Bacillus aidingensis sp. nov., a moderately halophilic bacterium isolated from Ai-Ding salt lake in China

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A Gram-positive, halophilic bacterium was isolated from a sediment sample from Ai-Ding salt lake in China. The isolate, designated strain 17-5^T, grew at salinities of 8–33 % (w/v) NaCl (optimally at 12 %, w/v). The genomic DNA G + C content of strain 17-5^T was 48.1 mol%. The predominant isoprenoid quinone was MK-7(H₂) and the cell-wall peptidoglycan contained *meso*-diaminopimelic acid. The major polar lipids were diphosphatidylglycerol and an unidentified glycolipid. The major cellular fatty acids were anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and C_{16:0}. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 17-5^T was a member of the genus *Bacillus*, being most closely related to *Bacillus qingdaonensis* JCM 14087^T (96.0 % sequence similarity) and *Bacillus salarius* DSM 16461^T (95.6 %). The levels of 16S rRNA gene sequence similarity with respect to other *Bacillus* species were less than 91.7 %. Comparative analysis of the 16S rRNA gene sequence data, chemotaxonomy and phenotypic features of the novel isolate and related species of *Bacillus* indicated that strain 17-5^T represents a novel species within the genus *Bacillus*, for which the name *Bacillus aidingensis* sp. nov. is proposed. The type strain is 17-5^T (=CGMCC 1.3227^T=DSM 18341^T).

Moderately halophilic bacteria that grow optimally in media containing 3–15 % (w/v) NaCl are widely distributed throughout various types of saline environments, such as salt lakes, salterns and salty foods (Ventosa *et al.*, 1998). Lake Ai-Ding (89° 10' 32"–83° 54' 32" E 42° 32' 10"–42° 49' 13" N) is a typical chloride–sulphate saline lake with a neutral pH and a salt concentration of 20–26 % (w/v). Previous studies on the microbial diversity of Ai-Ding salt lake have demonstrated the presence of a variety of halophilic micro-organisms (Cui *et al.*, 2006a, b; Ren & Zhou, 2005a, b). In this paper, we describe a novel moderately halophilic bacterium, designated strain 17-5^T, isolated from Lake Ai-Ding. Phenotypic and chemotaxonomic characteristics, as well as data from a phylogenetic analysis based on 16S rRNA gene sequence comparisons, showed that strain 17-5^T represents a novel species within the genus *Bacillus*.

The sample collection/treatment and the enrichment and isolation of strain 17-5^T were performed as described by

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 17-5^T is DQ504377.

Photomicrographs of cells of strain 17-5^T are available as supplementary material with the online version of this paper.

Ren & Zhou (2005a). Strain 17-5^T was routinely grown on HM medium (Ventosa *et al.*, 1982) containing 12 % (w/v) NaCl instead of 17.8 % NaCl (modified HM medium). Cellular morphology was examined using light microscopy and transmission electron microscopy. Gram staining was performed as described by Gerhardt *et al.* (1981), in parallel with the KOH test (Gegersen, 1978). Motility was determined in wet mounts by using phase-contrast microscopy; flagella were demonstrated using negative staining (Kodaka *et al.*, 1982) and transmission electron microscopy. The NaCl, temperature and pH ranges for growth, the utilization of carbon and energy sources (added at 0.5 %, w/v) and the hydrolysis of starch, casein, gelatin and Tweens 20, 40, 60 and 80 were determined as described previously (Ren & Zhou, 2005a). General biochemical tests (including those for nitrate reduction, urease activities, H₂S production, catalase and oxidase activities, citrate utilization and indole production and the Voges–Proskauer reaction and the methyl red test) were performed as described by Smibert & Krieg (1981). Susceptibility to antibiotics was tested by spreading bacterial suspensions on agar plates containing modified HM medium, placing antibiotic-impregnated paper discs (7 mm in diameter and 1 mm in thickness) on the agar

surface, incubating the plates for 48 h and checking for a clear zone of growth inhibition around each disc (representing antibiotic sensitivity). Strain 17-5^T showed an obligatory halophilic response, growing in the presence of 8–33 % (w/v) NaCl. Cells of strain 17-5^T were endospore-forming, motile, short rods with peritrichous flagella (see Supplementary Fig. S1, available in IJSEM Online). On modified HM medium strain 17-5^T formed creamy white, slightly centre-convex and circular colonies after cultivation at 37 °C for 24 h. Additional phenotypic properties are presented in the species description and in Table 1.

Preparation of the cell wall and determination of the peptidoglycan composition were performed by using the methods described by Schleifer & Kandler (1972), but with the modification that TLC on cellulose sheets was used instead of paper chromatography. Respiratory quinones were extracted according to the method of Collins *et al.* (1977) and were analysed by using reversed-phase HPLC (Groth *et al.*, 1996). Polar lipids were extracted and identified by using one-dimensional TLC followed by spraying with the appropriate detection reagents (Kates, 1986). Cellular fatty acids were extracted, methylated and analysed by GC using the standard Sherlock MIDI

(Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The genomic DNA G + C content was determined by means of the thermal denaturation method, according to Marmur & Doty (1962). Strain 17-5^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The polar lipid extract contained diphosphatidylglycerol and an unidentified glycolipid. The major isoprenoid quinone of strain 17-5^T was MK-7(H₂). The cellular fatty acids of strain 17-5^T were as follows: anteiso-C_{15:0} (30.6 %), C_{16:0} (16.4 %), anteiso-C_{17:0} (16.2 %), iso-C_{16:0} (15.1 %), iso-C_{15:0} (6.8 %), iso-C_{14:0} (4.7 %), iso-C_{17:0} (4.7 %), C_{15:0} (2.9 %) and C_{17:0} (2.4 %). These chemotaxonomic features of strain 17-5^T were typical of those found in members of the genus *Bacillus* (Priest *et al.*, 1988; Heyrman *et al.*, 2004, 2005; Albert *et al.*, 2005; Wieser *et al.*, 2005; Lim *et al.*, 2006a, b). The genomic DNA G + C content of strain 17-5^T was 48.1 mol%. This value is within the range for the genus *Bacillus*.

The 16S rRNA gene of strain 17-5^T was amplified by PCR with universal primers, as described previously (Duckworth *et al.*, 1996). The almost-complete nucleotide sequence (1562 bp) was determined by direct sequencing and was compared with 16S rRNA gene sequences available

Table 1. Differential features for strain 17-5^T (*Bacillus aidingensis* sp. nov.) with respect to the two most closely related *Bacillus* species

+, Positive; –, negative; ND, no data available.

Characteristic	Strain 17-5 ^T *	<i>B. qingdaonensis</i> JCM 14087 ^{T†}	<i>B. salarius</i> DSM 16461 ^{T‡}
Flagellation	+	–	–
Endospore formation	+	–	+
pH range for growth	6.0–9.5	6.5–10.5	6.8–9.5
Optimum pH	7.2	9.0	8.0
Temperature range (°C)	22–44	25–45	15–40
Optimum temperature (°C)	37	37	30
NaCl range for growth (% w/v)	8–33	2.5–20	3–20
Optimum NaCl conc. (% w/v)	12	12	10–12
Oxidase	–	–	+
Acid production from:			
Sucrose	–	+	ND
D-Xylose	–	+	+
D-Lactose	–	+	+
Gelatin hydrolysis	+	–	ND
Urease	–	+	–
Reduction of nitrate to nitrite	+	+	–
H ₂ S production	+	–	ND
Major fatty acids (% of total)	anteiso-C _{15:0} (30.6), anteiso-C _{17:0} (16.2), C _{16:0} (16.4), iso-C _{16:0} (15.1)	anteiso-C _{15:0} (34.7), anteiso-C _{17:0} (21.4), iso-C _{16:0} (13.9), C _{16:0} (7.7)	anteiso-C _{15:0} (53.1), anteiso-C _{17:0} (18.6), iso-C _{15:0} (8.9), iso-C _{16:0} (6.6)
Major menaquinone	MK-7(H ₂)	MK-7(H ₂)	MK-7
DNA G + C content (mol%)	48.1	48.0	43.0

*Data from this study.

†Data from Wang *et al.* (2007).

‡Data from Lim *et al.* (2006b).

in the GenBank database, using the BLAST program. Multiple alignment with closely related species was performed using the CLUSTAL W program (Thompson *et al.*, 1994). Ambiguous and unalignable bases were omitted manually and then the phylogenetic trees were constructed using the neighbour-joining, minimum-evolution and maximum-parsimony methods in MEGA, version 3.1 (Kumar *et al.*, 2004). The robustness of the resultant tree topology was evaluated by means of bootstrap resampling analysis with 1000 replicates. The 16S rRNA gene sequence analysis showed that strain 17-5^T was phylogenetically related to members of the family *Bacillaceae* and belonged within the phyletic group classically defined as the genus *Bacillus*. Strain 17-5^T was most closely related to *Bacillus qingdaonensis* JCM 14087^T and *Bacillus salarius* DSM 16461^T, with 96.0 and 95.6 % sequence similarity, respectively. The sequence similarities with respect to other known species of the genus *Bacillus* were much lower (<91.7 %). The neighbour-joining phylogenetic tree (Fig. 1) also showed that strain 17-5^T formed a coherent cluster with *B. qingdaonensis* JCM 14087^T and *B. salarius* DSM 16461^T within the genus *Bacillus* and was distantly related to other members of the genus. Similar tree topologies were observed when other algorithms were used. The high levels of sequence similarity divergence (>4.0 %) with respect to the type strains of the known species of the genus *Bacillus* suggested

that strain 17-5^T represents a novel taxon within the genus *Bacillus* (Stackebrandt *et al.*, 2002).

The characteristics that serve to differentiate strain 17-5^T from related *Bacillus* species are summarized in Table 1. The differences in some features, such as nitrate reduction, H₂S production, oxidase and urease activities, salt range for growth, optimal pH for growth, and acid production from xylose and lactose, as well as the fatty acid composition, can be used to distinguish this strain from phylogenetically related taxa (Table 1). Therefore, on the basis of the taxonomic data presented here, strain 17-5^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus aidingensis* sp. nov. is proposed.

Description of *Bacillus aidingensis* sp. nov.

Bacillus aidingensis (ai.din.gen'sis. N.L. masc. adj. *aidingensis* from Lake Ai-Ding, a saline lake in China).

Cells are Gram-positive, aerobic, short rods 0.2–0.4 × 1.3–2.5 µm in size and motile by means of peritrichous flagella. Ellipsoidal endospores are formed subterminally or centrally. Colonies are creamy white, slightly centre-convex, circular, 4–5 mm in diameter and have regular margins after cultivation at 37 °C on modified HM medium for 24 h. Growth occurs at temperatures in the range 22–44 °C (optimally at 37 °C) and at NaCl concentrations in the range 8–33 % (w/v) (optimally at 12 %). No growth occurs

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships among strain 17-5^T (*Bacillus aidingensis* sp. nov.) and related species. Numbers at nodes are bootstrap percentages, based on an analysis of 1000 resampled datasets. Bar, 1 % sequence divergence.

in the absence of NaCl. pH range for growth is 6.0–9.5 (optimum, pH 7.2). Positive in the Voges–Proskauer reaction and for catalase, gelatin and aesculin hydrolysis, nitrate reduction and H₂S production, but negative in the methyl red test and for oxidase, urease, DNase, phosphatase, NH₃ production and hydrolysis of casein, starch, cellulose and Tweens 20, 40, 60 and 80. The following compounds are utilized as sole carbon and energy sources: D-glucose, D-mannose, D-galactose, L-sorbose, D-sorbose, D-fructose, lactose, sucrose, maltose, cellobiose, melibiose, trehalose, raffinose, melezitose, D-mannitol, inositol, dulcitol, erythritol, glycerol, inulin and salicin. L-Rhamnose, D-arabinose and D-xylose are not used as carbon sources. Acid is produced from D-glucose, D-galactose, D-mannose, D-fructose, maltose, cellobiose, trehalose, D-mannitol and glycerol, but not from L-sorbose, D-sorbose, raffinose, melezitose, lactose, sucrose, melibiose, dulcitol, erythritol, inulin or salicin. Sensitive to the following antibiotics (µg, unless indicated otherwise): ampicillin (10), oxacillin (1), penicillin G (10 U), cefazolin (30), tetracycline (30), chloramphenicol (30), clindamycin (2), erythromycin (15), sulfamethoxazole (300), nitrofurantoin (300), norfloxacin (10), vancomycin (30), ciprofloxacin (5), clarithromycin (15), kanamycin (30), leucomycin (15), acetylspiramycin (15), rifampicin (5), spectinomycin (100), ampicillin/sulbactam (10/10), azithromycin (15), josamycin (15), medicamycin (15), bacitracin (0.04 U) and novobiocin (5). Resistant to the following antibiotics (µg, unless indicated otherwise): gentamicin (10), streptomycin (10), tobramycin (10), neomycin (30) and polymyxin B (300 U). Major polar lipids are diphosphatidylglycerol and an unidentified glycolipid. 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 diagnostic diamino acid in the cell-wall peptidoglycan is *meso*-diaminopimelic acid. The predominant menaquinone is MK-7(H₂). The genomic DNA G+C content of the type strain is 48.1 mol% (*T*_m).

The type strain, 17-5^T (=CGMCC 1.3227^T=DSM 18341^T), was isolated from a sediment sample from Lake Ai-Ding, a salt lake in Xin-Jiang Province, China.

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