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. Subsequently, 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 M Mg2+. 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,
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 H₂S, 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 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 (Tm) (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 neighbor-joining methods of De Ley et al. (1970) as modified by Huß et al. (1983).

The profile of the major polar lipids of strain AJ2ᵀ, comprising C₂₀C₂₀ and C₂₀C₂₅ 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 AJ2ᵀ clustered with Natrinema species with validly published names. The results indicated that strain AJ2ᵀ belongs to the genus Natrinema.

Strain AJ2ᵀ, 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 AJ2ᵀ possessed a major glycolipid, which ran very slowly. The glycolipid spot was also found in N. pellirubrum JCM 10476ᵀ. The amount of this glycolipid in AJ2ᵀ was observably less than that in N. pellirubrum JCM 10476ᵀ, and the amount of phosphatidylglycerol sulfate in AJ2ᵀ was more than that in N. pellirubrum JCM 10476ᵀ (McGenity et al., 1998). Moreover, strain AJ2ᵀ did not contain glycolipids found in N. pallidum JCM 8980ᵀ and N. versiforme AS 1.2365ᵀ. Therefore, the polar lipid profiles among Natrinema species also served to distinguish them (see Supplementary Fig. B in IJSEM Online).

Overall, our data indicate that strain AJ2ᵀ 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 AJ2ᵀ 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.](image-url)
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$_2$S produced from thiosulfate. The following substrates are utilized for growth: glucose, glycerol, maltose, maltulose, alanine, arginine, lysine, ornithine, acetate, fumarate, malate, propionate, pyruvate and succinate. Acid is produced from glucose, glycerol, maltose and mannoose. 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 phosphatidyglycerol, phosphatidyglycerol phosphate methyl ester, phosphatidyglycerol 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**


