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ORIGINAL PAPER

Halorubrum halophilum sp. nov., an extremely halophilic archaeon isolated from a salt-fermented seafood

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Abstract A novel, red-pigmented, pleomorphic and short rod-shaped haloarchaeon, designated B8^T, was isolated from a salt-fermented seafood. Strain B8^T was found to be able to grow at 20–45 °C, in the presence of 15–30 % (w/v) NaCl and at pH 7.0–9.0. The optimum requirements were found to be a temperature range of 35–40 °C, pH 8.0 and the presence of 25 % NaCl. The cells of strain B8^T were observed to be Gram-staining negative and lysed in distilled water. Anaerobic growth did not occur in the presence of nitrate, L-arginine, dimethyl sulfoxide or trimethylamine *N*-oxide. The catalase and oxidase activities were found to be positive and nitrate was reduced in aerobic conditions. Tween 20, 40 and 80 were found to be hydrolyzed, whereas casein, gelatin and starch were not hydrolyzed. Indole or H_2S was not formed and urease activity was not detected. A phylogenetic analysis based on the 16S rRNA gene sequences indicated that strain B8^T is most closely related to members of the genus *Halorubrum* in the family *Halobacteriaceae*. Strain B8^T was found to have three 16S rRNA genes, *rrnA*, *rrnB* and *rrnC*; similarities between the 16S rRNA gene sequences are 99.0–99.8 %. Strain B8^T shared 99.0 % 16S rRNA gene sequence similarity with *Halorubrum* (*Hrr.*) *lipolyticum* JCM 13559^T and *Hrr. saccharovorum* DSM 1137^T, 98.8 % with *Hrr. kocurii* JCM 14978^T, 98.3 % with *Hrr. lacusprofundi* DSM 5036^T, 98.0 % with *Hrr. arcis* JCM 13916^T, 97.7 %

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with *Hrr. aidingense* JCM 13560^T and 97.0 % with *Hrr. aquaticum* JCM 14031^T, as well as 93.7–96.5 % with other type strains in the genus *Halorubrum*. The RNA polymerase subunit B′ gene sequence similarity of strain B8^T with *Hrr. kocurii* JCM 14978^T is 97.2 % and lower with other members of the genus *Halorubrum*. DNA–DNA hybridization experiments showed that strain B8^T shared equal or lower than 50 % relatedness with reference species in the genus *Halorubrum*. The genomic DNA G+C content of strain B8^T was determined to be 64.6 mol%. The major isoprenoid quinone of strain B8^T was identified as menaquinone-8 and the major polar lipids as phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate, sulfated mannosyl glucosyl diether and an unidentified phospholipid. Based on this polyphasic taxonomic study, strain B8^T is considered to represent a new species in the genus *Halorubrum*, for which the name *Hrr. halophilum* sp. nov. is proposed. The type strain is B8^T (=JCM 18963^T = CECT 8278^T).

Keywords Haloarchaea · *Halorubrum halophilum* · Polyphasic taxonomy · Salt-fermented seafood

Introduction

High salinity is toxic to most cells but many extremely halophilic archaea, i.e. haloarchaea, have been isolated in hypersaline environments (Grant 2004). Well known hypersaline environments for the isolation of haloarchaea are soda lakes, salt lakes and solar salterns, but some haloarchaea have been isolated from a salt-fermented seafood made from shrimp (Roh et al. 2007a, b, 2009; Roh and Bae 2009). All haloarchaea are classified within the family *Halobacteriaceae* in the order *Halobacteriales* of the phylum *Euryarchaeota*. This family currently contains 40 genera based on the List of Prokaryotic Names with Standing in Nomenclature (Euzéby 1997). In particular, the genus *Halorubrum*, which was proposed formally by McGenity and Grant (1995), currently includes 25 validly named species: *Halorubrum* (*Hrr.*) *aidingense* (Cui et al. 2006), *Hrr. alkaliphilum* (Feng et al. 2005), *Hrr. aquaticum* (Gutiérrez et al. 2011), *Hrr. arcis* (Xu et al. 2007), *Hrr. californiense* (Pesenti et al. 2008), *Hrr. chaoviator* (Mancinelli et al. 2009), *Hrr. cibi* (Roh and Bae 2009), *Hrr. coriense* (Oren and Ventosa 1996), *Hrr. distributum* (Oren and Ventosa 1996), *Hrr. ejinorense* (Castillo et al. 2007), *Hrr. ezzenmoulense* (Kharroub et al. 2006), *Hrr. kocurii* (Gutiérrez et al. 2008), *Hrr. lacusprofundi* (McGenity and Grant 1995), *Hrr. lipolyticum* (Cui et al. 2006), *Hrr. litoreum* (Cui et al. 2007), *Hrr. luteum* (Hu et al. 2008), *Hrr. orientale* (Castillo et al. 2006), *Hrr. saccharovorum* (Tomlinson and Hochstein 1976; McGenity and Grant 1995), *Hrr. sodomense* (McGenity and Grant 1995), *Hrr. tebenquichense* (Lizama et al. 2002), *Hrr. terrestre* (Ventosa et al. 2004), *Hrr. tibetense* (Fan et al. 2004), *Hrr. trapanicum* (McGenity and Grant 1995), *Hrr. vacuolatum* (Mwatha and Grant 1993; Kamekura et al. 1997) and *Hrr. xinjiangense* (Feng et al. 2004). Cells of the genus *Halorubrum* are rods or irregular-shaped, motile or non-motile, strictly aerobic and oxidase and catalase-positive. The major polar lipids are phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulfate (PGS) and/or sulfated mannosyl glucosyl diether (S-DGD-3) (McGenity and Grant 1995). The G+C content of the genomic DNA is in the range of 61.7–71.2 mol% (McGenity and Grant 2001; Roh and Bae 2009). The present study characterized a new haloarchaeon, strain B8^T and determined the taxonomic position of this strain based on phenotypic, phylogenetic and chemotaxonomic analyses, according to the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren et al. 1997). Based on this polyphasic taxonomic study, strain B8^T is considered to represent a new species in the genus *Halorubrum*, for which the name *Hrr. halophilum* sp. nov. is proposed here.

Materials and methods

Archaeal strain and culture conditions

A strain, designated B8^T, was isolated from a salt-fermented seafood made from shrimp. A sample was

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serially diluted and spread onto a complex medium (DSM medium no. 954), which was adjusted to pH 7.0 and contained the following (l^{-1}): 5 g casamino acids (BD), 5 g yeast extract (BD), 20 g $MgCl_2 \cdot 6H_2O$, 2 g KCl, 12 g Tris, 0.2 g $CaCl_2 \cdot 2H_2O$, and 200 g NaCl. A solid medium was prepared by adding 2 % (w/v) agar. A single colony was streaked repeatedly to obtain a pure culture at 37 °C. For long-term preservation, strain B8^T was frozen at −80 °C in medium 954 supplemented with 5 % (v/v) dimethyl sulfoxide (DMSO). *Hrr. lipolyticum* JCM 13559^T, *Hrr. kocurii* JCM 14978^T, *Hrr. saccharovorum* DSM 1137^T, *Hrr. lacusprofundi* DSM 5036^T *Hrr. aidingense* JCM 13560^T, *Hrr. arcis* JCM 13916^T and *Hrr. aquaticum* JCM 14031^T were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) or Japan Collection of Microorganisms (JCM) and used in the comparative taxonomic analyses.

Morphological, physiological and biochemical characterization

All of the phenotypic tests were performed using medium 954 or a halophile medium (HMD) that contained (l^{-1}): 20 g $MgCl_2 \cdot 6H_2O$, 5 g K_2SO_4, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.1 g yeast extract, 0.5 g NH_4Cl, 0.05 g KH_2PO_4, 0.5 g casamino acids as a carbon source and 180 g NaCl (Savage et al. 2007), unless indicated otherwise. The cell morphology and size were determined using a transmission electron microscope (SUPRA 55 VP; Carl Zeiss). Gram staining was performed using the published method for haloarchaea (Dussault 1955). Cell lysis in distilled water was tested by incubating cells in distilled water for 1 week before transfer to medium 954. The optimal conditions for growth in medium 954 with various NaCl concentrations were tested using 0–30 % (w/v) NaCl at intervals of 5 %. The optimal pH range for growth was assayed from pH 5.0–11.0 at intervals of 1.0 using HMD with the following buffers: 10 mM 2-(*N*-morpholino) ethanesulfonic acid for pH 5.0 and 6.0, 10 mM bis–Tris propane for pH 7.0–9.0 and 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid for pH 10.0 and 11.0. Growth was also tested at different temperatures, i.e. 5–60 °C at intervals of 5 °C. The standard phenotypic tests for nitrate and nitrite reduction in aerobic conditions, indole formation, urease activity and the hydrolysis of casein and starch were performed as described by Benson (2002) using medium 954 as the basal medium. The hydrolysis of Tween 20, 40 and 80 were tested as described by González et al. (1978) and the hydrolysis of gelatin was tested according to Smibert and Krieg (1994) using medium 954 as the basal medium. Anaerobic growth was tested in filled, stoppered tubes using medium 954 in the presence of 30 mM nitrate, 5 g L-arginine, 5 g DMSO or 5 g trimethylamine *N*-oxide (TMAO) at 37 °C in an anaerobic chamber (Coy), where the atmosphere comprised $N_2/CO_2/H_2$ (90:5:5, by vol.). H_2S formation was tested according to Cui et al. (2007). Acid production from D-glucose was tested by growing strain B8^T in HMD supplemented with 1 % D-glucose. Methyl red and Voges–Proskauer tests were determined using MR-VP Broth (BD) and Simmon's citrate test was performed using Simmons citrate agar (DB), for which each medium was supplemented with 20 % (w/v) NaCl. Arginine, lysine and ornithine decarboxylases tests were carried out using Moeller Decarboxylase Broth Base (BD) containing 20 % (w/v) NaCl as the basal medium. To assess the utilization of sole carbon and energy sources, HMD was supplemented with 10 mM bis–Tris propane and 1 % of the following substrates: acetate, L-alanine, L-arginine, L-aspartate, citrate, D-fructose, fumarate, D-galactose, D-glucose, L-glutamate, glycerol, glycine, DL-lactate, lactose, L-lysine, L-malate, maltose, mannitol, D-mannose, L-ornithine, pyruvate, D-ribose, sorbitol, L-sorbose, starch, succinate, sucrose or D-xylose. For testing antibiotic sensitivity, strain B8^T was inoculated on agar medium plates using antibiotic discs with the following amounts (μg per disc, unless indicated): ampicillin (20), bacitracin (0.1 IU), chloramphenicol (50), ciprofloxacin (10), erythromycin (25), neomycin (50), norfloxacin (20), novobiocin (50), penicillin G (20 IU) and rifampin (10). The strain was incubated for 2 weeks at 37 °C.

Determination of the 16S rRNA and RNA polymerase subunit B′ (*rpoB′*) gene sequences and phylogenetic analysis

To obtain chromosomal DNA, cells of strain B8^T were harvested, extracted and purified using a DNA extraction kit (G-spin^{TM}; iNtRON Biotechnology). The 16S rRNA gene of strain B8^T was amplified using a PCR pre-Mix (iNtRON Biotechnology) and the primer set Arch21F and 1492R (DeLong 1992), as

described previously (Roh et al. 2008). To check the heterogeneous 16S rRNA gene sequences, PCR products were ligated and transformed using an All-in PCR cloning kit (BioFact) according to the manufacturer's protocol. Multiple clones were picked randomly and then sequenced. PCR-mediated amplification and sequencing of the *rpoB′* genes were carried out according to Minegishi et al. (2010). The almost full-length 16S rRNA and *rpoB′* gene sequences were determined using SeqMan (DNASTAR). The phylogenetic neighbours and the pairwise sequence similarities were determined using EzTaxon-e (Kim et al. 2012). The 16S rRNA gene sequences of strain B8^T and validly named related species were aligned using the SILVA Incremental Aligner (Pruesse et al. 2012). The *rpoB′* gene sequences of the related taxa were obtained from GenBank (http://www.ncbi.nlm.nih.gov) and multiple sequence alignment was performed using the Clustal_W program. The phylogenetic trees were constructed using the neighbor-joining (NJ) (Saitou and Nei 1987), minimum-evolution (ME) (Nei et al. 1998) and maximum-likelihood (ML) (Felsenstein 1981) algorithms based on Kimura's two-parameter model (Kimura 1980) with 1,000 randomly selected bootstrap replicates using MEGA5 (Tamura et al. 2011).

Determination of the DNA–DNA hybridization (DDH), DNA G+C content, quinones and polar lipid analysis

The DDH experiments were performed using the fluorometric method with photobiotin-labeled DNA probes and microwell plates (MaxiSorp, FluoroNunc), as described by Ezaki et al. (1989). The genomic DNA G+C content was determined as described by González and Saiz-Jimenez (2002).

Polar lipids were extracted and detected using thin layer chromatography on a silica gel 60 F254 plate (Merck), according to the method of Minnikin et al. (1984). The compositions of the polar lipid spots were determined by spraying each plate with specific detection reagents as follows: sulfuric acid–ethanol (1:2, by vol) for total lipids, molybdenum blue for phospholipids and α-naphthol-sulfuric acid for glycolipids. The quinones of strain B8^T were analyzed using an HPLC system (UltiMate 3000; Dionex) coupled to a diode array detector and a single quadrupole mass spectrometer (HCT Ion-Trap MS; Bruker).

Results and discussion

Cells of strain B8^T were observed to be Gram-stain negative, pleomorphic, short rod or oval shaped, 0.5–0.6 μm in width and 0.9–1.1 μm in length (Supplementary Fig. S1). The colonies were red with a smooth and rounded shape on solid medium. Strain B8^T was found to grow in the presence of 15–30 % (w/v) NaCl, at 20–45 °C and at pH 7.0–9.0, with optimum growth in the presence of 25 % NaCl, at 35–40 °C and pH 8.0. The cells of strain B8^T lysed in distilled water. Strain B8^T was found to reduce nitrate in aerobic conditions and to be positive for acid production from D-glucose, catalase and oxidase. The novel strain was found to be able to hydrolyze Tween 20, 40 and 80, but not casein, gelatin and starch. H_2S was not produced from Na_2SO_3. Indole was not formed and no urease activity was observed. Strain B8^T could not grow in anaerobic conditions using nitrate, L-arginine, DMSO or TMAO. The Methyl red, Voges-prokauer and Simmon's citrate tests were negative. Strain B8^T did not produce arginine dihydrolase, lysine decarboxylase or ornithine decarboxylase. Strain B8^T was found to be sensitive to novobiocin, bacitracin, erythromycin, rifampin, neomycin and ciprofloxacin, but resistant to penicillin G, ampicillin, chloramphenicol and norfloxacin. Detailed results of the phenotypic tests and the nutritional features of this strain are presented in the species description. Table 1 shows the different characteristics of strain B8^T compared with those of closely related type strains of members of the genus *Halorubrum*, which indicates that strain B8^T can be distinguished from closely related members of the genus *Halorubrum*.

Sequence comparisons indicated that strain B8^T has three rRNA genes, *rrnA*, *rrnB* and *rrnC*. The almost complete 16S rRNA of the three rRNA genes and the *rpoB′* gene sequences of strain B8^T were obtained (1,430, 1,450, 1,450 and 1,830 bp, respectively; GenBank accession numbers EF077637, KF848218, KF848217 and KF700332, respectively). Similarities between the three 16S rRNA gene sequences were 99.0–99.8 %. A comparison with related sequences showed that strain B8^T (based on the 16S rRNA *rrnA* gene sequence) shares the highest levels of similarity with the following strains with validly published names: *Hrr. lipolyticum* JCM 13559^T (99.0 % 16S rRNA gene sequence similarity), *Hrr. saccharovorum* DSM 1137^T (99.0 %), *Hrr. kocurii* JCM 14978^T

Table 1 Differential characteristics of strain B8^T and closely related species in the genus *Halorubrum*

Characteristic	1	2	3	4	5
NaCl range for growth (%, w/v)	15–30	15–25	20–25	15–30	20–25
Optimum NaCl (%)	25	20	25	25	25
Optimum temperature (°C)	35–40	25	40	45	35
pH range for growth	7.0–9.0	7.0–9.0	7.0–9.0	7.0–9.0	7.0–8.0
Optimum pH	8.0	7.0	8.0	7.0	8.0
Hydrolysis of Tween 80	+	+	−	−	−
Nitrate reduction	+	+	−	+	+
Carbon and energy source					
Acetate	+	+	+	+	−
L-Aspartate	−	+	−	+	−
Fumarate	+	−	−	−	+
D-Galactose	+	+	−	−	−
Lactose	−	+	−	−	−
L-Malate	−	−	−	−	+
L-Ornithine	+	+	+	−	−
Succinate	+	−	−	−	+
Sucrose	−	−	−	+	−
DNA G+C content	64.6	65.9[a]	71.2[b]	69.4[c]	65.3[b]

Taxa: 1, *Hrr. halophilum* B8^T sp. nov.; 2, *Hrr. lipolyticum* JCM 13559^T; 3, *Hrr. saccharovorum* DSM 1137^T; 4, *Hrr. kocurii* JCM 14978^T; 5, *Hrr. lacusprofundi* DSM 5036^T. All data were from this study, unless otherwise indicated. All strains were found to be positive for catalase and oxidase and utilization of L-arginine, L-glutamate, D-glucose, DL-lactate, D-mannose and pyruvate; negative for hydrolysis of casein, starch, gelatin, indole formation and utilization of L-alanine, citrate, D-fructose, glycerol, glycine, L-lysine, maltose, mannitol, D-ribose, sorbitol, L-sorbose, starch and D-xylose. Symbols: + positive, − negative

[a] Data from Cui et al. (2006)

[b] Data from McGenity and Grant (1995)

[c] Data from Gutiérrez et al. (2008)

(98.8 %), *Hrr. lacusprofundi* DSM 5036^T (98.3 %), *Hrr. arcis* JCM 13916^T (98.0 %), *Hrr. aidingense* JCM 13560^T (97.7 %), *Hrr. aquaticum* JCM 14031^T (97.0 %) and other type strains in the genus *Halorubrum* (93.7–96.5 %). Strain B8^T clustered with *Hrr. lipolyticum*, *Hrr. saccharovorum Hrr. kocurii, Hrr. lacusprofundi* and *Hrr. aidingense* in the phylogenetic trees based on the 16S rRNA gene sequences (Fig. 1). The phylogenetic analyses based on the *rpoB′* gene sequences showed that strain B8^T is closely related to the following members: *Hrr. kocurii* JCM 14978^T (97.2 % *rpoB′* gene sequence similarity), *Hrr. saccharovorum* DSM 1137^T (96.8 %), *Hrr. lipolyticum* JCM 13559^T (96.3 %), *Hrr. lacusprofundi* DSM 5036^T (94.9 %), *Hrr. arcis* JCM 13916^T (94.2 %), *Hrr. aidingense* JCM 13560^T (93.2 %) and *Hrr. aquaticum* JCM 14031^T (92.2 %). The phylogenetic trees based on the *rpoB′* gene sequences showed a similar topology compared with trees of the 16S rRNA gene (Fig. 1b).

The DDH values of strain B8^T with the type strains of *Hrr. lipolyticum* JCM 13559^T, *Hrr. saccharovorum* DSM 1137^T, *Hrr. kocurii* JCM 14978^T, *Hrr. lacusprofundi* DSM 5036^T, *Hrr. arcis* JCM 13916^T, *Hrr. aidingense* JCM 13560^T and *Hrr. aquaticum* JCM 14031^T were 50 ± 9, 48 ± 11, 23 ± 2, 17 ± 3, 23 ± 4, 12 ± 2 and 14 ± 6 %, respectively. DDH values of <70 % represent distinct species according to current cut-offs employed in prokaryotic systematics (Wayne et al. 1987; Stackebrandt and Goebel 1994), so strain B8^T can be considered a distinct genospecies in the genus *Halorubrum*. The DNA G+C content of strain B8^T was determined to be 64.6 mol% (T_m), which is similar to the levels found in the closely related strain *Hrr. lipolyticum* JCM 13559^T (65.9 mol%) (Cui et al. 2006). The polar lipids

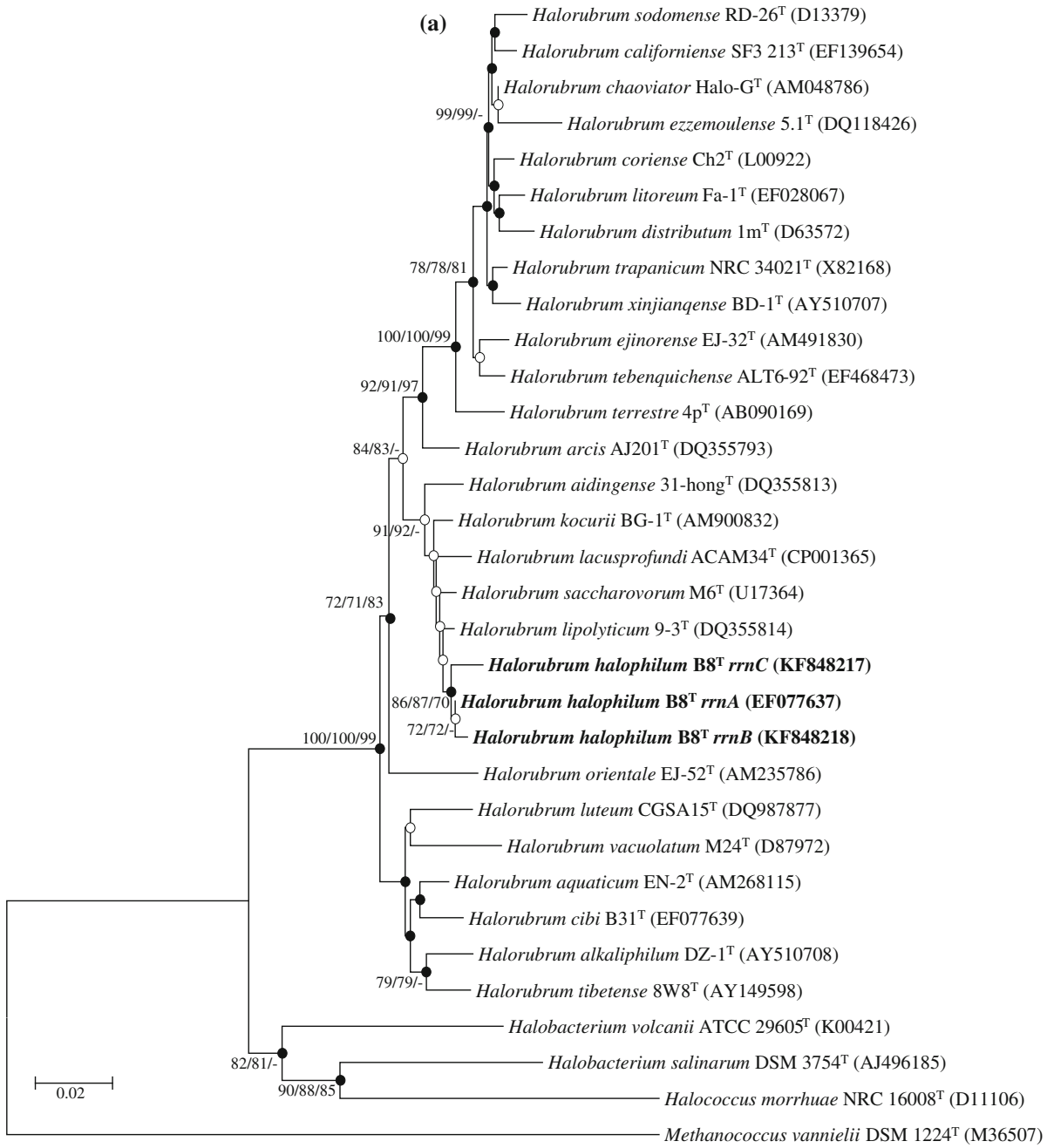

Fig. 1 Phylogenetic tree based on the neighbour-joining (NJ) algorithm for the 16S rRNA (**a**) and *rpoB′* (**b**) gene sequences of strain B8^T and closely related taxa. The *numbers* on the nodes indicate the bootstrap values (>70 %), which were calculated using the NJ/minimum-evolution (ME)/maximum-likelihood (ML) probabilities. The *closed circles* represent the nodes obtained using both the ME and ML methods, whereas the *open circles* indicate nodes recovered using either the ME or ML method. *Methanococcus vannielii* DSM 1224^T and *Haloquadratum walsbyi* DSM 16790 were used as the outgroup for the phylogenetic trees based on the 16S rRNA and *rpoB′* gene sequences, respectively. *Bar*, 0.02 (**a**) and 0.05 (**b**) accumulated changes per nucleotide

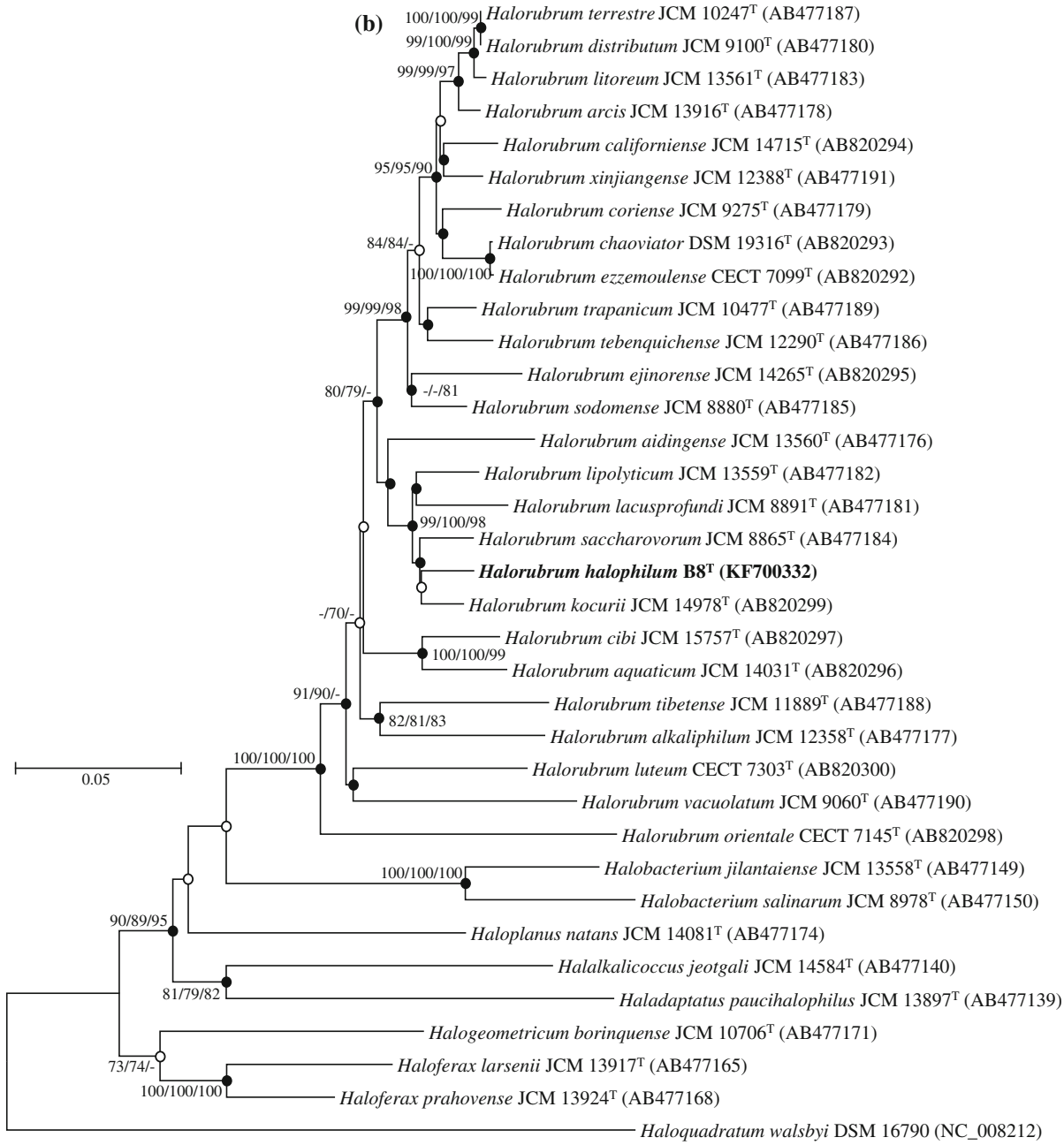

Fig. 1 continued

detected in strain B8^T comprised PG, PGP-Me, PGS, S-DGD-3 and an unidentified phospholipid (Supplementary Fig. S2). The major polar lipid profile of strain B8^T resembles that of the closely related *Halorubrum* species *Hrr. lipolyticum*, *Hrr. saccharovorum*, *Hrr. lacusprofundi* and *Hrr. arcis* which contain PG, PGP-Me, PGS and S-DGD-3 (McGenity and Grant 1995; Cui et al. 2006; Xu et al. 2007). However, the presence of PGS and S-DGD-3 in strain B8^T help distinguish the strain from *Hrr. kocurii* and *Hrr. aidingense*, respectively (Cui et al. 2006; Gutiérrez et al. 2008). The major isoprenoid quinone

detected in strain B8^T was menaquinone (MK)-8 and minor ones were MK-7(H_4) and MK-8(H_2).

In conclusion, the results of the phenotypic, phylogenetic and chemotaxonomic analyses showed that the haloarchaeal strain B8^T belongs to the genus *Halorubrum.* Table 1 shows that strain B8^T exhibits some differences compared with closely related type strains in the genus *Halorubrum*. Thus, based on this polyphasic taxonomic study, strain B8^T is considered to represent a novel species in the genus *Halorubrum*, for which the name *Hrr. halophilum* nov. is proposed.

Description of *Halorubrum halophilum* sp. nov.

Halorubrum halophilum (ha.lo'phi.lum. Gr. n. *hals*, *halos*, salt; Gr. adj. *philos*, loving; N.L. neut. adj. *halophilum* salt-loving)

Cells are Gram-stain negative, pleomorphic, short rod or oval shaped and 0.5–0.6 μm in width and 0.9–1.1 μm in length. Colonies are red, smooth and round in shape. Cell lysis occurs in distilled water. Growth occurs in the presence of 15–30 % (w/v) NaCl (optimum, 25 %), at 20–45 °C (optimum, 35–40 °C) and pH 7.0–9.0 (optimum, pH 8.0). Anaerobic growth does not occur in the presence of nitrate, L-arginine, DMSO or TMAO. Cells are positive for nitrate reduction in aerobic conditions, catalase and oxidase activity, acid production from D-glucose and the hydrolysis of Tween 20, 40 and 80, but negative for nitrite reduction in aerobic conditions, indole formation, H_2S production, urease activity, Methyl red, Voges–Proskauer, Simmon's citrate and the hydrolysis of casein, gelatin or starch. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are not produced. Acetate, L-arginine, fumarate, D-galactose, D-glucose, L-glutamate, DL-lactate, D-mannose, L-ornithine, pyruvate and succinate are utilized as sole carbon and energy sources, whereas L-alanine, L-aspartate, citrate, D-fructose, glycerol, glycine, lactose, L-lysine, L-malate, maltose, mannitol, D-ribose, sorbitol, L-sorbose, starch, sucrose and D-xylose are not utilised. The polar lipids are phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate, sulfated mannosyl glucosyl diether and an unidentified phospholipid. The major isoprenoid quinone is MK-8. The DNA G+C content of the type strain is 64.6 mol%.

The type strain, B8^T (=JCM 18963^T = CECT 8278^T), was isolated from a salt-fermented seafood made from shrimp. The GenBank/EMBL/DDBJ accession number for the 16S rRNA *rrnA*, *rrnB* and *rrnC* and *rpoB′* gene sequences of strain B8^T are EF077637, KF848218, KF848217 and KF700332, respectively.

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References

Benson HJ (2002) Microbiological applications: a laboratory manual in general microbiology. McGraw-Hill, New York

Castillo AM, Gutiérrez MC, Kamekura M, Xue Y, Ma Y, Cowan DA, Jones BE, Grant WD, Ventosa A (2006) *Halorubrum orientale* sp. nov., a halophilic archaeon isolated from Lake Ejinor, Inner Mongolia, China. Int J Syst Evol Microbiol 56(11):2559–2563

Castillo AM, Gutiérrez MC, Kamekura M, Xue Y, Ma Y, Cowan DA, Jones BE, Grant WD, Ventosa A (2007) *Halorubrum ejinorense* sp. nov., isolated from Lake Ejinor, Inner Mongolia, China. Int J Syst Evol Microbiol 57(11):2538–2542

Cui HL, Tohty D, Zhou PJ, Liu SJ (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 HL, Lin ZY, Dong Y, Zhou PJ, Liu SJ (2007) *Halorubrum litoreum* sp. nov., an extremely halophilic archaeon from a solar saltern. Int J Syst Evol Microbiol 57(10):2204–2206

DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89(12):5685–5689

Dussault HP (1955) An improved technique for staining red halophilic bacteria. J Bacteriol 70(4):484–485

Euzéby JP (1997) List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. Int J Syst Bacteriol 47(2):590–592

Ezaki T, Hashimoto H, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39:224–229

Fan H, Xue Y, Ma Y, Ventosa A, Grant WD (2004) *Halorubrum tibetense* sp. nov., a novel haloalkaliphilic archaeon from Lake Zabuye in Tibet, China. Int J Syst Evol Microbiol 54(4):1213–1216

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17(6):368–376

Feng J, Zhou PJ, Liu SJ (2004) *Halorubrum xinjiangense* sp. nov., a novel halophile isolated from saline lakes in China. Int J Syst Evol Microbiol 54(5):1789–1791

Feng J, Zhou P, Zhou YG, Liu SJ, 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(1):149–152

González JM, Saiz-Jimenez C (2002) A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. Environ Microbiol 4(11):770–773

González C, Gutiérrez C, Ramirez C (1978) *Halobacterium vallismortis* sp. nov. An amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. Can J Microbiol 24(6):710–715

Grant WD (2004) Life at low water activity. Philos Trans R Soc Lond B 359:1249–1267

Gutiérrez MC, Castillo AM, Pagaling E, Heaphy S, Kamekura M, Xue Y, Ma Y, Cowan DA, Jones BE, Grant WD, Ventosa A (2008) *Halorubrum kocurii* sp. nov., an archaeon isolated from a saline lake. Int J Syst Evol Microbiol 58(9):2031–2035

Gutiérrez MC, Castillo AM, Corral P, Kamekura M, Ventosa A (2011) *Halorubrum aquaticum* sp. nov., an archaeon isolated from hypersaline lakes. Int J Syst Evol Microbiol 61(5):1144–1148

Hu L, Pan H, Xue Y, Ventosa A, Cowan DA, Jones BE, Grant WD, Ma Y (2008) *Halorubrum luteum* sp. nov., isolated from Lake Chagannor, Inner Mongolia, China. Int J Syst Evol Microbiol 58(7):1705–1708

Kamekura M, Dyall-Smith ML, 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(3):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(7):1583–1588

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62(3):716–721

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16(2):111–120

Lizama C, Monteoliva-Sánchez M, Suárez-García A, Rosello-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(1):149–155

Mancinelli RL, Landheim R, Sánchez-Porro C, Dornmayr-Pfaffenhuemer M, Gruber C, Legat A, Ventosa A, Radax C, Ihara K, White MR, Stan-Lotter H (2009) *Halorubrum chaoviator* sp. nov., a haloarchaeon isolated from sea salt in Baja California, Mexico, Western Australia and Naxos, Greece. Int J Syst Evol Microbiol 59(8):1908–1913

McGenity TJ, Grant WD (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 TJ, Grant WD (2001) Genus VII. *Halorubrum*. In: Boone DR (ed) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 320–324

Minegishi H, Kamekura M, Itoh T, Echigo A, Usami R, Hashimoto T (2010) Further refinement of the phylogeny of the *Halobacteriaceae* based on the full-length RNA polymerase subunit B' (*rpoB'*) gene. Int J Syst Evol Microbiol 60(10):2398–2408

Minnikin DE, O'Donnell AG, Goodfellow M (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2(5):233–241

Mwatha WE, Grant WD (1993) *Natronobacterium vacuolata* sp. nov., a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. Int J Syst Bacteriol 43(3):401–404

Nei M, Kumar S, Takahashi K (1998) The optimization principle in phylogenetic analysis tends to give incorrect topologies when the number of nucleotides or amino acids used is small. Proc Natl Acad Sci USA 95(21):12390–12397

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 WD (1997) Proposed minimal standards for description of new taxa in the order *Halobacteriales*. Int J Syst Bacteriol 47(1):233–238

Pesenti PT, Sikaroodi M, Gillevet PM, Sánchez-Porro C, Ventosa A, Litchfield CD (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(12):2710–2715

Pruesse E, Peplies J, Glockner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28(14):1823–1829

Roh SW, Bae JW (2009) *Halorubrum cibi* sp. nov., an extremely halophilic archaeon from salt-fermented seafood. J Microbiol 47(2):162–166

Roh SW, Nam Y-D, Chang H-W, Kim K-H, Lee H-J, Oh H-M, Bae J-W (2007a) *Natronococcus jeotgali* sp. nov., a halophilic archaeon isolated from shrimp jeotgal, a traditional fermented seafood from Korea. Int J Syst Evol Microbiol 57(9):2129–2131

Roh SW, Nam YD, Chang HW, Sung Y, Kim KH, Oh HM, Bae JW (2007b) *Halalkalicoccus jeotgali* sp. nov., a halophilic archaeon from shrimp jeotgal, a traditional Korean fermented seafood. Int J Syst Evol Microbiol 57(10):2296–2298

Roh SW, Sung Y, Nam YD, Chang HW, Kim KH, Yoon JH, Jeon CO, Oh HM, Bae JW (2008) *Arthrobacter soli* sp.

nov., a novel bacterium isolated from wastewater reservoir sediment. J Microbiol 46(1):40–44

Roh SW, Nam Y-D, Chang H-W, Kim K-H, Sung Y, Kim M-S, Oh H-M, Bae J-W (2009) *Haloterrigena jeotgali* sp. nov., an extremely halophilic archaeon from salt-fermented food. Int J Syst Evol Microbiol 59(9):2359–2363

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425

Savage KN, Krumholz LR, Oren A, Elshahed MS (2007) *Haladaptatus paucihalophilus* gen. nov., sp. nov., a halophilic archaeon isolated from a low-salt, sulfide-rich spring. Int J Syst Evol Microbiol 57(1):19–24

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Kreig NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 607–654

Stackebrandt E, Goebel BM (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, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10): 2731–2739

Tomlinson GA, Hochstein LI (1976) *Halobacterium saccharovorum* sp. nov., a carbohydrate-metabolizing, extremely halophilic bacterium. Can J Microbiol 22(4):587–591

Ventosa A, Gutiérrez MC, Kamekura M, Zvyagintseva IS, Oren A (2004) Taxonomic study of *Halorubrum distributum* and proposal of *Halorubrum terrestre* sp. nov. Int J Syst Evol Microbiol 54(2):389–392

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Truper HG (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 XW, Wu YH, Zhang HB, 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(5):1069–1072