**Natronorubrum sulfidifaciens** sp. nov., an extremely haloalkaliphilic archaeon isolated from Aiding salt lake in Xin-Jiang, China

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An extremely haloalkaliphilic archaeon, strain AD2T, was isolated from Aiding salt lake in Xin-Jiang, China. Strain AD2T required at least 12 % NaCl for growth. MgCl₂ was not required. The isolate was able to grow over a pH range of 8.0–10.0 and temperature range of 20–55 °C, with optimal growth at pH 8.7–9.2 and 44–47 °C. The major polar lipids of strain AD2T were phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester; glycolipids were not detected. Analysis of its 16S rRNA gene sequence indicated that strain AD2T was phylogenetically related to members of the genus *Natronorubrum*, with sequence similarities to the type strains of *Natronorubrum bangense*, *Natronorubrum tibetense* and *Natronorubrum aibiense* of 97.1, 95.9 and 96.1 %, respectively. The G+C content of its DNA was 60.9 mol% (Tm). Levels of DNA–DNA relatedness between strain AD2T and the type strains of *Nrr. bangense*, *Nrr. tibetense* and *Nrr. aibiense* were 49, 38 and 41 %, respectively. It was concluded that strain AD2T represents a novel species of the genus *Natronorubrum*, for which the name *Natronorubrum sulfidifaciens* sp. nov. is proposed. The type strain is AD2T (= CGMCC 1.6307T = JCM 14089T).

The genus *Natronorubrum* was established by Xu et al. (1999) to accommodate two novel haloalkaliphilic archaeal species, *Natronorubrum bangense* and *Natronorubrum tibetense*, which were isolated from the Bange soda lake in Tibet, China. Recently, *Natronorubrum aibiense* was added to the genus (Cui et al., 2006a). Members of the genus *Natronorubrum* have been frequently isolated from several salt lakes in China (Fan et al., 2003; Pan et al., 2006). The Aiding salt lake (42° 32′ 10″–42° 49′ 13″ N 89° 10′ 32″–89° 54′ 32″ E), the lowest point in China (155 m below sea-level) and after the Dead Sea the second lowest inland depression in the world, has been a target for the study of halophilic archaeal diversity under extremely high salt conditions for many years (Tohty & Xu, 2001; Cui et al., 2006b). Here we describe a haloalkaliphilic strain isolated from Aiding salt lake, which we propose to classify as representing a novel species of the genus *Natronorubrum*.

Strain AD2T was isolated from sediment of the Aiding salt lake. The medium and method used for isolation were as described by Xu et al. (1999, 2001). The strain was routinely grown aerobically at 45 °C in a complex medium containing the following ingredients (per litre distilled water): 7.5 g Casamino acids (Difco), 10 g yeast extract (Difco), 3.0 g trisodium citrate, 0.1 g MgSO₄·7H₂O, 2.0 g KCl, 0.036 g FeCl₂·7H₂O, 180 g NaCl and 10 g Na₂CO₃.

Phenotypic tests were performed according to the proposed minimal standards for the description of novel taxa in the order *Halobacteriales* (Oren et al., 1997). Colony morphology was observed on salt-milk agar medium (Kocur & Hodgkiss, 1973), the final pH of which was adjusted to
Cells of strain AD2<sup>T</sup> were motile, pleomorphic (rods, triangular or disc-shaped), Gram-negative and were able to grow over a wide range of salinities (12–28 % NaCl; optimal growth at 18 %). Colonies on salt-milk agar medium were red-pigmented. Detailed results of phenotypic tests and nutritional features of strain AD2<sup>T</sup> are given in the species description below and some differential properties in comparison with recognized members of the genus *Natronorubrum* are listed in Table 1.

Polar lipid analysis indicated that strain AD2<sup>T</sup> contained phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester (Kates, 1986; see Supplementary Fig. S1 available in IJSEM Online), which are the major phospholipids found in members of the genus *Natronorubrum*. No glycolipids were detected (Supplementary Fig. S1).

The DNA G + C content of strain AD2<sup>T</sup> was 60.9 mol%. Phylogenetic analysis based on the 16S rRNA gene according to the neighbour-joining method (Kumar et al., 2004) indicated that strain AD2<sup>T</sup> was closely related to *Nrr. bangense*, *Nrr. tibetense* and *Nrr. aibiense* (Fig. 1), with 16S rRNA gene sequence similarities to the type strains of these species of 97.1, 95.9 and 96.1 %, respectively. Levels of DNA–DNA relatedness between strain AD2<sup>T</sup> and the type strains of *Nrr. bangense*, *Nrr. tibetense* and *Nrr. aibiense* were 49, 38 and 41 %, respectively.

Based on these results, it is concluded that strain AD2<sup>T</sup> represents a novel species of the genus *Natronorubrum*, for which the name *Natronorubrum sulfidifaciens* sp. nov is proposed.

**Table 1. Differential characteristics between strain AD2<sup>T</sup> and other members of the genus *Natronorubrum***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Optimum pH</td>
<td>8.7–9.2</td>
<td>9.5</td>
<td>9.0</td>
<td>7.5–8.0</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;S formation</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Hydrolys of Tweens 40, 60 and 80</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Gelatin liquefaction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Sensitivity to antibiotics:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tetracycline</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Utilization of:</td>
<td></td>
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<tr>
<td>Galactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Succinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>60.9</td>
<td>59.9</td>
<td>60.1</td>
<td>61.2</td>
</tr>
<tr>
<td>Presence of glycolipids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure 1.** Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain AD2<sup>T</sup>, recognized members of the genus *Natronorubrum* and related genera within the family *Halobacteriaceae*. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 70 % support. Bar, 0.02 expected changes per site.

**Description of *Natronorubrum sulfidifaciens* sp. nov.**


Cells are motile, pleomorphic (rods, triangular or disc-shaped) and Gram-negative. Colonies on agar plates containing 3.1 M NaCl are red, elevated and round. Chemorganotrophic and aerobic. Growth occurs at NaCl concentrations of 2.1–4.8 M, at an Mg<sup>2+</sup> concentration of 0.1 M, at pH 8.0–10.0 and at 20–55 °C. The optimal NaCl concentration, pH and temperature for growth are 3.1 M, pH 8.7–9.2 and 44–47 °C. Catalase- and oxidase-positive. Anaerobic growth with nitrate, arginine and DMSO does not occur. Nitrate reduction to nitrite is observed. H<sub>2</sub>S is 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 not hydrolysed. Negative for caseinase, amylase and gelatinase. The following substrates are utilized as carbon sources: glucose, sucrose, maltose, glycerol, lactate, malate, succinate, acetate, pyruvate, fumarate and glutamate. Mannose, galactose, fructose, sorbose, xylose, D-ribose, lactose, starch, mannitol, sorbitol,
citrate, glycine, L-alanine, L-arginine, L-aspartic acid, L-lysine and L-ornithine are not utilized as carbon sources. Sensitive to the following antibiotics (µg per disc): erythromycin (15), rifampicin (5), novobiocin (30), tetracycline (30) and ciprofloxacin (5). Resistant to the following antibiotics (µg per disc, unless otherwise indicated): ampicillin (10), chloramphenicol (30), kanamycin (30), neomycin (30), vancomycin (30), norfloxacin (10), streptomycin (10), bacitracin (0.04 IU per disc) and penicillin G (10 IU per disc). The major polar lipids are phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester. The DNA G+C content is 60.9% (Tm).

The type strain, AD2T (= CGMCC 1.6307T = JCM 14089T), was isolated from Aiding salt lake in Xin-Jiang, China.

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References


