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Paenibacillus insulae sp. nov., isolated from soil[§]

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A Gram-stain-positive, motile, endospore-forming, and strictly aerobic rod-shaped bacterium designated DS80^T was isolated from an island soil. The strain DS80^T grew at temperatures between 15 and 40°C (optimum = 30°C) and at pH values ranging from 5.0 to 9.0 (optimum = 7.0). The phylogenetic analysis based on the comparisons of the 16S rRNA gene sequences showed that the isolate was affiliated to the genus *Paenibacillus* and was mostly related to *Paenibacillus assamensis* GPTSA11^T (with the sequence similarity of 96.33%) and *Paenibacillus urinalis* 5402403^T(95.48%). The G+C content of the genomic DNA was 44.0 mol% and the major fatty acids were *anteiso*-$C_{15:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, and $C_{16:1}$ *ω*11*c*. Strain DS80^T contained MK-7 as the major menaquinone, and phosphatidylglycerol, phosphatidylethanolamine, and diphosphatidylglycerol as the major polar lipids. The peptidoglycan contained a major amount of *meso*-diaminopimelic acid. The chemotaxonomic profile of strain DS80^T was consistent with that of *Paenibacillus*. However, the phenotypic properties clearly separated the strain from other species of the genus. Accordingly, a new species, *Paenibacillus insulae* sp. nov., is proposed (type strain =DS80^T =JCM 17278^T =KCTC 13833^T).

Keywords: *Paenibacillus insulae*, Gram-positive, Dokdo Island, 16S rRNA

Introduction

The genus *Paenibacillus* is a group of aerobic or facultative anaerobic, endospore-forming bacteria, originally included within the genus *Bacillus* and then reclassified as a separate genus in 1993 (Ash *et al.,* 1993). Thereafter, its description was amended by Shida *et al.* (1997). As of July 2015, over 160 species are recognized as members of the genus *Paenibacillus* (http://www.bacterio.net/paenibacillus.html). While soil is considered to be the normal habitat for the genus, species belonging to the genus *Paenibacillus* have also been isolated from various ecological niches, such as soil, rhizosphere, wastewater, estuarine wetlands, diseased insect larvae, food, cow feces, blood cultures, Antarctic sediment, roots of winter crops, and phyllosphere (Priest, 2009). Members of *Paenibacillus* are characterized by the production of rod-shaped cells and ellipsoidal endospores in swollen sporangia, Gram-stain-positive cell walls, motility by flagella, possession of anteiso-C15:0 as the major cellular fatty acid, and genomic DNA G+C contents in the range of 39–54 mol% (Shida *et al.*, 1997; Montes *et al.*, 2004; Takeda *et al.*, 2005; De Vos *et al.*, 2009). The genus *Paenibacillus* usually produce small, translucent, light brown or white, and sometimes pink or yellowish colonies on agar plates (Shida *et al.*, 1997; Priest, 2009).

Numerous species of the genus *Paenibacillus* exhibit interesting metabolic and physiological features, such as the ability to fix nitrogen, hydrolysis of a variety of carbohydrates, and insecticidal activity, implying its potential as a valuable resource (Priest, 2009). Some *Paenibacillus* strains also produce antibiotic compounds, for example, polymyxins and lidopeptides, while others show plant growth promoting potential (Lal and Tabacchioni, 2009; Cochrane and Vederas, 2014).

In this study, a bacterial strain was isolated during a survey of microbial diversity in an island soil. The polyphasic analysis suggests that the isolate belongs to the genus *Paenibacillus*, but exhibits novel taxonomic properties; accordingly, a novel species in the genus *Paenibacillus* is proposed.

Materials and Methods

Strain isolation

Strain DS80^T was isolated from the soil of Dokdo Island in the East Sea (37°14′ 18″ N 131°52′ 22″ E). The standard dilution plating technique using tryptic soy agar (TSA, BD) was employed and isolation was carried out after incubation at 28°C for 2 days. Subcultivation was routinely performed on TSA at 28°C for 2 days under aerobic condition and the biomass was stored at -70°C in tryptic soy broth (TSB, BD) supplemented with 20% (v/v) glycerol for preservation.

Phylogenetic analysis

Bacterial genomic DNA was extracted using a commercial genomic DNA extraction kit (Bioneer). The 16S rRNA gene was amplified using the universal bacterial primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) (Lane, 1991); the purified PCR products were sequenced. The almost com-

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plete 16S rRNA gene sequence of strain DS80^T was manually aligned with those of the type strains of related *Paenibacillus* species, which were determined using EzTaxon server (http://ezbiocloud.net/eztaxon/). Secondary structural information implemented in the BioEdit program version 7.1.3 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) was used for accurate alignment. Phylogenetic trees were inferred by using three algorithms, namely, neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1981), and maximum-parsimony (Fitch, 1971) methods using the BioEdit version 7.0 and MEGA4 (Tamura *et al*., 2007) packages. The topology of the obtained tree was evaluated by a bootstrap analysis (Felsenstein, 1981) based on 1,000 resamplings.

Phenotypic characterization

The phenotypic properties of strain DS80^T were tested using standard procedures. Cell morphology was examined with a scanning electron microscope (S-4800+EDS, HORIBA) using cells grown for 24 h at 30°C on TSA. The optimal temperature and temperature range for growth was tested on TSA at 10, 15, 20, 25, 30, 35, 37, and 40°C. Growth in a medium with NaCl was tested in TSA agar supplemented with 0–15% NaCl (at 1% intervals). The pH range for growth was assessed on TSB medium, the pH of which was adjusted to 3.0–11.0 at intervals of 1.0 pH unit with HCl or NaOH. Catalase and oxidase activities were determined using previously described methods (Barrow and Feltham, 1993). Enzymatic hydrolysis of casein and starch were examined using TSA amended with 1% substrates as described by Wollum (1982). Phenotypic and enzymatic characterizations were also conducted using the API 50CHB and API 20E kits (bioMérieux).

Chemotaxonomic analysis

The total cellular fatty acid compositions of the strain and related species were analyzed using the Microbial Identification System (MIDI) with cells grown on TSA at 30°C for 3 days. Menaquinones were extracted from a 200-mg dry cell mass with a 10% aqueous solution of 0.3% (w/v) NaCl in methanol petroleum ether (boiling point, 60–80°C) at the ratio of 1:1. The upper phase was collected and dried in a vacuum evaporator and the residue was dissolved in 100 µl acetone. The extract was applied on a TLC plate (20 × 20 Silica gel 60 F_{254}; Merck) using petroleum ether (boiling point, 60–80°C) and diethyl ether (85:15, v/v). Purified menaquinones were dissolved in 2-propanol and analyzed by reverse-phase TLC (Collins and Jones, 1980). Polar lipids were extracted and examined by a two-dimensional TLC on aluminum-backed thin-layer plates (20 × 20 Silica gel 60 F_{254}; Merck) according to the previously described procedures (Collins and Jones, 1980; Kates, 1986). The G+C content of genomic DNA was determined by the thermal denaturation method (Marmur and Doty, 1962) using an Ultrospec 2100 spectrophotometer (Pharmacia). DNA from *P. assamensis* GPTSA11^T was used as a control. The cell-wall amino acid was determined by TLC as described by Staneck and Roberts (1974).

Results and Discussion

The cells of strain DS80^T were Gram-stain-positive and rod-shaped with the dimensions of 1.7–2.1 × 0.4–0.8 mm (Supplementary data Fig. S1). The colonies on TSA were flat with undulate margins and light yellowish-white colored. Ellipsoidal endospores were formed centrally within swollen sporangia.

The analysis of 16S rRNA gene sequences indicated that strain DS80^T is placed in the phylogenetic lineage occupied by the genus *Paenibacillus*. The closest relatives were *P. assamensis* GPTSA11^T (with the sequence similarity of 96.33%) and *P. urinalis* 5402403^T (95.48%). Strain DS80^T was clustered with *P. assamensis* GPTSA11^T in the phylogenetic tree and the close relationship between the two was supported by

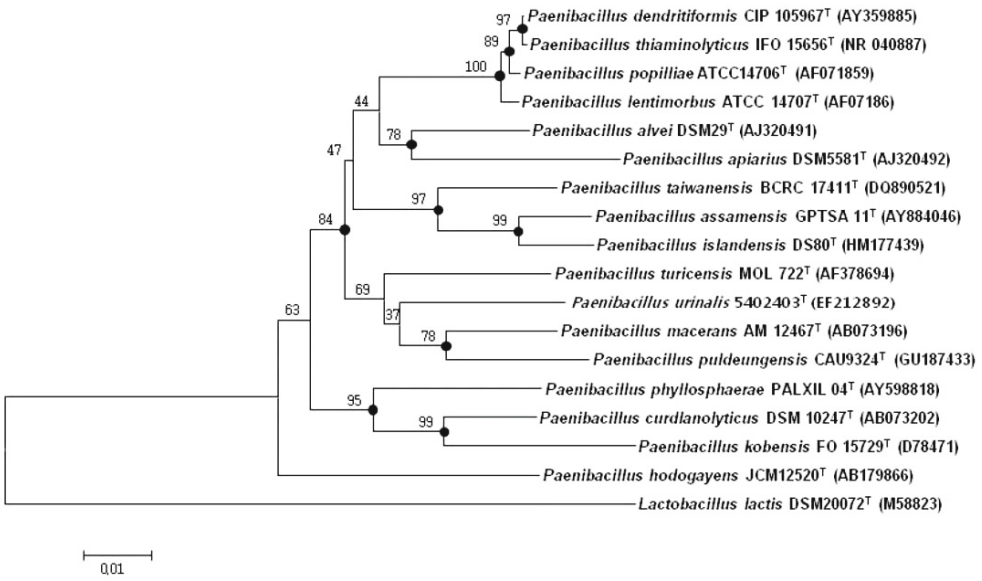

Fig. 1. Phylogenetic tree showing the taxonomic position of strain DS80^T within the genus *Paenibacillus*. The tree was constructed from a Kimura two-parameter distance matrix and the neighbor-joining method. Percentage bootstrap values (>50%, 1,000 replications) are given at branching points, and filled circles indicate that the corresponding nodes (groupings) were also recovered in maximum-parsimony and maximum-likelihood trees. The bar represents 0.01% estimated sequence divergence.

Table 1. Differential characteristics of strain DS80^T and the related type strains of *Paenibacillus* species

Strains: 1, strain DS80^T; 2, *P. polymyxa* KCTC 3627^T; 3, *P. alvei* ATCC 6344^T; 4, *P. assamensis* GPTSA 11^T; 5, *P. taiwanensis* G-soil-2-3^T; 6, *P. urinalis* 5402403^T.

Characteristic	Strains					
	1	2	3	4	5	6
Growth at[a]:						
10°C	-	+	-	-	+	-
45°C	+	-	+	-	+	-
pH 5	-	-	-	-	+	-
Indole production	-	-	+	-	-	-
Acid production from:						
Glycerol	+	+	-	+	+	-
Adonitol	-	-	+	-	-	-
D-Galactose	-	+	-	-	-	+
Inositol	-	-	-	-	-	-
Methyl α-D-glucoside	+	+	-	+	-	+
Amygdalin	+	+	-	-	+	+
Arbutin	-	+	+	-	-	+
D-Cellobiose	+	+	-	-	+	+
D-Melibiose	-	+	+	+	-	+
Sucrose	-	+	-	-	+	+
D-Trehalose	+	+	-	-	+	-
D-Melezitose	-	-	-	-	-	-
Glycogen	+	+	-	+	-	-
Hydrolysis of:						
Starch	+	+	+	+	-	-
Casein	+	+	+	+	+	+
Urea	-	-	+	-	-	-
DNA G+C content (mol%)[a]	44.0	43–46[b]	45–47[b]	41.2	44.6	ND

[a] Data for the marked properties were taken from previous studies (data taken from Lee *et al.*, 2007; Roux *et al.*, 2008; Priest, 2009).
[b] variable for species, not of type strains.
+, Positive; -, negative; ND, not determined.

different treeing algorithms, as well as by a high bootstrap value (Fig. 1). However, the sequence similarity was considerably below the suggested cutoff values for species distinction (Stackebrandt and Ebers, 2006; Kim *et al.*, 2014).

The phenotypic properties also distinguished strain DS80^T from other related species (Table 1). DS80^T and the type strain of *P. assamensis*, the closest species, differed in the growth temperature range and acid production from selected substrates. Other detailed results of morphological, physiological, and biochemical tests are provided in the species description in Table 1.

The cellular fatty acid composition of strain DS80^T is shown in Table 2 together with those of related *Paenibacillus* species. Major cellular fatty acids of strain DS80^T were *anteiso*-$C_{15:0}$ (41.4%), *iso*-$C_{15:0}$ (12.4%), *iso*-$C_{16:0}$ (8.7%), and $C_{16:1}$ *ω11c* (6.1%). The dominance of *anteiso*-$C_{15:0}$ is a characteristic of *Paenibacillus* (Priest, 2009). The major menaquinone of strain DS80^T was MK-7 and the major polar lipids were phosphatidylglycerol (PG), phosphatidylethnolamine (PE), diphosphatidylglycerol (DPG), and a minor amount of phosphatidylinositol (PI) was also present, which was comparable to those of *P. assamensis* GPTSA11^T (Supplementary data Fig. S2). The diagnostic diamino acid in the cell wall was *meso*-diaminopimelic acid. The DNA G+C content was 44.0 mol%.

The chemotaxonomic properties of DS80^T were consistent with its classification as *Paenibacillus* (Priest, 2009), but the fatty acid profiles and DNA G+C content, as well as many other phenotypic properties, distinguished the strain from related species (Tables 1 and 2).

Based on the combined phylogenetic, phenotypic and chemotaxonomic properties, strain DS80^T evidently deserves recognition as a new species of *Paenibacillus*, for which the name *Paenibacillus insulae* sp. nov. is proposed.

Description of *Paenibacillus insulae* sp. nov.

Paenibacillus insulae (L. fem. gen. n. *insulae*, of an island, referring to the source of isolation of the type strain).

Cells are strictly aerobic, rod-shaped (1.7–2.1 × 0.4–0.8 μm), Gram-stain-positive, and motile. Endospores are formed in swollen sporangia. Colonies are flat with undulate margins and light yellowish-white on TSA. Grows at 15–40°C (optimum 30°C), at pH from 5.0–9.0 (optimum pH 7.0) and in the presence of 1% NaCl (w/v). Oxidase and catalase activities are positive. Nitrate is not reduced. Hydrolyses starch

Table 2. Cellular fatty acid composition (%) of strain DS80^T and related *Paenibacillus* species

Strains: 1, strain DS80^T; 2, *P. polymyxa* KCTC 3627^T; 3, *P. alvei* ATCC 6344^T; 4, *P. assamensis* GPTSA 11^T; 5, *P. taiwanensis* G-soil-2-3^T; 6, *P. urinalis* 5402403^T. All data were obtained from this study.

Fatty acid	1	2	3	4	5	6
$C_{10:0}$	-	-	tr	-	-	-
anteiso-$C_{11:0}$	tr	-	-	-	-	-
$C_{11:0}$ 2OH	tr	-	-	-	-	-
iso-$C_{12:0}$	-	-	-	-	tr	-
$C_{12:0}$	tr	-	0.5	0.8	tr	tr
iso-$C_{13:0}$	-	-	tr	0.9	0.6	tr
anteiso-$C_{13:0}$	0.6	-	0.9	1.2	0.8	-
$C_{13:0}$	-	-	0.6	-	-	-
iso-$C_{14:0}$	2.4	tr	4.6	1.1	6.2	4.1
$C_{14:0}$	1.2	tr	2.3	1.4	1.5	tr
iso-$C_{15:0}$	12.4	5.6	10.4	26.6	11.1	4.1
anteiso-$C_{15:0}$	41.4	52.2	41.0	37.5	37.7	49.0
$C_{15:1}$ *ω5c*	-	-	-	1.3	-	-
$C_{15:0}$	-	-	-	-	-	6.3
$C_{16:1}$*ω7c* alcohol	3.1	-	5.6	0.9	1.0	-
iso-$C_{16:0}$	8.7	9.6	9.2	5.4	19.1	15.4
$C_{16:1}$ *ω11c*	6.1	-	8.2	2.8	2.6	1.3
$C_{16:0}$	5.7	11.3	3.7	6.6	6.7	7.6
iso-$C_{17:1}$ *ω10c*	3.3	-	1.9	2.4	0.7	-
iso-$C_{17:0}$	2.7	4.3	1.6	-	4.5	2.9
anteiso-$C_{17:0}$	4.3	12.2	3.0	4.7	5.8	5.7
$C_{17:1}$ *ω9c*	2.2	-	2.3	tr	-	-
$C_{17:0}$	1.2	tr	1.16	5.1	tr	-
$C_{17:0}$ 10-methyl	tr	-	-	-	-	-
iso-$C_{18:0}$	-	-	-	-	tr	-
$C_{18:1}$ *ω9c*	tr	-	-	-	tr	-
$C_{18:0}$	-	-	-	-	tr	-
Summed Feature 3[a]	tr	-	tr	-	tr	-
Summed Feature 4[b]	2.8	-	2.0	1.0	tr	-

[a] Summed Feature 3 consists of $C_{16:1}$ *ω7c* and/or 16:1 *ω7c*.
[b] Summed Feature 4 consists of $C_{17:1}$ I, and/or *anteiso*-$C_{17:1}$ B.
tr, Trace amount (<0.5% of total); -, not detected.

and casein. Acid is produced from glycerol, ribose, glucose, methyl α-D-glucoside, *N*-acetyl-glucosamine, esculin, D-cellobiose, D-maltose, D-trehalose, starch, glycogen, gentiobiose, and 2-nitrophenyl-β-D-lactopyranoside, but not from erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, methyl-β-D-xylopyranside, galactose, fructose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-α-D-mannopyranside, amygdalin, arbutin, salicin, lactose, melibiose, inulin, melezitose, raffinose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-koto-gluconate, and 5-keto-gluconate. Based on API 50CHB test, glycerol, ribose, glucose, mannose, α-methyl-D-glucoside, *N*-acetyl glucosamine, esculin, cellobiose, maltose, sucrose, trehalose, starch, glycogen, gentibiose, D-turanose, gluconate (weak), and 2-keto-gluconate (weak) are used, but the remaining substrates are not. Based on the API 20E test, arginine dihydrolase, ornithine decarboxylase, citrate utilization, H_2S production, indole production (weak), gelatinase, and cytochrome oxidase are positive, while β-galactosidase, lysine decarboxylase, urease, tryptophan deaminase, and acetoin production are negative.

The major fatty acids (>5%) are *anteiso*-$C_{15:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, and $C_{16:1}$ *ω*11*c*. The major respiratory quinone is MK-7 and the major polar lipids are phosphatidylglycerol (PG), phosphatidylethnolamine (PE), and diphosphatidylglycerol (DPG). The diagnostic diamino acid in the cell wall is *meso*-diaminopimelic acid. The DNA G+C content is 44.0 mol%.

The type strain DS80^T (=KCTC 13833^T =JCM 17278^T) was isolated from the soil of Dokdo Island, Republic of Korea.

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