on the growth stage; ng, no growth. All strains are positive for hydrolysis of starch and Voges-Proskauer test but negative for H_2S production, indole production, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophane deaminase, fermentation of D-glucose, D-manitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose. All strains assimilate D-glucose, methyl-α-D-glucopyranoside, esculin ferric citrate, D-cellobiose, D-maltose, D-saccharose, and D-trehalose but not assimilate arabinose, capric acid, adipic acid, citrate, phenyl acetic acid, L-xylose, D-adonitol, L-sorbose, dulcitol, inulin, D-melezitose, D-lyxose, D-tagatose, L-arabitol, gluconate, 2-ketogluconate, and 5-ketogluconate.

Characteristics	1	2	3	4	5	6
Anaerobic growth	-	+[a]	-[b]	+[c]	+[d]	-[e]
Oxidase	-	+[a]	v[b]	-[c]	+[d]	+[e]
Hydrolysis of						
Casein	-	ng	+	+	+	-
Lipid	-	+	+	+	+	+
Chitin	-	-	-	-	+	-
CM-cellulose	+	-	+	+	-	-
Tween 20	ng	ng	+	+	+	+
Tween 40	+	-	+	+	+	+
Tween 60	+	-	+	+	+	+
Tween 80	-	-	+	-	+	-
Esculin	-	+	+	+	-	+
Nitrate reduction	-	-	+	+	-	-
Assimilation of						
D-Mannose	+	-	-	+	+	+
D-Manitol	+	-	-	+	-	+
N-Acetyl-glucosamine	-	-	+	+	-	+
Potassium gluconate	+	-	+	+	+	+
Erythritol	-	+	-	-	-	-
D-Galactose	-	-	+	+	-	+
D-Fructose	-	-	-	+	-	-
D-Mannose	-	-	-	+	+	-
D-Mannitol	-	-	-	+	-	-
D-Sorbitol	-	-	-	+	-	-
Xylitol	-	-	+	-	-	-
D-arabitol	-	-	-	+	-	-
Enzyme activity						
ß-Galactosidase	+	+	+	+	-	+
Gelatinase	-	-	+	+	+	-
Urease	-	-	-	+	-	-
DNA G+C content (mol%)	48.5	51.2[a]	55.4-55.5[b]	51.7[c]	53[d]	53.2[e]

[a] Data are taken from Chou *et al.* (2009).
[b] Data are taken from Wu *et al.* (2011).
[c] Data are taken from Kim *et al.* (2004).
[d] Data are taken from Yoon *et al.* (2002).
[e] Data are taken from Kim *et al.* (2009).

Morphological, physiological, and biochemical characteristics

Strain CO 4-7^T formed yellowish-pink, irregular, convex colonies with entire margins, 1.0–2.0 mm in diameter, on R2A agar after a 3 day-incubation at 28°C. Cells of strain CO 4-7^T were strictly aerobic, Gram-staining-positive, and motile rods, 0.4–0.7 μm wide and 2.5-3.2 μm long and have a middle or terminal ellipsoidal spore in a swollen sporangium (Supplementary data Fig. S1). In addition to R2A agar, strain CO 4-7^T showed growth on TSA, NA, LB, and MA media. The temperature range for growth was 10–45°C with an optimum at 30°C. The pH range for growth was 6.0–7.5 with an optimum at pH 6.5. Growth was observed in the presence of 0–5% NaCl with optimum at 0.5%. Other phenotypic characteristics of strain CO 4-7^T and selected type strains of *Paenibacillus* species are shown in Table 1. Strain CO 4-7^T and *P. tianmuensis* KACC 16677^T did not grow in anaerobic condition and was negative for oxidase activity. Lipid was not hydrolyzed only by strain CO 4-7^T and esculin was not hydrolyzed by strain CO 4-7^T and *P. chinjuensis* KACC 12279^T.

Chemotaxonomy

Strain CO 4-7^T had menaquinone 7 (MK-7) as the major isoprenoid quinone and the polar lipids of the strain were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified aminophospholipids, an unidentified phospholipid, and an unidentified polar lipid (Supplementary data Fig. S2), similar to what is reported for the *P. contaminans* strain CKOBP-6^T (Chou *et al.*, 2009). Strain CO 4-7^T had anteiso-$C_{15:0}$ (51.8%) as the major fatty acid, which is characteristic of the genus *Paenibacillus* (Priest, 2009). Strain CO 4-7^T, *P. contaminans* KACC 17159^T, and *P. chinjuensis* KACC 12279^T had $C_{16:0}$ as the second most abundant fatty acid (12.7–19.9%) while *P. tianmuensis* KACC 16677^T, *P. elgii* KACC 15271^T, and *P. filicis* KACC 14197^T had iso-$C_{15:0}$ as the second most abundant fatty acid (13.8-19.0%). $C_{18:1}\omega 9c$ was present only in strain CO 4-7^T and *P. chinjuensis* KACC 12279^T. The complete fatty acid composition is given in Table 2. Strain CO 4-7^T contained *meso*-diaminopimelic acid in the cell wall.

Taxonomic conclusion

In conclusion, strain CO 4-7^T is similar to other type strains of the genus *Paenibacillus* because it clusters with them with high confidence based on 16S rRNA gene sequence similarity and has anteiso-$C_{15:0}$ as the major fatty acid and *meso*-diaminopimelic acid as the cell-wall diamino acid, which are characteristic of the genus *Paenibacillus*. However, it can be distinguished from them because of its low sequence similarities with the 16S rRNA genes of other type strains, and distinct phenotypic characteristics including a lack of oxidase activity, no lipid hydrolysis, and a distinct fatty acids profile. Thus, strain CO 4-7^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus cucumis* sp. nov. is proposed.

Description of *Paenibacillus cucumis* sp. nov.

Paenibacillus cucumis (cu´cu.mis. L. gen. n. *cucumis* of the cucumber, referring to the isolation source of the type strain, a soil cultivated with cucumber plants).

Cells are strictly aerobic, Gram-staining-positive, endo-

Table 2. Cellular fatty acid compositions of strain CO 4-7^T and selected type strains of *Paenibacillus* speices

Strains: 1, CO 4-7^T; 2, *P. contaminans* KACC 17159^T; 3, *P. tianmuensis* KACC 16677^T; 4, *P. elgii* KACC 15271^T; 5, *P. chinjuensis* KACC 12279^T; 6, *P. filicis* KACC 14197^T. All data are from this study. Only fatty acids that represent more than 1% of the total fatty acids are indicated. All strains were grown on R2A agar at 28°C for 3 days. Values are percentages of total fatty acids. -, Not detected.

Fatty acids	1	2	3	4	5	6
$C_{12:0}$	-	-	-	-	1.8	-
$C_{12:0}$ 3-OH	-	-	-	-	2.7	-
$C_{12:0}$ 2-OH	-	-	-	-	5.1	-
iso-$C_{13:0}$ 3-OH	-	-	-	-	2.6	-
$C_{14:0}$	2.8	3.6	-	3.9	4.0	1.8
iso-$C_{14:0}$	2.0	1.6	2.7	2.6	-	4.4
anteiso-$C_{15:0}$	51.8	63.8	58.2	44.9	34.8	60.3
iso-$C_{15:0}$	3.7	1.2	19.0	14.6	1.5	13.8
iso-$C_{15:1}$ G	-	-	-	-	2.3	-
$C_{16:0}$	12.7	17.6	3.4	11.2	19.9	6.5
iso-$C_{16:0}$	8.6	6.0	3.7	5.4	4.0	7.5
$C_{16:1}\omega 11c$	2.9	-	2.3	4.3	-	-
$C_{16:1}\omega 7c$ OH	-	-	1.5	-	-	-
Anteiso-$C_{17:0}$	4.8	4.1	3.5	3.3	3.1	2.6
iso-$C_{17:0}$	1.4	-	1.8	-	-	2.5
iso-$C_{17:1}\omega 10c$	-	-	2.3	-	-	-
$C_{18:0}$	3.6	-	1.7	4.1	7.0	-
$C_{18:1}\omega 9c$	1.3	-	-	-	6.3	-
Summed feature 1[a]	-	-	-	-	1.0	-
Summed feature 4[a]	-	-	-	5.7	-	-
Summed feature 8[a]	-	-	-	-	2.2	-

[a] Summed features are groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 1 comprised iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH; summed feature 4 comprised iso-$C_{17:1}$ I and/or anteiso $C_{17:1}$ B; summed feature 8 comprised $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$.

spore-forming, motile rods, 0.4–0.7 μm wide and 2.5–3.2 μm long. Grows at 10–45°C (optimum, 30°C), at pH 6.0–7.5 (optimum, pH 6.5) and in the presence of 0–5% NaCl (optimum, 0.5%). Colonies on R2A medium are yellowish pink, irregular, and convex with entire margins. Growth occurs on TSA, NA, LB, and MA media. Positive for catalase and negative for oxidase. Starch, CM-cellulose, and Tweens 40 and 60 are hydrolyzed. Casein, lipid, and chitin are not hydrolyzed. Positive for acetoin production, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urease, tryptophan deaminase, indole production, gelatinase, and acid production from D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, and L-arabinose (API 20E). Positive for *ß*-galactosidase but negative for nitrate reduction, esculin hydrolysis, and gelatin hydrolysis based on the results from the API 20NE.

Assimilates glucose, D-mannose, D-mannitol, D-maltose, gluconate, and malic acid and does not assimilate *N*-acetyl-glucosamine, capric acid, adipic acid, citrate, and phenylacetic acid (API 20NE). Assimilates D-arabinose, L-arabinose, D-ribose, D-xylose, methyl-*ß*-D-xylopyranoside, methyl-*α*-D-mannopyranoside, methyl-*α*-D-glucopyranoside, amygdalin, salcin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-raffinose, glycogen, gentiobiose, D-turanose, D-fucose, and L-fucose, and does not assimilate glycerol, erythritol, L-xylose, D-adonitol, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, D-melezitose, amidon, xylitol, D-lyxose, D-tagatose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, and 5-ketogluconate (API 50CH). The major isoprenoid quinone is MK-7. The cellular phospholipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified aminophospholipids, an unidentified phospholipid, and an unidentified polar lipid. The predominant fatty acids (>5%) are anteiso-$C_{15:0}$, $C_{16:0}$, and iso-$C_{16:0}$. The diamino acid in the cell wall is *meso*-diaminopimelic acid. The DNA G+C content of the type strain is 48.5 mol%. The type strain, CO 4-7^T (KACC 17444^T = JCM 19515 ^{T}), was isolated from a greenhouse soil cultivated with cucumber in Cheongsong, Korea.

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References

Ash, C., Priest, F., and Collins, M.D. 1993. Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. *Antonie van Leeuwenhoek* **64**, 253–260.

Chou, J.H., Lee, J.H., Lin, M.C., Chang, P.S., Arun, A.B., Young, C.C., and Chen, W.M. 2009. *Paenibacillus contaminans* sp. nov., isolated from a contaminated laboratory plate. *Int. J. Syst. Evol. Microbiol.* **59**, 125–129.

Gomori, G. 1955. Preparation of buffers for use in enzyme studies. *Methods Enzymol.* **1**, 138–146.

Gonzalez, J.M. and Saiz-Jimenez, C. 2002. A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ. Microbiol.* **4**, 770–773.

Groth, I., Schumann, P., Weiss, N., Martin, K., and Rainey, F.A. 1996. *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int. J. Syst. Bacteriol.* **46**, 234–239.

Kim, D.S., Bae, C.Y., Jeon, J.J., Chun, S.J., Oh, H.W., Hong, S.G., Baek, K.S., Moon, E.Y., and Bae, K.S. 2004. *Paenibacillus elgii* sp. nov., with broad antimicrobial activity. *Int. J. Syst. Evol. Microbiol.* **54**, 2031–2035.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., and *et al.* 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **62**, 716–721.

Kim, B.C., Kim, M., Lee, K., Kwon, S., Bae, K., and Shin, K.S. 2009. *Paenibacillus filicis* sp. nov., isolated from the rhizosphere of the fern. *J. Microbiol.* **47**, 524–529.

Kwon, S.W., Kim, J.S., Park, I.C., Yoon, S.H., Park, D.H., Lim, C.K., and Go, S.J. 2003. *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. *Int. J. Syst. Evol. Microbiol.* **53**, 21–27.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., and *et al.* 2004. ARB: a software environment for sequence data. *Nucleic Acids Res.* **32**, 1363–1371.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Priest, F.G. 2009. Genus I. *Paenibacillus*, pp. 269–295. *In* De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H., and Whitman, W.B. (eds.), Volume three the *Firmicutes*, Bergey's manual of systematic bacteriology, Springer, New York, USA.

Pruesse, E., Peplies, J., and Glöckner, F.O. 2012. SINA: accurate high throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics.* **28**, 1823–1829.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188–7196.

Rhuland, L.E., Work, E., Denman, R.F., and Hoare, D.S. 1955. The behavior of the isomers of *α*,*ε*-diaminopimelic acid on paper chromatograms. *J. Am. Chem. Soc.* **77**, 4844–4846.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization, pp. 607–654. *In* Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.), Methods for general and molecular bacteriology, American Society for Microbiology, Washington, D.C., USA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Wu, X., Fang, H., Qian, C., Wen, Y., Shen, X., Li, O., and Gao, H. 2011. *Paenibacillus tianmuensis* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.* **61**, 1133–1137.

Yoon, J.H., Seo, W.T., Shin, Y.K., Kho, Y.H., Kang, K.H., and Park, Y.H. 2002. *Paenibacillus chinjuensis* sp. nov., a novel exopolysaccharide-producing bacterium. *Int. J. Syst. Evol. Microbiol.* **52**, 415–421.