Paenibacillus gansuensis sp. nov., isolated from desert soil of Gansu Province in China

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A Gram-positive, endospore-forming, rod-shaped bacterium, designated strain B518^T, was isolated from a desert-soil sample from Gansu Province in China. The isolate was strictly aerobic and was motile by means of several flagella. The strain grew optimally at 35–40 °C and at pH 7.0–7.5. The predominant fatty acids of strain B518^T were anteiso-C_{15:0}, C_{16:0}, anteiso-C_{17:0}, iso-C_{16:0} and iso-C_{15:0}. The G + C content of the genomic DNA was 50 mol% and the predominant quinone was MK-7. Comparative 16S rRNA gene sequence analyses showed that strain B518^T formed a distinct phyletic line within the genus *Paenibacillus* and was most closely related to *Paenibacillus chitinolyticus* IFO 15660^T, with a 16S rRNA gene sequence similarity of 95.8 %. The levels of 16S rRNA gene sequence similarity with respect to other type strains of *Paenibacillus* species were below 95.1 %. On the basis of the chemotaxonomic data and molecular properties, strain B518^T represents a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus gansuensis* sp. nov. is proposed. The type strain is B518^T (=KCTC 3950^T=DSM 16968^T).

The genus *Paenibacillus* was created from the group 3 bacilli on the basis of a 16S rRNA gene sequence analysis conducted 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), rice fields (Sánchez *et al.*, 2005), 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) and the phyllospheres and rhizospheres of trees (Rivas *et al.*, 2005a, b; Lim *et al.*, 2006). In this report, we describe the taxonomic characterization of a novel aerobic, mesophilic, Gram-positive *Paenibacillus* species that produces an ellipsoidal terminal endospore in a swollen sporangium.

Strain B518^T was isolated from a desert-soil sample (1000 m above sea level) collected in northwest China (Sunan

County, Gansu Province) by serial dilution plating on R2A agar (Difco) at 30 °C for 3 days. Subcultivation was done on R2A agar at 35 °C for 2–3 days. Gram staining was performed using a bioMérieux Gram-stain kit according to the manufacturer's instructions. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described by Jeon *et al.* (2003, 2005). Endospores were stained according to the Schaeffer–Fulton method (Smibert & Krieg, 1981). The physiological characteristics of strain B518^T were examined by growing the isolate on R2A medium. Growth was tested at different pH values (6.0–12.0) and temperatures (4–50 °C) on R2A broth. R2A broths with different pH values were prepared as described previously (Gomori, 1955). Oxidase activity was tested by assessing the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), and catalase activity was evaluated by assessing the production of O₂ bubbles in a 3 % (v/v) aqueous H₂O₂ solution. The hydrolysis of compounds was checked on R2A agar according to methods described previously (Lanyi, 1987; Smibert & Krieg, 1994). Nitrate reduction was performed according to the method of Lanyi (1987), and acid production from carbohydrates was tested as described by Leifson (1963). When grown on R2A agar at 35 °C for 2 days, strain B518^T formed pale

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B518^T is AY839866.

Transmission electron micrographs showing the morphology, cell wall and spore structures of strain B518^T, and a neighbour-joining phylogenetic tree for strain B518^T and related taxa are available as supplementary figures in IJSEM Online.

yellow, glistening, translucent, slightly sticky, irregular and slightly raised colonies. Growth was observed at temperatures between 10 and 45 °C; optimum growth occurred at 35–40 °C and pH 7.0–7.5. The cells of the isolate were motile rods (0.7–0.9 µm wide and 1.7–2.4 µm long) possessing several flagella (see Supplementary Fig. S1a available in IJSEM Online). The cells of strain B518^T were Gram-positive, oxidase-negative, catalase-negative, and did not reduce nitrate to nitrite. The isolate produced an ellipsoidal terminal endospore in a swollen sporangium. Anaerobic growth was not observed under anaerobic conditions for 5 days at 35 °C on R2A agar.

Analysis of the fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Analyses of peptidoglycan and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The G+C content of the genomic DNA of strain B518^T was determined, using HPLC apparatus fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM), according to the method of Tamaoka & Komagata (1984). The major respiratory lipoquinone of strain B518^T was MK-7. The fatty acid profile of the strain showed a predominance of saturated fatty acids such as anteiso-C_{15:0} (48.7%), C_{16:0} (10.1%), anteiso-C_{17:0} (9.6%), iso-C_{16:0} (9.5%) and iso-C_{15:0} (9.1%). Similar fatty acid profiles have also been reported for type strains of the genus *Paenibacillus* (Table 1). Cell-wall peptidoglycan could not be extracted from strain B518^T, which was confirmed by several re-examinations and analysis by another institute (Deutsche

Table 1. Cellular fatty acid content of strain B518^T and related taxa

Taxa: 1, strain B518^T; 2, *P. chitinolyticus* IFO 15660^T (Lee *et al.*, 2004); 3, *P. daejeonensis* AP-20^T (Lee *et al.*, 2002); 4, *Paenibacillus ehimensis* KCTC 3748^T (Lee *et al.*, 2004); 5, *Paenibacillus polymyxa* DSM 36^T (Enright *et al.*, 2003; Kuroshima *et al.*, 1996). Data are expressed as percentages of total fatty acids. Fatty acids representing less than 0.5% are not included.

Fatty acid	1	2	3	4	5
Saturated					
C _{14:0}	1.5	–	2.8	–	2.9
C _{15:0}	1.0	–	15.9	–	0.8
C _{16:0}	10.1	6.9	14.3	7.1	15.6
iso-C _{14:0}	1.5	–	2.4	–	2.4
iso-C _{15:0}	9.1	3.6	7.2	8.1	9.7
iso-C _{16:0}	9.5	8.4	10.2	8.6	8.6
iso-C _{17:0}	6.0	2.0	2.5	3.3	8.5
anteiso-C _{15:0}	48.7	58.7	36.4	52.9	41.3
anteiso-C _{17:0}	9.6	7.3	2.7	8.0	8.4
Others					
C _{16:1} ω11c	0.8	–	2.6	5.2	1.5
iso-C _{17:0} ω10c	0.9	–	–	–	–

Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). However, examination of thin sections using transmission electron microscopy revealed the presence of a typical single, thick, Gram-positive cell-wall ultrastructure and a typical spore structure (see Supplementary Fig. S1b, c in IJSEM Online). The G+C content of the genomic DNA of strain B518^T was 50 mol%. In Table 2, the typical phenotypic characteristics of strain B518^T are summarized and compared with those of the type strains of closely related taxa. Members of the genus *Paenibacillus* 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% (Shida *et al.*, 1997; Montes *et al.*, 2004; Takeda *et al.*, 2005). Strain B518^T also possesses these phenotypic characteristics typical of the genus *Paenibacillus*.

The sequencing and assembly of the 16S rRNA gene sequence were carried out as described by Bakermans & Madsen (2002). The sequence of strain B518^T was compared with 16S rRNA gene sequences available in GenBank by using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine the approximate phylogenetic affiliation, and was aligned with close relatives by using CLUSTAL W software (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three different methods, namely the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms available in PHYLIP, version 3.6

Table 2. Characteristics of strain B518^T and some related taxa

Taxa: 1, strain B518^T; 2, *P. chitinolyticus* IFO 15660^T (Lee *et al.*, 2004); 3, *P. polymyxa* DSM 36^T (Enright *et al.*, 2003; Kuroshima *et al.*, 1996); 4, *P. daejeonensis* AP-20^T (Lee *et al.*, 2002); 5, *P. ehimensis* KCTC 3748^T (Lee *et al.*, 2004). Symbols: +, positive; –, negative; V, variable; ND, not determined.

Characteristic	1	2	3	4	5
Anaerobic growth	–	–	+	ND	–
Oxidase	–	V	–	+	+
Catalase	–	+	+	+	ND
Nitrate reduction	–	+	V	+	+
Motility	+	+	ND	+	ND
Growth at 50 °C	–	–	–	ND	+
Acid production from:					
Arabinose	+	–	–	ND	+
Glucose	+	+	ND	ND	+
Lactose	+	ND	+	+	ND
Mannitol	–	–	+	+	+
Xylose	+	–	+	ND	+
Hydrolysis of:					
Aesculin	+	ND	+	+	ND
Casein	+	+	+	ND	V
Starch	–	–	+	ND	+
DNA G+C content (mol%)	50	51–53	43–46	53	52–53

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing phylogenetic relationships of strain B518^T and related taxa. Bootstrap values (percentages of 1000 replicates) are shown where greater than 50%. *Bacillus subtilis* NCDO 1769^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

(Felsenstein, 2002). By using the FASTA3 program in EBI, 16S rRNA gene sequence comparisons for similarity calculations were made between the novel strain and related members of the genus *Paenibacillus*. A bootstrap analysis was performed using the algorithm of the Kimura two-parameter model (Kimura, 1980) of the neighbour-joining method in the PHYLIP package. An almost-complete 16S rRNA gene sequence (1529 nt) of strain B518^T was obtained, and a phylogenetic analysis based on 16S rRNA gene sequences showed that strain B518^T formed a distinct cluster with *Paenibacillus chitinolyticus* IFO 15660^T and *Paenibacillus daejeonensis* KCTC 3745^T (see Fig. 1; an extended version of this tree is available as Supplementary Fig. S2 in IJSEM Online). The topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms also supported the notion that the isolate belongs to the genus *Paenibacillus* (data not shown). The 16S rRNA gene sequence of strain B518^T also contained two signature sequences found in most *Paenibacillus* species, i.e. PAEN 862F (5'-TCGATACCCTTGCGTCCGAAGT-3') and PAEN 515F (5'-GAGTAAGTCTCTCGGAATGACG-GTACTTGAGAAGAAAGCCCC-3') (Saha *et al.*, 2005). Comparative 16S rRNA gene sequence analyses showed that the isolate was most closely related to *P. chitinolyticus* IFO 15660^T, having a similarity of 95.8%. Sequence similarities to other members included in the phylogenetic analysis were below 95.1%. Thus, on the basis of the physiological, biochemical and phylogenetic properties of strain B518^T, this isolate represents a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus gansuensis* sp. nov. is proposed.

Description of *Paenibacillus gansuensis* sp. nov.

Paenibacillus gansuensis (gan.su.en'sis. N.L. masc. adj. *gansuensis* belonging to Gansu, from where the type strain was isolated).

Cells are Gram-positive rods, 0.7–0.9 µm wide and 1.7–2.4 µm long at 35 °C on R2A agar. Motile by means of several flagella. Colonies are glistening, translucent,

semi-sticky, irregular, slightly raised and pale yellow on R2A agar. Growth occurs at 10–45 °C (optimum, 35–40 °C). Nitrate is not reduced to nitrite. Catalase- and oxidase-negative. Aesculin, casein and Tween 80 are hydrolysed. Hypoxanthine, tyrosine, starch and xanthine are not hydrolysed. Acids are produced from D-glucose, D-fructose, D-ribose, D-xylose, lactose, D-trehalose, maltose, L-arabinose, D-raffinose, D-mannose, *myo*-inositol, glycerol, sucrose, melibiose and salicin, but not from D-mannitol, adonitol or arbutin. The major isoprenoid quinone is MK-7. The major fatty acids are anteiso-C_{15:0}, C_{16:0}, anteiso-C_{17:0}, iso-C_{16:0} and iso-C_{15:0}. The DNA G+C content is 50 mol% (HPLC).

The type strain, B518^T (= KCTC 3950^T = DSM 16968^T), was isolated from a desert-soil sample from Gansu Province in China.

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