Natronorubrum aibiense sp. nov., an extremely halophilic archaeon isolated from Aibi salt lake in Xin-Jiang, China, and emended description of the genus *Natronorubrum*

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An extremely halophilic archaeon (strain 7-3^T) was isolated from Aibi salt lake in Xin-Jiang, China. It formed small (<1.0 mm), red, elevated colonies on salt-milk agar medium. Strain 7-3^T was able to grow at pH 6.5–9.5 (optimum pH 8.0) and at 20–50 °C (optimum 45 °C). Analysis of its 16S rRNA gene sequence indicated that strain 7-3^T was phylogenetically related to members of the genus *Natronorubrum*, with sequence similarities of 97.0% to *Natronorubrum bangense* A33^T and 95.2% to *Natronorubrum tibetense* GA33^T. The G + C content of its DNA was 61.2 mol% (*T_m*). Levels of DNA–DNA relatedness to *Nrr. bangense* and *Nrr. tibetense* were 45 and 37%, respectively. It is concluded that strain 7-3^T (=CGMCC 1.4299^T=JCM 13488^T) should be classified as the type strain of a novel species of the genus *Natronorubrum*, for which the name *Natronorubrum aibiense* sp. nov. is proposed. Based on the properties of *Nrr. aibiense* given here and of those of *Nrr. bangense* and *Nrr. tibetense* described previously, an emended description of the genus *Natronorubrum* is presented.

The genus *Natronorubrum* was established by Xu *et al.* (1999) to accommodate two novel species, *Natronorubrum bangense* and *Natronorubrum tibetense*. These two species were isolated from the Bange soda lake in Tibet, China, and are able to grow organotrophically under highly saline and alkaline conditions and at moderately high temperatures. Recently, it has been suggested that the genus *Natronorubrum* might also contain neutrophilic members that potentially have roles in heterotrophic oxidation of thiosulfate in hypersaline habitats (Sorokin *et al.*, 2005). The highly saline lake Aibi (82° 35′–83° 16′ E 44° 05′–45° 08′ N) in Xin-Jiang, China, has been a long-term target for the study of element cycling and microbial flora under extremely high-salinity conditions (Cui *et al.*, 2006), and many representative halophilic species have been described, such as *Natrinema versiforme* (Xin *et al.*, 2000) and

Haloterrigena saccharevitans (Xu *et al.*, 2005). Here we report the taxonomic characterization and identification of a halophilic archaeal organism.

Strains 7-3^T, 7-1, 7-2, D7 and ABH7 were isolated from sediment of the Aibi salt lake. The medium and method used for isolation were as described by Xin *et al.* (2000) and Feng *et al.* (2004). The strains were routinely grown aerobically at 37 °C in a complex medium containing the following (per litre distilled water): 7.5 g Casamino acids (Difco), 10 g yeast extract (Difco), 3.0 g trisodium citrate, 10.2 g MgCl₂·6H₂O, 20 g KCl, 0.05 g FeSO₄·7H₂O, 180 g NaCl (pH adjusted to 8.0 with 1 M NaOH solution).

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) after incubation at 45 °C for 6 days. Cell morphology was described and growth characteristic tests, miscellaneous biochemical tests, nutrition tests, sensitivity to antimicrobial agents, lipid analysis and nucleic acid characterization were performed as reported and cited by Feng *et al.* (2004, 2005).

Abbreviations: PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; TGD, triglycosyl diether.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 7-3^T is DQ205516.

A one-dimensional thin-layer chromatogram of total polar lipids from strain 7-3^T and related taxa is available as a supplementary figure in IJSEM Online.

Table 1. Characteristics that differentiate *Natronorubrum aibiense* sp. nov. from the two other members of the genus

Strains: 1, *Nrr. aibiense* 7-3^T (strains 7-1, 7-2, D7 and ABH7 gave identical results); 2, *Nrr. bangense* A33^T; 3, *Nrr. tibetense* GA33^T. +, Positive; –, negative; ND, not determined. Data for reference strains were taken from Xu *et al.* (1999).

Property	1	2	3
Cell shape	Rod	Pleomorphic	Pleomorphic
Motility	+	–	–
NaCl concentration for growth (%)			
Optimum	18	22.5	20
Range	12–25	12–25	12–30
Mg ²⁺ concentration for growth (M)			
Optimum	0.05	ND	ND
Range	0–0.5	ND	ND
pH for growth			
Optimum	8.0	9.5	9.0
Range	6.5–9.5	8.0–11.0	8.5–11.0
Nitrate reduction	+	–	–
Hydrolysis of Tweens 40 and 60	–	–	+
Gelatin liquefaction	–	–	+
Sensitivity to:			
Erythromycin	–	+	+
Tetracycline	–	+	+
DNA G+C content (mol%)	61.2	59.9	60.1

Cells of strains 7-3^T, 7-1, 7-2, D7 and ABH7 were rod-shaped, motile, Gram-negative and able to grow over a wide range of salinities (12–25 % salt; optimal growth at 18 %). Cells lysed in distilled water. Colonies on salt-milk agar medium were red-pigmented. Results of phenotypic tests and nutritional features of these strains are given in the species description below and in Table 1. As listed in Table 1, the strains had very similar phenotypic properties. Minor differences among the strains were that strains 7-1, 7-2, D7 and ABH7 produced smaller colonies on complex medium than strain 7-3^T, and that strain 7-3^T grew faster than the others.

The nearly entire 16S rRNA genes of strains 7-3^T, 7-1, 7-2, D7 and ABH7 were PCR-amplified and the sequences

were used for construction of a phylogenetic tree (Fig. 1). Sequence similarity calculations using Kimura's two-parameter and pairwise-deletion model gave values of 99.7–99.9 %, indicating that strains 7-3^T, 7-1, 7-2, D7 and ABH7 represented a monophyletic group. The most closely related species were *Nrr. bangense* and *Nrr. tibetense*, with 16S rRNA gene sequence similarities of 95.2 and 97.0 %, respectively. Levels of DNA–DNA relatedness between strain 7-3^T and *Nrr. bangense* A33^T and *Nrr. tibetense* GA33^T were 45 and 37 %, respectively. The DNA G+C content of strain 7-3^T was 61.2 mol%.

The phospholipids of strain 7-3^T, *Nrr. bangense* A33^T and *Nrr. tibetense* GA33^T were determined to be C₂₀–C₂₀ and C₂₀–C₂₅ derivatives of phosphatidylglycerol (PG) and

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between members of the genus *Natronorubrum* and related genera within the family *Halobacteriaceae*. Bootstrap values are based on 1000 replicates; only values >80 % are shown. Bar, 0.02 expected changes per site.

phosphatidylglycerol phosphate (PGP) methyl ester. In addition, three glycolipids, namely triglycosyl diether-1 (TGD-1) and unidentified glycolipids 1 and 2, were detected for strain 7-3^T, by comparison with the glycolipid profile of *Halobacterium salinarum* ATCC 33170 (Torreblanca *et al.*, 1986), but these were not detected for *Nrr. bangense* A33^T or *Nrr. tibetense* GA33^T (see Supplementary Fig. S1 in IJSEM Online).

Based on these results it is concluded that strain 7-3^T represents a novel species of the genus *Natronorubrum*, for which the name *Natronorubrum aibiense* sp. nov. is proposed.

Emended description of the genus *Natronorubrum* Xu *et al.* 1999

Cells are Gram-negative, rod-shaped or pleomorphic flat-shaped (triangular, square, disc and other polygonal shapes). Colonies are red-pigmented. Cells are non-motile or motile, strictly aerobic, oxidase- and catalase-positive. Require at least 12 % NaCl for growth; cells are lysed in distilled water. Alkaliphilic or neutrophilic. Mesophilic and chemo-organotrophic. Many sugars (glucose, fructose, sucrose, maltose and lactose) are utilized, sometimes with acid production. Polar lipids are C₂₀–C₂₀ and C₂₀–C₂₅ derivatives of PG and PGP. Cells may also contain TGD-1 and other unidentified glycolipids. The type species is *Natronorubrum bangense*.

Description of *Natronorubrum aibiense* sp. nov.

Natronorubrum aibiense (ai.bi.en'se. N.L. neut. adj. *aibiense* from Aibi salt lake, China).

Cells are rod-shaped (0.8–1.0 × 1.4–3.6 µm) and Gram-negative. Colonies on salt-milk agar plates containing 18 % (w/v) NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at NaCl concentrations of 2.0–4.3 M, at pH 6.5–9.5 and at 20–50 °C. Optimal NaCl concentration, pH and temperature for growth are 2.6–3.1 M, pH 8.0 and 45 °C, respectively. Catalase- and oxidase-positive. Anaerobic growth with nitrate, arginine or DMSO does not occur. Nitrate reduction to nitrite is observed. H₂S is not produced from Na₂S₂O₃. Indole formation is positive. Tween 80, casein and starch are not hydrolysed. Gelatin is not liquefied. Casamino acids are required for growth. Glucose, galactose, sucrose and maltose are utilized and acids are produced. No growth or acid production is observed on fructose, sorbose, xylose, mannose, lactose, D-ribose, mannitol or sorbitol. Sensitive to rifampicin and novobiocin. Resistant to erythromycin, neomycin,

chloramphenicol, bacitracin, ampicillin, penicillin G, norfloxacin, ciprofloxacin, streptomycin, kanamycin, tetracycline and vancomycin. Cells contain C₂₀–C₂₀ and C₂₀–C₂₅ derivatives of PG and PGP, TGD-1 and two unidentified glycolipids. The DNA G+C content of the type strain is 61.2 mol% (T_m).

The type strain, 7-3^T (=CGMCC 1.4299^T=JCM 13488^T), was isolated from Aibi salt lake in Xin-Jiang, China.

Acknowledgements

This work was supported by grants from the Ministry of Science and Technology (2004CB719601) and Chinese Academy of Sciences (KJCX1-SW-07).

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