Paenibacillus harenae sp. nov., isolated from desert sand in China

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A Gram-positive, endospore-forming, rod-shaped bacterium, designated strain B519^T, was isolated from a desert sand sample of Gansu Province, China. Strain B519^T was strictly aerobic and cells were motile by means of peritrichous flagella. The strain grew optimally at 32–35 °C and pH 6.5–7.0. Chemotaxonomic data supported the affiliation of the new isolate to the genus *Paenibacillus*, including menaquinone-7 (MK-7) as the major isoprenoid quinone, DNA G + C content of 49.9 mol%, cell-wall type A1 γ (meso-diaminopimelic acid as the diagnostic diamino acid) and anteiso-C_{15:0}, iso-C_{15:0}, C_{16:0} and iso-C_{16:0} as the major fatty acids. Comparative 16S rRNA gene sequence analysis showed that strain B519^T was most closely related to *Paenibacillus alkaliterrae* KSL-134^T (98.0 % similarity). DNA–DNA relatedness between strain B519^T and *P. alkaliterrae* KSL-134^T was about 12.3 %. On the basis of phenotypic characteristics and molecular properties, strain B519^T is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus harenae* sp. nov. is proposed. The type strain is B519^T (=KCTC 3951^T =DSM 16969^T).

The genus *Paenibacillus* was delineated from members of *Bacillus* group 3 on the basis of 16S rRNA gene sequence analysis by Ash *et al.* (1993). Since the description of the genus, species belonging to the genus *Paenibacillus* have been isolated from various ecological habitats, including warm springs (Saha *et al.*, 2005; Chou *et al.*, 2007), alkaline soils (Yoon *et al.*, 2005), petroleum-hydrocarbon-contaminated sediment (Daane *et al.*, 2002), the surface of sterilized seeds of garden peas (Šmerda *et al.*, 2005), Antarctic sediments (Montes *et al.*, 2004), the rhizosphere of trees (Rivas *et al.*, 2005; Sánchez *et al.*, 2005; Park *et al.*, 2007; Yoon *et al.*, 2007) and desert sand (Lim *et al.*, 2006). Here we describe the taxonomic characterization of a novel aerobic, mesophilic, Gram-positive *Paenibacillus* species isolated from desert sand.

Strain B519^T was isolated on R2A agar (Difco) at 30 °C for 3 days from a desert sand sample from Gansu Province,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B519^T is AY839867.

A transmission electron micrograph showing the general morphology of negatively stained cells of strain B519^T and extended 16S rRNA gene sequence-based neighbour-joining and maximum-parsimony trees are available as supplementary material with the online version of this paper.

China. Subcultivation was done on R2A agar at 32 °C for 2 days. Gram staining was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Cell morphology and motility were studied by using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described by Jeon *et al.* (2005). Endospores were stained according to the Schaeffer–Fulton method (Smibert & Krieg, 1981). Physiological characteristics of the isolate were examined by cultivation on R2A medium at different temperatures and pH values (Gomori, 1955). Requirement for and tolerance of NaCl were determined in R2A medium supplemented with NaCl. Oxidase activity was tested by oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Catalase activity was determined by production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Hydrolysis of aesculin, casein, hypoxanthine, starch, Tween 80, L-tyrosine, xanthine and urea was determined on R2A according to the methods of Lányi (1987) and Smibert & Krieg (1994). Nitrate reduction was determined according to the method of Lányi (1987). Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann &

Baumann (1981), with each substrate supplemented with 2 % (v/v) Hunter's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Additional enzyme activities were determined by using the API ZYM (bioMérieux) system at 32 °C. On R2A agar, strain B519^T formed sticky, glistening, translucent, raised and circular/slightly irregular colonies when grown at 32 °C for 2 days. Growth was observed at 10–40 °C, with optimum growth at 32–35 °C. The strain grew at pH 6.0–10.0 (optimum growth at pH 6.5–7.0). Cells of the new isolate were rod-shaped (width of 0.8–1.2 µm and length of 2.5–3.5 µm) that were motile by means of peritrichous flagella (see Supplementary Fig. S1 in IJSEM Online). Strain B519^T grew on R2A medium with the addition of 0–3 % (w/v) NaCl; optimum growth occurred on R2A without the addition of NaCl. The isolate produced an ellipsoidal terminal endospore in a swollen sporangium. No growth was observed under anaerobic conditions after 7 days at 32 °C on R2A.

Analysis of fatty acid methyl esters of strain B519^T and reference strains was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID, Inc.) after cultivation for 2 days on R2A agar. Preparation of cell walls from strain B519^T and analysis of the peptidoglycan were carried out by using the methods described by Schleifer (1985), with the modification that TLC was performed on cellulose sheets rather than paper chromatography. Isoprenoid quinones were analysed according to Komagata & Suzuki (1987). The DNA G+C content of strain B519^T was determined by HPLC according to the method of Tamaoka & Komagata (1984), with the chromatograph fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM). The fatty acid profile of the strain was characterized as containing saturated fatty acids such as anteiso-C_{15:0} (41.1 %), iso-C_{15:0} (12.4 %), C_{16:0} (11.6 %) and iso-C_{16:0} (10.1 %) as the major components; its fatty acid profile resembles those of related *Paenibacillus* species (Table 1). The major respiratory lipoquinone of strain B519^T was menaquinone-7 (MK-7). Analysis of the cell-wall peptidoglycan indicated that it was of the A1γ type, i.e. meso-diaminopimelic acid as the diagnostic diamino acid. The genomic DNA G+C content of strain B519^T was 49.9 mol%. Typical phenotypic characteristics of strain B519^T are summarized and compared with those of the type strains of closely related *Paenibacillus* species in Table 2. Recognized members of the genus *Paenibacillus* generally produce an ellipsoidal, terminal endospore in a swollen sporangium, possess anteiso-C_{15:0} as the major fatty acid and have DNA G+C contents in the range 39–55 mol% (Shida *et al.*, 1997; Montes *et al.*, 2004; Takeda *et al.*, 2005). In this regard, strain B519^T possessed phenotypic characteristics typical of the genus *Paenibacillus*.

16S rRNA gene sequence analysis was carried out as described by Lane (1991). The resultant 16S rRNA gene sequence of strain B519^T was compared with available 16S rRNA gene sequences from GenBank by using the BLAST

Table 1. Cellular fatty acid compositions of strain B519^T and related members of the genus *Paenibacillus*

Strains: 1, B519^T; 2, *P. alkaliterrae* KSL-134^T; 3, *Paenibacillus agarexedens* DSM 1327^T; 4, *Paenibacillus agaridevorans* DSM 1355^T. Data are expressed as percentages of total fatty acids. ND, not detected. All data are from the present study.

Fatty acid	1	2	3	4
Straight chain				
C _{14:0}	1.5	2.1	2.1	1.0
C _{15:0}	4.2	3.5	5.3	4.7
C _{16:0}	8.6	12.3	12.8	10.9
C _{17:0}	ND	ND	2.3	1.3
C _{18:0}	ND	2.9	1.0	0.8
Branched				
iso-C _{14:0}	2.6	3.0	1.5	5.5
iso-C _{15:0}	10.2	5.8	4.3	4.3
anteiso-C _{15:0}	49.1	48.4	46.9	41.7
iso-C _{16:0}	13.1	12.7	11.6	22.5
iso-C _{17:0}	4.2	1.9	5.0	1.9
anteiso-C _{17:0}	5.5	4.5	7.3	4.4
Unsaturated				
C _{16:1ω11c}	0.9	1.3	ND	ND
C _{16:1ω7c} alcohol	ND	ND	ND	0.9
Hydroxy				
C _{16:0} 2-OH	ND	1.7	ND	ND

program (<http://www.ncbi.nlm.nih.gov/BLAST/>) and gene sequences were aligned with those of closely related species by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were constructed according to three different methods, the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms available in the PHYLIP program, version 3.6 (Felsenstein, 2002). The resultant tree topologies were evaluated by bootstrap analyses based on 1000 resamplings of the neighbour-joining and maximum-parsimony data in the PHYLIP package. By using the FASTA3 program (<http://www.ebi.ac.uk/Tools/fasta33/>), 16S rRNA gene sequence similarity calculations were made between strain B519^T and the type strains of other members of the *Paenibacillaceae*. DNA–DNA hybridization was carried out to evaluate levels of genomic DNA–DNA relatedness between strain B519^T and *Paenibacillus alkaliterrae* KSL-134^T according to the fluorometric microplate method (Ezaki *et al.*, 1989). Fluorometric data recorded after 30 min of incubation were used for calculation of levels of DNA–DNA relatedness. The highest and lowest values in each sample were excluded and the remaining three values were used for calculation of the mean level of DNA–DNA relatedness.

The almost-complete 16S rRNA gene sequence (1523 nt) of strain B519^T was obtained and was used for initial BLAST searches in GenBank and for phylogenetic analysis. Phylogenetic analyses based on 16S rRNA gene sequences by using the neighbour-joining and maximum-parsimony methods showed that strain B519^T formed a cluster with *P.*

Table 2. Characteristics of strain B519^T and related members of the genus *Paenibacillus*

Strains: 1, B519^T (data from the present study); 2, *P. alkaliterrae* KSL-134^T (unless indicated, data from Yoon *et al.*, 2005); 3, *P. agarixedens* DSM 1327^T (Uetanabaro *et al.*, 2003); 4, *P. agaridevorans* DSM 1355^T (van der Maarel *et al.*, 2000). +, Positive; –, negative; *m*-DAP, meso-diaminopimelic acid; ND, no data available. All strains are positive for motility, catalase, oxidase and formation of swollen sporangia and negative for anaerobic growth, urease, indole production, nitrate reduction, the Voges–Proskauer reaction, casein hydrolysis and growth at pH 5.7 and in the presence of 5 % (w/v) NaCl.

Characteristic	1	2	3	4
Cell size (µm)	0.8–1.2 × 2.5–3.5	0.4–0.5 × 1.5–3.0	0.5–1.4 × 2.0–8.0	0.6–0.8 × 2.0–5.0
Colony colour*	Whitish	Ivory	Whitish	Whitish
Hydrolysis of:*				
Agar	–	–	+	+
Starch	–	+	+	(–)
Utilization of:*				
D-Fructose	+	+	–	–
D-Mannose	+	+	–	–
D-Xylose	+	(–)	+	–
Cell-wall type	A1γ (<i>m</i> -DAP)	A1γ (<i>m</i> -DAP)	ND	ND
DNA G + C content (mol%)	49.9	49.4	47	51

*Data for these tests, and tests for catalase, oxidase and urease activities, indole production, nitrate reduction and casein hydrolysis, were determined in the present study. Results in parentheses are given as positive in reference sources.

alkaliterrae KSL-134^T (100 % bootstrap support) within the genus *Paenibacillus* (Fig. 1; see also Supplementary Fig. S1). The topologies of phylogenetic trees built according to the maximum-likelihood and maximum-parsimony algorithms also supported the suggestion that strain B519^T

belonged to the genus *Paenibacillus* (Fig. 1). The level of DNA–DNA relatedness between strain B519^T and *P. alkaliterrae* KSL-134^T was 12.3 %, which was clearly below the 70 % threshold generally accepted for species delineation (Rosselló-Mora & Amann, 2001; Wayne *et al.*, 1987).

The physiological, biochemical and phylogenetic properties of strain B519^T suggest that it represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus harenae* sp. nov. is proposed.

Description of *Paenibacillus harenae* sp. nov.

Paenibacillus harenae (ha.re'nae. L. gen. n. *harenae* of sand, referring to the isolation of the type strain from desert sand).

Cells are strictly aerobic, Gram-positive, spore-forming, motile rods. Oxidase- and catalase-positive. Nitrate is not reduced to nitrite. Grows between 10 and 40 °C (optimum 32–35 °C) and from pH 6.0 to 10.0 (optimum pH 6.5–7.0). Positive for esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase and α-fucosidase (API ZYM). Aesculin is hydrolysed, but hypoxanthine, casein, starch, Tween 80, L-tyrosine, xanthine and urea are not. Acid is produced from D-glucose, D-fructose, D-mannitol, trehalose, α-D-lactose, maltose, rhamnose, raffinose, inositol, sucrose, arbutin, D-salicin, melibiose, D-xylose and L-arabinose, but not from D-ribose, glycerol, adonitol or D-mannose. The cell-wall peptidoglycan is of the A1γ type, with meso-diaminopimelic acid as the diagnostic diamino acid. The major isoprenoid quinone is MK-7. The DNA G + C content of the type strain is 49.9 mol%.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain B519^T and related taxa. Bootstrap values at branch points are shown as percentages of 1000 replicates; only values >50 % are shown. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Brevibacillus brevis* JCM 2503^T was used as an outgroup. Bar, 0.01 changes per nucleotide position. Extended neighbour-joining and maximum-parsimony trees are shown in Supplementary Fig. S1.

The type strain, B519^T (=KCTC 3951^T =DSM 16969^T), was isolated from a desert sand sample from Gansu Province, China.

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