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 AD2^T, was isolated from Aiding salt lake in Xin-Jiang, China. Strain AD2^T 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 AD2^T were phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester; glycolipids were not detected. Analysis of its 16S rRNA gene sequence indicated that strain AD2^T 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% (*T_m*). Levels of DNA–DNA relatedness between strain AD2^T and the type strains of *Nrr. bangense*, *Nrr. tibetense* and *Nrr. aibiense* were 49, 38 and 41 %, respectively. It was concluded that strain AD2^T represents a novel species of the genus *Natronorubrum*, for which the name *Natronorubrum sulfidifaciens* sp. nov. is proposed. The type strain is AD2^T (=CGMCC 1.6307^T=JCM 14089^T).

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 AD2^T 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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AD2^T is DQ535889.

A two-dimensional thin-layer chromatogram of the phospholipids from strain AD2^T is available as supplementary material in IJSEM Online.

9.0–9.5 with 1 M NaOH, after incubation at 45 °C for 7–10 days. The production of H₂S was tested by growing the isolate in a tube with the above-described liquid complex medium supplemented with 0.5 % Na₂S₂O₃; a filter paper strip impregnated with lead acetate was used for detection of H₂S. Cell morphology and growth characteristic tests, miscellaneous biochemical tests, nutrition tests, sensitivity to antimicrobial agents and polar lipid analysis were performed as described and cited by Xu *et al.* (1999, 2001). Nucleic acid characterization was performed as described and cited by Feng *et al.* (2005) and the DNA G + C content was determined by the thermal denaturation method (Marmur & Doty, 1962).

Cells of strain AD2^T 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^T 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^T 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^T was 60.9 mol%. Phylogenetic analysis based on the 16S rRNA gene according to the neighbour-joining method (Kumar *et al.*, 2004)

Table 1. Differential characteristics between strain AD2^T and other members of the genus *Natronorubrum*

Taxa: 1, strain AD2^T; 2, *Nrr. bangense* A33^T; 3, *Nrr. tibetense* GA33^T; 4, *Nrr. aibiense* 7-3^T.

Characteristic	1	2	3	4
Optimum pH	8.7–9.2	9.5	9.0	7.5–8.0
H ₂ S formation	+	–	–	–
Hydrolysis of Tweens 40, 60 and 80	–	–	+	–
Gelatin liquefaction	–	–	+	–
Sensitivity to antibiotics:				
Erythromycin	+	+	+	–
Ciprofloxacin	+	–	–	–
Tetracycline	+	+	+	–
Utilization of:				
Galactose	–	–	–	+
Succinate	+	+	+	–
DNA G + C content (mol%)	60.9	59.9	60.1	61.2
Presence of glycolipids	–	–	–	+

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain AD2^T, 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.

indicated that strain AD2^T 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^T 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^T represents a novel species of the genus *Natronorubrum*, for which the name *Natronorubrum sulfidifaciens* sp. nov. is proposed.

Description of *Natronorubrum sulfidifaciens* sp. nov.

Natronorubrum sulfidifaciens (sul.fi.di.fa'ci.ens. N.L. n. sulfidum sulfide; L. part. faciens making, producing; N.L. part. adj. sulfidifaciens sulfide-producing).

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 Mg²⁺ 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. H₂S is produced from Na₂S₂O₃. 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 % (T_m).

The type strain, AD2^T (= CGMCC 1.6307^T = JCM 14089^T), was isolated from Aiding salt lake in Xin-Jiang, China.

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