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Paenibacillus seodonensis sp. nov., isolated from a plant of the genus *Campanula*

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Strain DCT-19^T, representing a Gram-stain-positive, rod-shaped, aerobic bacterium, was isolated from a native plant belonging to the genus *Campanula* on Dokdo, the Republic of Korea. Comparative analysis of the 16S rRNA gene sequence showed that this strain was closely related to *Paenibacillus amylolyticus* NRRL NRS-290^T (98.6%, 16S rRNA gene sequence similarity), *Paenibacillus tundrae* A10b^T (98.1%), and *Paenibacillus xylanexedens* NRRL B-51090^T (97.6%). DNA-DNA hybridization indicated that this strain had relatively low levels of DNA-DNA relatedness with *P. amylolyticus* NRRL NRS-290^T (30.0%), *P. xylanexedens* NRRL B-51090^T (29.0%), and *P. tundrae* A10b^T (24.5%). Additionally, the genomic DNA G + C content of DCT-19^T was 44.8%. The isolated strain grew at pH 6.0–8.0 (optimum, pH 7.0), 0–4% (w/v) NaCl (optimum, 0%), and a temperature of 15–45°C (optimum 25–30°C). The sole respiratory quinone in the strain was menaquinone-7, and the predominant fatty acids were $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{16:0}$. In addition, the major polar lipids were diphosphatidylglycerol and phosphatidylethanolamine. Based on its phenotypic properties, genotypic distinctiveness, and chemotaxonomic features, strain DCT-19^T is proposed as a novel species in the genus *Paenibacillus*, for which the name *Paenibacillus seodonensis* sp. nov. is proposed (=KCTC 43009^T =LMG 30888^T). The type strain of *Paenibacillus seodonensis* is DCT-19^T.

Keywords**:** *Paenibacillus seodonensis* sp. nov., Dokdo, *Campanula*, polyphasic, novel species

Introduction

Paenibacillus was historically first defined by Ash *et al.* (1991) from the genus *Bacillus* based on the 16S rRNA molecular analysis. Finally, Ash (1993) reclassified the members of *Bacillus* group 3 to the genus *Paenibacillus*. They are generally Gram-stain-positive, aerobic bacterial strains. Presently, the genus comprises more than 239 species isolated from various environments (http://www.bacterio.net/paenibacillus.html), including estuarine wetlands, cold springs, alkaline soils, animals, acidic soil, and plant roots. In addition, interestingly, some strains of the genus *Paenibacillus* have a capability for nitrogen fixation and degrading activity for xylan, which is a component of the plant cell wall (Xie *et al.*, 2014). This information may indicate that this process is an important step in the nitrogen cycle and decomposition of plant-derived organic matter in soil (Morales *et al.*, 1995).

In the course of screening for novel bacteria, strain DCT-19^T was isolated from a native plant belonging to the genus *Campanula* in Dokdo, the Republic of Korea. Based on the 16S rRNA gene sequence analysis, we determined the isolate to be closely related to the genus *Paenibacillus*. Strain DCT-19^T was subjected to more detailed experiments using polyphasic taxonomic approaches with genomic traits. Based on the results obtained in this research, we propose that strain DCT-19^T should be placed in the genus *Paenibacillus* as the type strain of a novel species.

Materials and Methods

Isolation and culture conditions

Campanula takesimana plants were sampled in April 2017 from Dokdo (37°14′N 131°51′E). The root portions of *C. takesimana* were excised using a sterilized knife and were put into plastic bags without other soil debris. Then, the residual soil particles were harvested using a fine brush and were thoroughly suspended in phosphate-buffered saline (PBS, pH 7.5) and serially diluted (to five-fold); then, 100 µl of each dilution was spread onto Reasoner's 2A (R2A, Difco) agar plates. Sequentially, the agar plates were incubated at 30°C for 7 days. To obtain a pure colony, single colony was repetitively transferred onto fresh R2A agar plates and incubated again under the same conditions. Finally, one creamy colony, designated DCT-19^T, was routinely cultured on R2A agar at 30°C and was maintained in a glycerol suspension (final concentration, 15%, w/v) at -70°C. The isolated colony was deposited in the Korean Collection for Type Cultures (KCTC 43009^T) and the Belgian Co-ordinated Collection of Microorganisms (LMG 30888^T). Based on the 16S rRNA gene sequence similarity and phylogenetic relationship, we selected three reference strains – *Paenibacillus amylolyticus* NRRL NRS-290^T, *Paenibacillus tundrae* NRRL B-51094^T (= A10b^T), and *Paenibacillus xylanexedens* NRRL B-51090^T – which were

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obtained from the Agricultural Research Service Culture Collection (Northern Regional Research Laboratory, NRRL). Unless otherwise noted, morphological, physiological, and biochemical characteristics of the novel isolate and reference strains were investigated with routine cultivation on R2A agar at 30°C for 3 days.

Phylogenetic analysis

To determine the phylogenic position of strain DCT-19^T, genomic DNA (gDNA) extraction was performed using a commercial genomic DNA extraction kit (GeneAll Biotechnology Co. Ltd.). PCR amplification of the 16S rRNA gene was performed with the universal bacterial primer set 27F and 1492R (Weisburg *et al.*, 1991). Amplified PCR products were purified and sequenced by Macrogen Co. Ltd. using the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturer protocols. To cover the entire region of the 16S rRNA gene, single-sequencing was performed on each template using 27F, 518F, 900R, and 1492R primers. To elaborate, the fluorescent-labeled fragments were purified by the method recommended by Applied Biosystems as it removes the unincorporated terminators and dNTPs. The samples were injected for electrophoresis in an ABI 3730xl DNA Analyzer (Applied Biosystems). The almost complete DCT-19^T 16S rRNA gene sequence (about 1.4 kb) was obtained by assembling the sequences with the SeqMan software (DNASTAR), and the sequence was then compared with the 16S rRNA gene sequences of related taxa obtained from the GenBank database and the EzBioCloud server (https://www.ezbiocloud.net) (Yoon *et al.*, 2017). Sequence alignments were done with CLUSTAL X (Thompson *et al.*, 1997). Gaps between sequences were edited with the BioEdit program, and evolutionary distances were calculated with the Kimura two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed by the neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1981), and maximum-parsimony methods (Fischer and Thatte, 2010) in the MEGA7 software (Kumar *et al.*, 2016). Bootstrap analysis was performed based on 1,000 resampled datasets using programs within the software.

Phenotypic characteristics

Single morphology and cell size were observed with a JEOL JEM1010 Transmission Electron Microscope (80 kV) after negative staining with 1% (w/v) phosphotungstic acid. The Gram reaction was performed with the BD Gram staining kit according to the manufacturer's instructions. The growth condition in the presence of 0–10% (w/v) NaCl (1% concentration increments) was determined as previously described (Koh *et al.*, 2017) after four weeks at 30°C. Growth at 5–45°C, with increments of 5°C, at pH 4.0–10.0, with increments of 0.5 pH unit (pH was adjusted with 1 M NaOH and 1 M HCl), was assessed on R2A broth after three weeks. For the pH growth condition experiments, four buffers were used as previously described (Koh *et al.*, 2015a): 10-mM homopiperazine-1,4-bis(2-ethanesulfonic acid) (pH 4.0–5.0), 10-mM 2-(N-morpholino)ethanesulfonic acid (pH 5.0–6.5), 10-mM 1,3-bis[tris(hydroxymethyl)methylamino]propane (bispropane, pH 7.0–8.5), and 10-mM 3-(cyclohexylamino)-1-propanesulfonic acid (pH 9.0–10.0). Carbon source utilization and enzyme activities were tested with the API 20NE, 32GN, and ZYM systems (bioMérieux), according to the manufacturer's instructions, and tests were carried out in duplicate and repeated twice to ensure reproducibility. An anaerobic growth test was conducted with the GasPak™ EZ anaerobe pouch system (BD) over two weeks. The tests for catalase and oxidase activities and for the hydrolysis of DNA, cellulose, starch, skim milk, and Tween 60 were done with R2A medium as the basal medium as previously described (Koh *et al.*, 2015b).

Chemotaxonomic analyses

The peptidoglycan of strain DCT-19^T was analyzed as described by Schleifer and Kandler (1972). Cellular fatty acids of strain DCT-19^T, *P. amylolyticus* NRRL NRS-290^T, *P. tundrae* NRRL B-51094, and *P. xylanexedens* NRRL B-51090^T were analyzed with cells grown on R2A agar for 3 days at 30°C. Fatty acid methyl ester mixtures were prepared according to a protocol published for the Sherlock Microbial Identification System (MIDI), and the profiles were determined according to the MIDI/Hewlett Packard Microbial Identification System using GC (6890N and 7683 autosampler; Agilent Technologies), based on the manufacturer's instructions. Polar lipids were extracted from freeze-dried cells (100 mg) derived from the isolated strains and were analyzed as previously described (Koh *et al.*, 2017). Quinones were extracted using a chloroform/methanol mixture (2:1, v/v) (Hu *et al.*, 1999), evaporated under vacuum, and re-extracted three times with n-hexane/water (1:1, v/v). Then, they were concentrated and applied to a Sep-Pak Plus silica column (Waters). Quinone components were separated and identified by reversed-phase HPLC equipped with a photodiode array detector and internal and external quinone standards, as previously described (Koh *et al.*, 2015a).

Genomic analysis

Whole-genome sequence of strain DCT-19^T was determined by PacBio RS II sequencing platform and was assembled by the hierarchical genome-assembly process (HGAP4) *de novo* assembler, representing ca. 220-fold coverage. The genome annotation was performed by the NCBI prokaryotic genome annotation pipeline (PGAP).

DNA-DNA Hybridization

The DNA-DNA Hybridization (DDH) assay was done with strain DCT-19^T as described previously (Ezaki *et al.*, 1990). The genomic DNAs of strain DCT-19^T and three reference strains were extracted with a genomic DNA extraction kit (GeneAll, Biotechnology Co. Ltd) and used as probe DNA. Probe DNA was biotinylated with photobiotin and hybridized with single-stranded, unlabeled genomic DNA fragments of the reference or test microorganisms. The mean values from three independent determinations of the DDH experiments were reciprocally obtained.

Nucleotide sequence accession numbers

The GenBank/EMBL/DDBJ accession numbers for the 16S

rRNA gene and whole-genome sequence of strain DCT-19^T are MH718803 and CP029639, respectively.

Results and Discussion

Phylogenetic analysis

The 16S rRNA gene sequence of strain DCT-19^T was a continuous stretch of approximately 1400 nucleotide base pairs. The sequence similarity search for its 16S rRNA gene in the EzBioCloud database revealed that the isolate was the most closely related to *P. amylolyticus* NRRL NRS-290^T (98.6%), followed by *P. tundrae* A10b^T (98.1%), *P. dongdonensis* KUDC0114^T (98.0%), *P. tylopili* MK2^T (97.9%), *P. cucumis* AP-115^T (97.8%), and *P. xylanexedens* B22a^T (97.6%) (Fig. 1). Strain DCT-19^T has not yet been identified as an already recognized species within the genus *Paenibacillus*, and thus, it could be considered to represent a novel species belonging to the genus *Paenibacillus*.

Phenotypic and physiological characteristics

The cells of strain DCT-19^T are Gram-stain-positive, strictly aerobic, catalase-positive, and oxidase-negative, and the colony morphology is circular, raised, entire margins and creamy. Cells were rod-shaped (2.0 μm length and 1.0 μm width) with peritrichous flagella. Cell growth occurred at 15–40°C (optimum 25–30°C), pH 6.0–8.0 (optimum pH 7.0), and 0–4% (w/v) NaCl (optimum growth with 0%). Other physiological and biological characteristics of strain DCT-19^T are summarized in the species description, and a comparison of the selective characteristics of strain DCT-19^T and selected type strains is given in Table 1. Additionally, strain DCT-19^T exhibits degradation activities for skim milk and starch but not for DNA, cellulose, and Tween 60. As shown in Table 1, there are numerous other phenotypic characteristics that could be used to distinguish strain DCT-19^T from its closest phylogenetic neighbors *P. amylolyticus* NRRL NRS-290^T, *P. tundrae* NRRL B-51094^T, and *P. xylanexedens* NRRL B-51090^T.

Chemotaxonomic analyses

As a major diamino acid, *meso*-diaminopimelic acid (*meso*-DAP) was detected in the peptidoglycan of strain DCT-19^T. The presence of *meso*-DAP in the peptidoglycan is a marker for the members of the genus *Paenibacillus* (Shida *et al.*, 1997). The major fatty acid in strain DCT-19^T was $C_{15:0}$ anteiso (51.8%), followed by $C_{16:0}$ anteiso (15.2%) and $C_{16:0}$ (12.8%). This $C_{15:0}$ anteiso was also identified as a major fatty acid of the selected reference strains (54.7%–63.4%). In addition, we found that the proportions of other fatty acids slightly varied among all the strains (Table 2). The major

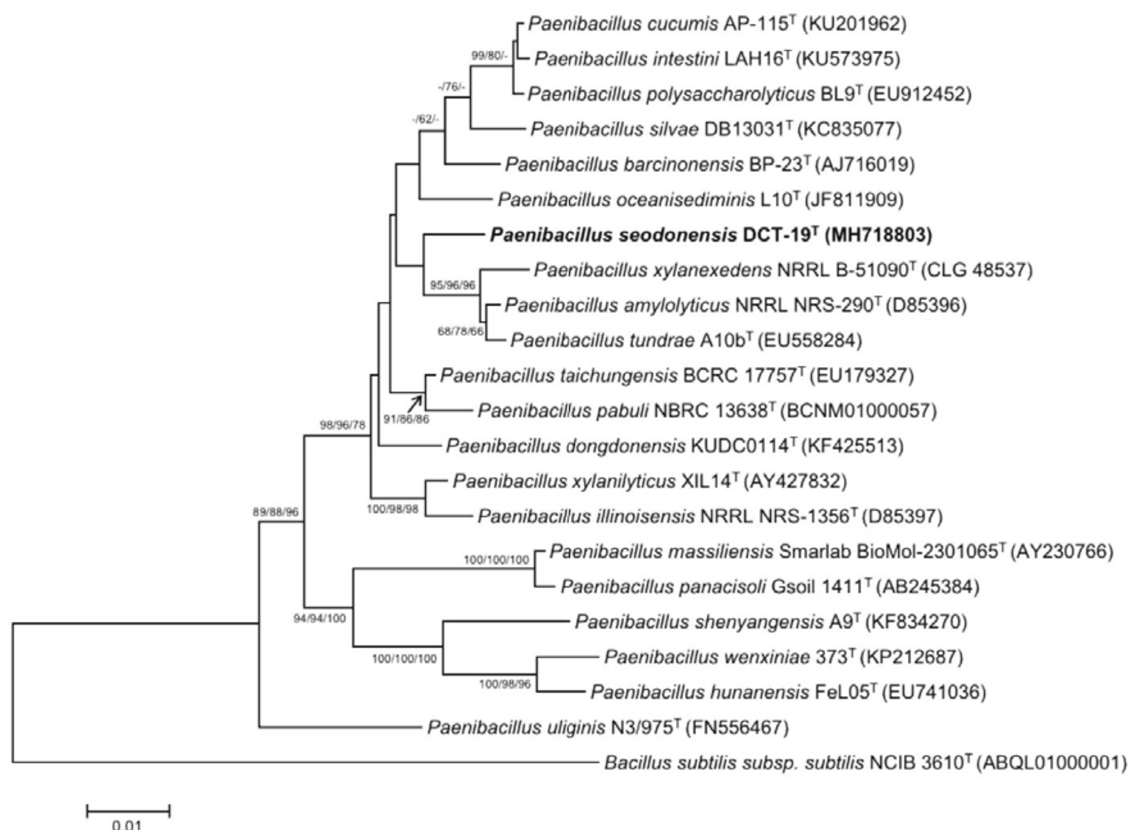

Fig. 1. A neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain DCT-19^T among the related strains of the genus *Paenibacillus* and other representative members of *Paenibacillus*. Bootstrap values (based on 1,000 replications) greater than 60% are shown at the branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with maximum likelihood method and maximum parsimony method with *Bacillus subtilis* subsp. *subtilis* NCIB 3610^T included as an out-group. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of strain DCT-19^T and phylogenetically related strains of the genus *Paenibacillus*

Strains: 1, strain DCT-19^T; 2, *Paenibacillus amylolyticus* NRRL NRS-290^T; 3, *Paenibacillus tundrae* NRRL B-51094^T; 4, *Paenibacillus xylanexedens* NRRL B-51090^T.

All data were obtained in this study, unless otherwise noted. All strains were positive for the enzymatic activity of catalase, β-glucosidase (esculin hydrolysis), β-galactosidase (PNPG), esterase (C4), esterase lipase (C8), and acid phosphatase and were positive for the utilization of D-glucose, L-arabinose, D-mannitol, gluconate, salicin, D-ribose, D-sucrose, and glycogen, but negative for oxidase, indole production, glucose acidification, arginine dihydrolase, urease, protease, caprate, adipate, malate, citrate, phenyl-acetate, L-fucose, D-sorbitol, propionate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, itaconate, suberate, malonate, DL-lactate, L-alanine, 5-ketogluconate, 3-hydroxy-benzoate, and L-serine.

Characteristics	1	2	3	4
Cell size (μm):				
Length	2.0	3.0–5.0[a]	2.5[b]	2.5[c]
Width	1.0	0.7–0.9[a]	0.5[b]	0.5[c]
Nitrate reduction	+	-[a]	-[b]	-[c]
Temperature range (°C)	15–40	10–40[a]	13-37[b]	13–32[c]
pH range	6.0–8.0	4.5–9.0[a]	5.2-8.8[b]	5.7–8.8[c]
NaCl range (%, w/v)	0–4	0–4[a]	ND*	ND
Enzyme activity:				
Alkaline phosphatase	-	+	+	+
Trypsin	-	+	+	-
α-Chymotrypsin	-	+	+	+
Naphtol-AS-BI-phosphohydrolase	+	+	-	+
α-Galactosidase	-	+	+	+
α-Glucosidase	-	+	+	+
N-Acetyl-glucosamine	-	-	-	+
α-Mannosidase	-	-	+	-
Assimilation of:				
D-Mannose	+	-	+	-
N-Acetyl-glucosamine	-	-	-	+
D-Maltose	-	+	+	+
D-Melibiose	-	+	+	+
Valerate	+	-	-	-
N-Acetyl-glucosamine	-	-	-	+
Inositol	+	-	-	+
D-Maltose	-	+	+	+
Acetate	+	-	-	+
DNA G + C content (mol%)	44.8**	46.3–46.6[a]	50.3[b]	46.4[c]

+, positive reaction; –, negative reaction.
[a] Data from Shida *et al*. (1997).
[b,c] Data from Nelson *et al*. (2009).
* ND, not determined.
** Estimated by whole-genome sequencing.

polar lipids of strain DCT-19^T were diphosphatidylglycerol and phosphatidylethanolamine. Additionally, phosphatidylglycerol, four unidentified lipids, two unidentified phospholipids, and three unidentified aminophospholipids were detected by two-dimensional TLC of the total polar lipids. These results support the conclusion that strain DCT-19^T represents a species distinct from the known species of the genus *Paenibacillus*.

Genomic traits

The genome size of strain DCT-19^T was about 6.67 Mb, and the genome was predicted to have 6,375 coding sequences (CDSs), 33 rRNA and tRNA genes. Unexpectedly, 2261 CDSs (about 35.5%) were determined as a hypothetical protein. In addition, about 30 genes were classified into viral defense and/or phage-relatives. Taken together, it may indicate that strain DCD-19^T might be under stress for gene shuffling by horizontal gene transfers or phage infection. Based on the cluster orthologous groups (COGs), most CDSs were classified to functional unknown (35.2% of total assigned COGs), followed by those identified as having roles in carbohydrate transport and metabolism (11.0%) and transcription (9.4%). Genes coding for nitrite reductase (*nirBDK*) and nitrate reductase (*narGH*) were identified. Approximately 120 genes involved in ATP binding cassette (ABC) transporters, including branched-chain amino acids, iron (III), phosphate, sulfate, thiamine, zinc, and oligopeptide were determined. Moreover, we confirmed several genes as having degradation capabilities, such as starch (*amyCD*). The genomic G + C content of strain DCT-19^T was 44.8%, which is within the range of the values of other recognized species of the genus *Paenibacillus* (Table 1).

DNA-DNA Hybridization

The levels of DNA-DNA relatedness of strain DCT-19^T with *P. amylolyticus* NRRL NRS-290^T, *P. xylanexedens* NRRL

Table 2. Cellular fatty acid profiles of strain DCT-19^T and phylogenetically related members of the genus *Paenibacillus*

Strains: 1, strain DCT-19^T; 2, *Paenibacillus amylolyticus* NRRL NRS-290^T; 3, *Paenibacillus tundrae* NRRL B-51094^T; 4, *Paenibacillus xylanexedens* NRRL B-51090^T

All data are from the present study. All strains were grown on R2A agar at 30°C for 3 days. Values are percentages of the total fatty acids, and only fatty acids accounting for more than 0.5% in at least one of the strains are indicated.

Fatty acid	1	2	3	4
Saturated				
$C_{12:0}$	2.77	0.55	1.16	1.15
$C_{14:0}$	2.37	4.29	4.2	1.32
$C_{16:0}$	12.8	5.18	8.73	3.47
$C_{17:0}$	0.67	TR	TR	TR
Unsaturated				
$C_{16:1}$ $\omega 11c$	0.68	TR	1.57	TR
$C_{17:1}$ $\omega 11c$	TR	4.56	TR	TR
Branched-chain fatty acid				
$C_{14:0}$ iso	4.72	7.51	9.04	4.51
$C_{15:0}$ iso	2.01	3.83	2.87	5.14
$C_{16:0}$ iso	15.2	8.32	12	8.8
$C_{17:0}$ iso	1.35	0.8	0.75	1.81
$C_{16:1}$ $\omega 7c$ alcohol	TR	TR	0.51	1.26
$C_{15:0}$ anteiso	51.8	57.8	54.7	63.4
$C_{17:0}$ anteiso	5.12	3.66	2.94	5.96

TR, trace (less than 0.5%)

B-51090^T, and *P. tundrae* NRRL B-51094^T were 30.0 ± 2.60%, 29.0 ± 1.76, and 24.5 ± 1.45%, respectively.

Taxonomic conclusion

Based on the phylogenetic tree, strain DCT-19^T is proposed to belong to the genus *Paenibacillus*. The phenotypic, chemotaxonomic, and genotypic results distinguish strain DCT-19^T from other closely related strains. Therefore, strain DCT-19^T has been proposed as a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus seodonensis* sp. nov. has been proposed.

Description of *Paenibacillus seodonensis* sp. nov.

Paenibacillus seodonensis (seo.do.nen.sis. N.L. masc. adj. *seodonensis* of Seodo in the Dokdo Islands, Korea, where the type strain was isolated).

Cells are Gram-stain-positive, rod shaped, aerobic, 2.0 μm in length, and 1.0 μm in width. Growth occurs at pH 6.0–8.0 (optimum, pH 7.0), 0–4% (w/v) NaCl (optimum, 0%), and at a temperature of 15–40°C (optimum 25–30°C). Cells can grow on tryptic soy agar and nutrient agar and reduce nitrate to nitrite but not nitrogen. Cells are positive for enzymatic activities of esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, β-galactosidase, and β-glucosidase but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, and α-glucosidase. Cells can use D-glucose, L-arabinose, D-mannose, D-mannitol, salicin, valerate, D-ribose, inositol, D-sucrose, acetate, and glycogen but not D-melibiose, L-fucose, D-sorbitol, propionate, caprate, citrate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, N-acetyl-glucosamine, D-maltose, itaconate, suberate, malonate, DL-lactate, L-alanine, 5-ketogluconate, 3-hydroxy-benzoate, and L-serine. *Meso*-diaminopimelic acid was detected in the peptidoglycan of cells. The predominant respiratory quinone is MK-7. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ anteiso, and $C_{16:0}$. The polar lipid pattern comprises diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, four unidentified lipids, two unidentified phospholipids, and three unidentified aminophospholipids.

The G + C content of the type strain is 44.8% as determined by genome sequencing. The type strain DCT-19^T (= KCTC 43009^T = LMG 30888^T) was isolated from a native plant that belongs to the genus *Campanula* on Dokdo, the Republic of Korea.

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Conflict of Interest

The authors declare to have no conflicts of interest.

References

Ash, C., Farrow, J.A., Dorsch, M., Stackebrandt, E., and Collins, M.D. 1991. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int. J. Syst. Bacteriol.* **41**, 343–346.

Ash, C., Priest, F.G., and Collins, M.D. 1993. Molecular identification of rRNA group 3 *Bacilli* (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* **64**, 253–260.

Ezaki, T., Adnan, S., and Miyake, M. 1990. Quantitative microdilution plate hybridization to determine genetic relatedness among bacterial strains. *Nihon Saikingaku Zasshi* **45**, 851–857.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376.

Fischer, M. and Thatte, B. 2010. Revisiting an equivalence between maximum parsimony and maximum likelihood methods in phylogenetics. *Bull. Math. Biol.* **72**, 208–220.

Hu, H.Y., Fujie, K., and Urano, K. 1999. Development of a novel solid phase extraction method for the analysis of bacterial quinones in activated sludge with a higher reliability. *J. Biosci. Bioeng.* **87**, 378–382.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.

Koh, H.W., Hong, H., Min, U.G., Kang, M.S., Kim, S.G., Na, J.G., Rhee, S.K., and Park, S.J. 2015a. *Rhodanobacter aciditrophus* sp. nov., an acidophilic bacterium isolated from mine wastewater. *Int. J. Syst. Evol. Microbiol.* **65**, 4574–4579.

Koh, H.W., Rani, S., Kim, S.J., Moon, E., Nam, S.W., Rhee, S.K., and Park, S.J. 2017. *Halomonas aestuarii* sp. nov., a moderately halo-

philic bacterium isolated from a tidal flat. *Int. J. Syst. Evol. Microbiol.* **67**, 4298–4303.

Koh, H.W., Song, H.S., Song, U., Yim, K.J., Roh, S.W., and Park, S.J. 2015b. *Halolamina sediminis* sp. nov., an extremely halophilic archaeon isolated from solar salt. *Int. J. Syst. Evol. Microbiol.* **65**, 2479–2484.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**, 1870–1874.

Morales, P., Sendra, J.M., and Perez-Gonzalez, J.A. 1995. Purification and characterization of an arabinofuranosidase from *Bacillus polymyxa* expressed in *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.* **44**, 112–117.

Nelson, D.M., Glawe, A.J., Labeda, D.P., Cann, I.K., and Mackie, R.I. 2009. *Paenibacillus tundrae* sp. nov. and *Paenibacillus xylanexedens* sp. nov., psychrotolerant, xylan-degrading bacteria from Alaskan tundra. *Int. J. Syst. Evol. Microbiol.* **59**, 1708–1714.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Schleifer, K.H. and Kandler, O. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**, 407–477.

Shida, O., Takagi, H., Kadowaki, K., Nakamura, L.K., and Komagata, K. 1997. Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int. J. Syst. Bacteriol.* **47**, 289–298.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703.

Xie, J.B., Du, Z., Bai, L., Tian, C., Zhang, Y., Xie, J.Y., Wang, T., Liu, X., Chen, X., Cheng, Q., *et al.* 2014. Comparative genomic analysis of N_2-fixing and non-N_2-fixing *Paenibacillus* spp.: Organization, evolution and expression of the nitrogen fixation genes. *PLoS Genet.* **10**, e1004231.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., and Chun, J. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int. J. Syst. Evol. Microbiol.* **67**, 1613–1617.