Terribacillus aidingensis sp. nov., a moderately halophilic bacterium

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Three Gram-positive, moderately halophilic bacteria, designated YI7-61T, IA7 and DB2, were isolated from sediments of Aiding salt lake in the Xinjiang region of China. Cells of the strains were rod-shaped, motile by means of peritrichous flagella and produced ellipsoidal spores. Colonies were pale yellow in colour. The strains grew optimally at 30–37 °C, pH 6–7 and 3–7 % (w/v) NaCl. The diamino acid in the murein was meso-diaminopimelic acid and the major quinone system was MK-7. The major cellular fatty acids were anteiso-C15 : 0 and anteiso-C17 : 0. The DNA G+C content was 44.6–45.0 mol%. 16S rRNA gene sequence analysis revealed that strains YI7-61T, IA7 and DB2 were closely related to members of the genus Terribacillus and showed 96.8–97.6, 96.4–97.2 and 95.4–95.5 % 16S rRNA gene sequence similarity with Terribacillus halophilus 002-051T, Terribacillus saccharophilus RB589 and Terribacillus goriensis CL-GR16T, respectively. DNA–DNA relatedness among the isolates was 88–92 % and strain YI7-61T shared 24, 18 and 18 % DNA–DNA relatedness with T. halophilus JCM 21760T, T. saccharophilus JCM 21759T and T. goriensis DSM 18252T, respectively. On the basis of phenotypic and phylogenetic distinctiveness, the three isolates should be placed in the genus Terribacillus as representatives of a novel species, for which the name Terribacillus aidingensis sp. nov. is proposed. The type strain is YI7-61T (=CGMCC 1.8913T =NBRC 105790T).

Gram-positive, rod-shaped, spore-forming, moderately halophilic bacteria have been isolated from various saline or hypersaline environments (Ventosa et al., 1998; Kim et al., 2007). They were originally placed in the genus Bacillus (Claus & Berkeley, 1986). Subsequently, molecular and chemotaxonomic analyses have shown that they form several phylogenetically distinct lineages within the family Bacillaceae and many new genera and novel species of this kind of bacteria have been reported. To date, the spore-forming, moderately halophilic bacteria that have been described include Halobacillus (Spring et al., 1996; Chen et al., 2009a), Virgibacillus (Heyndrickx et al., 1998; Chen et al., 2009b; Niederberger et al., 2009), Gracilibacillus (Waino et al., 1999; Tang et al., 2009), Oceanobacillus (Lu et al., 2001; Namwong et al., 2009), Filobacillus (Schlesner et al., 2001), Jeotgalibacillus (Yoon et al., 2001), Marinibacillus (Yoon et al., 2001), Lentibacillus (Yoon et al., 2002), Paralibacillus (Ishikawa et al., 2002), Halolactibacillus (Ishikawa et al., 2005), Pontibacillus (Lim et al., 2005; Chen et al., 2009c), Thalassobacillus (Garcia et al., 2005), Tenuibacillus (Ren & Zhou, 2005a), Salinibacillus (Ren & Zhou, 2005b), Salsuginibacillus (Carrasco et al., 2007a), Piscibacillus (Tanassupawat et al., 2007), Terribacillus (An et al., 2007), Sediminibacillus (Carrasco et al., 2008), Aquasalibacillus (Marquez et al., 2008) and Bacillus (Garabito et al., 1997; Aral et al., 1999; Carrasco et al., 2007b; Xue et al., 2008).

At present, the genus Terribacillus includes three species. Terribacillus saccharophilus and Terribacillus halophilus were isolated from field soil in Japan (An et al., 2007), and Terribacillus goriensis DSM 18252T was isolated from coastal water off the east coast of Korea (Kim et al., 2007; Krishnamurthi & Chakrabarti, 2008). The main phenotypic
characteristics of the genus *Terribacillus* are the formation of ellipsoidal endospores, gelatin liquefaction and the absence of H$_2$S and indole production and nitrate reduction. The DNA G+C content is 43.0–46.0 mol%, the major fatty acids are anteiso-C$_{15:0}$ and anteiso-C$_{17:0}$ and MK-7 is the major component of the quinone system (An et al. 2007; Krishnamurthi & Chakrabarti, 2008).

Three moderately halophilic bacterial strains, designated YI7-61$^T$, IA7 and DB2, were isolated from sediments of Aiding salt lake in Xinjiang, China (42° 32'–49' 10–13° N 89° 10–54' 32° E). At the time of sampling, the sediments were 17°C, 12.1–15.4% (w/v) NaCl and pH 7.1–7.3. For isolation, the samples were suspended in sterilized water supplemented with 5% NaCl, serially diluted and spread on plates of complex isolation and maintenance medium described previously (Liu et al. 2005). For cultivation of the isolates for characterization, the isolation medium was adjusted to pH 7.0 and incubation was at 35°C, unless indicated otherwise. Phylogenetic analysis of the 16S rRNA gene sequences showed that the isolates belonged to the genus *Terribacillus*. The reference strains *T. saccharophilus* JCM 21759$^T$, *T. halophilus* JCM 21760$^T$ and *T. goriensis* DSM 18252$^T$ were grown according to conditions described by An et al. (2007) and Kim et al. (2007), respectively.

To characterize the isolates phenotypically, standard tests were performed according to the proposed minimal standards for the description of aerobic, endospore-forming bacteria (Logan et al. 2009). Cell morphology and motility were examined after cultivation for 16 h. Endospores were observed after 48 h using phase-contrast microscopy (Eclipse 50i; Nikon). Bacterial flagellation was observed using transmission electron microscopy (JEM-1230; JEOL). Gram reaction was determined according to the methods described by Doetsch (1981) and Gregersen (1978). Colony morphology was examined after 3 days at 35°C on modified isolation medium (6% NaCl, pH 7.0). Conditions for growth were determined in liquid isolation medium by monitoring the increase in optical density at 600 nm. Growth was tested with 0, 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 21, 22, 23, 25 or 30% (w/v) NaCl at pH 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11, achieved using the appropriate biological buffers (pH 8.0, Na$_2$HPO$_4$/NaH$_2$PO$_4$; pH 9.0, Na$_2$CO$_3$/NaHCO$_3$; and pH 11.0, Na$_2$HPO$_4$/NaOH), as described by Gomori (1955), and at 4, 10, 15, 20, 25, 28, 30, 33, 35, 37, 40, 45, 50, 55 and 60°C. The API 20E and API 50C HB systems (bioMérieux) were used to determine the physiological and biochemical characteristics, such as the Voges–Proskauer test, fermentation of 49 carbohydrates and hydrolysis of gelatin. All suspension media were supplemented with 5% NaCl (w/v). Tests for catalase, urease and oxidase, hydrolysis of casein, starch, tyrosine, Tween 80 and ascorbic acid, utilization of citrate, reduction of nitrate, Voges–Proskauer reaction, production of indole and H$_2$S and arginine dihydrolase, tryptophan deaminase, phenylalanine deaminase, lysine decarboxylase and ornithine decarboxylase activities were performed for the isolates and the reference strains according to the conventional methods described by Dong & Cai (2001). Cells of strains YI7-61$^T$, IA7 and DB2 were motile, Gram-positive and rod-shaped. Non-swollen ellipsoidal endospores were formed. Catalase and β-galactosidase were negative. Urease and oxidase were positive. Starch could be hydrolysed. H$_2$S and indole were not produced. Nitrate was not reduced to nitrite. Gelatin was liquefied. The Voges–Proskauer test was negative. The results for the reference strains in this study were the same as those reported before except for total salt concentration for growth (Table 1). The morphological, physiological and biochemical characteristics are given in detail in Table 1, Supplementary Fig. S1, available in IJSEM Online, and the species description.

### Table 1. Differential characteristics of *Terribacillus aidingensis* sp. nov. and its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tr>
<td>Motility</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Spore shape</td>
<td>E</td>
<td>E, S</td>
<td>E, S</td>
<td>E, S*</td>
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<tr>
<td>Spore position</td>
<td>C, ST</td>
<td>ST, T</td>
<td>ST, T</td>
<td>C, ST*</td>
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<tr>
<td>Voges–Proskauer test</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Urease</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Oxidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Catalase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Growth with (% NaCl):</td>
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<tr>
<td>Range</td>
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<td>0–16</td>
<td>0–19</td>
<td>0–14*</td>
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<tr>
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<td>2–7</td>
<td>2–7</td>
<td>0–2*</td>
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<td>Growth at (°C):</td>
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<tr>
<td>Range</td>
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<td>5–45</td>
<td>5–45</td>
<td>15–43*</td>
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<td>Acid production from:</td>
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<tr>
<td>Amygdalin</td>
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<td>–</td>
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<tr>
<td>Fructose</td>
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<td>Galactose</td>
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<td>+</td>
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<td>–</td>
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<td>Casein</td>
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<td>+</td>
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<td>Starch</td>
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<td>+</td>
<td>–</td>
<td>+</td>
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<td>m-DAP</td>
<td>m-DAP</td>
<td>m-DAP</td>
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<td>DNA G+C content</td>
<td>44.6–45.0</td>
<td>44.0</td>
<td>45.8</td>
<td>43.0*</td>
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</table>

*Data were taken from Kim et al. (2007) and Krishnamurthi & Chakrabarti (2008).
Biomass for chemotaxonomic analysis was harvested from cultures after incubation on complex medium at 35 °C for 18 h. The analysis of the cell-wall peptidoglycan was carried out using the method described by Schleifer & Kandler (1972) and Schleifer (1985). Cell-wall hydrolysates were separated by one-dimensional chromatography on microcellulose thin layers, using \textit{T. halophilus} JCM 21760\textsuperscript{T} as a reference. Menaquinones were analysed as described by Collins (1985) using reversed-phase HPLC (HP-1050), using \textit{T. halophilus} JCM 21760\textsuperscript{T} and \textit{Arthrobacter nicotinovorans} CGMCC C.1933\textsuperscript{T} as references. The fatty acid compositions of strain YI7-61\textsuperscript{T}, \textit{T. halophilus} JCM 21760\textsuperscript{T} and \textit{T. saccharophilus} JCM 21759\textsuperscript{T} were determined under the same laboratory conditions for cells grown in complex medium supplemented with 5 % (w/v) NaCl (pH 7.0) on a rotary shaker (200 r.p.m.) at 35 °C for 18 h, according to the manufacturer’s instructions for the Microbial Identification System (MIDI). The diamino acid in the murein and the menaquinone of strain YI7-61\textsuperscript{T} were \textit{meso}-diaminopimelic acid (\textit{m}-DAP) and MK-7, respectively, which were the same as those of \textit{T. halophilus} JCM 21760\textsuperscript{T}. The major cellular fatty acids of strains YI7-61\textsuperscript{T}, IA7 and DB2 were anteiso-C\textsubscript{15:0} (67.9, 58.8 and 48.6 %, respectively) and anteiso-C\textsubscript{17:0} (19.4, 21.4 and 34.8 %, respectively). Detailed cellular fatty acid compositions of strain YI7-61\textsuperscript{T}, \textit{T. halophilus} JCM 21760\textsuperscript{T}, \textit{T. saccharophilus} JCM 21759\textsuperscript{T} and \textit{T. goriensis} DSM 18252\textsuperscript{T} are given in Supplementary Table S1.

Chromosomal DNA was extracted and purified according to standard methods (Marmur, 1961). The determination of DNA G + C content was carried out using the thermal denaturation method according to Marmur & Doty, (1962) with a BIO-20 UV spectrophotometer. For the calculation of DNA G + C content, the equation of Owen & Hill (1979) was used. The DNA G + C contents of strains YI7-61\textsuperscript{T}, IA7 and DB2 were 45.0, 44.6 and 44.9 mol%, respectively. These results are within the emended G + C content range (43–46 mol%) for the genus \textit{Terribacillus} Krishnamurthi & Chakrabarti (2008).

The 16S rRNA gene sequence was amplified as described by Duckworth \textit{et al.} (1996). PCR products were sequenced with a DNA sequencer (ABI 373A; Applied Biosystems) and the software provided by the manufacturer. The almost-complete 16S rRNA gene sequences of strains YI7-61\textsuperscript{T}, IA7 and DB2 (1472 bp, 1464 bp and 1485 bp) were compared with sequences from public databases using the BLAST program via the National Center for Biotechnology Information. Pairwise sequence similarities were calculated using the BioEdit software package (Hall, 1999). Phylogenetic trees were reconstructed using \textit{MEGA} version 3.1 (Kumar \textit{et al.}, 2004). Distances were calculated by using distance options according to Kimura’s two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining, maximum-parsimony and minimum-evolution methods. The stability of the relationships was assessed by 1000 bootstrap analyses. A neighbour-joining phylogenetic tree is shown in Fig. 1. The topologies of the trees generated with the minimum-evolution and maximum-parsimony methods were very similar to the neighbour-joining tree (data not shown). The phylogenetic analysis revealed that the three isolates belonged to the same phylogenetic lineage within the genus \textit{Terribacillus}. 16S rRNA gene sequence similarities among the isolates
were 98.5 % (YI7-61T and IA7), 98.7 % (YI7-61T and DB2) and 98.7 % (IA7 and DB2). Strains YI7-61T, IA7 and DB2 showed 16S rRNA gene sequence similarities of 97.6, 96.8 and 96.9 % with *T. halophilus* 002-051T, 97.2, 96.4 and 96.7 % with *T. saccharophilus* RB589 and 95.5, 95.4 and 95.4 % with *T. goriensis* CL-GR16T, respectively. On the basis of 16S rRNA gene sequence analysis, it was evident that the novel isolates should be assigned to the genus *Terribacillus*.

To determine levels of DNA–DNA relatedness, extracted DNA was sheared by sonication (Braun Melsungen) at 50 W for three periods of 10 s, rehybridized in 2 x SSC at 70 °C and analysed spectrophotometrically (De Ley et al., 1970; Huß et al., 1983). DNA–DNA relatedness among the isolates was 88 % (YI7-61T and IA7), 90 % (YI7-61T and DB2) and 92 % (IA7 and DB2). Strain YI7-61T showed DNA–DNA relatedness of 24, 18 and 18 % with *T. halophilus* JCM 21760T, *T. saccharophilus* JCM 21759T and *T. goriensis* DSM 18252T, respectively. Since <70 % DNA–DNA relatedness is a key marker for the delineation of a novel species (Wayne et al., 1987), these results showed that the three isolates belonged to the same species, which was different from the previously recognized members of the genus *Terribacillus*.

On the basis of morphological, physiological and chemotaxonomic characteristics, 16S rRNA gene sequence comparison and DNA–DNA relatedness, strains YI7-61T, IA7 and DB2 should be treated as representatives of a novel species within the genus *Terribacillus*, for which the name *Terribacillus aidingensis* sp. nov. is proposed.

**Description of *Terribacillus aidingensis* sp. nov.**

*Terribacillus aidingensis* (ai.ding.en’sis. N.L. masc. adj. aidingensis from Aiding salt lake, where the type strain was isolated).

Cells are rod-shaped, 0.3–0.7 x 1.2–3.5 μm, Gram-positive, occurring singly, in pairs or in short chains, and motile by means of peritrichous flagella. Non-swellen ellipsoidal endospores are located mainly in a central position; subterminal endospores observed occasionally. Colonies on solid complex medium are circular, smooth, entire, slightly raised, pale yellow and 1–3 mm in diameter after 3 days. Growth occurs with 0.5–21 % (w/v) NaCl (optimum 3–7 % total salts), at 4–48 °C (optimum 30–37 °C) and at pH 5–9 (optimum pH 6–7). With API 20E and conventional methods, urease and oxidase are produced, but catalase, β-galactosidase, arginine dihydrodolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are not. Tween 80, gelatin and casein are hydrolysed, but tyrosine is not. Nitrate is not reduced to nitrite. Citrate is not utilized. H₂S and indole are not produced. Voges–Proskauer reaction (acetoin) is negative. With API 50CHB, acid is produced from N-acetylglucosamine, ascorcin, amygdalin, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, lactose, D-mannitol, melibiose, raffinose, trehalose, and sucrose, but not from DL-arabinose, DL-fucose, D-lyxose, maltoose, D-mannose, melezitose, L-rhamnose, D-ribose, L-sorbose, starch, D-tagatose, turanose, DL-xyllose, gluconate, glycojen, inulin, salcin, D-adonitol, dulcitol, DL-arabitol, erythritol, glycerol, inositol, D-sorbitol, xylitol, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyllose, 2-ketogluconate or 5-ketogluconate. The diamino acid in the murein is m-DAP and the menaquinone is MK-7. The major cellular fatty acids are anteiso-C₁₅:₀ and anteiso-C₁₇:₀. The DNA G+C content is 44.6–45.0 mol % and the DNA G+C content of the type strain is 45.0 mol %.

The type strain is YI7-61T (=CGMCC 1.8913T =NBRC 105790T), isolated from the Aiding Salt lake in Xinjiang, China.

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**References**


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