Halostagnicola larsenii gen. nov., sp. nov., an extremely halophilic archaeon from a saline lake in Inner Mongolia, China

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Strain XH-48^T was isolated from the sediment of Lake Xilinhote, a saline lake in Inner Mongolia (China). The organism is pleomorphic, neutrophilic and requires at least 2.5 M (15%) NaCl, but not MgCl₂, for growth; it exhibits optimal growth at 3.4 M (20%) NaCl. The G + C content of its DNA is 61 mol%. 16S rRNA gene sequence analysis revealed that strain XH-48^T is a member of the family *Halobacteriaceae*, but there were low levels of similarity with other members of this family. The highest sequence similarity values (94.5 and 93.3%) were obtained with the 16S rRNA genes of *Natrialba aegyptiaca* and *Natrialba asiatica*, respectively. Polar lipid analyses revealed that strain XH-48^T contains phosphatidylglycerol and phosphatidylglyceromethylphosphate, derived from both C₂₀C₂₀ and C₂₀C₂₅ glycerol diethers, and two unidentified glycolipids. On the basis of the data obtained, the novel isolate cannot be classified within any recognized genus. Strain XH-48^T should be placed within a novel genus and species within the family *Halobacteriaceae*, order *Halobacteriales*, for which the name *Halostagnicola larsenii* gen. nov., sp. nov. is proposed. The type strain of *Halostagnicola larsenii* is strain XH-48^T (=DSM 17691^T=CGMCC 1.5338^T=JCM 13463^T=CECT 7116^T).

The haloarchaea are a diverse group of extremely halophilic archaea that require at least 1.5 M NaCl for growth. They are positioned within the order *Halobacteriales*, family *Halobacteriaceae* (Grant *et al.*, 2001). Prior to the 1970s, haloarchaeal taxonomy was based mainly on standard biochemical tests and cell morphology (Gibbons, 1974). Recent sequence comparisons of haloarchaeal 16S rRNA genes as a basis for phylogeny as well as chemotaxonomic criteria, particularly polar lipid composition, have resulted in the recognition of great taxonomic diversity at the genus

level, and 21 genera in this family have now been described (Tindall *et al.*, 1984; Torreblanca *et al.*, 1986; Oren *et al.*, 1995; McGenity & Grant, 1995; Kamekura & Dyall-Smith, 1995; Kamekura *et al.*, 1997; McGenity *et al.*, 1998; Montalvo-Rodríguez *et al.*, 1998; Y. Xu *et al.*, 1999; Ventosa *et al.*, 1999; Wainø *et al.*, 2000; Grant 2001a, b; Oren *et al.*, 2002; Hezayen *et al.*, 2002; Vreeland *et al.*, 2002; Itoh *et al.*, 2005; Xue *et al.*, 2005; Castillo *et al.*, 2006).

The recent isolation of micro-organisms from hypersaline environments in different parts of China has led to the discovery of several novel species and genera belonging to the archaea (Y. Xu *et al.*, 1999, 2001; Xin *et al.*, 2000, 2001; Fan *et al.*, 2004; Feng *et al.*, 2004, 2005; Itoh *et al.*, 2005; X.-W. Xu *et al.*, 2005a, b, c; Xue *et al.*, 2005; Castillo *et al.*, 2006). In this paper, the almost complete 16S rRNA gene sequence from the halophilic strain XH-48^T, isolated from Lake Xilinhote in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain XH-48^T is AM117571.

A thin-layer chromatogram of the polar lipids of strain XH-48^T and some haloarchaea is available as a supplementary figure in IJSEM Online.

Inner Mongolia (China), is reported, along with its polar lipid composition and physiological and biochemical characteristics. On the basis of the data presented, we have concluded that strain XH-48^T is not closely related to any of the currently described haloarchaeal taxa and is sufficiently different to justify its classification as a novel species within a novel genus.

Strain XH-48^T was isolated from a sediment sample of the saline Lake Xilinhote (43° 55' N 115° 37' E). Samples were diluted in a 20 % salt solution and spread on nutrient agar plates containing the following (l⁻¹): NaCl, 195 g; MgCl₂·6H₂O, 32.5 g; MgSO₄·7H₂O, 50.8 g; CaCl₂, 0.8 g; KCl, 5 g; NaHCO₃, 0.16 g; NaBr, 0.6 g; yeast extract, 5 g. The pH of the medium was adjusted to 7.5 with 1 M NaOH. Sampling was carried out in September 2003. The water from Lake Xilinhote was at 23.6 °C and pH 8.5 and had a conductivity of 185 mS cm⁻¹. Strain XH-48^T grew at temperatures in the range 25–50 °C (optimum, 37 °C) and at pH values in the range 6.0–9.0 (optimum, pH 7.0–8.0). Routine cultivation was performed at 37 °C and pH 7.5. The requirements for NaCl and magnesium for growth were determined in media with 1.0–5.2 M NaCl or 0–0.5 M MgCl₂. Strain XH-48^T was capable of growing at a wide range of NaCl concentrations, from 2.5 (15 %) to 5.0 M (30 %). It grew optimally in the presence of 3.4 M (20 %) NaCl, as has been shown for most extremely halophilic archaea (Grant *et al.*, 2001). MgCl₂ was not required for growth.

Phenotypic tests were performed according to the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997). Tests for catalase and oxidase activities and for the hydrolysis of starch and Tween 80 were performed as described previously (Gonzalez *et al.*, 1978). Nitrate reduction, H₂S formation, indole production and the utilization of sugars, alcohols, amino acids and organic acids were assessed as described by Oren *et al.* (1997). Strain XH-48^T was oxidase- and catalase-positive. The results of methyl red, Voges–Proskauer, nitrate reduction, indole production from tryptone and Simmons' citrate tests were negative. Casein, aesculin and Tween 80 were not hydrolysed. Gelatin was not liquefied. The urease test was positive. Starch and DNA were hydrolysed. Susceptibility to antibiotics was determined on agar-medium plates by using absorbent paper discs impregnated with antibiotics at the following concentrations: ampicillin (10 µg), bacitracin (10 U), chloramphenicol (30 µg), erythromycin (15 µg); gentamicin (10 µg), nalidixic acid (30 µg), neomycin (10 µg), novobiocin (30 µg), penicillin G (10 U), rifampicin (30 µg), streptomycin (10 µg) and tetracycline (30 µg). The isolation medium, containing yeast extract at 0.05 % (w/v) and supplemented with 1 % (w/v) of the substrate being tested, was used to determine the utilization of different organic substrates as carbon and energy sources or as carbon, nitrogen and energy sources (Torreblanca *et al.*, 1986). The results of the tests for antibiotic susceptibility and utilization of different substrates are included in the

species description. The formation of acid from different sugars was assessed in media (in which the yeast extract concentration was 0.05 %, w/v) supplemented with 1 % (w/v) of the sugar being tested (sterilized separately).

Cell morphology and motility were examined using an Olympus BX41 microscope equipped with phase-contrast optics. For photography, drops of exponentially growing liquid cultures were mixed on a microscope slide with an equal volume of melted 1 % agarose containing 20 % NaCl and then covered with a coverslip. The cells were non-motile and pleomorphic, exhibiting rod-shaped, square or disc-shaped morphologies (Fig. 1). Colony morphology was observed on agar medium under optimal growth conditions after incubation at 37 °C for 10 days. Colonies of strain XH-48^T that formed on agar plates were circular, smooth, entire, opaque and pink-pigmented.

The polar lipids were extracted with chloroform/methanol as previously described (Kamekura, 1993). TLC was performed using Merck HPTLC silica gel 60 plates (art. 5641) with the solvent system chloroform/methanol/acetic acid/water (85:22.5:10:4, by vol.). Glycolipids were detected as purple spots by means of spraying with 0.5 % α -naphthol in methanol/water (1:1) and then with sulphuric acid/ethanol (1:1), followed by brief heating at 160 °C. TLC of the polar lipids (see Supplementary Fig. S1 available in IJSEM Online) suggested that strain XH-48^T contained phosphatidylglycerol and phosphatidylglyceromethylphosphate derived from both C₂₀C₂₀ and C₂₀C₂₅ glycerol diethers, as is shown by the two spots (Xin *et al.*, 2000).

Fig. 1. Phase-contrast micrograph of strain XH-48^T grown in liquid medium under optimum conditions. Bar, 10 µm.

Two glycolipid spots ($C_{20}C_{20}$ and $C_{20}C_{25}$ moieties, marked with pencil) were detected between S-TGA-1 and S-TeGA, which showed mobilities different from that of S2-DGA-1, the characteristic glycolipid for species of the genus *Natrinema*.

Chromosomal DNA of strain XH-48^T was isolated and purified according to the methods described by Wilson (1987) and Marmur (1961). The G + C content of the genomic DNA was determined from the mid-point (T_m) of the thermal denaturation profile (Marmur & Doty, 1962), using the equation of Owen & Hill (1979). The DNA G + C content of strain XH-48^T is 61.1 mol%. The 16S rRNA gene of strain XH-48^T was amplified by a PCR using three universal primers as described previously (Lopez-Garcia *et al.*, 2001; Arahall *et al.*, 1996), and an almost-complete nucleotide sequence (approx. 1400 bp) was determined. The ARB software package (Ludwig *et al.*, 2004) was used for 16S rRNA gene sequence analysis. Base-frequency filters were applied in the sequence-comparison analysis and the effects on the results were evaluated. The 16S rRNA gene phylogenetic analysis, performed using the neighbour-joining method (Saitou & Nei, 1987), showed the position of strain XH-48^T as a branch in the *Natrilalba* clade (Fig. 2). Similar topologies were obtained when other treeing methods (maximum parsimony and maximum likelihood) were used. In consequence, it was concluded that strain XH-48^T formed a new distinct branch related to *Natrilalba aegyptiaca* (94.5% 16S rRNA gene sequence similarity to the type strain) and *Natrilalba asiatica* (93.3%). The phenotypic characteristics of these two species of the genus *Natrilalba* are very different from those of strain XH-48^T, e.g. in terms of indole production, hydrolysis of gelatin, casein and Tween 80, acid production from carbon sources and susceptibility to antibiotics. In addition, the alignment of the 16S rRNA gene sequence with all published sequences of haloarchaea clearly shows that strain XH-48^T does not belong to the genus *Natrilalba*, since its sequence does not share the two signature bases, 403G and 560G, of those of members of the genus *Natrilalba* (Kamekura *et al.*, 2004).

The phenotypic, polar lipid profile and phylogenetic data based on the 16S rRNA sequence comparison clearly support the placement of strain XH-48^T in a novel genus and species within the haloarchaea, for which we propose the name *Halostagnicola larsenii* gen. nov., sp. nov. Table 1 shows the characteristics that differentiate the novel genus from other related haloarchaeal genera.

Description of *Halostagnicola* gen. nov.

Halostagnicola (Ha.lo.stag.ni'co.la. Gr. n. *hals*, *halos* salt; L. neut. n. *stagnum* a piece of standing water, pond, lake; L. suff. *-cola* inhabitant, dweller; N.L. fem. n. *Halostagnicola* a dweller of a saline lake).

Gram-negative. Cells are pleomorphic although most are rod-shaped. Colonies are pink-pigmented. Strictly aerobic; oxygen is used as the terminal electron acceptor. Growth occurs at pH values between 6.0 and 9.0, at temperatures between 25 and 50 °C and at salinities between 15 and 30% (2.5–5.0 M) NaCl. Optimal growth occurs at pH 7.0–8.0, 37 °C and 20% (3.4 M) NaCl. The DNA G + C content of the type strain of the only species in the genus is 61.0 mol% (T_m method). Polar lipids include phosphatidylglycerol and phosphatidylglyceromethylphosphate, derived from both $C_{20}C_{20}$ and $C_{20}C_{25}$ glycerol diethers, and two unidentified glycolipids. Phylogenetically affiliated to the *Halobacteriaceae*. The type species is *Halostagnicola larsenii*, which was first isolated from a salt lake.

Description of *Halostagnicola larsenii* sp. nov.

Halostagnicola larsenii (lar.se'ni.i. N.L. gen. n. *larsenii* of Larsen, named for the Norwegian microbiologist H. Larsen, one of the pioneers in the study of haloarchaea).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.5–1.0 µm wide and 1.0–3.0 µm long (Fig. 1). Colonies are circular and 1–2 mm in diameter after incubation for 10 days at 37 °C. Extremely halophilic and the cells lyse in water. Magnesium is not required for growth. Growth does not occur above 50 °C. The pH range for growth is 6.0–9.0. Amino acids are not required for growth. Catalase- and oxidase-positive. Negative for indole production and in methyl red, Voges–Proskauer and Simmons' citrate tests. H₂S is not produced from cysteine. Acid is produced from sucrose. Does not produce arginine dihydrolase, lysine decarboxylase or ornithine decarboxylase. Produces urease and phosphatase. Anaerobic growth with nitrate or arginine does not occur. Gelatin, Tween 80, casein and aesculin are not hydrolysed. Starch and DNA are hydrolysed. Nitrate is reduced to nitrite. The following substrates are utilized for growth: glycerol, fructose, maltose, trehalose, lactose, glucose, arabinose, galactose, mannitol, ribose, starch, xylose, propionate, glutamate and acetate. No growth occurs on mannitol, sorbitol, raffinose, succinate, malate or fumarate.

Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain XH-48^T and some related haloarchaeal species. Accession numbers are shown in parentheses. Bar, 1% sequence divergence.

Table 1. Characteristics that distinguish *Halostagnicola* gen. nov. from related haloarchaea

Data are from Castillo *et al.* (2006), Grant *et al.* (2001), Tindall (1992), Oren (2001) and this study. +, Positive; –, negative; ND, not determined; V, variable.

Characteristic	<i>Halostagnicola</i>	<i>Halovivax</i>	<i>Halorubrum</i>	<i>Haloarcula</i>	<i>Natronobacterium</i>	<i>Natronococcus</i>	<i>Natrialba</i>	<i>Natrinema</i>
Morphology	Rod/pleomorphic rod	Rod/pleomorphic rod	Rod/pleomorphic rod	Rod/pleomorphic rod	Rod	Coccus	Rod	Rod/pleomorphic rod
Cell size (µm)	0.5–1 × 1–3	0.4–0.5 × 4–5	0.12–1 × 0.5–7	0.2–2 × 0.5–5	0.5–0.7 × 10–15	1–2	0.5–1 × 1–5	0.6–1 × 1–6
NaCl optimum (M)	3.4	3.4	1.7–4.5/ND	2.5–4.3	3.0	2.5–3.8	3.5–4	3.4–4.3
NaCl range (M)	2.5–5.0	2.5–4.3	0.5–4.5	1.7–5.2	2.0–5.2	1.4–5.2	2.0–5.2	ND
pH optimum	7.0–8.0	7.0–7.5	Neutral–9.5	6.5–7.5	9.5	9.0–9.5	6.6–9.5	7.2–7.6
Temperature optimum (°C)	37	37	31–50	35–53	37	35–45	35–40	37–40/ND
Nitrite from nitrate	+	–	+	+ /ND	–	+	+	+
Anaerobic growth on nitrate	–	–	–	+ /ND	–	–/ND	–	ND
Acid production from carbohydrates	V	V	+ /ND	+	–	ND	V	–
Hydrolysis of:								
Starch	+	–	V	V	–	V	V	–
Gelatin	–	+	–/ND	V	+	V	+ /ND	+
Casein	–	+	–/ND	–/ND	ND	ND	+ /ND	ND
Tween 80	–	+	–/ND	V	ND	ND	ND	ND
DNase	+	–	ND	ND	ND	ND	ND	ND
Urease	–	+	ND	ND	ND	ND	ND	ND
H ₂ S production	+	–	–/ND	+ /ND	V	ND	V	V
Pigmentation	Pink	Pale pink	Red/purple	Red	Red	Red	White/red	Light red–pale orange
Major glycolipids	Unidentified new glycolipid	Unidentified glycolipid	S-DGD-3	TGD-2	Unknown	–	–	Unidentified
DNA G + C content (mol%)	61.0	60.3	62.7–72.1	62–65	65.0	63.5–64	60.0–63.1	69.9

Susceptible to bacitracin and novobiocin. Resistant to ampicillin, chloramphenicol, erythromycin, gentamicin, nalidixic acid, neomycin, penicillin G, rifampicin, streptomycin and tetracycline.

The type strain is strain XH-48^T (= DSM 17691^T = CGMCC 1.5338^T = JCM 13463^T = CECT 7116^T), isolated from the saline Lake Xilinhot in Inner Mongolia, China.

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References

- Arahal, D. R., Dewhirst, F. E., Paster, B. J., Volcani, B. E. & Ventosa, A. (1996). Phylogenetic analyses of some extremely halophilic archaea isolated from Dead Sea water, determined on the basis of their 16S rRNA sequences. *Appl Environ Microbiol* **62**, 3779–3786.
- Castillo, A. M., Gutiérrez, M. C., Kamekura, M., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2006). *Halovivax asiaticus* gen. nov., sp. nov., a novel extremely halophilic archaeon isolated from Inner Mongolia, China. *Int J Syst Evol Microbiol* **56**, 765–770.
- Fan, H., Xue, Y., Ma, Y., Ventosa, A. & Grant, W. D. (2004). *Halorubrum tibetense* sp. nov., a novel haloalkaliphilic archaeon from Lake Zabuye in Tibet, China. *Int J Syst Evol Microbiol* **54**, 1213–1216.
- Feng, J., Zhou, P. & Liu, S. (2004). *Halorubrum xinjiangense* sp. nov., a novel halophile isolated from saline lakes in China. *Int J Syst Evol Microbiol* **54**, 1789–1791.
- Feng, J., Zhou, P., Zhou, Y., Liu, S. & Warren-Rhodes, K. (2005). *Halorubrum alkaliphilum* sp. nov., a novel haloalkaliphile isolated from a soda lake in Xinjiang, China. *Int J Syst Evol Microbiol* **55**, 149–152.
- Gibbons, N. E. (1974). Family V. *Halobacteriaceae* fam. nov. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 269–270. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- Gonzalez, C., Gutierrez, C. & Ramirez, C. (1978). *Halobacterium vallismortis* sp. nov. An amyolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* **24**, 710–715.
- Grant, W. D. (2001a). Genus I. *Halobacterium* Elazari-Volcani 1957, 207, ^{AL} emend. Larsen and Grant 1989, 2222. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 301–305. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Grant, W. D. (2001b). Genus IV. *Halococcus* Schoop 1935a, 817^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 311–314. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Grant, W. D., Kamekura, M., McGenity, T. J. & Ventosa, A. (2001). Order I. *Halobacteriales* Grant and Larsen 1989b, 495^{VP} (Effective publication: Grant and Larsen 1989a, 2216). In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 294–299. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Hezayen, F. F., Tindall, B. J., Steinbüchel, A. & Rehm, B. H. A. (2002). Characterization of a novel halophilic archaeon, *Halobiforma haloterrestris* gen. nov., sp. nov., and transfer of *Natronobacterium nitratireducens* to *Halobiforma nitratireducens* comb. nov. *Int J Syst Evol Microbiol* **52**, 2271–2280.
- Itoh, T., Yamaguchi, T., Zhou, P. & Takashina, T. (2005). *Natronolimnobius baerhuensis* gen. nov., sp. nov. and *Natronolimnobius innermongolicus* sp. nov., novel haloalkaliphilic archaea isolated from soda lakes in Inner Mongolia, China. *Extremophiles* **9**, 111–116.
- 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. & Dyll-Smith, M. L. (1995). Taxonomy of the family *Halobacteriaceae* and the description of two genera *Halorubrobacterium* and *Natrialba*. *J Gen Appl Microbiol* **41**, 333–350.
- Kamekura, M., Dyll-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.
- Kamekura, M., Mizuki, T., Usami, R., Yoshida, Y., Horikoshi, K. & Vreeland, R. H. (2004). The potential use of signature bases from 16S rRNA gene sequences to aid the assignment of microbial strains to genera of halobacteria. In *Halophilic Microorganisms*, pp. 77–87. Edited by A. Ventosa. Heidelberg: Springer.
- Lopez-Garcia, P., Moreira, D., Lopez-Lopez, A. & Rodríguez-Valera, F. (2001). A novel haloarchaeal-related lineage is widely distributed in deep oceanic regions. *Environ Microbiol* **3**, 72–78.
- Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *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. & Grant, W. D. (1995). Transfer of *Halobacterium saccharovorum*, *Halobacterium sodomense*, *Halobacterium trapanicum* NRC 34021 and *Halobacterium lacusprofundi* to the genus *Halorubrum* gen. nov., as *Halorubrum saccharovorum* comb. nov., *Halorubrum sodomense* comb. nov., *Halorubrum trapanicum* comb. nov., and *Halorubrum lacusprofundi* comb. nov. *Syst Appl Microbiol* **18**, 237–243.
- 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.
- Montalvo-Rodríguez, R., Vreeland, R. H., Oren, A., Kessel, M., Betancourt, C. & Lopez-Garriga, J. (1998). *Halogeometricum borinquense* gen. nov., sp. nov., a novel halophilic archaeon from Puerto Rico. *Int J Syst Bacteriol* **48**, 1305–1312.
- Oren, A. (2001). The order *Halobacteriales*. In *The Prokaryotes. An Evolving Electronic Resource for the Microbiological Community*. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer. <http://141.150.157.117:8080/prokPUB/index.htm>
- Oren, A., Gurevich, P., Gemmell, R. T. & Teske, A. (1995). *Halobaculum gomorrense* gen. nov., sp. nov., a novel extremely halophilic archaeon from the Dead Sea. *Int J Syst Bacteriol* **45**, 747–754.
- 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.
- Owen, R. J. & Hill, L. R. (1979). The estimation of base compositions, base pairing and genome size of bacterial deoxyribonucleic acids. In *Identification Methods for Microbiologists*, 2nd edn, pp. 217–296. Edited by F. A. Skinner & D. W. Lovelock. 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.
- Tindall, B. J. (1992). The family *Halobacteriaceae*. In *The Prokaryotes. A Handbook of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 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., Ross, H. N. M. & Grant, W. D. (1984). *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaeobacteria. *Syst Appl Microbiol* **5**, 41–57.
- Torreblanca, M., Rodriguez-Valera, F., Juez, G., Ventosa, A., Kamekura, M. & Kates, M. (1986). Classification of non-alkaliphilic halobacteria based on numerical taxonomy and polar lipid composition and description of *Haloarcula* gen. nov. and *Haloferax* gen. nov. *Syst Appl Microbiol* **8**, 89–99.
- Ventosa, A., Gutiérrez, M. C., Kamekura, M. & Dyal-Smith, M. L. (1999). Proposal to transfer *Halococcus turkmenicus*, *Halobacterium trapanicum* JCM 9743 and strain GSL-11 to *Haloterrigena turkmenica* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 131–136.
- Vreeland, R. H., Straight, S., Krammes, J., Dougherty, K., Rosenzweig, W. D. & Kamekura, M. (2002). *Halosimplex carlsbadense* gen. nov., sp. nov., a unique halophilic archaeon, with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate. *Extremophiles* **6**, 445–452.
- Wainø, M., Tindall, B. J. & Ingvorsen, K. (2000). *Halorhabdus utahensis* gen. nov., sp. nov., an aerobic, extremely halophilic member of the *Archaea* from Great Salt Lake, Utah. *Int J Syst Evol Microbiol* **50**, 183–190.
- Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.2. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, Y. (2000). *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.
- Xin, H., Itoh, T., Zhou, P., Suzuki, K. & Nakase, Y. (2001). *Natronobacterium nitratreducens* sp. nov., a haloalkaliphilic archaeon isolated from a soda lake in China. *Int J Syst Evol Microbiol* **51**, 1825–1829.
- 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.
- Xu, Y., Wang, Z., Xue, Y., Zhou, P., Ma, Y., Ventosa, A. & Grant, W. D. (2001). *Natrialba hulunbeirensis* sp. nov. and *Natrialba chahannaoensis* sp. nov., novel haloalkaliphilic archaea from soda lakes in Inner Mongolia Autonomous Region, China. *Int J Syst Evol Microbiol* **51**, 1693–1698.
- Xu, X.-W., Ren, P.-G., Liu, S.-J., Wu, M. & Zhou, P.-J. (2005a). *Natrinema altunense* sp. nov., an extremely halophilic archaeon isolated from a salt lake in Altun Mountain of Xin-Jiang, China. *Int J Syst Evol Microbiol* **55**, 1311–1314.
- Xu, X.-W., Wu, M., Zhou, P.-J. & Liu, S.-J. (2005b). *Halobiforma lacisalsi* sp. nov., isolated from a lake in China. *Int J Syst Evol Microbiol* **55**, 1949–1952.
- Xu, X.-W., Liu, S.-J., Tohty, D., Oren, A., Wu, M. & Zhou, P.-J. (2005c). *Haloterrigena saccharevitans* sp. nov., an extremely halophilic archaeon from Xin-Jiang, China. *Int J Syst Evol Microbiol* **55**, 2539–2542.
- Xue, Y., Fan, H., Ventosa, A., Grant, W. D., Jones, B. E., Cowan, D. A. & Ma, Y. (2005). *Halalkalicoccus tibetensis* gen. nov., sp. nov., representing a novel genus of haloalkaliphilic archaea. *Int J Syst Evol Microbiol* **55**, 2501–2505.