**Paraliobacillus quinghaiensis** sp. nov., isolated from salt-lake sediment in China

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A Gram-positive, moderately halophilic, endospore-forming, catalase- and oxidase-positive, obligately aerobic bacterium, designated strain YIM-C158T, was isolated from sediment of a salt lake in the Qaidam Basin, north-west China. Cells were motile with peritrichous flagella and rod-shaped, with meso-diaminopimelic acid in the cell-wall peptidoglycan. Strain YIM-C158T grew in the presence of 1–20 % (w/v) NaCl and pH 6.0–10.0, with optimum growth at 5 % (w/v) NaCl and pH 8.0. The strain grew at 4–50 °C, with optimum growth at 37 °C. The major cellular fatty acids were anteiso-C15 : 0, iso-C14 : 0, C16 : 0, anteiso-C17 : 0, iso-C16 : 0, C16 : 1ω7c alcohol and C16 : 1ω11c. Strain YIM-C158T contained menaquinone MK-7 as the sole respiratory quinone and phosphatidylglycerol and phosphatidylglycerol as the polar lipids. The genomic DNA G+C content was 39.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM-C158T was most closely related to *Paraliobacillus ryukyuensis* DSM 15140T (96.8 % similarity), and the two strains formed a distinct branch in the phylogenetic tree. The level of DNA–DNA relatedness between the two strains was 15.6 %. The combination of phylogenetic analysis, phenotypic characteristics, chemotaxonomic differences and DNA–DNA hybridization data supported the view that strain YIM-C158T represents a novel species of the genus *Paraliobacillus*, for which the name *Paraliobacillus quinghaiensis* sp. nov. is proposed. The type strain is YIM-C158T (= DSM 17857T = CGMCC 1.6333T).

The genus *Paraliobacillus* was first proposed by Ishikawa et al. (2002) to accommodate a Gram-positive, facultatively anaerobic, chemo-organotrophic, spore-forming, rod-shaped, motile (with peritrichous flagella) and slightly halophilic bacterium, with meso-diaminopimelic acid in the cell-wall peptidoglycan. At the time of writing, this genus comprised only one species with a validly published name, *Paraliobacillus ryukyuensis* (Ishikawa et al., 2002, 2003), which phylogenetically occupied an independent lineage within a group composed of halophilic/halotolerant/alkali-tolerant and/or alkali-tolerant species (Spring et al., 1996; Garabito et al., 1997; Heyndrickx et al., 1999; Waino et al., 1999; Lu et al., 2001; Schlesner et al., 2001; Zhilina et al., 2001; Yoon et al., 2002; García et al., 2005; Ishikawa et al., 2005; Jeon et al., 2005; Lim et al., 2005; Ren & Zhou, 2005a, b; Mayr et al., 2006; Nunes et al., 2006; An et al., 2007a; Carrasco et al., 2007; Echigo et al., 2007; Kim et al., 2007) (henceforth referred to as the HA group in this paper) in Bacillus rRNA group 1 (Ash et al., 1991), with the highest similarity values to the genera *Halolactibacillus* (Ishikawa et al., 2005) and *Amphibacillus* (Niimura et al., 1990). In a recent study of the microbial diversity of the Qaidam Basin in Qinghai Province, north-west China, a moderately halophilic strain, designated YIM-C158T, was isolated from a sediment sample collected from the Dabuxun salt lake in August 2002. This lake is located at 36° 50′ N–37° 06′ N, 94° 55′ E–95° 18′ E and the temperature, pH and salinity of the water were 18 °C, 5.8–6.2 and 27.0 % NaCl (w/v), respectively. Data from a polyphasic taxonomic study showed that strain YIM-C158T represents a novel species of the genus *Paraliobacillus*. Strain YIM-C158T was isolated from a salt-lake sediment sample by platting 1 : 10 serial dilutions of the sample on...
marine agar 2216 (MA, pH 7.2; Difco) (Atlas, 1993), supplemented with 10 % (w/v) NaCl at 28 °C. The strain was maintained on a slant of MA supplemented with 5 % (w/v) NaCl (MA5) at 4 °C and in marine broth 2216 (MB; Difco) supplemented with 20 % (v/v) glycerol at −80 °C. The reference strain P. ryukyuensis DSM 15140T was obtained from the DSMZ. Unless indicated otherwise, morphological and physiological studies were performed with cells grown on a modified MA5 medium (MMA5; pH 8.0), containing MA5 plus (1−): 1 g glucose, 2 g pancreatic digest of casein, 1 g lactalbumin hydrolysate (Oxoid) and 1 g malt extract. For chemotaxonomic and molecular systematic studies, the organism was grown in a modified MB medium (MMB5; pH 8.0) in flasks on a rotary shaker at 200 r.p.m. and 37 °C. The composition of MMB5 was the same as MMA5, but without the agar. The biomass was harvested by centrifugation and washed twice with distilled water and freeze-dried.

Cell morphology was examined by using light microscopy (model BH 2; Olympus). Gram staining was carried out using the standard Gram reaction combined with the KOH lysis test method (Gregersen, 1978). Flagella and endospores were stained according to the methods of Leifsson and Scheaffer-Fulton, respectively (Smibert & Krieg, 1981). Motility was observed as described previously (Chen et al. 2007).

Cells of strain YIM-C158T were Gram-positive, thin rods, approximately 3–5 × 0.4–0.6 μm, occurring singly, in pairs or in chains. Terminal or subterminal ellipsoidal spores were observed in swollen sporangia. Colonies were circular, translucent with slightly irregular margins and 1–2 mm in diameter after incubation for 4–5 days at 37 °C on MMA5. Colonies grown for less than 4 days were creamy white, but became pale yellow after several days. No diffusible pigments were produced.

DNA was isolated according to Hopwood et al. (1985) and the DNA G+C content was determined by using the thermal denaturation method (Mandel & Marmur, 1968) with a Shimadzu UV-visible spectrophotometer (UV1601). The genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were done as described previously (Cui et al., 2001). Phylogenetic analysis was performed using the software package MEGA version 3.1 (Kumar et al., 2004) after multiple alignment of sequence data using CLUSTAL_X (Thompson et al., 1997). Distances (corrected according to the Kimura two-parameter model; Kimura, 1980) were calculated and clustering was performed using the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees (not shown) were generated using the treeing algorithms contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1000 resamplings (Felsenstein, 1985). DNA–DNA hybridization was carried out using photo-biotin-labelled probes in microplate wells as described by Ezaki et al. (1989). A microplate spectrofluorimeter (GeminiXPS; Molecular Devices) was employed for fluorescence measurements.

The DNA G+C content of strain YIM-C158T was 39.5 mol%. An almost-complete 16S RNA gene sequence (1521 bp) was determined. Phylogenetic analysis based on 16S rRNA gene sequences showed that, phylogenetically, strain YIM-C158T was most closely related to the type strain of the only recognized species of the genus Paraliobacillus, P. ryukyuensis DSM 15140T, with a comparably high 16S rRNA gene sequence similarity value of 96.8 %. The two strains constituted an independent lineage in the phylogenetic tree within the HA group in rRNA group 1 of the phyletic assemblage of bacteria classically defined as the genus Bacillus, and occupied a phylogenetic position that was closely related to the genera Halolactibacillus (95.1–95.7 % similarity) and Amphibacillus (93.8–93.5 %) (Fig. 1). The level of DNA–DNA relatedness between strain YIM-C158T and P. ryukyuensis DSM 15140T was 15.6 %, which was far below the threshold value of about 70 % recommended by Wayne et al. (1987) for assigning strains to the same species. The results of the phylogenetic analysis and the DNA–DNA hybridization data showed that strain YIM-C158T could be considered as representing a member of the genus Paraliobacillus, but did not belong to the recognized species of the genus.

Growth was tested at various temperatures (4–60 °C) on MMA5 and pH values (5.0–10.0) in MMB5. The buffer solutions described by Chen et al. (2007) were used for the pH experiments. Tolerance of requirement for salts was determined on MA supplemented with 0–30 % (w/v) NaCl, and on some other media as controls, i.e. nutrient agar, tryptic soy agar (TSA; BBL) and ISP medium 2 agar (Shirling & Gottlieb, 1966). Hydrolysis of polymers, urease activity, nitrate reduction and Voges–Proskauer and methyl red tests were determined as described by Gerhardt et al. (1981), Ventosa et al. (1982) and Atlas (1993). Growth under anaerobic conditions, resistance to antibiotics and catalase and oxidase activities were determined as described by Chen et al. (2007). Substrate utilization as sole carbon and energy source, activities of constitutive enzymes and other physiological characteristics were examined using API 20E, API 50 CH (with API 50 CH B/E medium) and API ZYM strips (bioMérieux) and GP2 MicroPlates (Biolog), according to the manufacturers’ instructions. All suspension media were supplemented with 5 % (w/v) NaCl and incubated at 37 °C.

Strain YIM-C158T was found to be catalase- and oxidase-positive and obligately aerobic. The strain was moderately halophilic, as the optimum NaCl concentration for growth was 5 %, with a NaCl concentration range for growth of 1–20 % (Kushner, 1993). No growth was observed on nutrient agar, TSA or ISP medium 2 agar. The results of other phenotypic tests are given in the species description and in Table 1.
Amino acids of whole-cell hydrolysates were determined as described by Hasegawa et al. (1983). Polar lipids were extracted by using the method of Minnikin et al. (1979) and were identified by using two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Isoprenoid quinones were analysed by HPLC as described by Groth et al. (1996). The fatty acids were determined for the novel isolate, as well as for \( P. \) ryukyuensis DSM 15140\(^T \), Fig. 1. Phylogenetic dendrogram, based on 16S rRNA gene sequence analysis, constructed using the neighbour-joining method, showing the position of strain YM-C158\(^T \) within the genus \( \text{Paraliobacillus} \). \( \text{Exiguobacterium undae} \) DSM 14481\(^T \) was used as the outgroup. ‘\( m \)’ and ‘\( p \)’ indicate branches that were also obtained using the maximum-likelihood (Felsenstein, 1981) or maximum-parsimony (Kluge & Farris, 1969) algorithms, respectively; asterisks indicate branches that were recovered with all three methods. Numbers at nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values greater than 50% are given. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics of strain YM-C158\(^T \) (\( \text{Paraliobacillus quinghaiensis} \) sp. nov.) and related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony pigmentation</td>
<td>Creamy white to pale yellow</td>
<td>Yellow</td>
<td>White</td>
<td>White</td>
<td>Yellow</td>
<td>Pinkish white</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
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<tr>
<td>Facultatively anaerobic</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Spore formation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>4–50</td>
<td>10–47.5</td>
<td>17–55</td>
<td>25–45</td>
<td>18–56</td>
<td>18–56</td>
<td>5–40</td>
<td>5–45</td>
</tr>
<tr>
<td>NaCl (%), w/v</td>
<td>1–20</td>
<td>0–22</td>
<td>0–6</td>
<td>&lt;6</td>
<td>1.0–19.7</td>
<td>1.0–20.9</td>
<td>0–24</td>
<td>0–25.5</td>
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<tr>
<td>Optimum</td>
<td>5</td>
<td>0.75–3.0</td>
<td>ND</td>
<td>ND</td>
<td>10.8</td>
<td>5.4–10.8</td>
<td>2.0–3.0</td>
<td>2.5–3.0</td>
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<tr>
<td>pH</td>
<td>6.0–10.0</td>
<td>5.5–9.5</td>
<td>7.0–9.0</td>
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<td>7.0–10.5</td>
<td>8.5–11.5</td>
<td>6.5–9.5</td>
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</tr>
<tr>
<td>Optimum</td>
<td>8.0</td>
<td>7.0–8.5</td>
<td>8.5</td>
<td>ND</td>
<td>8.5–9.0</td>
<td>9.5–9.7</td>
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<td>9.5</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
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<td>Major isoprenoid quinone</td>
<td>MK-7</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>39.5</td>
<td>35.6</td>
<td>42.3</td>
<td>36.0</td>
<td>41.5</td>
<td>39.2</td>
<td>40.2</td>
<td>38.5</td>
</tr>
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Y.-G. Chen and others

\( International Journal of Systematic and Evolutionary Microbiology \) 59
as described by Sasser (1990) using the Microbial Identification System (MIDI; Microbial ID), with cells grown in MMB5 (pH 8.0) in flasks on a rotary shaker at 200 r.p.m. and 37 °C for 2 days.

Chemotaxonomic data for strain YIM-C158T were compatible with its assignment to the genus *Paraliobacillus* (Ishikawa et al., 2002). The peptidoglycan of strain YIM-C158T contained *meso*-diaminopimelic acid and menaquinone MK-7 was the sole respiratory quinone. The polar lipid profile comprised phosphatidylmethylethanolamine, phosphatidylcholine and three unknown phospholipids (see Supplementary Fig. S1, available in IJSEM Online). The fatty acid profile of strain YIM-C158T was similar to that of *P. ryukyuensis* (see Supplementary Table S1, in IJSEM Online). The major fatty acids of strain YIM-C158T were anteiso-C15:0, iso-C14:0, C16:0, anteiso-C17:0, iso-C16:0 and C16:1ω7c alcohol and C16:1ω11c.

Similarities to *P. ryukyuensis* DSM 15140T supported the placement of strain YIM-C158T in the genus *Paraliobacillus* (Ishikawa et al., 2002), i.e. having MK-7 as the respiratory quinone, *meso*-diaminopimelic acid in the cell-wall peptidoglycan and exhibiting catalase activity; the novel strain also had cellular fatty acid contents that were similar to those of *P. ryukyuensis* DSM 15140T (Table 1 and Supplementary Table S1). However, strain YIM-C158T differed markedly from *P. ryukyuensis* DSM 15140T by having a comparatively higher NaCl concentration for optimum growth of 5 % (w/v), a greater growth temperature range of 4–50 °C and a higher DNA G+C content of 39.5 mol%, and by reducing nitrate to nitrite and not being able to grow under anaerobic conditions (Table 1). Strain YIM-C158T also differed from the recognized *Paraliobacillus* species by its discriminative fatty acid pattern, in which there were comparatively significant amounts of unbranched saturated fatty acids (making up 12.18% of the total) as well as iso-C14:0 whereas the amount of anteiso-C17:0 was noticeably reduced and hydroxy fatty acids were not detected in strain YIM-C158T (Supplementary Table S1, in IJSEM Online). In addition, strain YIM-C158T and *P. ryukyuensis* DSM 15140T could be clearly distinguished from members of the genera *Halobactibacillus* and *Amphibacillus* by the presence of quinones (Table 1) and their noticeably smaller amounts of unbranched saturated fatty acids, a larger amount of anteiso-C15:0 and a relatively low content of C16:0 (Supplementary Table S1), even though both strains exhibited high 16S rRNA gene sequence similarity values to organisms of these two genera.

In conclusion, on the basis of the genotypic data (phylogenetic analysis, DNA–DNA relatedness and genomic DNA G+C content) and the phenotypic distinctiveness and chemotaxonomic data presented above, we propose that strain YIM-C158T represents a novel species of the genus *Paraliobacillus*, with the name *Paraliobacillus quinghaiensis* sp. nov.

**Description of *Paraliobacillus quinghaiensis* sp. nov.**

*Paraliobacillus quinghaiensis* (quing.hai.en’sis. N.L. masc. adj. *quinghaiensis* pertaining to Qinghai, western province of China, where the type strain was isolated).

Cells are Gram-positive, catalase- and oxidase-positive, obligately aerobic, thin rods (3–5 × 0.4–0.6 μm), occurring singly, in pairs or in chains, and motile with peritrichous flagella. Bear ellipsoidal endospores that lie in terminal or subterminal swollen sporangia. After incubation for 4–5 days on MMA5 at 37 °C, colonies are circular, translucent with slightly irregular margins, with a tiny raised point at the centre, and 1–2 mm in diameter. Colonies grown for less than 4 days are creamy white, but become pale yellow after several days. No diffusible pigments are produced. Growth occurs at 4–50 °C (optimum, 37 °C) and pH 6.0–10.0 (optimum, pH 8.0). Moderately halophilic, with growth occurring at 1–20% (w/v) NaCl (optimum, 5% NaCl, w/v). Positive for hydrolysis of starch, but negative for hydrolysis of casein, cellulose, chitin, gelatin, Tween 20, Tween 80 and urea. Nitrate is reduced to nitrite. Methyl red test is positive, but the Voges–Proskauer test is negative. H₂S and indole are not produced. Positive for oxidation of arabinose, but negative for citrate utilization and fermentation/oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose and amygdalin (API 20E). Acid is produced from D-arabinose, maltose and potassium 2-ketogluconate (API 50 CH). Oxidizes L-arabinose, arbutin, cellobiose, glyceral, α-D-lactose, maltose, D-mannitol, methyl α-D-glucoside, D-ribose, sucrose, trehalose, D-xylose, L-alanine, L-asparagine and L-serine (Biolog GP2). Constitutive enzymes expressed are catalase, cytochrome oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, acid phosphatase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and naphthol-AS-BI-phosphohydrolase (API ZYM). Has MK-7 as the sole respiratory quinone and contains *meso*-diaminopimelic acid in the cell-wall peptidoglycan. Polar lipid profile consists of phosphatidylmethylethanolamine, phosphatidylcholine and three unknown phospholipids. Major cellular fatty acids (making up 85.46% of the total) are anteiso-C15:0, iso-C14:0, C16:0, anteiso-C17:0, iso-C16:0, C16:1ω7c alcohol and C16:1ω11c. The DNA G+C content of the type strain is 39.5 mol%.

The type strain, YIM-C158T (=DSM 17857T=CGMCC 1.6333T), was isolated from a sediment sample collected from the Dabuxun salt lake in the Qaidam Basin in Qinghai Province, north-west China.

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Paraliobacillus quinghaiensis sp. nov.


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