Halorubrum lipolyticum sp. nov. and Halorubrum aidingense sp. nov., isolated from two salt lakes in Xin-Jiang, China

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Two extremely halophilic archaea, strains 9-3T and 31-hongT, were isolated from Aibi salt lake and Aiding salt lake in Xin-Jiang, China. Their morphology, physiology, biochemical features, polar lipid compositions and 16S rRNA gene sequences were characterized in order to elucidate their taxonomic status. The results from this study indicated that strains 9-3T and 31-hongT are members of the genus Halorubrum. Their physiological properties and polar lipid compositions are clearly different from those of the currently described species of Halorubrum. DNA–DNA relatedness values for strain 9-3T with respect to its closely related neighbours Halorubrum saccharovorum JCM 8865T and Halorubrum lacusprofundi JCM 8891T were 51.6 and 25.1%, respectively, DNA–DNA relatedness values for strain 31-hongT with respect to its closely related neighbours Hrr. saccharovorum JCM 8865T and Hrr. lacusprofundi JCM 8891T were 29.4 and 44.9%, respectively, and DNA–DNA relatedness between strains 9-3T and 31-hongT was 54%. Thus, two novel species of the genus Halorubrum are proposed, Halorubrum lipolyticum sp. nov. (type strain 9-3T = CGMCC 1.5332T = JCM 13559T) and Halorubrum aidingense sp. nov. (type strain 31-hongT = CGMCC 1.2670T = JCM 13560T).

To date, the genus Halorubrum contains 12 species with validly published names: Halorubrum lacusprofundi (Franzmann et al., 1988), Hrr. saccharovorum (Tomlinson & Hochstein, 1976), Hrr. coriense (Kamekura & Dyall-Smith, 1995; McGenity & Grant, 1995), Hrr. distributum (Zvyagintseva & Tarasov, 1987; Oren & Ventosa, 1996), Hrr. sodomense (Oren, 1983; Oren & Ventosa, 1996), Hrr. tebenquichense (Lizama et al., 2002), Hrr. terrestre (Ventosa et al., 2004), Hrr. trapanicum (McGenity & Grant, 1995), Hrr. xinjiangense (Feng et al., 2004), Hrr. alkaliphilum (Feng et al., 2005), Hrr. tibetense (Fan et al., 2004) and Hrr. vacuolatum (Mwatha & Grant, 1993; Kamekura et al., 1997). A phylogenetic tree based on the 16S rRNA gene sequences of these species indicates that the 12 species belonged to three distinct clusters (Fig. 1). Cluster 1 contains neutrophilic species, including Hrr. coriense, Hrr. distributum, Hrr. sodomense, Hrr. tebenquichense, Hrr. terrestre, Hrr. trapanicum and Hrr. xinjiangense. Cluster 2 consists of two other neutrophilic species, Hrr. lacusprofundi and Hrr. saccharovorum. Cluster 3 contains three alkaliophilic species, Hrr. alkaliphilum, Hrr. tibetense and Hrr. vacuolatum. During our surveys on the halophilic archaeal diversity of two salt lakes in the Xin-Jiang region of China (Cui et al., 2006), two strains that clustered tightly with members of cluster 2 were obtained. In this paper, we present the taxonomy of the two strains, strains 9-3T and 31-hongT.

Strains 9-3T and 31-hongT were isolated from sediments of Aibi salt lake and Aiding salt lake, respectively. The isolation procedure was as described previously (Xin et al., 2000; Feng et al., 2004). Characterization of strains 9-3T and 31-hongT was performed according to the proposed minimal standards for the description of novel taxa in the order Halobacteriales (Oren et al., 1997). The morphology and growth characteristics were assessed, miscellaneous biochemical and nutrition tests were performed, sensitivity to antimicrobial agents was determined, lipids were analysed and nucleic acid characterization and DNA–DNA hybridization (thermal

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences of strains 9-3T and 31-hongT are DQ355814 and DQ355813, respectively.

A thin-layer chromatogram showing the glycolipids/phospholipids of strains 9-3T and 31-hongT and members of the genus Halorubrum is available as a supplementary figure in IUSEM Online.
denaturation and renaturation method) were performed as described and cited by Feng et al. (2004, 2005).

The cells of strains 9-3\textsuperscript{T} and 31-hong\textsuperscript{T} are motile, Gram-negative rods. Both strains are able to grow in a wide range of salinities (1.7–4.3 M salt; optimal growth at 2.6 M). The cells lyse in distilled water. Colonies on agar medium are red-pigmented. Details of the morphology, growth characteristics, nutrition, antibiotic sensitivity and biochemistry of strains 9-3\textsuperscript{T} and 31-hong\textsuperscript{T} are given in the species descriptions. Differences between strains 9-3\textsuperscript{T} and 31-hong\textsuperscript{T} and other members of the genus Halorubrum are shown in Table 1.

Phylogenetic analyses based on the 16S rRNA gene sequences of strain 9-3\textsuperscript{T} and strain 31-hong\textsuperscript{T} indicated that they were most closely related to those of Hrr. lacusprofundi ACAM 34\textsuperscript{T} (98.2 and 98.5 %, respectively) and Hrr. saccharovorum JCM 8865\textsuperscript{T} (98.1 and 97.3 %, respectively). The 16S rRNA gene sequence similarity between strains 9-3\textsuperscript{T} and 31-hong\textsuperscript{T} was 97.6 %.

Polar lipid analysis indicated that both strains contained C\textsubscript{20}–C\textsubscript{20} derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulfate. Glycolipids of strain 9-3\textsuperscript{T} migrated with the same mobility as C20–C20 derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl-glucosyl glycerol diether. The DNA G+C content of the type strain is 65 %, respectively. The DNA–DNA relatedness values for strain 9-3\textsuperscript{T} and 31-hong\textsuperscript{T} were 51.6 and 25.1 %, and those for strain 31-hong\textsuperscript{T} with respect to Hrr. saccharovorum JCM 8865\textsuperscript{T} and Hrr. lacusprofundi JCM 8891\textsuperscript{T} were 29.4 and 44.9 %, respectively. The DNA–DNA relatedness between strains 9-3\textsuperscript{T} and 31-hong\textsuperscript{T} was 54 %.

On the basis of these results, it is concluded that strains 9-3\textsuperscript{T} and 31-hong\textsuperscript{T} represent two novel species of the genus Halorubrum, for which the names Halorubrum lipolyticum sp. nov. and Halorubrum aidingense sp. nov. are proposed.

**Description of Halorubrum lipolyticum sp. nov.**

*Halorubrum lipolyticum* (li.po.ly'tic.um. Gr. n. *lipos* fat; N.L. neut. adj. *lyticum* from Gr. adj. *luktos* able to loosen, able to dissolve; N.L. neut. adj. *lipolyticum* fat-dissolving, referring to the property of being able to hydrolyse lipids).

Cells are motile, rod-shaped (0.9–1.2 × 1.8–5.5 μm) and Gram-negative. Colonies on agar plates containing 2.6 M NaCl are red, elevated and rounded. Chemo-organotrophic and aerobic. Growth occurs at NaCl concentrations of 1.7–4.8 M, at pH 7.0–9.0 and temperatures of 25–58 °C. Optimal NaCl concentration, pH and temperature for growth are 2.6 M, pH 7.5 and 45–48 °C, respectively. Catalase- and oxidase-positive. Anaerobic growth with nitrate, arginine or DMSO does not occur. Nitrate reduction to nitrite is observed. H\textsubscript{2}S is not produced from Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}. Catalase-negative. Amylase- and gelatinase-positive. Glucose, fructose, galactose, mannose, sucrose and maltose are utilized and acids are produced. No growth or acid production is observed on sorbose, xylose, lactose, D-ribose, mannitol or sorbitol. Sensitive to rifampicin, bacitracin and novobiocin. Resistant to erythromycin, neomycin, chloramphenicol, ampicillin, penicillin G, norfloxacin, ciprofloxacin, streptomycin, kanamycin, tetracycline and vancomycin. Cells contain C\textsubscript{20}–C\textsubscript{20} derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl-glucosyl glycerol diether. The DNA G+C content of the type strain is 65.9 mol% (T\textsubscript{m}).

The type strain, 9-3\textsuperscript{T} (=CGMCC 1.5332\textsuperscript{T} = JCM 13559\textsuperscript{T}), was isolated from Aibi salt lake in Xin-Jiang, China.
**Table 1.** Differentiation of *Halorubrum lipolyticum* sp. nov. 9-3<sup>T</sup> and *Halorubrum aidingense* sp. nov. 31-hong<sup>T</sup> from other *Halorubrum* species

<table>
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<th>Characteristic</th>
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<td>Motility</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mg&lt;sup&gt;2+&lt;/sup&gt; required</td>
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<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;S formation</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>DNA G+C content (mol%)</td>
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<td>64-2</td>
<td>65-3</td>
<td>71-2</td>
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<td>66</td>
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<td>Presence of sulfated mansnosyl-glucosyl glycerol diether</td>
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</table>

**Description of *Halorubrum aidingense* sp. nov.**

*Halorubrum aidingense* (ai.ding.en’se. N.L. neut. adj. aidingense from Aiding salt lake in China, where the type strain was isolated).

Cells are motile, rod-shaped (0.9–1.0 × 3.6–6.4 μm) and Gram-negative. Colonies on agar plates containing 2.6 M NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at NaCl concentrations of 1.7–4.3 M, at pH 7.0–9.0 and at temperatures of 25–52 °C. Optimal NaCl concentration, pH and temperature for growth are 2.6 M, pH 7.5 and 40–42 °C, respectively. Catalase- and oxidase-positive. Anaerobic growth with nitrate, arginine or DMSO does not occur. Nitrate reduction to nitrite is observed. H<sub>2</sub>S is not produced from Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Positive for indole formation. Tweens 20, 40, 60 and 80 are hydrolysed. Casein and starch are not hydrolysed. Gelatin is not liquefied. Glucose, fructose, galactose, mannose, sucrose, maltose and manniitol are utilized and acids are produced. No growth or acid production is observed on sorbose, xylose, lactose, D-ribose or sorbitol. Sensitive to rifampicin, bacitracin and novobiocin. Resistant to erythromycin, neomycin, chloramphenicol, ampicillin, penicillin G, norfloxacin, ciprofloxacin, streptomycin, kanamycin, tetracycline and vancomycin. Cells contain C<sub>20</sub>–C<sub>29</sub> derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate and phosphatidylglycerol phosphate sulfate. Sulfated mansnosyl-glucosyl glycerol diether was not detected. The DNA G+C content of the type strain is 64.2 mol% (T<sub>m</sub>).

The type strain, 31-hong<sup>T</sup> (=CGMCC 1.2670<sup>T</sup> = JCM 13560<sup>T</sup>), was isolated from Aiding salt lake in Xin-Jiang, China.

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**References**


