Natrinema altunense sp. nov., an extremely halophilic archaeon isolated from a salt lake in Altun Mountain in Xinjiang, China

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A novel extremely halophilic strain, AJ2^T, was isolated from Ayakekum salt lake located in the Altun Mountain National Nature Reserve in Xinjiang, China. This isolate was neutrophilic, motile and grew in a wide range of $MgCl_2$ concentrations (0·005–1·0 M). The major polar lipids of the isolate were $C_{20}C_{20}$ and $C_{20}C_{25}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulfate. A comprehensive 16S rRNA gene sequence analysis revealed that the isolate shared 96·6–97·7 % sequence identity with *Natrinema* species. The isolate, however, could be genetically differentiated from these species by DNA–DNA hybridization analysis and on the basis of its physiological properties. On the basis of the polyphasic evidence, strain AJ2^T (=AS 1.3731^T=JCM 12890^T) represents the type strain of a novel species, for which the name *Natrinema altunense* sp. nov. is proposed.

On the basis of 16S rRNA gene sequences, salt tolerance and chemotaxonomic and physiological characteristics, the genus *Natrinema* was created in 1998 to accommodate *Natrinema pellirubrum* (formerly *Halobacterium salinarum* NCIMB 786) and *Natrinema pallidum* (formerly *Halobacterium halobium* NCIMB 777) (McGenity *et al.*, 1998). In a phylogenetic tree based on 16S rRNA gene sequences, *Natrinema* species formed an independent cluster with respect to *Halobacterium* species. *Natrinema* species could be cultured at low salt concentrations, and possessed a specific protein profile and polar lipid composition. Sub sequently, a novel species of this genus, *Natrinema versiforme*, was described (Xin *et al.*, 2000). Thus, to date there are three species in the genus *Natrinema*. In this study, we describe a novel extremely halophilic archaeon isolated from Ayakekum salt lake (37° 37′ N, 89° 29′ E; 3884 m altitude) located in the Altun Mountain National Nature Reserve in Xinjiang, China, and propose a novel species, *Natrinema altunense* sp. nov.

The low temperature, low nutrient levels, abundant sunlight and remote geographical location of Ayakekum salt lake make it a relatively isolated ecosystem. A water sample (approx. 400 ml) was collected from the edge of Ayakekum salt lake in summer. The pH of the water (determined using a pH meter) was slightly alkali, at approximately pH 7·8. The isolate was routinely grown aerobically at 37 °C in rich medium (Oesterhelt & Stoeckenius, 1974). Pure cultures were obtained by restreaking several times. The organism was grown and maintained on S-G medium (Sehgal & Gibbons, 1960).

The phenotypic tests were performed according to the proposed minimal standards for the description of new taxon of the order *Halobacteriales* (Oren *et al.*, 1997). The optimal conditions for growth were determined in S-G medium modified with 0·85–5·1 M NaCl or 0–1·0 M Mg^{2+}. To determine the pH required for growth (using increments of 0·5 pH units, from pH 5·0 to pH 9·5), 50 mM MES (pH 5·0–6·0), 50 mM PIPES (pH 6·5–7·0), 50 mM Tricine (pH 7·5–8·5) and 50 mM CHES (pH 9·0–9·5) were employed as buffers. Cell morphology and motility were examined by using light microscopy (BX40; Olympus) and transmission electron microscopy (S-570; Hitachi). Gram staining was performed using samples fixed with acetic acid,

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AJ2^T is AY208972.

Thin-layer chromatograms showing the lipid content of strain AJ2^T and other *Natrinema* species, along with a table of some characteristics distinguishing this strain from *Natrinema versiforme* AS 1.2365^T, are available as supplementary material in IJSEM Online.

as described by Dussault (1955). Anaerobic growth was tested in the presence of nitrate, L-arginine or DMSO (each at 5 g l^{-1}) in filled, stoppered tubes. Gelatin hydrolysis was determined as described by Oren *et al.* (2002). Hydrolysis of starch, casein and Tweens 20, 40 and 80, reduction of nitrate and nitrite, production of indole and H_2S, activities of catalase and oxidase, and utilization of sugars, alcohols, amino acids and organic acids were tested according to Xin *et al.* (2000), as described by Oren *et al.* (1997).

Total lipids were extracted by using the modified method of Kamekura & Kates (1988). Phospholipids and glycolipids were separated on silica-gel plates (10 × 10 cm) by TLC and were analysed according to Xin *et al.* (2000). Genomic DNA was prepared by the method of Marmur (1961) and the purity was checked spectrometrically. The G+C content of the DNA was determined by thermal denaturation (T_m) (Marmur & Doty, 1962). The 16S rRNA gene sequence was amplified under conditions like those described by Gupta *et al.* (1983). The sequence was analysed along with sequences of closely related reference organisms from the FASTA network service. Sequence data were aligned with CLUSTAL W software, version 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by using neighbour-joining methods (Saitou & Nei, 1987) with the MEGA 3 program package (Kumar *et al.*, 2004). DNA–DNA hybridization was performed by using the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983).

The profile of the major polar lipids of strain AJ2^T, comprising $C_{20}C_{20}$ and $C_{20}C_{25}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulfate, was similar to that of *Natrinema* species (see Supplementary Fig. A in IJSEM Online). In a phylogenetic tree based on 16S rRNA gene sequences (Fig. 1), strain AJ2^T clustered with *Natrinema* species with validly published names. The results indicated that strain AJ2^T belongs to the genus *Natrinema*.

Strain AJ2^T, however, could be distinguished from *Natrinema* species with validly published names on the basis of some phenotypic characteristics (Table 1) (additional distinguishing characteristics are available in a Supplementary Table in IJSEM Online). Two-dimensional TLC revealed that strain AJ2^T possessed a major glycolipid, which ran very slowly. The glycolipid spot was also found in *N. pellirubrum* JCM 10476^T. The amount of this glycolipid in AJ2^T was observably less than that in *N. pellirubrum* JCM 10476^T, and the amount of phosphatidylglycerol sulfate in AJ2^T was more than that in *N. pellirubrum* JCM 10476^T (McGenity *et al.*, 1998). Moreover, strain AJ2^T did not contain glycolipids found in *N. pallidum* JCM 8980^T and *N. versiforme* AS 1.2365^T. Therefore, the polar lipid profiles among *Natrinema* species also served to distinguish them (see Supplementary Fig. B in IJSEM Online). The 16S rRNA gene sequence similarities between strain AJ2^T and *N. pallidum* JCM 8980^T, *N. pellirubrum* JCM 10476^T and *N. versiforme* AS 1.2365^T were 97·06, 96·64 and 97·71 %, respectively. Furthermore, the DNA–DNA relatedness values for strain AJ2^T with respect to *N. pallidum* JCM 8980^T, *N. pellirubrum* JCM 10476^T and *N. versiforme* AS 1.2365^T were 49·3 % (SD = 4·3 %), 35·5 % (SD = 2·6 %) and 51·4 % (SD = 1·3 %), respectively. The values were based on three independent determinations.

Overall, our data indicate that strain AJ2^T represents a novel species of the genus *Natrinema*, for which we propose the name *Natrinema altunense* sp. nov.

Description of *Natrinema altunense* sp. nov.

Natrinema altunense (al.tu.nen′se. N.L. neut. adj. *altunense* of Altun, referring to isolation of the organism from Altun Mountain, China).

Cells are rods that measure 0·8–1·2 × 3–7 μm and become pleomorphic under unfavourable conditions. Cells are motile and Gram-negative. Colonies are orange or red,

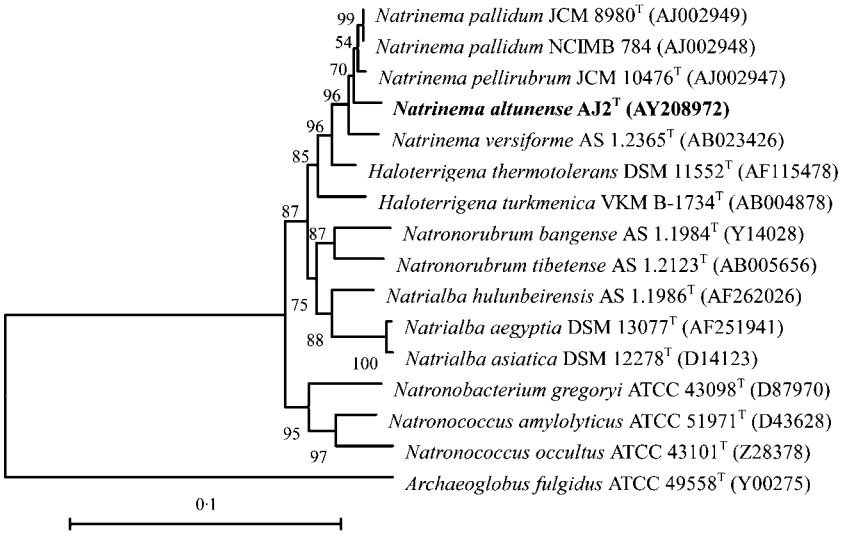

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences from strain AJ2^T and other related organisms. The tree was constructed using the neighbour-joining method, with bootstrap values calculated from 1000 resamplings. The number at each branch point represents the percentage bootstrap support. Bar, 10 substitutions at any nucleotide position per 100 nucleotide positions.

Table 1. Some characteristics that distinguish *N. altunense* sp. nov. from other *Natrinema* species

Taxa: 1, strain AJ2^T; 2, *N. versiforme* AS 1.2365^T; 3, *N. pellirubrum* JCM 10476^T; 4, *N. pallidum* JCM 8980^T. Symbols: +, positive; −, negative; W, weak; NR, not reported.

Characteristic	1	2*	3†	4†
Colony colour	Orange or red	Light red	Light red or orange	Pale orange, beige or almost colourless
NaCl range (M)	>1·7	>1·5	>2·0	>1·7
Mg^{2+} range (M)	0·005–1·000	0·005–0·500	NR	NR
pH range	6·0–8·0	6·0–8·0	6·0–8·6	6·0–8·4
pH optimum	7·0–7·7	6·5–7·0	7·2–7·8	7·2–7·6
Motility	+	−	NR	NR
Anaerobic growth with nitrate	W	+	−	−
Gas formation from:				
Nitrate	+	+	−	−
Nitrite	+	NR	−	NR
H_2S formation	+ (From thiosulfate)	+ (From sulfur)	− (From cysteine)	− (From cysteine)
Indole formation	−	+	−	−
Hydrolysis of:				
Gelatin	+	−	+	+
Tween 80	+	W	NR	NR
Utilization of:				
Fructose	−	+	+	+
Mannose	W	+	NR	NR
Lactose	−	−	+	+
Galactose	−	+	NR	NR
D-Ribose	−	+	+	−
Sucrose	−	+	NR	NR
D-Xylose	−	+	NR	NR
Glycolipid content	One major glycolipid‡	One major glycolipid	One sulfated glycolipid‡	Several unidentified glycolipids

*Data from Xin *et al.* (2000).

†Data from McGenity *et al.* (1998).

‡The glycolipid ran very slowly on TLC plates.

smooth, circular and elevated. Growth requires at least 1·7 M NaCl, optimally 3·0–4·3 M NaCl. Growth occurs at 0·005–1 M $MgCl_2$, optimally at around 0·05–0·2 M $MgCl_2$. The pH range for growth is 6·0–8·0, with an optimum at pH 7·0–7·7. Chemo-organotrophic. Grows anaerobically in the presence of nitrate. Oxidase- and catalase-positive. Nitrate and nitrite are reduced, and gas is produced. Indole formation is negative. Starch and casein are not hydrolysed. Gelatin and Tweens 20, 40 and 80 are hydrolysed. H_2S is produced from thiosulfate. The following substrates are utilized for growth: glucose, glycerol, maltose, glutamate, alanine, arginine, lysine, ornithine, acetate, fumarate, malate, propionate, pyruvate and succinate. Acid is produced from glucose, glycerol, maltose and mannose. Sensitive to norfloxacin, but not to erythromycin, neomycin, ciprofloxacin, streptomycin, kanamycin, ampicillin or vancomycin. The major polar lipids are $C_{20}C_{20}$ and $C_{20}C_{25}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and some unidentified glycolipids. The G+C content of the DNA is 65·6 mol% (T_m).

The type strain, AJ2^T (=AS 1.3731^T=JCM 12890^T), was isolated from a salt lake in Altun Mountain in China.

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References

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.

Gupta, R., Lanter, J. M. & Woese, C. R. (1983). Sequence of the 16S ribosomal RNA from *Halobacterium volcanii*, an archaebacterium. *Science* **221**, 656–659.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Kamekura, M. & Kates, M. (1988). Lipids of halophilic archaebacteria. In *Halophilic Bacteria II*, pp. 25–54. Edited by F. Rodriguez-Valera. Boca Raton, FL: CRC Press.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

McGenity, T. J., Gemmell, R. T. & Grant, W. D. (1998). Proposal of a new halobacterial genus *Natrinema* gen. nov., with two species *Natrinema pellirubrum* nom. nov. and *Natrinema pallidum* nom. nov. *Int J Syst Bacteriol* **48**, 1187–1196.

Oesterhelt, D. & Stoeckenius, W. (1974). Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. *Methods Enzymol* **31**, 667–678.

Oren, A., Ventosa, A. & Grant, W. D. (1997). Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int J Syst Bacteriol* **47**, 233–238.

Oren, A., Elevi, R., Watanabe, S., Ihara, K. & Corcelli, A. (2002). *Halomicrobium mukohataei* gen. nov., comb. nov., and emended description of *Halomicrobium mukohataei*. *Int J Syst Evol Microbiol* **52**, 1831–1835.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sehgal, S. N. & Gibbons, N. E. (1960). Effect of metal ions on the growth of *Halobacterium cutirubrum*. *Can J Microbiol* **6**, 165–169.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, T. (2000). *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.