Halopiger xanaduensis gen. nov., sp. nov., an extremely halophilic archaeon isolated from saline Lake Shangmatale in Inner Mongolia, China

M. C. Gutiérrez,¹ A. M. Castillo,¹ M. Kamekura,² Y. Xue,³ Y. Ma,³
D. A. Cowan,⁴ B. E. Jones,⁵ W. D. Grant⁶ and A. Ventosa¹

Correspondence
A. Ventosa
ventosa@us.es

¹Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain

²Noda Institute for Scientific Research, 399 Noda, Noda-shi, Chiba-ken 278-0037, Japan

³State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, 100080 Beijing, China

⁴Department of Biotechnology, University of the Western Cape, Bellville 7535, Cape Town, South Africa

⁵Genencor International BV, Archimedesweg 30, 2333 CN Leiden, The Netherlands

⁶Department of Infection, Immunity and Inflammation, University of Leicester, Leicester LE1 9HN, UK

Strain SH-6^T was isolated from the sediment of Lake Shangmatale, a saline lake in Inner Mongolia (China). Cells were pleomorphic. The organism was neutrophilic and required at least 2.5 M (15 %) NaCl, but not MgCl₂, for growth; optimal growth occurred at 4.3 M (25 %) NaCl. The G + C content of its DNA was 63.1 mol%. 16S rRNA gene sequence analysis revealed that strain SH-6^T is a member of the family *Halobacteriaceae*, but there was a low level of similarity with other members of this family. Highest sequence similarity (94.6 %) was obtained with the 16S rRNA genes of the type strains of *Natronolimnobius innermongolicus* and *Natronolimnobius baerhuensis*. Polar lipid analyses revealed that strain SH-6^T contains phosphatidylglycerol and phosphatidylglyceromethylphosphate, derived from both C₂₀C₂₀ and C₂₀C₂₅ glycerol diethers together with the glycolipid S₂-DGD-1. On the basis of the data obtained, the new isolate could not be classified in any recognized genus. Strain SH-6^T is thus considered to represent a novel species in a new genus within the family *Halobacteriaceae*, order *Halobacteriales*, for which the name *Halopiger xanaduensis* gen. nov., sp. nov. is proposed. The type strain of *Halopiger xanaduensis* is SH-6^T (=CECT 7173^T=CGMCC 1.6379^T=JCM 14033^T).

The haloarchaea constitute a large group of extremely halophilic, aerobic archaea that are placed in the order *Halobacteriales*, family *Halobacteriaceae* (Grant *et al.*, 2001). Classically, they were easily differentiated microscopically as rods or cocci that were, respectively, included within the genera *Halobacterium* or *Halococcus* (Gibbons, 1974). In recent years, the numbers of halobacterial genera and species have increased due to the use of different isolation media and culture conditions, combined with the study of a wide variety of hypersaline environments. The extremely halophilic archaea are the dominant microbial populations of

hypersaline environments (Ventosa, 2006). The current classification of halophilic archaea is based on three kinds of data: phenotypic features, chemical data (polar lipid composition) and genetic data (16S rRNA gene sequence information and DNA–DNA hybridization) (Oren *et al.*, 1997; Grant *et al.*, 2001). At the time of writing, the aerobic, extremely halophilic archaea are classified within 22 different genera with a large number of species. These genera are *Halalkalicoccus*, *Haloarcula*, *Halobacterium*, *Halobaculum*, *Halobiforma*, *Halococcus*, *Haloferax*, *Halogeometricum*, *Halomicrobium*, *Halorhabdus*, *Halorubrum*, *Halosimplex*, *Halostagnicola*, *Haloterrigena*, *Halovivax*, *Natrialba*, *Natri-nema*, *Natronobacterium*, *Natronococcus*, *Natronolimnobius*, *Natronomonas* and *Natronorubrum* (Tindall *et al.*, 1984; Torrealba *et al.*, 1986; Oren *et al.*, 1995, 2002; Kamekura & Dyal-Smith, 1995; Kamekura *et al.*, 1997; McGinity *et al.*, 1998; Montalvo-Rodriguez *et al.*, 1998; Ventosa *et al.*, 1999;

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SH-6^T is AM268114.

A thin-layer chromatograph of the polar lipids of strain SH-6^T and related haloarchaea is available as a supplementary figure with the online version of this paper.

Xu *et al.*, 1999; Wainø *et al.*, 2000; Grant, 2001a, b; Hezayen *et al.*, 2002; Vreeland *et al.*, 2002; Itoh *et al.*, 2005; Xue *et al.*, 2005; Castillo *et al.*, 2006a, b).

Here we describe a halophilic archaeal strain, designated SH-6^T, which was isolated from Lake Shangmatala, located in Inner Mongolia Autonomous Region, China. Preliminary 16S rRNA gene sequence comparisons indicated that the isolate was a member of the family *Halobacteriaceae*. The aim of the present work was to determine the exact taxonomic position of strain SH-6^T by using a polyphasic taxonomic characterization that combined phenotypic, chemotaxonomic and phylogenetic analyses.

Strain SH-6^T was isolated from a sediment sample from Shangmatala salt lake (43° 12' N 114° 01' E) by enrichment in liquid medium after 15 days incubation and subsequent plating of the enriched culture until purity was obtained on the same medium but with 2 % agar added. The medium contained (per litre distilled water): 174 g NaCl, 30 g MgCl₂·6H₂O, 45.4 g MgSO₄·7H₂O, 0.8 g CaCl₂, 4.5 g KCl, 0.16 g NaHCO₃, 0.5 g NaBr and 5 g yeast extract; the pH was adjusted to 8 with 1 M NaOH solution. At the time of sampling, the water in the lake had a salinity of 16.7 %, a temperature of 21.8 °C and a pH of 8.5. Strain SH-6^T grew at a temperature range of 28–45 °C (optimum 37 °C) and a pH range of 6.0–11 (optimum pH 7.5–8). Routine cultivation was conducted at 37 °C and pH 8. Growth ranges and optima for NaCl and MgCl₂ were determined by using the growth medium containing various concentrations of NaCl (0.9–5.2 M) and MgCl₂ (0–0.5 M), respectively. Strain SH-6^T was capable of growing over a wide range of NaCl concentrations: 2.5 M (15 %) to 5 M (30 %). It grew optimally in the presence of 4.3 M (25 %) NaCl, as has been shown for most extremely halophilic archaea. 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). Cell motility and morphology were observed under a phase-contrast light microscope (Olympus BX41) from an early exponential phase liquid culture. Cells of strain SH-6^T were non-motile and pleomorphic, although long rod-shaped cells were most common (Fig. 1). Rod-shaped cells were 3–13 × 0.5–1 µm in size. Colony morphology was observed on agar medium under optimal growth conditions after incubation at 37 °C for 10 days. Anaerobic growth was tested in the presence of 5 g nitrate or L-arginine l⁻¹ in filled, stoppered tubes. Tests for catalase and oxidase activities and hydrolysis of starch, casein, gelatin and Tween 80 were performed as described by Gonzalez *et al.* (1978). Nitrate reduction, H₂S formation, indole formation and the utilization of sugars, alcohols, amino acids and organic acids were carried out as described by Oren *et al.* (1997). Antibiotic sensitivity tests were performed by spreading bacterial suspensions on culture plates and applying discs impregnated with the following antibiotics (amounts in parentheses): ampicillin (10 µg), bacitracin (10 U), cephalothin (30 µg), chloramphenicol

Fig. 1. Phase-contrast micrograph of cells of strain SH-6^T grown in liquid medium under optimum conditions. Bar, 10 µm.

(30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), neomycin (10 µg), novobiocin (30 µg), penicillin G (10 U), rifampicin (30 µg), polymyxin (300 U), streptomycin (10 µg), sulfamethoxazole (25 µg), tetracycline (30 µg) and vancomycin (30 µg). The physiological and biochemical characteristics and antibiotic susceptibility of strain SH-6^T are given in the species description below.

Polar lipids were extracted with chloroform/methanol as described by Kamekura (1993). TLC was performed by using Merck HPTLC silica gel 60 plates (Art. 5641) in the solvent system, which comprised chloroform/methanol/acetic acid/water (85:22.5:10:4, by vol.). Glycolipids were detected as purple spots by spraying with 0.5 % α -naphthol in methanol/water (1:1) and then with sulfuric acid/ethanol (1:1), followed by heating at 160 °C. TLC of polar lipids (see Supplementary Fig. S1 available in IJSEM Online) suggested that strain SH-6^T contained phosphatidylglycerol and phosphatidylglyceromethylphosphate derived from both C₂₀C₂₀ and C₂₀C₂₅ glycerol diethers as shown from the two spots (Xin *et al.*, 2000). The glycolipid S₂-DGD-1 was also detected.

Chromosomal DNA of strain SH-6^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) by using the equation of Owen & Hill (1979). The DNA G + C content of strain SH-6^T was 63.1 mol%. The 16S rRNA gene of strain SH-6^T was amplified by PCR by using three universal primers as described by Lopez-Garcia *et al.* (2001) and Arahall *et al.* (1996) and the near-full-length nucleotide sequence (approximately 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. Comparison of the sequence with members of the family *Halobacteriaceae* based on the neighbour-joining method (Saitou & Nei, 1987) revealed that strain SH-6^T was distantly related to the other haloarchaeal genera investigated (Fig. 2). The type strains of *Natronolimnobius innermongolicus* and *Natronolimnobius baerhuensis* were shown to be the closest relatives of strain SH-6^T, having a 16S rRNA sequence similarity value of 94.6%. However, the similarity values were almost identical for several aerobic, extremely halophilic members of the *Archaea* representing various genera, e.g. the type strains of *Natrialba aegyptiaca* (94.5%), *Natronorubrum tibetense* (93.5%) and *Natronococcus occultus* (94.1%). Similar topologies were obtained when other treeing methods (maximum-parsimony and maximum-likelihood) were used. In consequence, it was concluded that strain SH-6^T formed a new distinct branch related to *Natronolimnobius innermongolicus* and *Natronolimnobius baerhuensis*. The phenotypic characteristics of these two species of the genus *Natronolimnobius* are very different from those of strain SH-6^T, i.e. their morphology (Fig. 1), optimum salinity and pH for growth, indole production, their ability to use carbon sources such as D-raffinose, D-arabinose, glycerol, fumarate and L-glutamate and their susceptibility to antibiotics (Table 1). Additionally, members of *Natronolimnobius* do not have detectable amounts of glycolipids, while strain SH-6^T contains the glycolipid S₂-DGD-1. It is worth noting that polar lipid composition has been found to be an excellent taxonomic marker for the delineation of haloarchaeal genera (Oren *et al.*, 1997; Grant *et al.*, 2001).

The phenotypic, polar lipid composition and phylogenetic data (based on 16S rRNA gene sequence comparisons) indicate that strain SH-6^T represents a novel species in a new genus within the haloarchaea, for which we propose the name *Halopiger xanaduensis* gen. nov., sp. nov. Table 1 details the characteristics that differentiate the new genus from other related haloarchaeal genera.

Description of *Halopiger* gen. nov.

Halopiger (Ha.lo.pi'ger. Gr. n. *hals*, *halos* salt; L. masc. adj. *piger* lazy; N.L. masc. n. *Halopiger* lazy halophile, referring to the slow growth under laboratory conditions).

Gram-negative. Cells are pleomorphic although most are long rods. Colonies are red pigmented. Strictly aerobic; oxygen is used as the terminal electron acceptor. Growth occurs at pH 6.0–11.0, at 28–45 °C and at 2.5–5.0 M (15–30%) NaCl. Optimal growth occurs at pH 7.5–8.0, 37 °C and 4.3 M (25%) NaCl. The DNA G + C content of the only species in the genus is 63.1 mol% (*T_m* method). Polar lipids include phosphatidylglycerol and phosphatidylglyceromethylphosphate derived from both C₂₀C₂₀ and C₂₀C₂₅ glycerol diethers and the glycolipid S₂-DGD-1. Isolated from salt lakes. Phylogenetically affiliated to the *Halobacteriaceae*. The type species is *Halopiger xanaduensis*. Recommended three-letter abbreviation of the genus: *Hpg*.

Description of *Halopiger xanaduensis* sp. nov.

Halopiger xanaduensis (xa.na.du.en'sis. N.L. masc. adj. *xanaduensis* referring to Xanadu, the lost city of Kublai Khan, located in Inner Mongolia, from where the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.5–1.0 µm wide and 3.0–13.0 µm long (Fig. 1). Colonies are circular, 1–2 mm in diameter after incubation for 10 days at 37 °C. Extremely halophilic. Cells lyse in water. Magnesium is not required. Growth does not occur above 45 °C. Amino acids are not required for growth. Catalase- and oxidase-positive. Anaerobic growth with arginine does not occur. Production of indole and methyl red, Voges–Proskauer and Simmons' citrate tests are negative. H₂S is not produced from cysteine. Acid is produced from D-arabinose, D-glucose and D-xylose, but not from D-fructose, D-galactose, glycerol, lactose,

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence comparisons, showing the position of strain SH-6^T and some other related haloarchaeal species. Accession numbers of the sequences used in this study are shown in parentheses after the strain designation. Bar, 1% sequence divergence.

Table 1. Characteristics that distinguish strain SH-6^T from members of other related haloarchaeal genera

Data were taken from Castillo *et al.* (2006a, b), Grant *et al.* (2001), Tindall (1992), Oren (2006), Itoh *et al.* (2005) and this study. +, Positive; –, negative; ND, no data available; V, variable.

Characteristic	Strain SH-6 ^T	<i>Natronolimnobius</i>	<i>Natrialba</i>	<i>Natronococcus</i>	<i>Natronobacterium</i>	<i>Natrinema</i>	<i>Halostagnicola</i>	<i>Halovivax</i>
Morphology	Rods/pleomorphic rods	Rods/pleomorphic rods	Rods	Cocci	Rods	Rods/pleomorphic rods	Rods/pleomorphic rods	Rods/pleomorphic rods
Cell size (µm)	3–13 × 0.5–1	1.5–6 × 0.5–0.6	0.5–1 × 1–5	1–2	0.5–0.7 × 10–15	0.6–1 × 1–6	0.5–1 × 1–3	0.4–0.5 × 4–5
Pigmentation	Red	Red	White/red	Red	Red	Light red to pale orange	Pink	Pale pink
NaCl optimum (M)	4.3	3.4	3.5–4	2.5–3.8	3.0	3.4–4.3	3.4	3.4
NaCl range (M)	2.5–5.0	1.7–4.3	2.0–5.2	1.4–5.2	2.0–5.2	ND	2.5–5.0	2.5–4.3
pH optimum	8.0	9	6.6–9.5	9.0–9.5	9.5	7.2–7.6	7.0–8.0	7.0–7.5
Temperature optimum (°C)	37	37–45	35–40	35–45	37	37–40	37	37
Nitrite from nitrate	–	V	+	+	–	+	+	–
Hydrolysis of:								
Starch	–	–	V	V	–	–	+	–
Gelatin	+	V	+ /ND	V	+	+	–	+
Casein	–	–	+ /ND	ND	ND	ND	–	+
Tween 80	+	V	ND	ND	ND	ND	–	+
DNA	–	ND	ND	ND	ND	ND	–	–
Indole production	–	+	ND	ND	ND	V	+	–
Urease	+	ND	ND	ND	ND	ND	–	+
H ₂ S production	–	V	V	ND	V	V	+	–
Major glycolipids	S ₂ -DGD-1	–	–/S ₂ -DGD-1	–	–	Unidentified	Unidentified	Unidentified
DNA G+C content (mol%)	63.1	59–63	60.0–63.1	63.5–64	65.0	69.9	61.0	60.3

maltose, D-mannitol, sucrose or D-trehalose. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are not produced. Urea, Tween 80, gelatin and aesculin are hydrolysed, whereas starch, casein, DNA and phosphatase are not. Nitrate and nitrite are reduced with gas production. The following substrates are utilized as sole carbon and energy sources: D-galactose, D-glucose, D-xylose, L-asparagine, L-serine, acetate and L-glutamate. No growth on D-arabinose, D-fructose, lactose, maltose, D-mannose, D-raffinose, D-ribose, starch, D-trehalose, glycerol, D-sorbitol, D-mannitol, glycine, isoleucine, L-lysine, L-threonine, fumarate, malate, propionate or succinate. Susceptible to bacitracin (10 U), novobiocin (30 µg) and sulfamethoxazole (25 µg). Resistant to ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), neomycin (10 µg), penicillin G (10 U), rifampicin (30 µg), polymyxin (300 U), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg). The G+C content of the DNA is 63.1 mol% (T_m).

The type strain, SH-6^T (=CECT 7173^T=CGMCC 1.6379^T=JCM 14033^T), was isolated from Shangmatala salt lake, Inner Mongolia, China.

Acknowledgements

This study was supported by grants from the Quality of Life and Management of Living Resources Programme of the European Commission (Project 'Multigenome Access Technology for Industrial Catalysts', QLK3-CT-2002-01972), Spanish Ministerio de Educación y Ciencia (BMC2003-01344 and BIO2006-06927) and the Junta de Andalucía.

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. (2006a). *Halovivax asiaticus* gen. nov., sp. nov., a novel extremely halophilic archaeon isolated from Inner Mongolia, China. *Int J Syst Evol Microbiol* **56**, 765–770.
- Castillo, A. M., Gutiérrez, M. C., Kamekura, M., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2006b). *Halostagnicola larsenii* gen. nov., sp. nov., an extremely halophilic archaeon from a saline lake in Inner Mongolia, China. *Int J Syst Evol Microbiol* **56**, 1515–1517.
- Gibbons, N. E. (1974). Family V. *Halobacteriaceae* fam. nov. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 269–273. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- Gonzalez, C., Gutierrez, C. & Ramirez, C. (1978). *Halobacterium vallismortis* sp. nov. An amylolytic 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 Bacteriol* **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. & Dyal-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., Dyal-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.
- 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., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & 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 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.
- Montalvo-Rodriguez, 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. (2006). The order *Halobacteriales*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 3, pp. 113–164. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer.
- Oren, A., Gurevich, P., Gemmell, R. T. & Teske, A. (1995). *Halobaculum gomorense* 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 *Haloferrax* gen. nov. *Syst Appl Microbiol* **8**, 89–99.
- Ventosa, A. (2006).** Unusual micro-organisms from unusual habitats: hypersaline environments. In *Prokaryotic Diversity: Mechanisms and Significance* (Society for General Microbiology Symposium no. 66), pp. 223–253. Edited by N. A. Logan, H. M. Lappin-Scott & P. C. F. Oyston. Cambridge: Cambridge University Press.
- Ventosa, A., Gutiérrez, M. C., Kamekura, M. & Dyall-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. 241–242. Edited by F. M. Ausubel, R. Bent, 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.
- 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.
- 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.