Haloarchaeobius iranensis gen. nov., sp. nov., an extremely halophilic archaeon isolated from a saline lake

A. Makhdoumi-Kakhki,¹ M. A. Amoozegar,^{1,2} M. Bagheri,² M. Ramezani² and A. Ventosa³

Correspondence

M. A. Amoozegar
amoozegar@ibrc.ir or
amoozegar@khayam.ut.ac.ir

¹Extremophile Laboratory, Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran

²Microorganisms Bank, Iranian Biological Resource Centre (IBRC), ACECR Tehran, Iran

³Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Sevilla, Spain

Strain EB21^T was isolated from a brine sample from Aran-Bidgol salt lake, a saline playa in Iran. Strain EB21^T was an orange–red-pigmented, motile rod and required at least 2 M NaCl but not MgCl₂ for growth. Optimal growth was achieved at 3.5 M NaCl and 0.2 M MgCl₂. The optimum pH and temperature for growth were pH 7.5 and 40 °C, while it was able to grow at pH 6.0–8.0 and 25–55 °C. Analysis of the 16S rRNA gene sequence revealed that strain EB21^T is a member of the family *Halobacteriaceae*, showing low levels of similarity to other members of the family. The highest sequence similarities, 91.8, 91.7 and 91.5%, were obtained with the 16S rRNA gene sequences of the type strains of *Halobiforma lacisalsi*, *Haloterrigena thermotolerans* and *Halalkalicoccus tibetensis*, respectively. Polar lipid analyses revealed that strain EB21^T contains phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulfate. Three unidentified glycolipids and one minor phospholipid were also observed. The only quinone present was MK-8(II-H₂). The G + C content of its DNA was 67.7 mol%. On the basis of the data obtained, the new isolate could not be classified in any recognized genus. Strain EB21^T is thus considered to represent a novel species in a new genus within the family *Halobacteriaceae*, order *Halobacteriales*, for which the name *Haloarchaeobius iranensis* gen. nov., sp. nov. is proposed. The type strain of *Haloarchaeobius iranensis* is EB21^T (=IBRC-M 10013^T =KCTC 4048^T).

The family *Halobacteriaceae* (order *Halobacteriales*, domain *Archaea*) contains micro-organisms that need at least 1.5 M NaCl for growth. They are rods, cocci or pleomorphic cells including flat discs, triangles and squares that occur ubiquitously in nature where the salt concentration is high, i.e. in salt lakes, soda lakes, salterns, etc. (Grant *et al.*, 2001). The classification of taxa belonging to this family is currently based on a polyphasic approach including phenotypic features, chemical data (polar lipid composition) and genotypic data (16S rRNA gene sequence analysis and DNA–DNA hybridization) (Oren *et al.*, 1997). At the time of writing (February 2011), members of this family are classified

within 32 different genera, with a large number of species (<http://www.the-icsp.org/taxa/halobacterlist.htm>).

Here we describe a halophilic archaeal strain, designated EB21^T, which was isolated from a salt lake located in the central desert of Iran. 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 EB21^T by using a polyphasic taxonomic characterization that combined phenotypic, chemotaxonomic, phylogenetic and genotypic analyses. These results indicate that strain EB21^T should be placed in a new genus as a representative of a novel species.

Sampling was carried out in November 2007 from Aran-Bidgol salt lake (34° 26' N 51° 48' E), a playa that can be considered as thalassohaline, with NaCl as the major salt. The lake water is neutral (about pH 7.0) and its salinity reaches saturation. For isolation, modified growth medium

Abbreviation: PGS, phosphatidylglycerol sulfate.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain EB21^T are JF293278 (*rrnA*) and JF293279 (*rrnB*).

Four supplementary figures are available with the online version of this paper.

(MGM) with 23 % total salt concentration, as described in the *Halohandbook* online protocol (Dyall-Smith, 2006), was used. This medium contains a 23 % salt mixture, prepared from 30 % stock solution, which consists of (g l⁻¹): NaCl, 240; MgSO₄ · 7H₂O, 35; MgCl₂ · 6H₂O, 30; KCl, 7; and CaCl₂ · 2H₂O, 1; supplemented with 1 % (w/v) peptone (Merck) and 0.2 % (w/v) yeast extract (Merck). Agar (1.5 % w/v) was used to solidify media if necessary. The medium was adjusted to pH 7.2–7.4 with 2 M Tris base (Merck). Samples were cultured in 23 % MGM solid medium after preparing the appropriate dilutions in the laboratory. Inoculated plates were incubated at 40 °C for up to 2 months. After successive cultivations, a pure isolate designated strain EB21^T was obtained. The characterization of this strain was achieved by following the minimal standards recommended by Oren *et al.* (1997) for describing novel taxa of the order *Halobacteriales*. The type strains *Haloferax volcanii* DSM 3754^T, *Halobiforma haloterrestis* DSM 13078^T and *Haloterrigena thermotolerans* DSM 11552^T were selected as reference strains in positive and negative testing.

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 used directly without fixing. Colony morphology was observed on agar medium under optimal growth conditions after incubation at 40 °C for 14 days. The Gram reaction was determined by following the method outlined by Dussault (1955). Strain EB21^T was motile and rod-shaped (Fig. S1, available in IJSEM Online) and stained Gram-negative. Colonies formed on agar plates were small (about 1.0–2.0 mm diameter), convex, round with an entire edge and orange–red-pigmented.

Physiological tests were conducted using liquid or solid (1.5 % agar) MGM medium unless stated otherwise. Liquid cultures were incubated at 40 °C on a shaking incubator at 200 r.p.m. Growth rates were determined by monitoring the increase in OD₆₀₀. The temperature range for growth was examined in liquid MGM medium at 20–60 °C at 5 °C intervals. Growth at pH 5.0–9.0 was tested; the buffers MES (pH 5–6.5), HEPES (pH 7–8) and CHES (pH 8.5–9) were added at a concentration of 50 mM. The requirements for NaCl and MgCl₂ for growth were determined in media containing 0–5 M NaCl (0.5 M increments) or 0–1 M MgCl₂ (0.05 M increments), respectively.

Strain EB21^T grew at 25–55 °C (optimum 40 °C) and pH 6–8 (optimum pH 7.5). Routine cultivation was conducted at 40 °C and pH 7.5. Strain EB21^T was capable of growing over a wide range of NaCl concentration: 2–5 M (12–30 %). It grew optimally in the presence of 3.5 M (20 %) NaCl, in the range that has been shown for most extremely halophilic archaea (Grant *et al.*, 2001). MgCl₂ was not required for growth, but optimal growth occurred in the presence of 0.2 M MgCl₂.

Acid production from substrates was tested in unbuffered MGM medium and was determined by measuring the

initial and final pH of the medium. The culture was considered positive for acid production if the pH decreased by at least 1 pH unit. To test for carbon-source utilization, peptone was omitted from the MGM medium and the concentration of yeast extract was reduced to 0.1 g l⁻¹ (Oren *et al.*, 1997). The ability of strain EB21^T to grow anaerobically in the presence of DMSO (5.0 g l⁻¹) and to ferment arginine (5.0 g l⁻¹) was tested in MGM medium prepared anaerobically in serum tubes according to the procedures described by Bryant (1972) and Balch & Wolfe (1976). Growth and gas formation with nitrate as electron acceptor were tested in 10 ml stoppered tubes, filled completely with liquid growth medium to which NaNO₃ (5 g l⁻¹) had been added, and containing an inverted Durham tube (Oren *et al.*, 1997). Tween hydrolysis activity was detected as described by Gutiérrez & González (1972). Casein, gelatin and starch hydrolysis was determined as described by Oren *et al.* (1997). Tests for catalase and oxidase activities were performed as described by González *et al.* (1978). Production of H₂S was tested by growing strain EB21^T in MGM liquid medium supplemented with 0.5 % (w/v) Na₂S₂O₃ (Oren *et al.*, 1997). Tryptone water medium supplemented with 23 % total salts was used for detection of indole production (Smibert & Krieg, 1994). Susceptibility to antimicrobial compounds was determined by the disc diffusion method after spreading the strain on solid MGM medium (Oren *et al.*, 1997).

Strain EB21^T was catalase- and oxidase-positive. It could hydrolyse gelatin and Tweens 20 and 40, but Tweens 60 and 80 and starch could not be hydrolysed. Strain EB21^T utilized D-fructose, D-galactose, D-glucose, maltose and lactose. Acid was produced from D-glucose and maltose but not from D-galactose, sucrose, D-fructose, D-xylose, D-ribose, D-mannitol, D-mannose, trehalose, L-arabinose or lactose. The detailed physiological and biochemical characteristics of strain EB21^T are listed in Table 1 and in the genus and species descriptions.

Genomic DNA of strain EB21^T was extracted as described by Wan Lam in the *Halohandbook* (Dyall-Smith, 2006) and the 16S rRNA gene was amplified using the archaeal universal primers 21F (5'-TTCCGGTTGATCCYGCC-GGA-3'; DeLong, 1992) and 1492R (5'-GGTACCTTGT-TACGACTT-3'; Lane *et al.*, 1985). PCR products were purified with a DNA purification kit (Roche), according to the manufacturer's protocol. Purified PCR products were then electrophoresed on a 1 % agarose gel to check their quality. Ligation of the PCR products with the pGEM-T vector, transformation of *Escherichia coli* DH5α and selection of transformants were carried out with a pGEM-T TA cloning kit (Promega), used according to the manufacturer's protocol. Multiple clones were picked randomly and then sequenced by the service of Macrogen (Seoul, South Korea) to determine whether the strain possessed multiple distinct 16S rRNA genes. Phylogenetic analysis was performed using the software package MEGA version 4 (Tamura *et al.*, 2004) after obtaining multiple alignments of data available from public databases by

Table 1. Differential characteristics between strain EB21^T and closely related genera within the order *Halobacteriales*

Taxa: 1, strain EB21^T (*Haloarchaeobius iranensis* gen. nov., sp. nov.); 2, *Halobiforma*; 3, *Haloterrigena*; 4, *Halalkalicoccus*; 5, *Natrialba*; 6, *Natronococcus*; 7, *Halobacterium*. Data were derived from this study and Hezayan *et al.* (2002), Ventosa *et al.* (1999), Xue *et al.* (2005), Kamekura & Dyll-Smith (1995) and Grant *et al.* (2001).

Characteristic	1	2	3	4	5	6	7
Cell shape	Rod	Coccus or rod	Coccus	Coccus	Coccobacillus or rod	Coccus	Small rod
Cell size (µm)	1.5–2.0 × 3–7	1.25–2.0 (coccus) or 0.5–1.5 × 2–8 (rod)	1.5–2.0	1.0–1.5	0.5–0.7 × 1–3	1.0–2.0	0.5–1.0 × 1.0–6.0
Pigmentation	Orange–red	Red	Red	Orange	Not pigmented	Pale brown	Red
Motility	+	+	–	–	+	–	+
Lysis in distilled water	+	– (coccus) or + (rod)	–	–	+	–	+
NaCl concentration for growth (M)							
Optimum	3.5	2.6–4.3	3–4	3.4	4	2.5–3.6	3.4–4.3
Range	2.0–5.0	>2.2	>2.0	1.4–5.2	2.0–5.2	1.4–5.2	3.0–5.2
Mg ²⁺ requirement	–	–	+	–	–	+	+
Optimum temperature (°C)	40	42–45	45	40	30–40	35–45	50
Growth at pH 7	+	+	+	–	+	–	+
Hydrolysis of gelatin	+	+	–	–	+	+	+
Indole production	–	+	–	–	+	–	+
Presence of PGS	+	–	–	–	–	–	+
Minor phospholipid(s)	PL1	–	–	–	–	PL1, PL2	–
DNA G + C content (mol%)*	67.7	66.9	59.8	61.5	62.3	61.2	67.1
16S rRNA gene sequence similarity to EB21 ^T (%)	(100)	<92.0	<91.9	<91.8	<91.4	<91.2	<89.2

*Values for the type strain of the type species.

CLUSTAL_X (Thompson *et al.*, 1997). Clustering was performed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and minimum-evolution (Rzhetsky & Nei, 1992) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

Fifteen almost-complete 16S rRNA gene sequences (1441 nt) were obtained from strain EB21^T. Sequence comparisons indicated that the strain has two rRNA genes, *rrnA* and *rrnB*, which have 98.4% sequence similarity to each other. 16S rRNA gene sequence analysis showed that strain EB21^T is a member of the family *Halobacteriaceae*, but there were low similarities to type species of other members of this family; the most closely related haloarchaeal taxa were *Halobiforma lacisalsi* AJ5^T (<92.0% similarity), *Haloterrigena thermotolerans* PR5^T (<91.9%) and *Halalkalicoccus tibetensis* DS12^T (<91.8%). Phylogenetic analysis using the neighbour-joining algorithm revealed that strain EB21^T clustered in a separate clade (Fig. 1); this phylogenetic position was also confirmed by trees generated using the minimum-evolution and maximum-parsimony algorithms (Figs S2 and S3).

The DNA G+C content was determined by the HPLC method (Mesbah *et al.*, 1989). The DNA G+C content of strain EB21^T was 67.7 mol%, which is within the range described for the family *Halobacteriaceae* (Grant *et al.*, 2001).

The polar lipid composition and respiratory quinones were determined using the services of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) and Dr Brian Tindall (DSMZ). Polar lipids were separated by two-dimensional silica gel TLC. Methods including solvents and detection reagents were described by Hezayen *et al.* (2001). Phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulfate (PGS) were present. Three glycolipids yet to be identified and one minor phospholipid were also observed

(Fig. S4). The presence of PGS in this novel archaeon allows it to be differentiated from phylogenetically related members of the *Halobacteriaceae*: *Halobiforma*, *Haloterrigena*, *Halalkalicoccus*, *Natrialba* and *Natronococcus*, which do not contain PGS (Hezayen *et al.*, 2002; Ventosa *et al.*, 1999; Xue *et al.*, 2005; Kamekura & Dyll-Smith, 1995; Grant *et al.*, 2001). Respiratory lipoquinones were determined as described previously (Wainø *et al.*, 2000); MK-8(II-H₂) was the only quinone present.

In conclusion, the morphological and physiological properties of the new isolate, the low levels of 16S rRNA gene sequence similarity with other genera within the family *Halobacteriaceae* and distinctive polar lipids suggest that strain EB21^T represents a novel species of a new genus within the family *Halobacteriaceae*, for which the name *Haloarchaeobius iranensis* gen. nov., sp. nov. is proposed.

Description of *Haloarchaeobius* gen. nov.

Haloarchaeobius [Ha.lo.ar.chae.o'bi.us. Gr. n. *hals*, *halos* salt; N.L. adj. *archaeos* from Gr. adj. *archaios* ancient; N.L. masc. n. *bios* from Gr. masc. n. *bios* life; N.L. masc. n. *Haloarchaeobius* halophilic ancient (archaeal) life].

Cells are motile rods. Gram-negative. Colonies are pigmented orange-red. Strictly aerobic; oxygen is used as the terminal electron acceptor. Growth occurs at pH 6.0–8.0, 25–50 °C and 2.0–5.0 M (12–30%) NaCl. MgCl₂ is not required. Optimal growth occurs at pH 7.5, 40 °C, 3.5 M (20%) NaCl and 0.2 M MgCl₂. Extremely halophilic. Polar lipids include phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate, three unidentified glycolipids and one minor phospholipid. MK-8(II-H₂) is the only respiratory lipoquinone present. The DNA G+C content of the type strain of the only species in the genus is 67.7 mol% (HPLC method). Affiliated phylogenetically to the *Halobacteriaceae*. The type species is *Haloarchaeobius iranensis*. Recommended three-letter abbreviation: *Hab*.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain EB21^T and close relatives within the family *Halobacteriaceae*. Accession numbers are given in parentheses. The sequence of the methanogenic archaeon *Methanospirillum hungatei* JF-1^T was used as an outgroup. Bootstrap values (%) are based on 1000 replicates. Bar, 0.02 substitutions per nucleotide position.

Description of *Haloarchaeobius iranensis* sp. nov.

Haloarchaeobius iranensis (i.ra.nen'sis. N.L. masc. adj. *iranensis* of or belonging to Iran, referring to the isolation of the type strain).

Exhibits the following properties in addition to those given in the genus description. Cells are $1.5\text{--}2.0 \times 3\text{--}7\ \mu\text{m}$ when grown in MGM liquid medium under optimum conditions. Colonies are small (about 1.0–2.0 mm diameter). Cells lyse in water. Catalase- and oxidase-positive. Does not grow under anaerobic conditions with nitrate, arginine or DMSO. Nitrate reduction to nitrite and gas formation from nitrate are not observed. Gelatin and Tweens 20 and 40 are hydrolysed, whereas Tweens 60 and 80, casein, starch and DNA are not. Indole is not produced from tryptophan. Acid is produced from D-glucose and maltose but not from L-arabinose, D-fructose, sucrose, D-mannitol, D-galactose, lactose or D-ribose. H_2S is not produced from thiosulfate. Does not produce arginine dihydrolase, lysine decarboxylase or ornithine decarboxylase. Methyl red and Voges–Proskauer tests are negative. D-Fructose, D-glucose, D-galactose, maltose, lactose, L-asparagine, L-glycine, L-proline, L-phenylalanine and L-tyrosine are utilized as sole source of carbon and energy, while L-arabinose, sucrose D-mannitol, D-ribose, L-arginine, L-methionine, L-aspartic acid, L-cysteine and L-valine are not. Susceptible to the following antimicrobial compounds: anisomycin (35 μg), bacitracin (10 U), nitrofurantoin (300 μg), novobiocin (5 μg) and rifampicin (5 μg). Resistant to amoxicillin (25 μg), ampicillin (10 μg), cephalotin (30 μg), carbenicillin (100 μg), cefoxitin (30 μg), chloramphenicol (30 μg), erythromycin (5 μg), gentamicin (10 μg), kanamycin (5 μg), nalidixic acid (30 μg), neomycin (30 μg), penicillin G (10 U), polymyxin B (100 U), streptomycin (10 μg), tetracycline (30 μg) and tobramycin (10 μg).

The type strain, EB21^T (=IBRC-M 10013^T =KCTC 4048^T), was isolated from Aran-Bidgol salt lake, Iran.

Acknowledgements

We thank J. Euzéby for his help with the etymology of the new names. This work was supported by grants from the Iranian Biological Resource Center (IBRC) (MI-1388-01) and the International Foundation for Science (IFS) (A/4527-1).

References

- Balch, W. E. & Wolfe, R. S. (1976). New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Appl Environ Microbiol* **32**, 781–791.
- Bryant, M. P. (1972). Commentary on the Hungate technique for culture of anaerobic bacteria. *Am J Clin Nutr* **25**, 1324–1328.
- DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* **89**, 5685–5689.
- Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.

- Dyall-Smith, M. L. (2006). *The Halo handbook: Protocols for Haloarchaeal Genetics*. <http://www.haloarchaea.com/resources/halo handbook>
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using bootstrap. *Int J Org Evol* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- González, C., Gutiérrez, C. & Ramírez, C. (1978). *Halobacterium vallismortis* sp. nov. An amyolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* **24**, 710–715.
- 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.
- Gutiérrez, C. & González, C. (1972). Method for simultaneous detection of proteinase and esterase activities in extremely halophilic bacteria. *Appl Microbiol* **24**, 516–517.
- Hezayen, F. F., Rehm, B. H. A., Tindall, B. J. & Steinbüchel, A. (2001). Transfer of *Natrialba asiatica* BIT to *Natrialba taiwanensis* sp. nov. and description of *Natrialba aegyptiaca* sp. nov., a novel extremely halophilic, aerobic, non-pigmented member of the Archaea from Egypt that produces extracellular poly(glutamic acid). *Int J Syst Evol Microbiol* **51**, 1133–1142.
- Hezayen, F. F., Tindall, B. J., Steinbüchel, A. & Rehm, B. H. A. (2002). Characterization of a novel halophilic archaeon, *Halobiforma haloterrestis* gen. nov., sp. nov., and transfer of *Natronobacterium nitratireducens* to *Halobiforma nitratireducens* comb. nov. *Int J Syst Evol Microbiol* **52**, 2271–2280.
- Kamekura, M. & Dyall-Smith, M. L. (1995). Taxonomy of the family *Halobacteriaceae* and description of two new genera *Halorubrobacterium* and *Natrialba*. *J Gen Appl Microbiol* **41**, 333–350.
- Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci U S A* **82**, 6955–6959.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- 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.
- Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* **9**, 945–967.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2004). MEGA4: molecular evolutionary genetics analysis MEGA software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. J. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**, 4876–4882.
- 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.

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.

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.