Salisediminibacterium halotolerans gen. nov., sp. nov., a halophilic bacterium from soda lake sediment

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An orange-pigmented, Gram-reaction-positive, non-spore-forming, halophilic, alkali-tolerant rod, designated strain halo-2T, was isolated from sediment of Xiarinaoer soda lake, in China’s Inner Mongolia Autonomous Region. Strain halo-2T grew in a complex medium with 3–30 % (w/v) NaCl and at pH 5–10. The cell-wall peptidoglycan contained meso-diaminopimelic acid and the major respiratory isoprenoid quinone was MK-7. The predominant cellular fatty acids were anteiso-C15 : 0 (43.6 %), anteiso-C17 : 0 (14.8 %) and iso-C15 : 0 (6.8 %) and the polar lipids consisted of diphasphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. The genomic DNA G+C content of the novel strain was 48.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain halo-2T was most closely related to Bacillus agaradhaerens DSM 8721T (93.9 % sequence similarity). However, strain halo-2T could be clearly differentiated from its closest phylogenetic relatives on the basis of several phenotypic, genotypic and chemotaxonomic characteristics. Strain halo-2T therefore represents a novel species in a new genus for which the name Salisediminibacterium halotolerans gen. nov., sp. nov. is proposed. The type strain of the type species is halo-2T (5CGMCC 1.7654T5NBRC 104935T).

Soda lakes, which represent the most alkaline naturally occurring environments on earth, contain dense populations of extremophilic micro-organisms from phylogenetically diverse lineages (Duckworth et al., 1996; Jones et al., 1998; Martins et al., 2001; Rees et al., 2004). Several alkaliphilic and/or halophilic bacteria that occur in such lakes are of considerable interest because of their biotechnological potential in the production of compatible solutes and/or hydrolytic enzymes (Horikoshi, 1996; Ventosa et al., 1998; Nogi et al., 2005).

During an investigation of alkaliphilic and halophilic bacteria in China, several bacterial strains were isolated from the sediment of Xiarinaoer soda lake (42°37’ N 115°28’ E), which is located in the Inner Mongolia Autonomous Region. At the time the samples were collected from the lake, the sediment was at −1 °C and pH 9.91 and the salinity of the overlying water was 84 g L−1. Two novel species of halophilic bacteria that were isolated from these samples, Halolactibacillus alkalophilus (Cao et al., 2008) and Salsuginibacillus halophilus (Cao et al., 2010), have already been reported. In this taxonomic study, which involved a polyphasic approach, a third novel species from the sediment samples, again in the family Bacillaceae, has been identified and described.

Strain halo-2T was isolated from the sediment of Xiarinaoer soda lake and routinely maintained, at 37 °C, in a basal complex medium (BCM) containing (l−1) 7.5 g casein hydrolysate, 10 g yeast extract, 3 g sodium citrate, 2 g KCl, 0.005 g FeSO4, 200 g NaCl and 10 g Na2CO3. The pH of this medium was adjusted to 8.0 using 30 % (w/v) Na2CO3 and, if required, the medium was solidified by the addition of agar (18 g l−1) (Pan et al., 2006).

Cell morphology was observed under a light microscope (Olympus) at ×1000. The Gram reaction was investigated using the KOH lysis method (Gregersen, 1978). Colony morphology was observed after growth on a plate of solidified BCM at 37 °C for 48 h. Tolerance to NaCl was assessed in liquid BCM containing no NaCl or 0.1 %, 0.5 % or 1–30 % (in increments of 1 %) (w/v) NaCl. The novel strain was also grown in liquid BCM at pH 5.0–13.0 (in increments of 0.5 pH units). The temperature range for growth was determined by incubation in the same liquid...
medium for 1 day to 3 weeks, at 4, 10, 15, 18, 20, 25, 30, 35, 37, 40, 45 or 50 °C. Growth was evaluated based on measurements of optical density at 600 nm (OD_{600}). Cultures grown at 37 °C and pH 8.0 on solidified BCM supplemented with CaCl₂ (1 mM), MnCl₂ (50 mM) and MgCl₂ (1 mM), nutrient agar (Difco) or marine agar 2216 (Difco) with or without 50 nM MnCl₂ were examined for spores under a light microscope. Catalase activity was detected by mixing a drop of a 24-h-old culture with a drop of 3 % (v/v) H₂O₂ on a microscope slide and seeing if gas bubbles (indicating a positive reaction) appeared. Oxidase activity was detected by daubing 24-h-old cultures on filter paper soaked in N,N-dimethyl-p-phenylenediamine solution and seeing if a red colour developed within 10 s (indicating a positive reaction; Cao et al., 2008). Growth under anaerobic conditions was evaluated on plates of solidified BCM, using the GasPak anaerobic system (BBL) according to the manufacturer’s instructions. Acid production from carbohydrates and utilization of carbon and nitrogen sources were investigated at pH 7.2 using liquid medium containing (1⁻¹) 2.0 g (NH₄)₂SO₄, 0.5 g NaH₂PO₄, 0.5 g K₂HPO₄, 0.2 g MgSO₄, 0.2 g yeast extract and 100 g NaCl. Carbohydrates, organic acids and amino acids were tested at concentrations of 0.5 % (w/v), 0.1 % (w/v) and 0.1 % (w/v), respectively, and growth was measured (as OD_{600}) after incubation for 3 days. Nitrate reduction, H₂S production and susceptibility to a range of antibiotics were investigated as described by Dong & Cai (2001).

Cell mass for the analyses of quinones, fatty acids, the cell-wall peptidoglycan and polar lipids was obtained by culture at 37 °C for 24 h in a modified liquid BCM that contained 9 % (w/v) NaCl and had been adjusted to pH 9.0. For the analysis of isoprenoid quinones, the method described by Collins (1985) was used to extract the lipids from cells that had been grown aerobically and then freeze-dried. Cellular fatty acids were extracted and fatty acid methyl ester mixtures were prepared according to the standard protocol of the MIDI Microbial Identification System (Sasser, 1990). The type of cell-wall peptidoglycan was determined by TLC (Hasegawa et al., 1983). Polar lipids were extracted by the method of Minnikin et al. (1979) and were identified by two-dimensional TLC and spraying with the appropriate detection reagents (Collins & Jones, 1980).

The spectral characteristics of any pigment produced by cells grown on solidified BCM were determined in a UV-1000 spectrophotometer (LabTech) following extraction with methanol (Hildebrand et al., 1994).

For comparison with the novel strain, Bacillus agaradhaerens DSM 8721ᵀ and Bacillus saliphilus DSM 15402ᵀ were grown under the same conditions and subjected to the same phenotypic tests.

Genomic DNA was extracted according to the method of Marmur (1961) for determination of G+C content in a Lambda Bio 20 spectrophotometer (Perkin Elmer), by the thermal denaturation method of De Ley et al. (1970). For this evaluation, the genomic DNA of Escherichia coli K-12 was used as a reference.

The 16S rRNA gene sequence of strain halo-2ᵀ was amplified by PCR, using the primers 27F (5‘-GAGAGT-TTGATCCTGGCTCAG) and 1495R (5‘-CTACGGCTA-CCTTGTAGCA) (Brosius et al., 1978). The amplicons produced were sequenced in a 3730 DNA sequencer (Applied Biosystems). The almost-complete 16S rRNA gene sequence of strain halo-2ᵀ (1443 bp) was then compared with the relevant sequences in GenBank using BLAST (Altschul et al., 1997). Sequences were aligned using version 1.8 of CLUSTAL W (Thompson et al., 1994). Levels of sequence similarity were also determined with CLUSTAL W by the method of Jukes & Cantor (1969). Version 4.1 of the MEGA package (Tamura et al., 2007) was used for the phylogenetic analysis of multiple sequence alignments, with evolutionary distances calculated according to Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were constructed by the neighbour-joining, minimum-evolution and maximum-parsimony methods. The topology of each tree was evaluated by bootstrap analysis with 1000 resamplings (Felsenstein, 1985).

In the phylogenetic analysis based on 16S rRNA gene sequences, strain halo-2ᵀ appeared most closely related to two unidentified isolates from soda lakes in Kenya: WE1 from Lake Elementeita (99 % sequence similarity) and 103NT4 from Lake Natron (98 %). The novel strain showed lower levels of 16S rRNA gene sequence similarity (91.0–93.9 %) with strains of established species in the genera Bacillus, Paraliobacillus, Alkalibacillus, Gracilibacillus, Piscibacillus, Halolactibacillus, Halobacillus, Lentibacillus, Virgibacillus and Thalassobacillus and even lower levels (<91 %) with members of other established species. These rather low levels of 16S rRNA sequence similarity with established species indicated that strain halo-2ᵀ belonged to a novel taxonomic group. The species that appeared most closely related to strain halo-2ᵀ were B. agaradhaerens DSM 8721ᵀ (93.9 % 16S rRNA gene sequence similarity) and B. saliphilus DSM 15402ᵀ (93.1 %). In the neighbour-joining tree based on 16S rRNA gene sequences, the novel strain formed a phyletic cluster with B. agaradhaerens DSM 8721ᵀ, Bacillus neizhouensis DSM 19794ᵀ, B. saliphilus DSM 15402ᵀ, Bacillus chagannorensis DSM 18086ᵀ, Bacillus cellulolyticus JCM 9156ᵀ, Bacillus velluderi DSM 9768ᵀ, Bacillus polygoni JCM 14604ᵀ, Bacillus clarkii DSM 8705ᵀ, Bacillus beveridgei DSM 22320ᵀ and Bacillus selentireducens ATCC 700615ᵀ (Fig. 1). The phylogenetic trees constructed using the minimum-evolution and maximum-parsimony algorithms reflected similar topologies to that seen in the neighbour-joining tree. (Fig. S1, available in IJSEM Online).

Cells of strain halo-2ᵀ were Gram-positive, facultatively anaerobic, non-motile, straight rods that were negative for oxidase and catalase activities. After 2 days’ incubation on solidified BCM at 37 °C, colonies were round, convex, entire and orange-coloured and measured 1–2 mm in diameter. Endospores were not observed (although B. agaradhaerens...
DSM 8721<sup>T</sup>, Paraliobacillus ryukyuensis DSM 15140<sup>T</sup>, Alkalibacillus salisculus DSM 16640<sup>T</sup>, Gracilibacillus borracitoleras DSM 17256<sup>T</sup>, Piscibacillus salipiscarius JCM 13188<sup>T</sup>, Halobacillus alkalophilus DSM 18525<sup>T</sup>, Lentibacillus salis DSM 16817<sup>T</sup> and Virgibacillus salarius JCM 18441<sup>T</sup>, which show 16S rRNA gene sequence similarities of 91.0–93.9 % with strain halo-2<sup>T</sup>, all produce endospores). The novel strain was halophilic and alkali-tolerant. It grew with 3–30 % (w/v) NaCl (optimum 9 %) but not in medium without NaCl. The pH range for growth was 5.0–10.0 (optimum pH 8.0). Other morphological and physiological characteristics of the novel strain are given in the species description and Table 1.

The cellular fatty acids of strain halo-2<sup>T</sup>, which were dominated by anteiso-C<sub>15:0</sub> (43.6 %), anteiso-C<sub>17:0</sub> (14.8 %) and iso-C<sub>15:0</sub> (6.8 %), differed markedly from those of B. agaradhaerens DSM 16540<sup>T</sup>, Piscibacillus salipiscarius JCM 13188<sup>T</sup>, Halobacillus alkalophilus DSM 18525<sup>T</sup>, Lentibacillus salis DSM 16817<sup>T</sup> and Virgibacillus salarius DSM 18441<sup>T</sup>, which show 16S rRNA gene sequence similarities of 91.0–93.9 % with strain halo-2<sup>T</sup>, all produce endospores). The novel strain was halophilic and alkali-tolerant. It grew with 3–30 % (w/v) NaCl (optimum 9 %) but not in medium without NaCl. The pH range for growth was 5.0–10.0 (optimum pH 8.0). Other morphological and physiological characteristics of the novel strain are given in the species description and Table 1.

Based on the phylogenetic, morphological and physiological characteristics described above, strain halo-2<sup>T</sup> represents a novel species of a new genus for which the name Salisediminibacterium halotolerans gen. nov., sp. nov. is proposed.

**Description of Salisediminibacterium gen. nov.**

*Salisediminibacterium* (Sa.li.se.din.i.mi.ni.bac.te.ri.um. L. n. *sal* salt; L. n. *sediment* -inis sediment; L. neut. n. *bacterium* a rod; N.L. neut. n. *Salisediminibacterium* a rod from salt sediment).

Cells are Gram-reaction-positive, non-spore-forming, non-motile rods that are facultative anaerobes. Negative for catalase and oxidase activities and nitrate reduction. Halophilic and alkali-tolerant; growth does not occur with NaCl at <3 % (w/v). The major cellular fatty acids are anteiso-C<sub>15:0</sub>, anteiso-C<sub>17:0</sub> and iso-C<sub>15:0</sub>. The cell-wall peptidoglycan contains the diagnostic diamino acid *meso*-diaminopimelic acid. The predominant menaquinone is MK-7 and
Table 1. Differential characteristics of strain halo-2\textsuperscript{T} and the type strains of related species

Strains: 1, halo-2\textsuperscript{T} (data from this study); 2, *Bacillus agaradhaerens* DSM 8721\textsuperscript{T} (this study); 3, *Bacillus saliphilus* DSM 15402\textsuperscript{T} (this study); 4, *Bacillus subtilis* DSM 10\textsuperscript{T} (this study); 5, *Paraliobacillus ryukyuensis* DSM 15140\textsuperscript{T} (Ishikawa et al., 2002); 6, *Alkalibacillus salicus* DSM 16460\textsuperscript{T} (Jeon et al., 2005); 7, *Gracilibacillus boraciitolerans* DSM 17256\textsuperscript{T} (Ahmed et al., 2007); 8, *Piscibacillus salipiscarius* JCM 13188\textsuperscript{T} (Tanasupawat et al., 2007); 9, *Halolactibacillus alkaliphilus* CGMCC AS 1.6843\textsuperscript{T} (Cao et al., 2008); 10, *Halobacillus alkaliphilus* DSM 18525\textsuperscript{T} (Romano et al., 2008); 11, *Lentibacillus salis* DSM 16817\textsuperscript{T} (Lee et al., 2008); 12, *Virgibacillus salarius* DSM 18441\textsuperscript{T} (Hua et al., 2008).

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<td>10</td>
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<td>7–10</td>
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<tr>
<td>Sampling site</td>
<td>Soda lake sediment</td>
<td>Soil</td>
<td>Algal mat</td>
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<td>Decomposing marine alga</td>
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<td>Soil</td>
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the polar lipid profile comprises diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. The type species is *Salisediminibacterium halotolerans*.

**Description of *Salisediminibacterium halotolerans* sp. nov.**

*Salisediminibacterium halotolerans* (ha.lo.to’le.rans. Gr. n. hals, halos salt; L. part. adj. tolerans tolerating; N.L. part. adj. halotolerans referring to the ability to tolerate high salt concentrations).

Displays the following properties in addition to those given in the genus description. In BCM, cells measure 0.5–0.8\(\times\)2.5–4.5 \(\mu\)m. After 48 h at 37\(\, ^oC\), colonies growing on agar-solidified BCM are 1–2 mm in diameter, round, convex, entire and orange-coloured. Grows at 18–50\(\, ^oC\) (optimum 37\(\, ^oC\)), with 3–30 % (w/v) NaCl (optimum 9 %) and at pH 5.0–10.0 (optimum pH 8.0). Hydrolyses gelatin and starch but not casein, DNA or Tween 80. Produces H2S but not indole. Negative for urease activity. Produces acid from D-arabinose, D-xylose, D-glucose, starch, sucrose, cellobiose, trehalose, melezitose, D-galactose, lactose, raffinose, L-rhamnose, salicin, sodium gluconate, sodium citrate and glycerol. Cellobiose, trehalose, inositol and mannitol are utilized as sole sources of carbon and energy or sole sources of carbon, nitrogen and energy. D-Ribose, D-fructose, sorbose, ethanol, D-mannose, raffinose, L-arabinose, inulin, D-sorbitol, succinic acid, L-lysine, L-tryptophan, L-threonine, L-methionine, L-phenylalanine, aspartic acid and tyrosine are not utilized. Resistant to chloramphenicol (30 \(\mu\)g), ampicillin (10 \(\mu\)g), tetracycline (30 \(\mu\)g) and carbenicillin (100 \(\mu\)g) but sensitive to streptomycin (10 \(\mu\)g) and kanamycin (30 \(\mu\)g). A water-insoluble orange pigment, with a major peak at 459 nm, is produced on solidified BCM.

The type strain, halo-2\(^T\) (=CGMCC 1.7654\(^T\)=NBRC 104935\(^T\)), was isolated from sediment of Xiarinaoer soda lake in Inner Mongolia, China. The genomic DNA G+C content of the type strain is 48.2 mol%.

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**References**


