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NOTE

Natronobacterium nitratireducens sp. nov., a haloalkaliphilic archaeon isolated from a soda lake in China

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Two novel haloalkaliphilic archaea, strains C231^T and C42, were isolated from a soda lake in China. Cells of the two strains were rod-shaped and Gram-negative and colonies were bright red. They required at least 2·5 M NaCl for growth, with an optimum at 3·5 M NaCl, and grew over a pH range from 8·0 to 10·5, with an optimum at pH 8·5. Hypotonic treatment with less than 1·5 M NaCl caused cell lysis. They had similar polar lipid compositions, possessing the diphytanyl (C20:C20) and phytanyl-sesterterpanyl (C20:C25) diether derivatives of phosphatidylglycerol and phosphatidylglycerophosphate methyl ester and a minor phospholipid, PL1. No glycolipids were detected. Comparison of 16S rDNA sequences and morphological features placed them in the genus *Natronobacterium*. Detailed phenotypic characterization and DNA–DNA hybridization studies revealed that the two strains belong to a new species in the genus *Natronobacterium*, for which the name *Natronobacterium nitratireducens* sp. nov. is proposed. The type strain is C231^T (= AS 1.1980^T = JCM 10879^T).

Keywords: *Natronobacterium nitratireducens* sp. nov., halobacteria, haloalkaliphilic, archaea

The haloalkaliphilic archaea are a distinct physiological group of halobacteria (the family *Halobacteriaceae*) due to their obligate alkaliphily (Grant & Larsen, 1989; Tindall, 1992). These archaea have been found in confined habitats such as soda lakes and soils of different geographical sites, e.g. Lake Magadi in Kenya (Tindall *et al*., 1980, 1984; Mwatha & Grant, 1993; Kanai *et al*., 1995; Duckworth *et al*., 1996), Wadi Natrun in Egypt (Soliman & Trüper, 1982; Morth & Tindall, 1985), Owens Lake in California (Morth & Tindall, 1985), soda lakes in China (Wang & Tang, 1989; Tian *et al*., 1997; Xu *et al*., 1999; Wang *et al*., 2000), soda solonchak soils in Russia (Zvyagintseva & Tarasov, 1987) and a soda lake in India (Upasani & Desai, 1990). Originally, obligate alkaliphily was thought to be a discriminating taxonomic criterion from neutrophilic halobacteria and two haloalkaliphilic genera were described, *Natronobacterium* and *Natronococcus*, that were differentiated mainly by cell morphology (Tindall *et al*., 1984; Mwatha & Grant, 1993; Kanai *et al*., 1995). A subsequent 16S rDNA sequence-based phylogenetic analysis, however, showed that these organisms don't belong to a monophyletic group and the species of rod-shaped haloalkaliphiles (species of former *Natronobacterium*) were split into four genera as *Halorubrum vacuolatum*, *Natrialba magadii*, *Natronomonas pharaonis* and the remaining species, *Natronobacterium gregoryi* (Kamekura *et al*., 1997). More recently, another new genus, *Natronorubrum*, was proposed to accommodate newly isolated haloalkaliphilic strains (Xu *et al*., 1999). Most of the currently described haloalkaliphilic species are included in the Natro group, which was defined phylogenetically as a rather diffuse cluster among the halobacteria (McGenity *et al*., 1998); however, two species (*Natronomonas pharaonis* and *Halorubrum vacuolatum*) are classified outside the Natro group (Kamekura *et al*., 1997; McGenity *et al*., 1998). Unlike

Abbreviations: PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerophosphate methyl ester; TMAO, trimethylamine *N*-oxide.

The DDBJ accession number for the 16S rDNA sequence of strain C231^T is AB045012.

neutrophilic halobacteria, the alkaliphilic halobacteria contain no or only a small amount of glycolipids. Accordingly, the current taxonomy of these haloalkaliphilic archaea depends greatly on phylogenetic analysis of 16S rRNA sequences.

In this paper, we describe the taxonomic properties of two novel haloalkaliphilic archaeal strains isolated from Chahannao soda lake, China, and propose a new species in the genus *Natronobacterium* to accommodate them.

Two organisms were isolated from clay samples collected from the near-edge floor of Chahannao (soda) lake in Inner Mongolia, China. The samples were incubated with enriched medium (described below) for 1–2 weeks and dilutions of the enriched cultures were spread on agar plates. Separate reddish colonies were transferred repeatedly onto agar plates and, finally, two strains designated C231^T and C42 were obtained. The purity of the strains was confirmed by the uniformity of colony appearance and cell morphology. The enrichment and growth medium contained (l^{-1}): 7·5 g Casamino acids (Difco), 10·0 g yeast extract (Difco), 3·0 g trisodium citrate dihydrate, 0·1–0·2 g $MgSO_4 . 7H_2O$, 2·0 g KCl, trace amounts of $FeCl_2 . 6H_2O$ and $MnCl_2 . 4H_2O$ and 200·0 g NaCl. The medium was adjusted to pH 8·5–9·2 with sterile Na_2CO_3. Agar slants and plates were prepared by adding 20·0 g agar l^{-1}. The organisms were cultivated at 37 °C with shaking at 180–210 r.p.m. in 300–500 ml Erlenmeyer flasks containing 100 ml medium. Inoculated agar plates were wrapped in plastic bags and incubated at 37 °C. *Natronobacterium gregoryi* JCM 8860^T and *Natronorubrum bangense* A33^T (= JCM 10635 ^{T}), which were used as reference strains, were cultivated in the same way. Cellular and colonial morphology was determined according to Oren *et al.* (1997). The range of NaCl concentrations for growth was determined in growth medium with 0·9–5·2 M NaCl. Likewise, the optimum magnesium concentration was determined in a range between 0 and 0·8 mM $MgSO_4 . 7H_2O$. For the determination of pH for growth (from pH 7·0 to 11·0 at intervals of 0·5), 50 mM Tricine (pH 7·5–8·5) and CHES (pH 9·0–10·0) were employed as buffers. Temperatures for growth were determined by using a temperature gradient incubator (model TN-3; ADVANTEC). Biochemical and physiological tests were conducted according to the standard or modified procedures of Oren *et al.* (1997) as described previously (Xin *et al.*, 2000). Liquid basal medium containing (l^{-1}) 0·1 g yeast extract (Difco), 1·0 g $NaNO_3$, 1·0 g KH_2PO_4, 2·0 g KCl, 0·2 g $CaCl_2 . 2H_2O$, 1·0 ml trace element solution of Imhoff & Trüper (1977) and 200·0 g NaCl (pH 8·5) was used to estimate the utilization of various substrates as carbon and energy sources. After 2 weeks to 1 month of cultivation at 37 °C without shaking, growth was determined by measuring the culture turbidity at 660 nm or by counting cell numbers. The growth-stimulation effect was also checked on agar plates. Total lipids were extracted by the modified method of Kamekura (1993) and separated by TLC on Merck Kieselgel 60-HPTLC by two-dimensional development as described by Ross *et al.* (1985). Phospholipids were detected with the Zinzadze reagent of Dittmer & Lester (1964). Glycolipids were detected by spraying the plate with 0·5 % 1-naphthol in methanol/water (1 : 1 v/v) and then with sulfuric acid/ethanol (1 : 1 v/v), followed by heating at 120 °C for 5–10 min. The cell-envelope fraction was prepared as described by Mescher *et al.* (1974) and glycoproteins were detected with the GelCode glycoprotein staining kit (Pierce). 16S rDNA sequences were determined and analysed phylogenetically as described previously (Xin *et al.*, 2000). The G+C content of whole DNA was determined by the HPLC method of Tamaoka (1994). DNA–DNA hybridization was conducted by the fluorometric method of Ezaki *et al.* (1989).

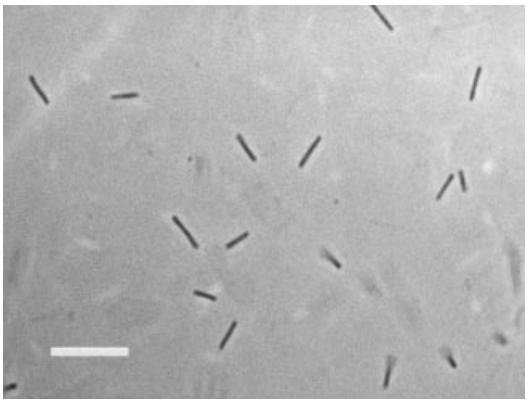

Fig. 1. Phase-contrast micrograph of strain C231^T. Bar, 10 μm.

Cells of strains C231^T and C42 were rod-shaped and 0·4–0·8 μm by 3–7 μm (Fig. 1). Cells were motile. No gas vacuoles were formed inside cells. Cells stained Gram-negative. Cell lysis occurred in diluted medium containing less than 1·5 M NaCl or in distilled water. Colonies formed on the agar plates were bright red, transparent, circular, 1·0–2·0 mm in diameter and convex.

The two strains required 2·5 M to saturated NaCl for growth, growing optimally at 3·5 M NaCl, and grew at pH values in the range 7·5–10·5, with optimal growth at pH 8·5. A concentration of 0·4–0·8 mM $MgSO_4$ supported optimal growth. Strain C231^T grew over a temperature range of 26–44 °C, with an optimum at 36–41 °C. The doubling time of strain C231^T under optimum growth conditions (3·5 M NaCl, 0·4 mM $MgSO_4$ at 37 °C and pH 8·5) was 10·2 h. Strains C231^T and C42 showed anaerobic growth in the presence of nitrate, DMSO and trimethylamine *N*-oxide (TMAO). They did not grow anaerobically with arginine. They reduced nitrate, but not nitrite. Gas was not formed from nitrate. Sulfide was formed from sulfur and thiosulfate. They exhibited positive catalase and oxidase reactions. No indole was formed. They showed

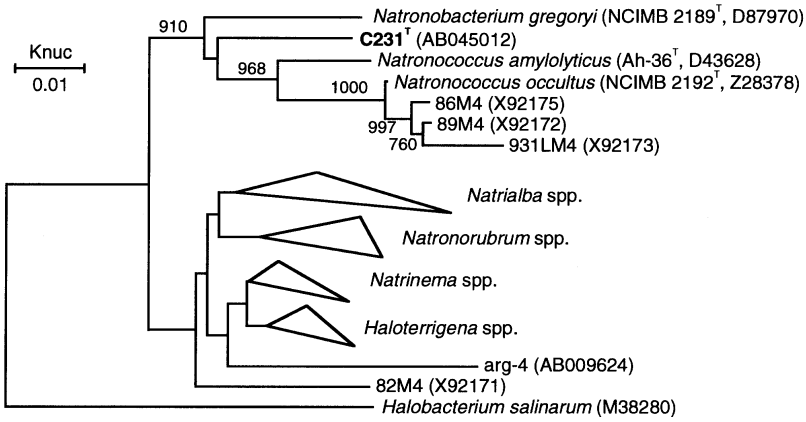

Fig. 2. Phylogenetic tree showing the position of strain C231^T in the Natro group (McGenity *et al.*, 1998). The tree was constructed by the neighbour-joining method, derived from 16S rRNA–DNA sequences. Numbers indicate the bootstrap scores of 1000 trials; values greater than 70% are shown. The clade of *Natrialba* spp. includes *Natrialba asiatica* 172P1^T (D14123), *Natrialba taiwanensis* B1T^T (D14124), *Natrialba magadii* NCIMB 2190^T (X72495) and strains C112 (AJ004806), HAM-2 (AF009601), SSL (D88256), Y21 (AJ001376) and 98NT (X92174). The clade of *Natronorubrum* spp. includes *Natronorubrum bangense* A33^T (Y14028) and *Natronorubrum tibetense* GA33^T (AB005656). The clade of *Natrinema* spp. includes *Natrinema pallidum* NCIMB 784 (AJ002948) and NCIMB 777^T (AJ002949), *Natrinema pellirubrum* NCIMB 786^T (AJ002947), *Natrinema versiforme* XF10^T (AB023426), *Haloterrigena turkmenica* NCIMB 767 (D14125) and GSL11 (D14126) and strains SR1.5 (AJ002945) and T5.7 (AJ002946). The clade of *Haloterrigena* spp. includes *Haloterrigena thermotolerans* PR5^T (AF115478) and *Haloterrigena turkmenica* VKM B-1743^T (AB004878).

hydrolysis of starch, gelatin and Tween 80, but not casein. Both strains utilized alanine, arginine, fructose, ornithine and pyruvate. Acetate, glucose and mannitol enhanced growth weakly. No or negligible growth enhancement occurred with arabinose, fumarate, galactose, glutamate, glycerol, glycine, lactose, lysine, malate, maltose, mannose, propionate, raffinose, rhamnose, ribose, sorbitol, starch, succinate, sucrose and xylose. The two strains were sensitive to anisomycin, aphidicolin, bacitracin, erythromycin, novobiocin and rifampicin and were insensitive to ampicillin, chloramphenicol, neomycin and penicillin G.

Polar lipid analysis showed that the two strains contained the diether derivatives of phosphatidylglycerol (PG) and phosphatidylglycerophosphate methyl ester (PGP-Me). The core diether lipids had diphytanyl (C20:C20) and phytanyl-sesterterpanyl (C20:C25) moieties, as suggested from the two spots of PG or PGP-Me on the two-dimensional TLC plate (not shown). In addition, strain C231^T had PL1 as a minor phospholipid. Strain C42 contained only a trace amount of PL1. No glycolipid was detected from the two strains. Glycoprotein was detected from the cell-envelope fraction of strain C231^T.

The nearly complete 16S rDNA sequence of strain C231^T determined was 1435 bases long. Strain C42 had a partial 16S rDNA sequence identical to that of strain C231^T (positions 20–306 in *Escherichia coli* numbering). Comparison of the 16S rDNA sequence of strain C231^T with those from other members of the Natro group and *Halobacterium salinarum* as a root organism was conducted on 1277 bases of each sequence excluding gaps, uncertain bases and unalignable regions (positions 183–192, 1000–1008, 1032–1039 and 1435–1465 according to *E. coli* numbering). On the phylogenetic tree constructed by the neighbour-joining method (Saitou & Nei, 1987), as shown in Fig. 2, strain C231^T clustered with *Natronobacterium gregoryi* NCIMB 2189^T, the two *Natronococcus* species and unidentified isolates from East African soda lakes (Duckworth *et al.*, 1996). This cluster was supported by a high bootstrap value of 91·0%. The topology was also supported by maximum-likelihood analysis using the fastDNAml program (Olsen *et al.*, 1994). Within this cluster, the two *Natronococcus* species and the related isolates formed a subcluster with high bootstrap values (96·8%), while strain C231^T and *Natronobacterium gregoryi* were separated from the subcluster. The sequence similarities between strain C231^T and the other members of this cluster ranged from 94·0 to 96·2% (*Natronobacterium gregoryi* NCIMB 2189^T, 96·0%; *Natronococcus amylolyticus* Ah-36^T, 96·2%; *Natronococcus occultus* NCIMB 2192^T, 95·5%; strain 86M4, 95·1%; 89M4, 95·0%; and 931LM4, 94·0%). When the comparison was made over an extended sequence range between strain C231^T and the former three species (*Natronobacterium gregoryi*, *Natronococcus amylolyticus* and *Natronococcus occultus*), strain C231^T showed 95·4, 95·5 and 94·6% similarities, respectively, in a comparison of 1397 positions.

The G+C contents of total DNA of strains C231^T and C42 were 63·8 and 63·5 mol% (mean values of three determinations). Strains C231^T and C42 showed very high DNA–DNA hybridization values (99–100%) to

each other, while they showed low values (< 12 %) to *Natronobacterium gregoryi* JCM 8860^T and *Natronorubrum tibetense* A33^T.

In the phylogenetic analysis, strain C231^T was included in the *Natronobacterium–Natronococcus* cluster; however, it was positioned somewhat distantly from the recognized species and was almost equidistant from *Natronobacterium gregoryi* and *Natronococcus amylolyticus*. The 16S rDNA sequence similarity values between strain C231^T and the latter two species were comparable to those observed between some species of different genera (e.g. *Natrialba magadii* and *Natronorubrum tibetense*: 96·4 %). In the absence of differential characters other than the 16S rDNA sequences, however, the creation of a new genus for strain C231^T would not be justified at present. In addition to the cell morphology, cell fragility against hypotonic treatment and the entirely Gram-negative reaction of the two strains agree well with the properties of *Natronobacterium gregoryi*, but not those of the two *Natronococcus* species: *Natronococcus* cells hold their shape even if they are suspended in distilled water and stain as mixtures of Gram-positive and Gram-negative cells (Tindall *et al*., 1984; Kanai *et al*., 1995). Thus, strains C231^T and C42 should be assigned to the genus *Natronobacterium*. The presence of glycoprotein in the cell-envelope fraction would not be differential between the two genera, as glycoprotein was also detected from *Natronococcus occultus* JCM 8859^T (unpublished work). In common with other haloalkaliphilic archaea, the two strains preferred lower magnesium concentrations for growth, lacked characteristic glycolipids and had derivatives of C20:C25 diether core lipids and high G+C content of total DNA.

Strains C231^T and C42 share similar phenotypic properties and very high DNA–DNA relatedness. Moreover, the partial 16S rDNA sequence of C42 is identical to that of strain C231^T. Therefore, both strains should be included in a single species. The two strains are distinct from *Natronobacterium gregoryi*, the sole member of this genus, in the following phenotypic properties: anaerobic growth in the presence of nitrate, DMSO or TMAO, nitrate reduction, indole formation, hydrolysis of starch and Tween 80. *Natronobacterium gregoryi* has two unidentified polar lipids, PL1 and PL3, whereas strains C231^T and C42 do not contain the latter. Moreover, low DNA–DNA hybridization values between the two strains and *Natronobacterium gregoryi* JCM 8860^T support the conclusion that the two strains are separated from *Natronobacterium gregoryi*. Accordingly, isolates C231^T and C42 are included in a novel species in the genus *Natronobacterium* and we propose the name *Natronobacterium nitratireducens* sp. nov. The type strain is C231^T. The two strains, C231^T and C42, have respectively been deposited in the Academy of Sciences, China General Microbiological Culture Collection, Beijing, China, as AS 1.1980^T and AS 1.1988 and in the Japan Collection of Microorganisms, RIKEN, Japan, as JCM 10879^T and JCM 10880.

Description of *Natronobacterium nitratireducens* sp. nov.

Natronobacterium nitratireducens (ni.tra.ti.re.du′cens. N.L. n. *nitratum* nitrate; L. v. *reduco* to reduce, to draw back; N.L. part. adj. *nitratireducens* nitrate-reducing).

Cells are Gram-negative and rod-shaped, 0·4–0·8 μm by 3–7 μm. Motile. Cells lyse in diluted medium containing less than 1·5 M NaCl or in distilled water. Colonies are bright red, circular, 1·0–2·0 mm in diameter and convex. Requires 2·5 M to saturated NaCl for growth, optimum 3·5 M. pH range for growth 8·0–10·5, optimum 8·5. Temperature range for growth 26–44 °C, optimum 36–41 °C. Chemo-organotrophic. Grows anaerobically in the presence of nitrate, DMSO or TMAO or aerobically. Utilizes alanine, ornithine and pyruvate. Reduces nitrate to nitrite. Forms sulfide from sulfur and thiosulfate. No indole formation. Hydrolyses starch, gelatin and Tween 80. Possesses C20:C20 and C20:C25 diether core lipids. No glycolipids. Possesses phosphatidylglycerol (PG), phosphatidylglycerophosphate methyl ester (PGP-Me) and a minor phospholipid, PL1. Sensitive to anisomycin, aphidicolin, bacitracin, erythromycin, novobiocin and rifampicin. Insensitive to ampicillin, chloramphenicol, neomycin and penicillin G. The type strain is C231^T (= AS 1.1980^T = JCM 10879^T).

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References

Dittmer, J. C. & Lester, R. L. (1964). A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. *J Lipid Res* **5**, 126–127.

Duckworth, A. W., Grant, W. D., Jones, B. E. & van Steenbergen, R. (1996). Phylogenetic diversity of soda lake alkaliphiles. *FEMS Microbiol Ecol* **19**, 181–191.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Grant, W. D. & Larsen, H. (1989). Extremely halophilic archaeobacteria. Order *Halobacteriales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2216–2219. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

Imhoff, J. F. & Trüper, H. G. (1977). *Ectothiorhodospira halochloris* sp. nov., a new extremely halophilic phototrophic bacterium containing bacteriochlorophyll b. *Arch Microbiol* **114**, 115–121.

Kamekura, M. (1993). Lipids of extreme halophiles. In *The Biology of Halophilic Bacteria*, pp. 135–161. Edited by R. H. Vreeland & L. I. Hochstein. Boca Raton, FL: CRC Press.

Kamekura, M., Dyall-Smith, M. L., Upasani, V., Ventosa, A. & Kates, M. (1997). Diversity of alkaliphilic halobacteria: proposals for transfer of *Natronobacterium vacuolatum*, *Natronobacterium magadii*, and *Natronobacterium pharaonis* to *Halorubrum*, *Natrialba*, and *Natronomonas* gen. nov., respectively, as *Halorubrum vacuolatum* comb. nov., *Natrialba magadii* comb. nov., and *Natronomonas pharaonis* comb. nov., respectively. *Int J Syst Bacteriol* **47**, 853–857.

Kanai, H., Kobayashi, T., Aono, R. & Kudo, T. (1995). *Natronococcus amylolyticus* sp. nov., a haloalkaliphilic archaeon. *Int J Syst Bacteriol* **45**, 762–766.

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.

Mescher, M. F., Strominger, J. L. & Watson, S. W. (1974). Protein and carbohydrate composition of the cell envelope of *Halobacterium salinarium*. *J Bacteriol* **120**, 945–954.

Morth, S. & Tindall, B. J. (1985). Variation of polar lipid composition within haloalkaliphilic archaebacteria. *Syst Appl Microbiol* **6**, 247–250.

Mwatha, W. E. & Grant, W. D. (1993). *Natronobacterium vacuolata* sp. nov., a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. *Int J Syst Bacteriol* **43**, 401–404.

Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. (1994). fastDNAmL: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput Appl Biosci* **10**, 41–48.

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.

Ross, H. N. M., Grant, W. D. & Harris, J. E. (1985). Lipids in archaebacterial taxonomy. In *Chemical Methods in Bacterial Systematics*, pp. 289–300. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

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

Soliman, G. S. H. & Trüper, H. G. (1982). *Halobacterium pharaonis* sp. nov., a new, extremely haloalkaliphilic archaebacterium with low magnesium requirement. *Zentbl Bakteriol Parasitenkd Infektionskr Hyg Abt I Orig* **C3**, 318–329.

Tamaoka, J. (1994). Determination of DNA base composition. In *Chemical Methods in Prokaryotic Systematics*, pp. 463–470. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Tian, X., Xu, Y., Liu, H. & Zhou, P. (1997). New species of *Natronobacterium*. *Acta Microbiol Sin* **37**, 1–6 (in Chinese).

Tindall, B. J. (1992). The family *Halobacteriaceae*. In *The Prokaryotes*, 2nd edn, vol. 1, pp. 768–808. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Tindall, B. J., Mills, A. A. & Grant, W. D. (1980). An alkalophilic red halophilic bacterium with a low magnesium requirement from a Kenyan soda lake. *J Gen Microbiol* **116**, 257–260.

Tindall, B. J, Ross, H. N. M. & Grant, W. D. (1984). *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaebacteria. *Syst Appl Microbiol* **5**, 41–57.

Upasani, V. N. & Desai, S. (1990). Sambhar salt lake. Chemical composition of the brines and studies on haloalkaliphilic archaebacteria. *Arch Microbiol* **154**, 589–593.

Wang, D. & Tang, Q. (1989). *Natronobacterium* from soda lakes of China. In *Recent Advances in Microbial Ecology*, pp. 68–72. Edited by T. Hattori, Y. Naruyama, R. Y. Morita & A. Uchida. Tokyo: Japan Scientific Societies Press.

Wang, Z., Xu, Y. & Zhou, P. (2000). Taxonomy of a new species of haloalkaliphilic archaeon. *Acta Microbiol Sin* **40**, 115–120.

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.

Xu, Y., Zhou, P. & Tian, X. (1999). Characterization of two novel haloalkaliphilic archaea, *Natronorubrum bangense* gen. nov., sp. nov. and *Natronorubrum tibetense* gen. nov., sp. nov. *Int J Syst Bacteriol* **49**, 261–266.

Zvyagintseva, I. S. & Tarasov, A. L. (1987). Extreme halophilic bacteria from saline soils. *Mikrobiologiya* **56**, 839–844.