Paenibacillus tarimensis sp. nov., isolated from sand in Xinjiang, China

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A Gram-positive, rod-shaped, motile, spore-forming bacterium, strain SA-7-6^T, was isolated from desert soil in China and was subjected to a polyphasic taxonomic study. The strain grew optimally at pH 7.5 and 37 °C. The G+C content of the genomic DNA of strain SA-7-6^T was 53.7 mol%. The predominant menaquinone was MK-7. The major cellular fatty acid was anteiso-C_{15:0}. Strain SA-7-6^T contained meso-diaminopimelic acid in the cell-wall peptidoglycan. 16S rRNA gene sequence analysis showed that the new isolate shared highest similarity with *Paenibacillus glycanilyticus* JCM 11221^T (96.6%) and *Paenibacillus daejeonensis* KCTC 3745^T (96.6%). Based on morphological, physiological, chemotaxonomic and phylogenetic characteristics, strain SA-7-6^T is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus tarimensis* sp. nov. is proposed. The type strain is SA-7-6^T (=CCTCC AB 206108^T=DSM 19409^T).

The genus *Bacillus* was first described by Cohn in 1872, and many of its members have been reclassified in recent years on the basis of 16S rRNA gene-based molecular analysis. Ash *et al.* (1993) transferred members of *Bacillus* group 3 to the genus *Paenibacillus*, which, at the time of writing, comprises 82 recognized species (<http://www.bacterio.cict.fr/p/paenibacillus.html>). Species belonging to the genus *Paenibacillus* have been isolated from different ecological habitats such as desert soil (Lim *et al.*, 2006a, b), honeybee larvae (Genersch *et al.*, 2006), warm springs (Saha *et al.*, 2005) and alkaline soil (Yoon *et al.*, 2005). Members produce ellipsoidal spores in swollen sporangia, are aerobic or facultatively anaerobic, and possess anteiso-C_{15:0} as the major cellular fatty acid and MK-7 as the predominant menaquinone (Shida *et al.*, 1997).

A novel bacterium, designated strain SA-7-6^T, was isolated from desert sand of the Tarim Basin, Xinjiang, China. The sand sample was suspended in sterile distilled water and inoculated on TGY (per litre distilled water: 5 g tryptone, 3 g yeast extract, 1 g glucose) agar plates for 48 h at 37 °C. For subsequent study, the isolate was cultured on trypticase soy agar (TSA) [per litre distilled water: 30.0 g trypticase soy broth (TSB; Difco), 16.0 g agar, adjusted to pH 7.5] at 37 °C for 16 h, which were the optimum conditions to gain cells in the exponential phase of growth.

Isolation of the genomic DNA of strain SA-7-6^T was carried out according to the methods described by Yoon *et al.* (1996). Fragments comprising the nearly full-length 16S rRNA gene were amplified by PCR with primers 12R (5'-GAGTTTGATCCTGGCTCAG-3') and 13F (5'-AGAAAGGAGGTGATCCAGCC-3'). The PCR products were then sequenced by Invitrogen Corporation, where a 1462-bp stretch was gained. Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity values were achieved by using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Strain SA-7-6^T showed highest 16S rRNA gene sequence similarity to *Paenibacillus glycanilyticus* JCM 11221^T (96.6%), *Paenibacillus daejeonensis* KCTC 3745^T (96.6%) and *Paenibacillus xinjiangensis* KCTC 3952^T (96.3%). Levels of sequence similarity to the type strains of other members of the genus *Paenibacillus* were below 96.3%. Multiple alignments were performed and a phylogenetic tree based on 16S rRNA gene sequences was constructed by using MEGA3.1 software (Kumar *et al.*, 2004). Sequence alignment was achieved by using the CLUSTAL W method (Thompson *et al.*, 1994). The model of Jukes & Cantor (1969) was used to compute evolutionary distances, based on which a phylogenetic tree was constructed by using the neighbour-joining method with bootstrap analysis of 1000 replications (Fig. 1) (Saitou & Nei, 1987) and by using the maximum-parsimony method (data not shown).

Stackebrandt & Goebel (1994) have stated that strains showing less than 97.0% 16S rRNA gene sequence similarity will have levels of DNA–DNA relatedness below

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SA-7-6^T is EF125184.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain SA-7-6^T and other related taxa within the genus *Paenibacillus*. Reference strains were selected from a comparison of 16S rRNA gene sequences from *Paenibacillus* species with validly published names. The sequence of *Bacillus subtilis* NCDO 1769^T was used to root the tree. Numbers at nodes indicate levels of bootstrap support based on 1000 resamplings; only values above 60 % are indicated. Bar, 1 % sequence divergence.

70%. Given this accepted standard for the definition of bacterial species, strain SA-7-6^T was not related to any recognized species of the genus *Paenibacillus*.

Cell morphology was examined by phase-contrast microscopy (Olympus). Flagellum type was observed by transmission electron microscopy by using cells from the exponential phase of growth (Fig. 2). Scanning electron microscopy was used to investigate the strain at different growth phases, namely vegetative cells, swollen cells and spores. When grown at 37 °C for 16 h, strain SA-7-6^T formed circular, smooth, opaque, ivory colonies on TSA plates, whereas after 24 h growth under the same conditions, colonies tended to be translucent. Cells were Gram-positive, rod-shaped and motile with peritrichous flagella. They produced ellipsoidal spores in swollen sporangia.

Physiological characteristics were examined by incubating the isolate in basal TSB medium at different temperatures (4, 25, 30, 37, 45 and 50 °C), pH and NaCl concentrations (Table 1). Growth was observed at 25–45 °C (optimum at 37 °C) but not at 4 or 50 °C. Growth at different pH

Fig. 2. Transmission electron micrograph of a cell of strain SA-7-6^T showing peritrichous flagella. Bar, 1 μm.

Table 1. Differential characteristics between strain SA-7-6^T and related *Paenibacillus* species

Strains: 1, SA-7-6^T (*P. tarimensis* sp. nov.); 2, *P. glycanilyticus* DSM 17608^T (data from Dasman *et al.*, 2002); 3, *P. mendelii* LMG 23002^T (Šmerda *et al.*, 2005); 4, *P. xinjiangensis* KCTC 3952^T (Lim *et al.*, 2006b); 5, *P. daejeonensis* KCTC 3745^T (Lee *et al.*, 2002). Acids were produced from D-lactose, melibiose and raffinose by all the strains. +, Positive; –, negative; w, weakly positive; v, variable; ND, not determined.

Characteristic	1	2	3	4	5
Gram stain	v	+	v	+	v
Anaerobic growth	–	–	+	–	ND
Oxidase	–	–	+	–	+
Optimum growth temperature (°C)	37	28–37	25–30	30–35	30
Growth at pH 5.7	–	+	ND	–	–
DNA G + C content (mol%)	53.7	50.5	50.8	47.0	53
Hydrolysis of starch	–	+	–	–	ND
Acid production from:					
Glycerol	–	+	+	+	ND
D-Arabinose	w	–	+	ND	ND
L-Arabinose	w	+	+	+	ND
D-Ribose	–	+	+	+	ND
D-Fructose	–	+	+	+	ND
D-Mannose	+	+	–	+	ND
L-Rhamnose	–	+	+	+	ND
Inositol	–	+	–	ND	ND
D-Mannitol	+	+	–	–	+
N-Acetylglucosamine	–	+	–	ND	ND
Amygdalin	+	+	–	ND	+
Arbutin	–	+	–	ND	ND
Salicin	+	+	+	–	ND
Melezitose	w	+	+	ND	ND
Glycogen	+	+	+	–	+
Turanose	w	+	+	ND	ND
D-Tagatose	+	–	–	ND	ND

(5.5–13.5) was investigated in tubes containing 5 ml TSB; Na₂HPO₄/NaH₂PO₄, Na₂CO₃/NaHCO₃ and Na₂HPO₄/NaOH buffers were added to adjust the pH of the growth medium. After incubation in a rotary shaker at 37 °C, strain SA-7-6^T grew at pH 6.0–9.0 (more rapidly between pH 7.0 and 8.0); pH 7.5 was regarded as the optimum pH for growth. The maximal NaCl concentration that the isolate was able to tolerate was 3 %.

Nitrate reduction, activities of catalase, oxidase, β-galactosidase, arginine decarboxylase and phenylalanine deaminase, H₂S production, indole production, the Voges–Proskauer reaction, methyl red test and hydrolysis of various compounds were tested on TSA by using the methods described by Smibert & Krieg (1994). Strain SA-7-6^T was positive for catalase, β-galactosidase, arginine decarboxylase (weakly), phenylalanine deaminase (weakly), the Voges–Proskauer reaction, methyl red test and hydrolysis of aesculin, but negative for oxidase, urease,

nitrate reduction, H₂S production, indole production, and hydrolysis of starch, casein, gelatin and Tween 80. Acid production was determined by using API 50 CH strips (bioMérieux) following the manufacturer's instructions. Differential characteristics between strain SA-7-6^T and the type strains of closely related *Paenibacillus* species are presented in Table 1. GP2 Biolog microplates were used to test for the utilization of carbon compounds by strain SA-7-6^T. Antibiotic sensitivity was tested by adding different antibiotics to tubes containing 5 ml TSB and incubating in a rotary shaker for 24 h at 37 °C. Strain SA-7-6^T was resistant to kanamycin (10 µg ml^{–1}), but not to ampicillin (20 µg ml^{–1}), carbenicillin (20 µg ml^{–1}) or chloramphenicol (25 µg ml^{–1}).

For fatty acid analysis, strain SA-7-6^T was grown on TSA at 37 °C for 16 h until the late exponential phase of growth. Fatty acids were extracted following the instructions of the Sherlock Microbial Identification System (MIDI). The major fatty acid was anteiso-C_{15:0} (47.22 %), which is in accordance with data for other members of the genus *Paenibacillus* (Shida *et al.*, 1997), followed by C_{16:0} (14.17 %), anteiso-C_{17:0} (13.47 %), iso-C_{15:0} (11.54 %) and iso-C_{17:0} (4.22 %) (Table 2). Distinct quantitative differences were found in the fatty acid profiles of strain SA-7-6^T and *P. xinjiangensis* KCTC 3952^T, which was also isolated from Xinjiang, China. Strain SA-7-6^T showed higher levels of C_{14:0}, iso-C_{15:0}, iso-C_{17:0}, anteiso-C_{17:0} and C_{16:1ω11c} but lower levels of iso-C_{14:0} and iso-C_{16:0} as compared with *P. xinjiangensis* KCTC 3952^T.

Cell walls were prepared according to the method of Komagata & Suzuki (1987). The diagnostic cell-wall amino

Table 2. Cellular fatty acid contents (%) of strain SA-7-6^T and related *Paenibacillus* species

Strains: 1, SA-7-6^T (*P. tarimensis* sp. nov.); 2, *P. xinjiangensis* KCTC 3952^T (data from Lim *et al.*, 2006b); 3, *P. polymyxa* DSM 36^T (data from the present study, as a positive control). ND, Not detected. Fatty acids comprising less than 0.5 % of the total were excluded. All strains were cultured on TSA at about 30 °C.

Fatty acid	1	2	3
Straight-chain saturated			
C _{14:0}	2.61	1.93	1.52
C _{15:0}	ND	3.44	ND
C _{16:0}	14.17	14.43	9.25
Iso-branched saturated			
C _{14:0}	0.84	2.12	2.01
C _{15:0}	11.54	9.32	5.91
C _{16:0}	3.74	9.62	9.64
C _{17:0}	4.22	3.54	4.35
Anteiso-branched saturated			
C _{15:0}	47.22	48.61	47.36
C _{17:0}	13.47	4.40	14.66
Unsaturated			
C _{16:1ω11c}	1.55	0.53	2.49

acid was *meso*-diaminopimelic acid. Extraction of menaquinone and determination of the menaquinone compositions were carried out by HPLC (Shimadzu) as described by Xie & Yokota (2003). The predominant menaquinone was MK-7.

The thermal denaturation method (Mandel & Marmur, 1968) was used to determine the G+C content of the genomic DNA spectrophotometrically (DU800 spectrophotometer; Beckman Coulter). The DNA G+C content of strain SA-7-6^T was 53.7 mol%.

On the basis of morphological, physiological, chemotaxonomic and phylogenetic characteristics, strain SA-7-6^T is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus tarimensis* sp. nov. is proposed.

Description of *Paenibacillus tarimensis* sp. nov.

Paenibacillus tarimensis (ta.ri.men'sis. N.L. masc. adj. *tarimensis* referring to Tarim Basin, China, the geographical origin of the type strain).

Cells are Gram-positive, aerobic rods, 3.0–6.0 µm in length and 0.5–0.8 µm in width. Motile by means of peritrichous flagella. Ellipsoidal endospores are formed in swollen sporangia. Colonies are circular, smooth, opaque and ivory on TSA plates after 16 h incubation at 37 °C. Growth occurs at 25–45 °C; optimum temperature for growth is 37 °C. Cells grow at pH 6.0–9.0; optimum pH is 7.5. Growth is not observed at NaCl concentrations above 3 %. Positive for catalase, β-galactosidase, arginine decarboxylase (weakly), phenylalanine deaminase (weakly), the Voges–Proskauer reaction, methyl red test and aesculin hydrolysis, but negative for oxidase, urease, nitrate reduction, H₂S production, indole production, and hydrolysis of starch, casein, gelatin and Tween 80. Acids are produced from D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-mannose, D-mannitol, amygdalin, aesculin, salicin, cellobiose, maltose, D-lactose, melibiose, sucrose, trehalose, raffinose, starch, glycogen, gentiobiose and D-tagatose, but not from glycerol, erythritol, D-ribose, L-xylose, D-adonitol, D-fructose, L-sorbose, L-rhamnose, inositol, D-sorbitol, methyl α-D-mannopyranoside, N-acetylglucosamine, arbutin, D-lyxose, D-arabitol or potassium 2-ketogluconate. Utilizes dextrin, glycogen, cellobiose, D-galacturonic acid, α-D-glucose, α-D-lactose, maltose, D-mannose, melibiose, methyl β-D-glucoside, raffinose, salicin, stachyose, sucrose, L-malic acid, succinamic acid, α-D-glucose 1-phosphate, D-glucose 6-phosphate, D-ribose, D-xylose, pyruvic acid methyl ester, succinic acid monomethyl ester and pyruvic acid, but not Tween 40, Tween 80, arbutin, D-sorbitol or α-ketoglutaric acid (Biolog GP2 plates). Major cellular fatty acids are anteiso-C_{15:0}, C_{16:0}, anteiso-C_{17:0} and iso-C_{15:0}. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid. MK-7 is the predominant menaquinone. The G+C content of the genomic DNA of the type strain is 53.7 mol%.

The type strain, SA-7-6^T (=CCTCC AB 206108^T=DSM 19409^T), was isolated from desert sand of the Tarim Basin, Xinjiang, China.

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