Paenibacillus puldeungensis sp. nov., isolated from a grassy sandbank

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The taxonomic position of a Gram-stain-positive, rod-shaped, endospore-forming, facultatively anaerobic bacterial strain, CAU 9324^T, isolated from a grassy sandbank was investigated by using a polyphasic approach. Strain CAU 9324^T grew optimally at 30 °C and pH 6.0. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid. The major isoprenoid quinone was menaquinone-7 (MK-7). The polar lipid profile consisted of diphosphatidylglycerol as the major component. The predominant cellular fatty acid was anteiso-C_{15:0}. The DNA G+C content of strain CAU 9324^T was 48.8 mol%. Phylogenetic analyses based on 16S rRNA gene sequences revealed that the strain belonged to the genus *Paenibacillus*, showing <96.4 % similarity to the type strains of all recognized *Paenibacillus* species. On the basis of phenotypic, chemotaxonomic and genotypic data, strain CAU 9324^T was considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus puldeungensis* sp. nov. is proposed. The type strain is CAU 9324^T (=KCTC 13718^T =CCUG 59189^T).

The genus *Paenibacillus* was proposed by Ash *et al.* (1993, 1994) and is the type genus of the family *Paenibacillaceae*. *Paenibacillus* species are widely distributed in the environment and play significant roles in microbial communities (Reva *et al.*, 1995). Generally, members of the genus produce endospores, possess anteiso-C_{15:0} as the major cellular fatty acid and have genomic DNA G+C contents in the range 39–54 mol% (Shida *et al.*, 1997; Saha *et al.*, 2005). In the course of screening micro-organisms from a sample of grassy sandbank from DaeYijac Island, Republic of Korea, a facultatively anaerobic, Gram-stain-positive, endospore-forming bacterium (designated strain CAU 9324^T) was isolated and characterized. On the basis of data from the present polyphasic investigation, strain CAU 9324^T is considered to represent a novel species of the genus *Paenibacillus*.

Strain CAU 9324^T was isolated on glucose-yeast extract agar (GYEA) (per litre: 10 g yeast extract, 10 g glucose, 15 g agar) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹) (Gordon & Mihm, 1962). A sample was diluted with sterilized distilled water. Serial dilutions were spread onto GYEA medium and incubated under aerobic conditions at 30 °C for 3 days.

Strain CAU 9324^T was preserved at –70 °C in GYE broth supplemented with 25 % (v/v) glycerol. The morphology of

the cells and spores was examined by light microscopy (model DM 1000; Leica). The presence of flagella was examined by scanning electron microscopy (model JSM-5410LV; JEOL) by using cells from an exponentially growing culture. Gram staining was carried out by using the procedure described by Doetsch (1981).

Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Oxidase activity was determined based on the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Acid production from carbohydrates was tested by using the API 50CHB system (bioMérieux). Enzyme activity was determined with the API ZYM system (bioMérieux), and other physiological and biochemical tests were performed with the API 20E system (bioMérieux) according to the manufacturer's instructions. Growth at 20, 30, 37 and 45 °C was determined on trypticase soy agar (TSA; Difco) in an aerobic incubator (model MIR-253; Sanyo) or anaerobic chamber (model Bactron; Sheldon). The pH range for growth was tested in trypticase soy broth (TSB) medium at pH 3.0–11.0. Growth with 0–15 % (w/v) NaCl at 30 °C was investigated in TSB.

Cellular fatty acid methyl esters were extracted by acid methanolysis (Minnikin *et al.*, 1980) after incubation for 3 days on TSA and were analysed by using an Agilent Technologies model 56890N gas chromatograph (Microbial Identification System Instrument) equipped with a cross-linked 5 % phenyl methyl silicon-fused silica capillary column (HP 19091B-102). Preparation of cell walls from the test strain and analysis of peptidoglycan

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CAU 9324^T is GU187433.

Two supplementary figures are available with the online version of this paper.

Table 1. Differential phenotypic properties between strain CAU 9324^T and the type strains of closely related *Paenibacillus* species

Strains: 1, CAU 9324^T; 2, *P. macerans* KCTC 1822^T; 3, *P. timonensis* KCTC 3995^T; 4, *P. motobuensis* KCTC 3992^T; 5, *P. sanguinis* KCTC 3993^T. Data were obtained in this study unless indicated. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5
Catalase	–	+	+	+	+
Oxidase	+	+	+	+	–
Nitrate reduction	+	+	–	–	–
Growth in 5% NaCl	+	–	–	w	+
Growth at 50 °C	–	+	+	+	+
Acid production from:					
Glycerol	+	+	–	–	–
D-Arabinose	–	+	+	–	+
L-Arabinose	+	+	+	–	–
Ribose	+	+	+	+	–
D-Xylose	+	+	+	+	–
Methyl β-D-xylopyranoside	+	–	+	+	–
Galactose	–	–	+	+	–
Glucose	+	+	+	–	+
Fructose	–	+	+	–	–
Mannose	–	+	+	–	–
Rhamnose	–	+	–	+	–
Mannitol	–	+	–	+	–
Sorbitol	–	+	–	–	–
Methyl α-D-mannopyranoside	–	–	+	–	–
Methyl α-D-glucoside	–	+	+	–	–
N-Acetylglucosamine	–	–	+	–	–
Amygdalin	–	+	+	–	–
Arbutin	–	+	+	–	–
Salicin	+	+	+	+	–
Maltose	+	+	+	+	–
Lactose	+	+	+	+	–
Melibiose	+	+	+	+	–
Sucrose	+	+	–	–	–
Trehalose	–	+	+	+	–
Inulin	+	+	–	+	–
Melezitose	–	+	–	–	–
Raffinose	+	+	+	–	–
Glycogen	–	+	+	+	–
Gentiobiose	+	–	+	w	–
Turanose	–	+	–	+	–
D-Arabitol	–	+	–	–	–
Gluconate	+	–	+	–	–
5-Ketogluconate	–	–	–	–	+
Hydrolysis of:					
Gelatin	–	–	–	+	+
Starch	+	+	+	+	–
Enzyme activity					
Esterase	–	+	+	+	–
Esterase lipase	–	–	+	+	+
Leucine arylamidase	+	w	+	+	–
Valine arylamidase	+	–	+	–	–
Cystine arylamidase	+	–	–	–	–
Trypsin	+	–	+	+	–

Table 1. cont.

Characteristic	1	2	3	4	5
α-Chymotrypsin	+	–	+	–	–
α-Galactosidase	+	+	+	+	–
β-Glucuronidase	+	+	+	+	–
α-Glucosidase	+	+	+	–	–
β-Glucosidase	+	+	+	+	–
N-Acetyl-β-glucosaminidase	–	–	+	+	+
DNA G+C content (mol%)	48.8	ND	ND	47*	ND

*Data taken from Iida *et al.* (2005).

structures were carried out by using the methods described by Schleifer (1985), with the modification that TLC was performed on cellulose sheets instead of paper chromatography. Menaquinones were analysed as described by Komagata & Suzuki (1987) by using reversed-phase HPLC. Polar lipids were extracted and analysed by two-dimensional TLC according to Minnikin *et al.* (1984).

The genomic DNA of strain CAU 9324^T was extracted according to the method of Marmur (1961). The 16S rRNA gene of the strain was amplified by PCR following established procedures (Nam *et al.*, 2004). The amplified 16S rRNA gene was sequenced directly by using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730; Applied Biosystems). Multiple alignments with sequences of a wide selection of *Paenibacillus* species and calculation of sequence similarities were carried out via the EzTaxon server (Chun *et al.*, 2007) and by using the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distance matrices were generated by the neighbour-joining method as described by Jukes & Cantor (1969). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) methods from the PHYLIP suite of programs (Felsenstein, 1989), and tree topology was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein, 1985) of the neighbour-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package. The G+C content of the genomic DNA was determined by HPLC according to the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

Cells of strain CAU 9324^T were Gram-stain-positive, spore-forming, non-motile and facultatively anaerobic. Colonies were white and flat, with a smooth surface and entire margins. Cells were short rods, approximately 0.3 × 1.3–2.3 µm, and the endospores were terminal and were formed in swollen sporangia (Supplementary Fig. S1, available in IJSEM Online). Growth was observed on GYEA medium at 20–45 °C, with optimal growth at 30 °C. In TSB, growth occurred at pH 5.0–11.0, with optimal

growth at pH 6.0. Strain CAU 9324^T grew in the presence of 0–15 % (w/v) NaCl, with optimal growth on medium containing 3 % (w/v) NaCl.

The phenotypic properties of strain CAU 9324^T are detailed in Table 1. The strain was oxidase-positive but catalase-negative. Acid production occurred from glycerol, L-arabinose, ribose, D-xylose, methyl β -D-xylopyranoside, glucose, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, raffinose, starch, gentiobiose and gluconate, but not from erythritol, D-arabinose, L-xylose, adonitol, galactose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, trehalose, melezitose, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, 2-ketogluconate or 5-ketogluconate. Activities were detected for leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase and β -glucosidase, but not alkaline phosphatase, esterase, esterase lipase, lipase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase.

Strain CAU 9324^T contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan, and whole-cell hydrolysates contained mainly ribose. The predominant isoprenoid quinone was unsaturated menaquinone-7 (MK-7). Strain CAU 9324^T displayed a polar lipid pattern that consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and two unknown aminophospholipids (Supplementary Fig. S2). The cellular fatty acid profile of strain CAU 9324^T grown on TSA was characterized as containing saturated branched and straight-chain components such as iso-C_{14:0} (5.2 %), C_{14:0} (3.0 %), iso-C_{15:0}

(3.9 %), anteiso-C_{15:0} (53.2 %), C_{15:0} (1.5 %), iso-C_{16:0} (9.0 %), C_{16:0} (17.0 %) and anteiso-C_{17:0} (3.9 %) (Table 2).

The almost-complete 16S rRNA gene sequence (1409 bp) of strain CAU 9324^T was determined and compared with the corresponding sequences of other bacterial strains in the GenBank database. Phylogenetic analyses indicated that the strain belonged to the genus *Paenibacillus*, with levels of 16S rRNA gene sequence similarity to all recognized *Paenibacillus* species ranging from 95.2 to 96.4 %. The phylogenetic tree obtained with the neighbour-joining method is shown in Fig. 1. Strain CAU 9324^T was related most closely to *Paenibacillus motobuensis* MC10^T (96.4 % 16S rRNA gene sequence similarity), *P. macerans* IAM 12467^T (96.2 %), *P. sanguinis* 2301083^T (96.2 %), *P. timonensis* 2301032^T (96.2 %) and *P. barengoltzii* SAFN-016^T (96.2 %). The DNA G + C content of strain CAU 9324^T was 48.8 mol%.

Therefore, on the basis of the data presented, strain CAU 9324^T is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus puldeungensis* sp. nov. is proposed.

Description of *Paenibacillus puldeungensis* sp. nov.

Paenibacillus puldeungensis (pul.deun.gen'sis. N.L. masc. adj. *puldeungensis* of/from puldeung, the Korean word for a

Table 2. Cellular fatty acid compositions of strain CAU 9324^T and the type strains of closely related *Paenibacillus* species

Strains: 1, CAU 9324^T; 2, *P. macerans* KCTC 1822^T; 3, *P. timonensis* KCTC 3995^T; 4, *P. motobuensis* KCTC 3992^T; 5, *P. sanguinis* KCTC 399^T. Data were obtained in this study and are percentages of total fatty acids. —, Not detected.

Fatty acid	1	2	3	4	5
Straight-chain					
C _{14:0}	3.0	2.4	3.5	1.8	1.4
C _{15:0}	1.5	2.4	2.0	1.7	8.4
C _{16:0}	17.0	8.3	10.7	8.0	15.3
Branched-chain					
anteiso-C _{15:0}	53.2	32.7	43.6	40.7	48.3
anteiso-C _{17:0}	3.9	12.8	4.4	8.2	3.5
iso-C _{14:0}	5.2	3.4	7.2	2.7	2.9
iso-C _{15:0}	3.9	9.2	11.4	15.0	3.5
iso-C _{16:0}	9.0	21.8	13.7	13.3	11.0
iso-C _{17:0}	—	7.1	2.4	8.8	1.8

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship between strain CAU 9324^T and the type strains of closely related members of the genus *Paenibacillus*. Branches of the tree that were also found by using the least-squares and/or maximum-likelihood treeing algorithms are indicated by asterisks (both algorithms) or by 'm' (maximum-likelihood only). Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >50 % are given. Bar, 0.01 substitutions per nucleotide position.

grassy sandbank such as the one from which the type strain was isolated).

Cells are Gram-stain-positive, facultatively anaerobic, non-motile, spore-forming rods ($0.3\text{--}0.4 \times 0.9\text{--}1.2\ \mu\text{m}$). Endospores are terminal and form within swollen sporangia. Colonies are white and flat with a smooth surface and have entire margins on GYEA medium after incubation at $30\ ^\circ\text{C}$ for 72 h. Growth occurs at $20\text{--}45\ ^\circ\text{C}$ (optimum $30\ ^\circ\text{C}$), at pH 5.0–11.0 (optimum pH 6.0) and in the presence of 0–15 % (w/v) NaCl (optimum 3 %) (concentrations above 15 % NaCl not tested). Positive for oxidase and nitrate reduction. Positive for activities of leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase and β -glucosidase. Produces acid from glycerol, L-arabinose, ribose, D-xylose, methyl β -D-xylopyranoside, glucose, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, raffinose, starch, gentiobiose and gluconate. The diagnostic amino acid in cell-wall hydrolysates is meso-diaminopimelic acid. Whole-cell hydrolysates contain mainly ribose. The major isoprenoid quinone is MK-7. The major polar lipid is diphosphatidylglycerol. The predominant cellular fatty acids are iso-C_{14:0}, C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, C_{15:0}, iso-C_{16:0}, C_{16:0} and anteiso-C_{17:0}. The DNA G + C content of the type strain is 48.8 mol%.

The type strain, CAU 9324^T (=KCTC 13718^T =CCUG 59189^T), was isolated from a sample taken from a grassy sandbank on DaeYiJac Island in the Republic of Korea.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program, Ministry of Education, Science & Technology (grant 11-2008-03-002-00), Republic of Korea.

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