Halorubrum aquaticum sp. nov., an archaeon isolated from hypersaline lakes

M. C. Gutiérrez,¹ A. M. Castillo,¹ P. Corral,¹ M. Kamekura² and A. Ventosa¹

Correspondence

M. C. Gutiérrez

mcg@us.es

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

²Halophiles Research Institute, 677-1 Shimizu, Noda-shi, Chiba-ken 278-0043, Japan

Two halophilic archaea, strains EN-2^T and SH-4, were isolated from the saline lakes Erliannor and Shangmatale, respectively, in Inner Mongolia, China. Cells were strictly aerobic, motile rods. Colonies were red. Strains EN-2^T and SH-4 were able to grow at 25–50 °C (optimum 35–40 °C), with 2.5–5.0 M NaCl (optimum 3.4 M NaCl) and at pH 6.0–9.0 (optimum pH 7.5). MgCl₂ was not required for growth. Cells lysed in distilled water and the lowest NaCl concentration that prevented cell lysis was 12 % (w/v). On the basis of 16S rRNA gene sequence analysis, strains EN-2^T and SH-4 were closely related to *Halorubrum cibi* B31^T (97.9 and 98.0 % similarity, respectively), *Hrr. tibetense* 8W8^T (97.3 and 97.7 %), *Hrr. alkaliphilum* DZ-1^T (96.8 and 97.1 %), *Hrr. luteum* CGSA15^T (96.8 and 97.0 %) and *Hrr. lipolyticum* 9-3^T (96.8 and 97.0 %). DNA–DNA hybridization showed that strains EN-2^T and SH-4 did not belong to the same species as any of these strains (≤45 % DNA–DNA relatedness) but that they are members of the same species (>70 % DNA–DNA relatedness). Polar lipid analysis revealed that strains EN-2^T and SH-4 contained phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, sulfated diglycosyl diethers and several unidentified glycolipids. The DNA G + C content of both isolates was 62.1 mol%. It was concluded that strains EN-2^T and SH-4 represent a novel species of the genus *Halorubrum*, for which the name *Halorubrum aquaticum* sp. nov. is proposed. The type strain is EN-2^T (=CECT 7174^T =CGMCC 1.6377^T =JCM 14031^T).

Members of the family *Halobacteriaceae*, the single recognized family within the order *Halobacteriales*, have long been known as the most abundant micro-organisms in hypersaline environments (Oren, 1994). At the time of writing, the family *Halobacteriaceae* comprises 27 genera (Oren *et al.*, 2009). The genus *Halorubrum* was established by McGenitty & Grant (1995) and currently contains 23 species: four haloalkaliphilic species, *Halorubrum vacuolatum* (Kamekura *et al.*, 1997), *Hrr. tibetense* (Fan *et al.*, 2004), *Hrr. alkaliphilum* (Feng *et al.*, 2005) and *Hrr. luteum* (Hu *et al.*, 2008), and 19 neutrophilic species, *Hrr. trapanicum* (Petter, 1931), *Hrr. saccharovorum* (Tomlinson & Hochstein, 1976), *Hrr. sodomense* (Oren, 1983), *Hrr. distributum* (Oren & Ventosa, 1996), *Hrr. lacusprofundi* (Franzmann *et al.*, 1988), *Hrr. coriense* (Kamekura & Dyall-Smith, 1995; McGenitty & Grant, 1995), *Hrr. tebenquichense* (Lizama *et al.*, 2002), *Hrr. terrestre* (Ventosa *et al.*, 2004), *Hrr. xinjiangense* (Feng *et al.*, 2004), *Hrr. ezzemoulense* (Kharroub *et al.*, 2006), *Hrr. lipolyticum* and *Hrr. aidingense* (Cui *et al.*, 2006), *Hrr. orientale* (Castillo *et al.*, 2006), *Hrr. arcis* (Xu *et al.*, 2007),

Hrr. litoreum (Cui *et al.*, 2007), *Hrr. ejinorensense* (Castillo *et al.*, 2007), *Hrr. kocurii* (Gutiérrez *et al.*, 2008), *Hrr. californiense* (Pesenti *et al.*, 2008) and *Hrr. cibi* (Roh & Bae, 2009).

In the present study, we characterized two strains, EN-2^T and SH-4, which were isolated from water samples of the saline lakes Erliannor (43° 44' N 112° 02' E) and Shangmatale (43° 12' N 114° 01' E), respectively, located in the Inner Mongolia Autonomous Region, China. At the time of sampling, the temperatures and pH of the lakes were 18.4 °C and 19.8 °C and pH 7.5 and 8.0, respectively. Water samples were plated on agar plates of halophilic medium (MH), containing (per litre distilled water) 195 g NaCl, 32.5 g MgCl₂·6H₂O, 50.8 g MgSO₄·7H₂O, 0.8 g CaCl₂, 5 g KCl, 0.16 g NaHCO₃, 0.6 g NaBr, 5 g yeast extract and 20 g agar (pH 7.5), and incubated at 37 °C for 1–2 weeks. Pure culture was obtained by repeated subcultivation on the same medium.

For comparative purposes, the following strains were obtained from the Japan Collection of Microorganisms (JCM) and the Spanish Type Culture Collection (CECT): *Hrr. cibi* JCM 15757^T, *Hrr. alkaliphilum* JCM 12358^T, *Hrr. lipolyticum* 9-3^T, *Hrr. luteum* CECT 7303^T, *Hrr. kocurii* JCM 14978^T and *Hrr. tibetense* JCM 11889^T. The *Hrr. cibi*,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EN-2^T is AM268115.

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

Hrr. kocurii and *Hrr. lipolyticum* strains were routinely cultivated on JCM medium no. 168, *Hrr. tibetense* JCM 11889^T and *Hrr. alkaliphilum* JCM 12358^T were cultivated on JCM medium no. 167 and *Hrr. luteum* CECT 7303^T was cultivated on CECT medium no. 248.

Phenotypic tests were performed according to the proposed minimal standards for the description of new taxa of the order *Halobacteriales* (Oren *et al.*, 1997). Motility and morphology of cells from exponentially growing liquid cultures were examined using an Olympus BX41 microscope equipped with phase-contrast optics. Cells of strains EN-2^T and SH-4 were motile rods (Supplementary Fig. S1, available in IJSEM Online). Colony morphology, colour and size were observed by growth on MH agar after incubation at 37 °C for 10 days. Growth with 0, 0.5, 1, 3, 5, 7, 10, 15, 20, 25 and 30 % (w/v) NaCl was determined on MH agar. Growth at pH 5.0–11.0 (at intervals of 0.5 pH unit) was determined in liquid MH with the pH readjusted after sterilization and growth was assessed by measuring the optical density at 600 nm. The temperature range for growth was assessed on MH agar at 15–55 °C (at intervals of 5 °C). Tests for catalase and oxidase, hydrolysis of starch, Tween 80, gelatin, casein, DNA and aesculin, reduction of nitrate and nitrite, formation of H₂S and indole and utilization of sugars, alcohols, amino acids and organic acids were carried out as described by Oren *et al.* (1997). Susceptibility to antibiotics was determined on MH agar using antibiotic discs containing (µg per disc unless otherwise stated): ampicillin (10) bacitracin (10 U), chloramphenicol (30), erythromycin (15), nalidixic acid (30), neomycin (30), novobiocin (30), rifampicin (5) and streptomycin (10). The physiological and biochemical characteristics as well as the antibiotic susceptibilities of strains EN-2^T and SH-4 are provided in the species description and Table 1.

Polar lipids of both isolates were extracted with chloroform/methanol as described previously (Kamekura, 1993) and separated by TLC using Merck HPTLC silica gel 60 plates (no. 5641) and 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. The polar lipid profiles of the isolates and the reference strains are shown in Supplementary Fig. S2. Strains EN-2^T and SH-4 contained phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, sulfated diglycosyl diethers and several unidentified glycolipids. Phosphatidylglycerol sulfate was not detected. This polar lipid profile is typical of neutrophilic species of the genus *Halorubrum* (McGenity & Grant, 2001).

Chromosomal DNA of strains EN-2^T and SH-4 was isolated and purified according to the method described by Marmur (1961). The G+C content of genomic DNA was determined from the mid-point (T_m) of the thermal denaturation profile (Marmur & Doty, 1962) using the

Table 1. Characters that differentiate *Halorubrum aquaticum* sp. nov. from other closely related species of the genus *Halorubrum*

Strains: 1, *Halorubrum aquaticum* sp. nov. EN-2^T and SH-4; 2, *Hrr. cibi* JCM 15757^T; 3, *Hrr. tibetense* JCM 11889^T (data for columns 1–3 from this study); 4, *Hrr. alkaliphilum* JCM 12358^T (data from Feng *et al.*, 2005 unless otherwise stated); 5, *Hrr. luteum* CECT 7303^T (Hu *et al.*, 2008); 6, *Hrr. lipolyticum* JCM 13559^T (Cui *et al.*, 2006); 7, *Hrr. saccharovororum* JCM 8865^T (Tomlinson & Hochstein, 1976); 8, *Hrr. kocurii* JCM 14978^T (this study). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Cell morphology*	RO	RO	RO	SR	PL	RO	RO	RO
Motility	–	+	–	+†	+	+	+	–
Colony colour‡	RE	RE	RE	PK†	O	RE	PK	RE
Mg ²⁺ requirement	–	+	–	–	–	+	+	–
Nitrate reduction	+	–	+	+†	+	+	+	+
Indole production	–	–	+	+	+	–	+	–
Oxidase	–	–	+	+	+	+	+	+
Starch hydrolysis	–	–	–	–	–	+	–	–
Tween 80 hydrolysis	+	–	+	–	–	+	–	–
Gelatin liquefaction	–	–	–	–	–	+	–	–
Utilization of:								
D-Fructose	+	–	–	+	–	+	–	–
D-Glucose	+	–	+	+	+	+	+	–
Lactose	+	+	+	–	+	–	+	–
D-Mannitol	–	–	+	–	–	–	–	–
Sucrose	+	–	+	–	–	+	+	–
Succinate	–	–	+	–	+	–	+	–
Presence of glycolipids	+	+	–	–	–	+	–	+
Sensitivity to (per disc):								
Bacitracin (10 U)	+	+	–	–	–	+	ND	+
Erythromycin (15 µg)	–	–	–	ND	+	–	+	–
Rifampicin (5 µg)	–	–	–	–	–	+	ND	+
DNA G+C content (mol%)	62.1	61.7	63.3	62.1	60.2	65.9	60.9	61.2

*PL, Pleomorphic; RO, rods; SR, short rods.

†Data obtained in this study.

‡O, Orange; PK, pink; RE, red.

equation of Owen & Hill (1979) as described previously (Ventosa *et al.*, 2004). The DNA G+C content of strains EN-2^T and SH-4 was 62.1 mol%. This value is within the range described for the genus *Halorubrum* (60.0–71.2 mol%; Grant *et al.*, 2001). The 16S rRNA genes of strains EN-2^T and SH-4 were sequenced by PCR using universal primers as described elsewhere (López-García *et al.*, 2001; Arahal *et al.*, 1996). The almost-complete 16S rRNA gene sequences of strains EN-2^T (1420 bp) and SH-4 (1381 bp) were determined. ARB software (Ludwig *et al.*, 2004) was used for sequence analysis. Following the

recommendations of Ludwig *et al.* (1998), phylogenetic trees were generated using alternative treeing methods (maximum parsimony, neighbour joining and maximum likelihood) (Saitou & Nei, 1987). Base-frequency filters were applied in the sequence comparison analysis and the effects on the results were evaluated. The topologies of the neighbour-joining and maximum-likelihood trees were similar to that of the maximum-parsimony tree (not shown). The identification of phylogenetic neighbours and calculation of pairwise sequence similarity were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The maximum-parsimony phylogenetic tree (Fig. 1) indicated that strains EN-2^T and SH-4 were closely related to *Hrr. cibi* B31^T (97.9 and 98.0% 16S rRNA gene sequence similarity, respectively), *Hrr. tibetense* 8W8^T (97.3 and 97.7%), *Hrr. alkaliphilum* DZ-1^T (96.8 and 97.1%), *Hrr. luteum* CGSA15^T (96.8 and 97.0%) and *Hrr. lipolyticum* JCM 13559^T (96.8 and 97.0%).

DNA–DNA hybridization between strains EN-2^T and SH-4 and the phylogenetically most closely related type strains of species of the genus *Halorubrum* was performed by the competition procedure of Johnson (1994), as described in detail by Gutiérrez *et al.* (2002). DNA–DNA relatedness between strains EN-2^T and SH-4 was 98 and 95% (reciprocal hybridizations). These values showed that the two isolates can be considered to represent the same species (Stackebrandt *et al.*, 2002). DNA–DNA relatedness between strains EN-2^T and SH-4 and *Hrr. cibi* JCM 15757^T, *Hrr. tibetense* JCM 11889^T, *Hrr. alkaliphilum* JCM 12358^T, *Hrr. luteum* CECT 7303^T and *Hrr. lipolyticum* JCM 13559^T was 38 and 27%, 45 and 39%, 37 and 43%, 23 and 30% and 25 and 18%, respectively. These levels of DNA–DNA relatedness are low enough to classify the two

isolates in a genotypically distinct species within the genus *Halorubrum*.

Differences in phenotypic characteristics (such as motility, oxidase, reduction of nitrate and nitrite, hydrolysis of different compounds and utilization of several substrates) (Table 1), polar lipid profiles and 16S rRNA gene sequences, together with the DNA–DNA hybridization data, justify the creation of a novel species within the genus *Halorubrum* to accommodate strains EN-2^T and SH-4 (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). The name *Halorubrum aquaticum* sp. nov. is proposed.

Description of *Halorubrum aquaticum* sp. nov.

Halorubrum aquaticum (a.qua'ti.cum. L. neut. adj. *aquaticum* living, growing or found in or by water, aquatic).

Cells are rods (1.0–1.2 × 2.0–6.0 µm). Non-motile. Colonies are circular, entire, smooth, red and 0.5–1.5 mm in diameter on MH agar after 7 days at 37 °C. Growth occurs with 2.5–5.0 M NaCl (optimum 3.4 M NaCl). MgCl₂ is not required. Strict aerobe. Growth occurs at 25–50 °C (optimum 35–40 °C) and pH 6.0–9.0 (optimum pH 7.5). Chemo-organotrophic. Oxidase-negative and catalase-positive. Nitrate and nitrite are reduced. Tween 80, urea and aesculin are hydrolysed, but casein, DNA, starch and gelatin are not. H₂S is not produced. Indole is not produced from tryptophan. Methyl red and Voges–Proskauer tests are negative. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are not produced. Anaerobic growth with nitrate or arginine does not occur. Acid is not produced from lactose, glycerol, D-glucose, D-fructose, D-arabinose, maltose, D-xylose,

Fig. 1. Maximum-parsimony phylogenetic tree based on 16S rRNA gene sequence comparison showing the relationships between strains EN-2^T and SH-4 and members of the genus *Halorubrum* and other archaea. Bootstrap values (>80%) based on 1000 replicates are shown at branch nodes. Bar, 5% sequence divergence.

D-galactose, trehalose or D-mannose. Growth occurs with glycerol, D-mannitol, D-fructose, lactose, maltose, sucrose, trehalose, D-glucose, D-arabinose, starch, L-glutamate and fumarate as single carbon and energy sources, but not with D-sorbitol, mannose, raffinose, D-ribose, D-xylose, succinate, propionate, malate or acetate. Growth occurs with glycine as a sole carbon, nitrogen and energy source, but not with L-asparagine, L-lysine, L-serine or L-threonine. Susceptible to (μg per disc unless otherwise stated) novobiocin (30), bacitracin (10 U), erythromycin (15) and streptomycin (10) and resistant to rifampicin (5), ampicillin (10), neomycin (30), chloramphenicol (30) and nalidixic acid (30). The polar lipids are phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, sulfated diglycosyl diethers and several unidentified glycolipids. The DNA G+C content of the type strain is 62.1 mol% (T_m).

The type strain, EN-2^T (=CECT 7174^T =CGMCC 1.6377^T =JCM 14031^T), was isolated from a saline lake, Lake Erliannor, in Inner Mongolia, China. Strain SH-4, from a similar source, is a second strain of the species.

Acknowledgements

This work was supported by grants from the Spanish Ministerio de Educación y Ciencia (grants no. BIO2009-10138 and CGL2010-19303), the National Science Foundation (grant no. DEB-0919290) and the Junta de Andalucía (grant no. P06-CVI-01829).

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., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2006). *Halorubrum orientale* sp. nov., a halophilic archaeon isolated from Lake Ejnor, Inner Mongolia, China. *Int J Syst Evol Microbiol* **56**, 2559–2563.
- Castillo, A. M., Gutiérrez, M. C., Kamekura, M., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2007). *Halorubrum ejnorense* sp. nov., isolated from Lake Ejnor, Inner Mongolia, China. *Int J Syst Evol Microbiol* **57**, 2538–2542.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Cui, H.-L., Tohty, D., Zhou, P.-J. & Liu, S.-J. (2006). *Halorubrum lipolyticum* sp. nov. and *Halorubrum aidingense* sp. nov., isolated from two salt lakes in Xin-Jiang, China. *Int J Syst Evol Microbiol* **56**, 1631–1634.
- Cui, H.-L., Lin, Z.-Y., Dong, Y., Zhou, P.-J. & Liu, S.-J. (2007). *Halorubrum litoreum* sp. nov., an extremely halophilic archaeon from a solar saltern. *Int J Syst Evol Microbiol* **57**, 2204–2206.
- 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.-J. & Liu, S.-J. (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.-G., Liu, S.-J. & 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.
- Franzmann, P. D., Stackebrandt, E., Sanderson, K., Volkman, J. K., Cameron, D. E., Stevenson, P. L., McMeekin, T. A. & Burton, H. R. (1988). *Halobacterium lacusprofundi* sp. nov., a halophilic bacterium isolated from Deep Lake, Antarctica. *Syst Appl Microbiol* **11**, 20–27.
- Grant, W. D., Kamekura, M., McGenity, T. J. & Ventosa, A. (2001). Order I. Halobacteriales Grant & Larsen 1989b, 495^{VP} (Effective publication: Grant & Larsen 1989a, 2216). In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 294–334. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Gutiérrez, M. C., Kamekura, M., Holmes, M. L., Dyal-Smith, M. L. & Ventosa, A. (2002). Taxonomic characterization of *Haloferax* sp. (“*H. alicantei*”) strain Aa 2.2: description of *Haloferax lucentensis* sp. nov. *Extremophiles* **6**, 479–483.
- Gutiérrez, M. C., Castillo, A. M., Pagaling, E., Heaphy, S., Kamekura, M., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E. & other authors (2008). *Halorubrum kocurii* sp. nov., an archaeon isolated from a saline lake. *Int J Syst Evol Microbiol* **58**, 2031–2035.
- Hu, L., Pan, H., Xue, Y., Ventosa, A., Cowan, D. A., Jones, B. E., Grant, W. D. & Ma, Y. (2008). *Halorubrum luteum* sp. nov., isolated from Lake Chagannor, Inner Mongolia, China. *Int J Syst Evol Microbiol* **58**, 1705–1708.
- Johnson, J. L. (1994). Similarity analysis of DNAs. In *Methods for General and Molecular Bacteriology*, pp. 655–681. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- 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 new genera Halorubrobacterium and Natrionalba. *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*, *Natrionalba*, and *Natronomonas* gen. nov., respectively, as *Halorubrum vacuolatum* comb. nov., *Natrionalba magadii* comb. nov., and *Natronomonas pharaonis* comb. nov., respectively. *Int J Syst Bacteriol* **47**, 853–857.
- Kharroub, K., Quesada, T., Ferrer, R., Fuentes, S., Aguilera, M., Boulahrouf, A., Ramos-Cormenzana, A. & Monteoliva-Sánchez, M. (2006). *Halorubrum ezzemoulense* sp. nov., a halophilic archaeon isolated from Ezzemoul sabkha, Algeria. *Int J Syst Evol Microbiol* **56**, 1583–1588.
- Lizama, C., Monteoliva-Sánchez, M., Suárez-García, A., Roselló-Mora, R., Aguilera, M., Campos, V. & Ramos-Cormenzana, A. (2002). *Halorubrum tebenquichense* sp. nov., a novel halophilic archaeon isolated from the Atacama Saltern, Chile. *Int J Syst Evol Microbiol* **52**, 149–155.
- López-García, P., Moreira, D., López-López, 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., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M. & Schleifer, K.-H. (1998). Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis* **19**, 554–568.

- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, 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 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. & Grant, W. D. (2001). Genus VII. *Halorubrum*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 320–324. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Oren, A. (1983). *Halorubrum sodomense* sp. nov., a Dead Sea halobacterium with an extremely high magnesium requirement. *Int J Syst Bacteriol* **33**, 381–386.
- Oren, A. (1994). The ecology of the extremely halophilic archaea. *FEMS Microbiol Rev* **13**, 415–439.
- Oren, A. & Ventosa, A. (1996). A proposal for the transfer of *Halorubrobacterium distributum* and *Halorubrobacterium coriense* to the genus *Halorubrum* as *Halorubrum distributum* comb. nov. and *Halorubrum coriense* comb. nov., respectively. *Int J Syst Bacteriol* **46**, 1180.
- 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., Arahal, D. R. & Ventosa, A. (2009). Emended descriptions of genera of the family *Halobacteriaceae*. *Int J Syst Evol Microbiol* **59**, 637–642.
- 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.
- Pesenti, P. T., Sikaroodi, M., Gillevet, P. M., Sánchez-Porro, C., Ventosa, A. & Litchfield, C. D. (2008). *Halorubrum californiense* sp. nov., an extreme archaeal halophile isolated from a crystallizer pond at a solar salt plant in California, USA. *Int J Syst Evol Microbiol* **58**, 2710–2715.
- Petter, H. F. M. (1931). On bacteria of salted fish. *Proc K Ned Akad Wet Amsterdam* **34**, 1417–1423.
- Roh, S. W. & Bae, J.-W. (2009). *Halorubrum cibi* sp. nov., an extremely halophilic archaeon from salt-fermented seafood. *J Microbiol* **47**, 162–166.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Tomlinson, G. A. & Hochstein, L. I. (1976). *Halobacterium saccharovorum* sp. nov., a carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* **22**, 587–591.
- Ventosa, A., Gutiérrez, M. C., Kamekura, M., Zvyagintseva, I. S. & Oren, A. (2004). Taxonomic study of *Halorubrum distributum* and proposal of *Halorubrum terrestre* sp. nov. *Int J Syst Evol Microbiol* **54**, 389–392.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Xu, X.-W., Wu, Y.-H., Zhang, H. B. & Wu, M. (2007). *Halorubrum arcis* sp. nov., an extremely halophilic archaeon isolated from a saline lake on the Qinghai-Tibet Plateau, China. *Int J Syst Evol Microbiol* **57**, 1069–1072.