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 17-5T, grew at salinities of 8–33 % (w/v) NaCl (optimally at 12 %, w/v). The genomic DNA G+C content of strain 17-5T 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-C15 : 0, anteiso-C17 : 0, iso-C16 : 0 and C16 : 0. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 17-5T was a member of the genus Bacillus, being most closely related to Bacillus qingdaonensis JCM 14087T (96.0 % sequence similarity) and Bacillus salarius DSM 16461T (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, chemotaxonomic and phenotypic features of the novel isolate and related species of Bacillus indicated that strain 17-5T represents a novel species within the genus Bacillus, for which the name Bacillus aidingensis sp. nov. is proposed. The type strain is 17-5T (=CGMCC 1.3227T =DSM 18341T).

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 17-5T, 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 17-5T represents a novel species within the genus Bacillus.

The sample collection/treatment and the enrichment and isolation of strain 17-5T were performed as described by Ren & Zhou (2005a). Strain 17-5T 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 (Gregersen, 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, b). 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 17-5T showed an obligatory halophilic response, growing in the presence of 8–33% (w/v) NaCl. Cells of strain 17-5T were endospore-forming, motile, short rods with peritrichous flagella (see Supplementary Fig. S1, available in IJSEM Online). On modified HM medium strain 17-5T 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 17-5T 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 17-5T was MK-7(H2). The cellular fatty acids of strain 17-5T were as follows: anteiso-C15:0 (30.6%), C16:0 (16.4%), anteiso-C17:0 (16.2%), iso-C16:0 (15.1%), iso-C15:0 (6.8%), iso-C14:0 (4.7%), iso-C17:0 (4.7%), C15:0 (2.9%) and C17:0 (2.4%). These chemotaxonomic features of strain 17-5T 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 17-5T was 48.1 mol%. This value is within the range for the genus Bacillus.

The 16S rRNA gene of strain 17-5T 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>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 17-5T*</th>
<th>B. qingdaonensis JCM 14087T†</th>
<th>B. salarius DSM 16461T‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagellation</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Endospore formation</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6.0–9.5</td>
<td>6.5–10.5</td>
<td>6.8–9.5</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>7.2</td>
<td>9.0</td>
<td>8.0</td>
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<td>Temperature range (°C)</td>
<td>22–44</td>
<td>25–45</td>
<td>15–40</td>
</tr>
<tr>
<td>Optimum temperature (°C)</td>
<td>37</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>NaCl range for growth (% w/v)</td>
<td>8–33</td>
<td>2.5–20</td>
<td>3–20</td>
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<tr>
<td>Optimum NaCl conc. (% w/v)</td>
<td>12</td>
<td>12</td>
<td>10–12</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td>–</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Sacrose</td>
<td>–</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>D-Xylose</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D-Lactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Gelatin hydrolysis</td>
<td>+</td>
<td>–</td>
<td>ND</td>
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<tr>
<td>Urease</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Reduction of nitrate to nitrite</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>H2S production</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Major fatty acids (% of total)</td>
<td>anteiso-C15:0(30.6%), anteiso-C17:0(16.2%), C16:0(16.4%), iso-C16:0(15.1%)</td>
<td>anteiso-C15:0(34.7%), anteiso-C17:0(21.4), iso-C16:0(13.9), C16:0(7.7)</td>
<td>anteiso-C15:0(53.1), anteiso-C17:0(18.6), iso-C16:0(8.9), iso-C17:0(6.6)</td>
</tr>
<tr>
<td>Major menaquinone</td>
<td>MK-7(H2)</td>
<td>MK-7(H2)</td>
<td>MK-7</td>
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<td>DNA G+C content (mol%)</td>
<td>48.1</td>
<td>48.0</td>
<td>43.0</td>
</tr>
</tbody>
</table>

*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\textsuperscript{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\textsuperscript{T} was most closely related to Bacillus qingdaonensis JCM 14087\textsuperscript{T} and Bacillus salarius DSM 16461\textsuperscript{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\textsuperscript{T} formed a coherent cluster with B. qingdaonensis JCM 14087\textsuperscript{T} and B. salarius DSM 16461\textsuperscript{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\textsuperscript{T} represents a novel taxon within the genus Bacillus (Stackebrandt et al., 2002).

The characteristics that serve to differentiate strain 17-5\textsuperscript{T} from related Bacillus species are summarized in Table 1. The differences in some features, such as nitrate reduction, H\textsubscript{2}S 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\textsuperscript{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. aiding-ensis from Lake Ai-Ding, a saline lake in China).

Cells are Gram-positive, aerobic, short rods 0.2–0.4 \(\times\) 1.3–2.5 µm in size and motile by means of peritrichous flagella. Ellipsoidal endospores are formed subterminally or centrically. 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 at 65 °C.

**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships among strain 17-5\textsuperscript{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 H$_2$S production, but negative in the methyl red test and for oxidase, urease, DNase, phosphatase, NH$_3$ production and hydrolysis of casein, starch, cellulose and Tweenes 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-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-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), nortioxacin (10), vancomycin (30), ciprofloxacin (5), clari-thromycin (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 diphasphatidyglycerol and an unidentified glycolipid. Major fatty acids are anteiso-C$_{15:0}$, C$_{16:0}$ anteiso, C$_{17:0}$ iso, C$_{16:1}$ω7c and iso-C$_{15:0}$. The diagnostic diamino acid in the cell-wall peptidoglycan is meso-diaminopimelic acid. The predominant menaquinone is MK-7(H$_2$). The genomic DNA G+C content of the type strain is 48.1 mol% ($T_m$).

The type strain, 17-5T ($T_m$), was isolated from a sediment sample from Lake Ai-Ding, a salt lake in Xin-Jiang Province, China.

Acknowledgements

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