**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 *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, 98°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 FeCl3·7H2O, 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.

**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. Chemoorganotrophic 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. Tween 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


