Halogeometricum rufum sp. nov., a halophilic archaeon from a marine solar saltern, and emended description of the genus *Halogeometricum*

Heng-Lin Cui,¹ Xin Yang,¹ Xia Gao,¹ Xin-Yi Li,¹ Xue-Wei Xu,² Yu-Guang Zhou,³ Hong-Can Liu³ and Pei-Jin Zhou³

Correspondence

Heng-Lin Cui
cuihenglin@sohu.com

¹School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, PR China

²Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China

³Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

Two halophilic archaea, strains RO1-4^T and RO1-64, were isolated from a marine solar saltern in Jiangsu, China. Cells of the two strains were pleomorphic, motile, and stained Gram-negative. Colonies were red-pigmented. Strains RO1-4^T and RO1-64 were able to grow at 25–55 °C (optimum 40–42 °C), at 2.1–5.1 M NaCl (optimum 3.9 M NaCl), at 0.05–0.7 M MgCl₂ (optimum 0.3 M MgCl₂) and at pH 6.0–8.5 (optimum pH 7.0). Cells lyse in distilled water and the minimal NaCl concentration to prevent cell lysis is 12 % (w/v). On the basis of 16S rRNA gene sequence analysis, strains RO1-4^T and RO1-64 were closely related to *Halogeometricum borinquense* PR3^T (98.0 and 98.2 % similarity, respectively) and *Halosarcina pallida* BZ256^T (97.8 and 97.9 %). The major polar lipids of the two strains were phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and two glycolipids (S-DGD-1 and DGD-1). The DNA G + C contents of strains RO1-4^T and RO1-64 are 64.9 and 62.4 mol%, respectively. The DNA–DNA hybridization value between strains RO1-4^T and RO1-64 was 83.0 % and both strains showed low DNA–DNA relatedness with *Halogeometricum borinquense* PR3^T (42.5 and 50.1 % relatedness, respectively) and *Halosarcina pallida* BZ256^T (37.6 and 42.1 % relatedness). It was concluded that strains RO1-4^T and RO1-64 represent a novel species of the genus *Halogeometricum*, for which the name *Halogeometricum rufum* sp. nov. is proposed. The type strain is RO1-4^T (=CGMCC 1.7736^T =JCM 15770^T).

The genus *Halogeometricum* was proposed by Montalvo-Rodríguez *et al.* (1998) to accommodate the extremely pleomorphic strain *Halogeometricum borinquense* PR3^T isolated from the solar salterns of Cabo Rojo, Puerto Rico. The colonies of this species are pink, small and very mucoid. The major glycolipid of *Halogeometricum borinquense* PR3^T is an unsulfated glycolipid. Recently, a sarcina-shaped strain, BZ256^T, showing 96.8 % 16S rRNA gene sequence similarity to *Halogeometricum borinquense*

PR3^T was isolated from Zodletone Spring, a sulfide- and sulfur-rich but low NaCl concentration spring in south-western Oklahoma, USA. On the basis of the salient characteristics of strain BZ256^T (cell morphology and division pattern, lack of motility, glycolipids, ability to tolerate low salt conditions), the novel genus *Halosarcina* was proposed by Savage *et al.* (2008) to accommodate the specific strain. At the time of writing, no other novel strains related to the genera *Halogeometricum* and *Halosarcina* have been described. During our surveys on halophilic archaeal diversity of marine solar salterns of Eastern China, two halophilic archaeal strains related to *Halogeometricum borinquense* PR3^T and *Halosarcina pallida* BZ256^T were obtained. In this study, we characterize the two strains, RO1-4^T and RO1-64, as a novel species of the genus *Halogeometricum*.

Strains RO1-4^T and RO1-64 were isolated from sediment of Rudong solar saltern (32.2699° N 121.3999° E), which is an artificial marine solar saltern located in Jiangsu Province, China. The neutral oligotrophic haloarchaeal medium (NOM) used for the isolation procedure was

Abbreviations: DGD-1, mannosyl glucosyl diether; MP, maximum-parsimony; NJ, neighbour-joining; PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerol phosphate methyl ester; S-DGD-1, sulfated mannosyl glucosyl diether.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains RO1-4^T and RO1-64 are EU887286 and GQ443609, respectively.

A scanning electron micrograph and a phase-contrast micrograph of strain RO1-4^T, two thin-layer chromatograms and a maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences of strain RO1-4^T, strain RO1-64 and some other halophilic archaea are available with the online version of this paper.

modified according to the DBCM2 medium from the online Halohandbook (Dyall-Smith, 2008) and contained the following ingredients (g l^{-1}): yeast extract, 0.05; fish peptone, 0.25; sodium pyruvate, 1.0; KCl, 5.4; K_2HPO_4 , 0.3; CaCl_2 , 0.25; NH_4Cl , 0.25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 26.8; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 23.0; NaCl, 184.0 (pH adjusted to 7.0–7.2 with 1 M NaOH solution). The medium was solidified with 2.0% agar. The strains were routinely grown aerobically at 37 °C in a modified R2A (Reasoner & Geldreich, 1985) medium (MR2A) with the following modifications (g l^{-1}): trisodium citrate, 3.0; KCl, 2.0; CaCl_2 , 0.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20.0; NaCl, 200.0.

Phenotypic tests were performed according to the proposed minimal standards for the description of novel taxa in the order *Halobacteriales* (Oren *et al.*, 1997). The type strains *Haloferox volcanii* CGMCC 1.2150^T, *Haloferox elongans* JCM 14791^T, *Halosarcina pallida* BZ256^T and *Halogeometricum borinquense* JCM 10706^T were selected as reference strains in positive and negative testing. Cell morphology and motility in exponentially growing liquid cultures were examined using a Nikon microscope equipped with phase-contrast optics (model E400). Minimal salt concentration to prevent cell lysis was tested by suspending washed cells in serial sterile saline solutions containing NaCl ranging from 0 to 15% (w/v), and the stability of the cells was detected by light microscopic examination.

The Gram stain was performed by following the method outlined by Dussault (1955). Most miscellaneous biochemical tests and nutritional tests were performed as described and cited by Bardavid *et al.* (2007). Briefly, growth and gas formation with nitrate as electron acceptor were tested in 10 ml stoppered tubes, completely filled with liquid growth medium to which 5 g $\text{NaNO}_3 \text{ l}^{-1}$ had been added, and containing an inverted Durham tube. The formation of nitrite was monitored colorimetrically. Anaerobic growth in the presence of L-arginine and 5 g DMSO l^{-1} was tested in completely filled 10 ml stoppered tubes. Starch hydrolysis was determined on NOM agar plates supplemented with 2 g soluble starch l^{-1} and detected by flooding the plates with Lugol's iodine solution. Gelatin hydrolysis was performed by growing colonies on MR2A agar plates amended with 0.5% (w/v) gelatin and flooding the plates with Frazier's reagent (McDade & Weaver, 1959) after growth was established. Esterase activity was detected as outlined by Gutiérrez & González (1972). Tests for catalase and oxidase activities were performed as described by Gonzalez *et al.* (1978). Production of H_2S was tested by growing the isolates and reference strains in a tube with MR2A liquid medium supplemented with 0.5% (w/v) sodium thiosulfate; a filter-paper strip impregnated with lead acetate was used for H_2S detection (Cui *et al.*, 2007). To test for growth on single carbon sources, fish peptone and sodium pyruvate were omitted from the NOM medium and the compound to be tested was added at a concentration of 5 g l^{-1} . Antibiotic susceptibility was determined by the method of Gutiérrez *et al.* (2008) on

MR2A agar plates with antibiotic discs containing the following concentrations (μg per disc, unless otherwise indicated): ampicillin (10), anisomycin (20), aphidicolin (20), bacitracin (0.04 IU per disc), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), norfloxacin (10), novobiocin (30), penicillin G (10 IU per disc), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30).

Polar lipids were extracted by using a chloroform/methanol system and analysed using one- and two-dimensional TLC, as described by Kates (1986). Merck silica gel 60 F₂₅₄ aluminium-backed thin-layer plates were used in TLC analysis. To detect phospholipids, the plate was subjected to two-dimensional development, with the first solvent of chloroform/methanol/water (65:25:4, by vol.) followed by the second solvent of chloroform/methanol/acetic acid/water (80:12:15:4, by vol.), then sprayed with the phosphate stain reagent (Vaskovsky & Kostetsky, 1968). To detect glycolipids, the plate was subjected to single development in the solvent chloroform/methanol/acetic acid/water (85:22.5:10:4, by vol.) and then sprayed with sulfuric acid/ethanol (1:2, v/v) followed by heating at 150 °C for 3 min to detect phospholipids and glycolipids.

Genomic DNA from halophilic archaeal strains was prepared as described by Ng *et al.* (1995). The 16S rRNA gene was amplified via PCR by using primers 0018F and 1518R (Cui *et al.*, 2009). PCR was performed in a thermal cycler (MJ Research PTC-150) for 30 cycles (5 min denaturing step at 95 °C in the first cycle; 1 min denaturing at 95 °C, 1 min annealing at 60 °C and 1.5 min elongation at 72 °C; final extension step at 72 °C for 10 min). PCR products were examined on a 1.0% (w/v) agarose gel and then cloned into the pEASY-T vector (TransGen Biotech) and transformed into cells of *Escherichia coli* Mach1. Sixteen transformants were randomly picked and sequenced at the Sino-GenoMax Company (Beijing, China), to determine whether the two strains possessed multiple distinct 16S rRNA gene sequences. Multiple sequence alignments were performed by using the CLUSTAL W program integrated in the MEGA4 software (Tamura *et al.*, 2007). Phylogenetic trees were reconstructed using the neighbour-joining (NJ; Saitou & Nei, 1987) and maximum-parsimony (MP; Fitch, 1971) algorithms in MEGA4. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. 16S rRNA gene sequence similarity was calculated by comparison with sequences of related halophilic archaea from the online EzTaxon server (Chun *et al.*, 2007). The DNA G+C content was determined by the HPLC method (Mesbah *et al.*, 1989). DNA–DNA hybridization analyses were performed according to the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983).

Cells of strains RO1-4^T and RO1-64 were motile, pleomorphic (short and long rods and irregular cocci; see Supplementary Fig. S1 available in IJSEM Online) when

grown in MR2A liquid medium. Cells stained Gram-negative; colonies were red-pigmented. Strains RO1-4^T and RO1-64 were able to grow at 25–55 °C (optimum 40–42 °C), at 2.1–5.1 M NaCl (optimum 3.9 M NaCl), at 0.05–0.7 M MgCl₂ (optimum 0.3 M MgCl₂) and at pH 6.0–8.5 (optimum pH 7.0). Cells lyse in distilled water and the minimal NaCl concentration to prevent cell lysis is 12 % (w/v). Both strains produced indole from tryptophan, but did not hydrolyse gelatin, Tweens 20, 40, 60 or 80, starch or casein. They were able to grow in defined and complex media; D-glucose, D-mannose, D-galactose, maltose, sucrose, lactose, glycerol, D-sorbitol, acetate, pyruvate and DL-lactate as single carbon sources yielded the best growth. Acid was produced during growth on carbohydrates. More detailed results of phenotypic tests and nutritional features of the two strains are given in the species description.

Strains RO1-4^T and RO1-64 contained phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), a major glycolipid (GL1) chromatographically identical to sulfated mannosyl glucosyl diether (S-DGD-1) and a minor glycolipid (GL2) chromatographically identical to mannosyl glucosyl diether (DGD-1) (Supplementary Fig. S2). On the glycolipid TLC of *Halogeometricum borinquense* JCM 10706^T, besides the major glycolipid GLb (an unsulfated glycolipid), a glycolipid chromatographically identical to S-DGD-1 can also be detected as a minor component, which indicates that *Halogeometricum borinquense* JCM 10706^T is somewhat related to *Halosarcina pallida* BZ256^T based on the polar lipid composition.

Sixteen complete 16S rRNA gene sequences (1472 nt, each) of strains RO1-4^T and RO1-64 were obtained. Sequence comparisons indicated that both strains have one kind of 16S rRNA gene sequence; they are 99.8 % similar to each other. Both strains were closely related to *Halogeometricum borinquense* PR3^T (98.0 and 98.2 % similarity, respectively) and *Halosarcina pallida* BZ256^T (97.8 and 97.9 % similarity). Phylogenetic analysis using the NJ algorithm revealed that strains RO1-4^T and RO1-64 clustered with *Halogeometricum borinquense* PR3^T, forming a branch in the cluster with a bootstrap value of 71 % (Fig. 1). This phylogenetic position was confirmed in the tree generated using the MP algorithm (Supplementary Fig. S3); strains RO1-4^T and RO1-64 clustered with *Halogeometricum*

borinquense, forming a branch in the cluster with a bootstrap value of 85 %. Two other tree-generating algorithms, minimum evolution and unweighted pair group method with arithmetic mean (UPGMA), were used. Both the minimum evolution tree and the UPGMA tree confirmed the same phylogenetic position of related taxa seen in the NJ and MP trees (data not shown). These 16S rRNA gene-based phylogenetic analyses supported the placement of strains RO1-4^T and RO1-64 in the genus *Halogeometricum* rather than in the genus *Halosarcina*.

The DNA G + C contents of strains RO1-4^T and RO1-64 are 64.9 mol% and 62.4 mol%, respectively. These values are lower than that of *Halosarcina* (65.4 mol%; Savage *et al.*, 2008) but higher than the value reported for *Halogeometricum borinquense* (59.1 mol%; Montalvo-Rodríguez *et al.*, 1998). The DNA relatedness between strains RO1-4^T and RO1-64 was 83.0 %, showing that the two strains should be classified in the same species since the generally accepted threshold value to separate two species is 70 % (Stackebrandt & Goebel, 1994). The two strains, RO1-4^T and RO1-64, showed low DNA–DNA relatedness with *Halogeometricum borinquense* PR3^T (42.5 and 50.1 % relatedness, respectively) and *Halosarcina pallida* BZ256^T (37.6 and 42.1 % relatedness). However, the two strains are more related to *Halogeometricum borinquense* PR3^T than to *Halosarcina pallida* BZ256^T at the DNA–DNA hybridization level and represent a distinct genospecies of the genus *Halogeometricum*.

This polyphasic taxonomic study provides evidence that strains RO1-4^T and RO1-64 represent a novel species of the genus *Halogeometricum*, for which the name *Halogeometricum rufum* sp. nov. is proposed. Characteristics that distinguish strains RO1-4^T and RO1-64 from *Halogeometricum borinquense* JCM 10706^T and *Halosarcina pallida* BZ256^T are shown in Table 1.

Emended description of the genus *Halogeometricum* Montalvo-Rodríguez *et al.* 1998

Cells are pleomorphic (short and long rods, squares, triangles, ovals and irregular cocci) under optimal growth conditions and stain Gram-negative. Cells are motile. Colonies are pink–red due to the presence of bacterio-

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the member of the genus *Halogeometricum* and related genera within the family *Halobacteriaceae*. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more 70 % bootstrap support. Bar, 0.02 expected changes per site.

Table 1. Characteristics that distinguish strains RO1-4^T and RO1-64 from *Halogeometricum borinquense* JCM 10706^T and *Halosarcina pallida* BZ256^T

Strains: 1, RO1-4^T; 2, RO1-64; 3, *Halogeometricum borinquense* JCM 10706^T (data from Montalvo-Rodríguez *et al.*, 1998); 4, *Halosarcina pallida* BZ256^T (Savage *et al.*, 2008). +, Positive; –, negative; UG, unidentified glycolipid.

Characteristic	1	2	3	4
Cell shape	Pleomorphic	Pleomorphic	Pleomorphic	Coccus
Motility	+	+	+	–
Optimum NaCl (M)	3.9	3.9	3.4–4.3	3.1
Optimum growth temperature (°C)	40–42	40	40	30
Optimum pH	7.0	7.0	7.0	7.0–7.5
Anaerobic growth with nitrate	–	–	+	–
Gas formation from nitrate	–	–	+	–
Nitrate reduction	+	+	+	–
Casein hydrolysis	–	–	+	–
Gelatin liquefaction	–	–	+	–
Utilization of:				
Mannose	+	+	+	–
Fructose	–	–	+	–
Xylose	–	–	+	–
L-Arginine	–	–	–	+
DNA G + C content (mol%)	64.9	62.4	59.1	65.4
Types of glycolipids	S-DGD-1, DGD-1	S-DGD-1, DGD-1	S-DGD-1, UG	S-DGD-1, DGD-1

rubrin carotenoids. Cells lyse in distilled water. Oxidase- and catalase-positive. Aerobic heterotrophs. Sugars are metabolized, in some cases with formation of acids. Cells contain PG and PGP-Me, but phosphatidylglycerol sulfate is absent. In some species, the major glycolipid is GLb (an unsulfated glycolipid), and S-DGD-1 may be present as a minor component. In other species, the major glycolipid is chromatographically identical to S-DGD-1 and DGD-1 may be present as a minor component. The DNA G + C content is between 59.1 and 64.9 mol%. Isolated from solar salterns. The type species is *Halogeometricum borinquense*.

Description of *Halogeometricum rufum* sp. nov.

Halogeometricum rufum (ru'fūm. L. neut. adj. *rufum* red).

Cells are motile, pleomorphic (short and long rods and irregular cocci) under optimal growth conditions and stain Gram-negative. Colonies on agar plates containing 3.9 M NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at 25–55 °C (optimum 40–42 °C), at 2.1–5.1 M NaCl (optimum 3.9 M NaCl), at 0.05–0.7 M MgCl₂ (optimum 0.3 M MgCl₂) and at pH 6.0–8.5 (optimum pH 7.0). Cells lyse in distilled water and the minimal NaCl concentration to prevent cell lysis is 12 % (w/v). Catalase- and oxidase-positive. Does not grow under anaerobic conditions with nitrate, arginine or DMSO. Nitrate reduction to nitrite is not observed. Nitrite is reduced. H₂S is produced from sodium thiosulfate. Positive for indole formation. Tweens 20, 40, 60 and 80, casein, starch and gelatin are not hydrolysed. The following substrates are utilized as single carbon and energy sources for growth: D-glucose, D-mannose,

D-galactose, maltose, sucrose, lactose, glycerol, D-sorbitol, acetate, pyruvate, DL-lactate, L-alanine and L-glutamate. D-Fructose, succinate, L-malate, fumarate, L-ornithine, L-sorbose, D-ribose, D-xylose, starch, D-mannitol, citrate, glycine, L-arginine, L-aspartate and L-lysine are not utilized as carbon sources. Sensitive to the following antibiotics (µg per disc, unless otherwise indicated): rifampicin (5), novobiocin (30), bacitracin (0.04 IU per disc), anisomycin (20) and aphidicolin (20). Resistant to the following antibiotics: erythromycin (15), neomycin (30), chloramphenicol (30), ampicillin (10), penicillin G (10 IU per disc), norfloxacin (10), ciprofloxacin (5), streptomycin (10), kanamycin (30), tetracycline (30), vancomycin (30), gentamicin (10) and nalidixic acid (30). The major polar lipids are PG, PGP-Me, S-DGD-1 and DGD-1. The DNA G + C content of the type strain is 64.9 % (as determined by HPLC).

The type strain is RO1-4^T (=CGMCC 1.7736^T =JCM 15770^T) and was isolated from Rudong solar saltern in Jiangsu province, China.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (no. 30970006), the National Science & Technology Infrastructure Program of China (no. 2005DKA2120611), a grant from Jiangsu Department of Education (no. 08 kJB180002), a startup grant from Jiangsu University (no. 08JDG016) and two scientific training grants for senior students from Jiangsu Department of Education and Jiangsu University. We are grateful to Dr Mostafa S. Elshahed and Ms Kristen N. Savage for kindly providing the type strain *Halosarcina pallida* BZ256^T.

References

- Bardavid, R. E., Mana, L. & Oren, A. (2007). *Haloplanus natans* gen. nov., sp. nov., an extremely halophilic, gas-vacuolate archaeon isolated from Dead Sea–Red Sea water mixtures in experimental outdoor ponds. *Int J Syst Evol Microbiol* **57**, 780–783.
- 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., 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.
- Cui, H.-L., Zhou, P.-J., Oren, A. & Liu, S.-J. (2009). Intraspecific polymorphism of 16S rRNA genes in two halophilic archaeal genera, *Haloarcula* and *Halomicrobium*. *Extremophiles* **13**, 31–37.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.
- Dyall-Smith, M. L. (2008). The Halohandbook: protocols for haloarchaeal genetics. <http://www.haloarchaea.com/resources/halohandbook>.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- 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.
- Gutiérrez, C. & González, C. (1972). Method for simultaneous detection of proteinase and esterase activities in extremely halophilic bacteria. *Appl Microbiol* **24**, 516–517.
- Gutiérrez, M. C., Castillo, A. M., Kamekura, M. & Ventosa, A. (2008). *Haloterrigena salina* sp. nov., an extremely halophilic archaeon isolated from a salt lake. *Int J Syst Evol Microbiol* **58**, 2880–2884.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kates, M. (1986). In *Techniques of Lipidology*, 2nd revised edn, pp. 106–107, 187–188 and 251–254. Amsterdam: Elsevier.
- McDade, J. J. & Weaver, R. H. (1959). Rapid methods for the detection of gelatin hydrolysis. *J Bacteriol* **77**, 60–64.
- 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.
- Montalvo-Rodríguez, R., Vreeland, R. H., Oren, A., Kessel, M., Betancourt, C. & López-Garriga, J. (1998). *Halogeometricum borinquense* gen. nov., sp. nov., a novel halophilic archaeon from Puerto Rico. *Int J Syst Bacteriol* **48**, 1305–1312.
- Ng, W.-L., Yang, C.-F., Halladay, J. T., Arora, A. & DasSarma, S. (1995). Protocol 25. Isolation of genomic and plasmid DNAs from *Halobacterium halobium*. In *Archaea: a Laboratory Manual: Halophiles*, pp. 179–180. Edited by S. DasSarma & E. M. Fleischmann. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- 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.
- Reasoner, D. J. & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* **49**, 1–7.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Savage, K. N., Krumholz, L. R., Oren, A. & Elshahed, M. S. (2008). *Halosarcina pallida* gen. nov., sp. nov., a halophilic archaeon from a low-salt, sulfide-rich spring. *Int J Syst Evol Microbiol* **58**, 856–860.
- 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.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Vaskovsky, V. E. & Kostetsky, E. Y. (1968). Modified spray for the detection of phospholipids on thin-layer chromatograms. *J Lipid Res* **9**, 396.