Paenibacillus xinjiangensis sp. nov., isolated from Xinjiang province in China

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Strain B538^T is a Gram-positive, motile, rod-shaped bacterium, which was isolated from Xinjiang province in China. This organism grew optimally at 30–35 °C and pH 8.0–8.5. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain B538^T belonged to the genus *Paenibacillus* and chemotaxonomic data (DNA G + C content, 47.0 mol%; major isoprenoid quinone, MK-7; cell wall type, A1γ; meso-diaminopimelic acid; major fatty acids, anteiso-C_{15:0} and C_{16:0}) supported affiliation of the isolate with the genus *Paenibacillus*. Comparative 16S rRNA gene sequence analyses showed that the isolate was most closely related to *Paenibacillus glycanilyticus* DS-1^T, with 16S rRNA gene sequence similarity of 98.1%; sequence similarities to other members of the genus *Paenibacillus* used in the phylogenetic tree were less than 96.5%. The DNA–DNA relatedness between strain B538^T and *P. glycanilyticus* DS-1^T was about 8.0%. On the basis of physiological and molecular properties, strain B538^T (=KCTC 3952^T = DSM 16970^T) is proposed as the type strain of a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus xinjiangensis* sp. nov. is proposed.

The genus *Paenibacillus* was delineated from the members of group 3 *Bacillus* on the basis of 16S rRNA gene sequence analysis by Ash *et al.* (1993). Since the description of the genus, quite a few 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), the rhizosphere of trees (Rivas *et al.*, 2005c) and air (Rivas *et al.*, 2005a). These species also differ physiologically and produce diverse degradation enzymes (Rivas *et al.*, 2005b; Takeda *et al.*, 2005; Shida *et al.*, 1997). These findings suggest that the members of the genus *Paenibacillus* may have important roles in environmental habitats. In this

report, the taxonomic characterization is described of a novel aerobic, mesophilic, Gram-positive *Paenibacillus* species, which produces a spherical terminal endospore in a swollen sporangium.

Strain B538^T was isolated from alkaline soil (pH 9.0, 1000 m above sea-level) collected in northern China (Heshuo area of Xinjiang province) by the serial dilution plating method with R2A agar (Difco) medium at 35 °C for 3 days. The isolate was routinely grown aerobically on TSB (tryptic soy broth; Difco) agar for 2 days at 32 °C, except where indicated otherwise. Gram staining was performed using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Cell morphology was studied using TEM (JEM-1010; JEOL) as described by Jeon *et al.* (2005a). Motility was observed at 12 and 36 h with a light microscope (Nikon E600) according to a method described previously (Jeon *et al.*, 2005b). Endospores were stained according to the method of Schaeffer-Fulton (Smibert & Krieg, 1981). Growth was tested at different temperatures (4–55 °C) on TSB agar and at different pH values in TSB. Media with different pH values were prepared using appropriate biological buffers; Na₂HPO₄/NaH₂PO₄ buffer, Na₂CO₃/NaHCO₃ buffer and Na₂HPO₄/NaOH buffer were used for pH below 8.0, pH 8.0–10.0 and pH 11.0, respectively (Gomori, 1955). Oxidase activity was tested

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Abbreviations: ML, maximum likelihood; MP, maximum parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B538^T is AY839868.

A table giving the fatty acid composition of strain B538^T and related strains and a TEM showing the general morphology of strain B538^T are available as supplementary material in IJSEM Online.

using Bactident Oxidase strips (Merck) and catalase activity was evaluated by production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Nitrate reduction and the hydrolysis of compounds were checked on TSB agar according to methods described previously (Lanyi, 1987; Smibert & Krieg, 1994). Acid production from D-glucose, lactose, glycerol, L-rhamnose, D-xylose, sucrose, D-melibiose, D-trehalose, maltose, D-raffinose, D-ribose, D-mannose, L-arabinose, D-fructose, D-mannitol, adonitol and salicin was evaluated in TSB containing 0.001 % (w/v) phenol red, 0.3 % (w/v) agar and 1 % (w/v) each carbohydrate according to the modified method of Leifson (1963) after 3 days cultivation at 32 °C. Strain B538^T formed creamy, circular/slightly irregular and slightly convex colonies on TSB agar when grown at 32 °C for 2 days. The strain grew optimally on TSB agar without added NaCl and TSB agar supplemented with 1 % (w/v) NaCl, but did not grow on TSB agar with the addition of 4 % (w/v) NaCl or more. Growth occurred between 10 and 40 °C (optimum of 30–35 °C) and at pH 6.5–9.8 (optimum of pH 8.0–8.5). Cells of the isolate were motile short rods (0.8–1.2 µm wide and 2.0–3.2 µm long) with peritrichous flagella (see Supplementary Fig. S1 available in IJSEM Online). Strain B538^T was Gram-positive, oxidase-negative, catalase-positive and did not reduce nitrate to nitrite. A spherical endospore was formed at a terminal position in a swollen sporangium. Anaerobic growth was not observed during anaerobic cultivation over 5 days at 32 °C on TSA.

Whole-cell fatty acids of strain B538^T were analysed according to the Microbial Identification System (MIDI; Microbial ID) after cultivation on R2A, TSB agar or twofold-diluted nutrient agar (Difco) for 2 days at 32 °C. Analysis of peptidoglycan and isoprenoid quinones was carried out as described by Komagata & Suzuki (1987). The genomic DNA G+C content of strain B538^T was determined by HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the

method of Tamaoka & Komagata (1984). The major isoprenoid quinone was menaquinone-7 (MK-7). The predominant cellular fatty acids of strain B538^T when grown on TSB agar were anteiso-C_{15:0} (48.61 %), C_{16:0} (14.43 %), iso-C_{16:0} (9.65 %) and iso-C_{15:0} (9.32 %); no significant differences in the fatty acid profile were found when the isolate was grown on R2A. The fatty acid profile was similar to those reported for other related type strains of the genus *Paenibacillus* (Supplementary Table S1 in IJSEM Online). Analysis of cell wall peptidoglycan showed that strain B538^T possessed type A1γ with meso-diaminopimelic acid as the diagnostic diamino acid. The genomic DNA G+C content of strain B538^T was 47.0 mol%. Typical phenotypic properties of strain B538^T are summarized in Table 1 along with those of the type strains of closely related taxa. The characteristics of strain B538^T are consistent with those described for members of the genus *Paenibacillus* (Table 1), i.e. they produce a terminal endospore in a swollen sporangium, possess anteiso-C_{15:0} as the major fatty acid and have a DNA G+C content in the range 39–55 mol% (Shida *et al.*, 1997; Montes *et al.*, 2004; Takeda *et al.*, 2005).

Sequencing and assembly of the 16S rRNA gene were carried out as described previously (Lane, 1991). The resultant 16S rRNA gene sequence of strain B538^T was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine an approximate phylogenetic affiliation and the gene sequences were aligned with closely related members using CLUSTAL W software (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three different methods, neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms, which are available in the PHYLIP software, version 3.6 (Felsenstein, 2002). Sequence similarity values were computed using Similarity Matrix version 1.1 (Ribosomal Database Project II; <http://35.8.164.52/html/>; Cole *et al.*, 2003). Bootstrap

Table 1. Characteristics of strain B538^T and some other related type strains

Strains: 1, strain B538^T; 2, *Paenibacillus glycanilyticus* DS-1^T (Dasman *et al.*, 2002); 3, *Paenibacillus agarexodens* DSM 1327^T (Uetanabaro *et al.*, 2003); 4, *Paenibacillus agaridevorans* DSM 1355^T (Uetanabaro *et al.*, 2003); 5, *Paenibacillus alkaliterrae* DSM 17040^T (Yoon *et al.*, 2005); 6, *Paenibacillus granivorans* A30^T (van der Maarel *et al.*, 2000). All strains are positive for catalase. NA, No data available.

Characteristic	1	2	3	4	5	6
Anaerobic growth	—	+	—	—	—	—
Oxidase	—	—	+	+	+	—
Nitrate reduction	—	—	—	—	—	+
Growth at pH 5.7	—	+	—	—	—	—
Hydrolysis of:						
Starch	—	+	+	—	+	+
Tween 80	—	—	—	—	+	NA
Agar	—	NA	+	+	—	NA
Major fatty acid	anteiso-C _{15:0}	anteiso-C _{15:0}	NA	NA	anteiso-C _{15:0}	anteiso-C _{15:0}
DNA G+C content (mol%)	47.0	50.5	47	51	49.4	47.8

analysis (1000 replications) was performed to evaluate the stability of the phylogenetic tree according to the algorithm of the Kimura two-parameter model (Kimura, 1980) of the NJ method in the PHYLIP package.

DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain B538^T and *Paenibacillus glycanilyticus* DS-1^T. Extracted genomic DNAs were fragmented with *Hae*III for slot hybridization. Digested DNAs were diluted serially and loaded into slots with three replications for each sample and their DNAs were used individually as labelled DNA probes for cross-hybridization. Random primed DNA labelling with digoxigenin-dUTP and hybridization (hybridization temperature, 46 °C; washing temperature, 65 °C) were performed using a DIG High Prime DNA labelling kit (Roche Applied Science) according to the manufacturer's instructions and standard procedures (Sambrook & Russell, 2001; Lim *et al.*, 2005). The signals of the series of dilutions were quantified using Bio-Rad GelDoc scanning software. The signals produced by self-hybridization were inferred as 100 % and hybridization values (%) were calculated from results of three samples. Phylogenetic analysis using a nearly complete 16S rRNA gene sequence (1521 nt) of strain B538^T indicated that the isolate formed a phylogenetic cluster with *P. glycanilyticus* DS-1^T with a 99.7 % bootstrap value within the genus *Paenibacillus* (Fig. 1). Topologies of phylogenetic trees built using the ML and MP algorithms were similar to that of the tree constructed by NJ analysis (data not shown).

Fig. 1. NJ tree showing phylogenetic relationships between strain B538^T and some members of the genus *Paenibacillus* based on 16S rRNA gene sequences. Bootstrap values are given as percentages of 1000 replicates; only values greater than 50 % are shown. *Brevibacillus brevis* JCM 2503^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

Comparative 16S rRNA gene sequence analyses showed that the isolate was most closely related to *P. glycanilyticus* DS-1^T, with a 16S rRNA gene sequence similarity of 98.1 %; sequence similarities to other members of the genus *Paenibacillus* used in the phylogenetic tree were less than 96.5 %. The DNA–DNA relatedness between strain B538^T and *P. glycanilyticus* DS-1^T was about 8 %, which is sufficient for strain B538^T to be classified within a different species (Stackebrandt *et al.*, 2002). Therefore, on the basis of physiological, biochemical and phylogenetic properties, strain B538^T should be considered as representing a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus xinjiangensis* sp. nov. is proposed.

Description of *Paenibacillus xinjiangensis* sp. nov.

Paenibacillus xinjiangensis (xin.ji.ang.en'sis. N.L. masc. adj. *xinjiangensis* pertaining to Xinjiang in China, where the type strain was isolated).

Cells are approximately 0.8–1.2 µm wide and 2.0–3.2 µm long. Strictly aerobic, spore-forming, motile rods. Catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite. Colonies are smooth, circular to slightly irregular, slightly convex and cream coloured on TSB agar. Growth occurs at 10–40 °C (optimum, 30–35 °C), 0–3 % (w/v) NaCl (optimum, 0–1 %) and pH 6.5–9.8 (optimum, pH 8.0–8.5). Hydrolyses aesculin. Does not hydrolyse casein, starch, Tweens 20 or 80, L-tyrosine, hypoxanthine, xanthine or urea. Acids are produced from D-glucose, lactose, glycerol, L-rhamnose, D-xylose, sucrose, D-melibiose, D-trehalose, maltose, D-raffinose, D-ribose, D-mannose, L-arabinose and D-fructose, but not from D-mannitol, adonitol or salicin. Cell wall contains *meso*-diaminopimelic acid (A1γ type). The predominant menaquinone is MK-7. The major cellular fatty acids on TSB agar are anteiso-C_{15:0} (48.61 %), C_{16:0} (14.43 %), iso-C_{16:0} (9.65 %) and iso-C_{15:0} (9.32 %). DNA G + C content is 47.0 mol% (HPLC).

The type strain is B538^T (=KCTC 3952^T = DSM 16970^T), isolated from Xinjiang province in China.

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