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Halolamina rubra sp. nov., a haloarchaeon isolated from non-purified solar salt

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Abstract Two Gram-stain negative, rod-shaped and motile extreme halophiles, designated CBA1107^T and CBA1108, were isolated from non-purified solar salt. Based on the phylogenetic analysis, strains CBA1107^T and CBA1108 were shown to belong to the genus *Halolamina*, with similarities for the 16S rRNA gene sequences between strains CBA1107^T and *Halolamina pelagica* TBN21^T, *Halolamina salina* WSY15-H3^T and *Halolamina salifodinae* WSY15-H1^T of 98.3, 97.6 and 97.3 %, respectively; the similarities for the *rpoB′* gene sequences between the same strains were 96.0, 95.3 and 94.6 %, respectively. The colonies of both strains were observed to be red pigmented on growth medium. Strain CBA1107^T was observed to grow at 20–50 °C, in the presence of 15–30 % NaCl, at pH 6.0–9.0, and with 0.005–0.5 M Mg^{2+}. The cells of both strains lysed in distilled water. The DNA–DNA hybridization experiments showed that strain CBA1107^T shared 97 % relatedness with CBA1108 and <50 % relatedness with *H. pelagica* JCM 16809^T, *H. salina* JCM 18549^T and *H. salifodinae* JCM 18548^T. The genomic DNA G+C content of strain CBA1107^T was determined to be 65.1 mol%. The major polar lipids of the two strains were phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and glycolipids

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including sulfated mannosyl glucosyl diether and mannosyl glucosyl diether. Based on the polyphasic taxonomic analyses, the strains are considered to represent a new taxon for which the name *Halolamina rubra* sp. nov. is proposed, with the type strain CBA1107^T (=CECT 8421^T =JCM 19436^T).

Keywords Haloarchaea · *Halolamina rubra* sp. nov. · Solar salt · Polyphasic taxonomy

Introduction

The members of the family *Halobacteriaceae* are distributed across various saline environments such as solar salterns, soda lakes, salt lakes, salt mines, fermented sea foods (Roh et al. 2007a, b, 2008; Roh and Bae 2009; Roh et al. 2009) and deep-sea brine (Antunes et al. 2008). What these environments share is an extremely high-salt condition that is harmful to most cells but to which halophilic archaea are adapted for growth (Grant 2004). The number of isolated and described halophilic archaeal strains is increasing, with those from hypersaline conditions being described at an accelerated pace. *Halolamina pelagica* was isolated from the hypersaline environment of a solar saltern and described by Cui et al. (2011). Subsequently, *Halolamina salifodinae* and *Halolamina salina* were isolated from a salt mine and described by Zhang et al. (2013). These *Halolamina* strains are pink or red pigmented and pleomorphic, and require a minimum of 1.4 M NaCl for growth, with an optimum of 3.4–3.9 M NaCl. The temperature and pH ranges for growth of *Halolamina* species are 20–50 °C and pH 5.5–9.5, with optimum growth at 37–42 °C and pH 7.0–7.5. Cells of *Halolamina* species lyse in distilled water and are positive for oxidase and catalase activity. The major polar lipids of *Halolamina* species are phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulphate (PGS) and several glycolipids (Cui et al. 2011; Zhang et al. 2013).

In this study, we report two novel strains, CBA1107^T and CBA1108, which were isolated from non-purified solar salt in the Republic of Korea, and we conclude they represent a novel halophilic species within the genus *Halolamina* based on polyphasic taxonomic analysis.

Materials and methods

Haloarchaeal strain and culture condition

The haloarchaeal isolates, designated CBA1107^T and CBA1108, were cultivated by suspending 5 g of a non-purified solar salt sample produced in a solar saltern (E126°17′15″, N34°36′33″) in the Republic of Korea, in 30 mL of DSM medium no. 372 (M372; 5 g casamino acid, 5 g yeast extract, 1 g sodium glutamate, 3 g trisodium citrate, 2 g KCl, 20 g $MgSO_4$·7H2O, 36 mg $FeCl{\cdot}4H_2O$, 0.36 mg $MnCl_2{\cdot}4H_2O$, and 200 g NaCl per litre, adjusted to pH 7.0–7.2 with 1 N NaOH). The aliquots of medium were serially diluted spread onto M372 agar (containing 2 % agar, w/v) plates that were incubated at 37 °C for 1 month. The colonies were successively streaked three or more times on to M372 agar to obtain pure cultures. From the resulting colonies, two isolates were designated as strains CBA1107^T and CBA1108. *H. pelagica* JCM 16809^T, *H. salina* JCM 18549^T and *H. salifodinae* JCM 18548^T were obtained from the Japan Collection of Microorganisms (JCM) and used as reference strains. The reference strains were routinely cultivated on M372 medium at 37 °C.

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

The genomic DNA from the strains was extracted and purified as described by Sambrook et al. (1989). The 16S rRNA gene of strains CBA1107^T and CBA1108 were amplified by PCR using an archaea-specific primer set as described previously (Cui et al. 2009), and the PCR product was sequenced. The amplification of *rpoB′* was performed with the HrpoB2 1420F and HrpoA 153R primers as described by Minegishi et al. (2010). The amplified PCR products were sequenced and assembled as previously described Roh et al. (2008). The 16S rRNA and *rpoB′* gene sequences were compared with EzTaxon-e (Kim et al. 2012) and NCBI BLAST (Altschul et al. 1990), respectively. The 16S rRNA gene sequence alignments were performed with the SINA alignment service, which considers the secondary structure of the rRNA gene (Pruesse et al. 2012). The multiple sequence alignments of the *rpoB′* gene sequences were performed with CLUSTAL_W software. Phylogenetic

trees were constructed with the 16S rRNA and *rpoB′* gene sequences of strains CBA1107^T, CBA1108 and reference strains using MEGA 5 software (Tamura et al. 2011). A distance matrix was constructed using the two-parameter model described by Kimura (1980). The phylogenetic trees were generated by the neighbour-joining (NJ) (Saitou and Nei 1987), minimum-evolution (ME) (Nei et al. 1998) and maximum-likelihood (ML) (Felsenstein 1981) methods with 1,000 bootstrap replications for each.

Physiological, morphological and biochemical characterisation

Strains CBA1107^T and CBA1108 were phenotypically characterized utilizing the proposed minimal standards for describing extremely halophilic archaea as defined by Oren et al. (1997). All tests were performed in triplicate unless stated otherwise. Optimal growth conditions were determined using M372 medium as a basal medium in the presence of 0–30 % NaCl (w/v) at 5 % intervals and from 5, 10, 15, 20, 25, 30, 37, 40, 45, 50 and 55 °C. The optimal pH was determined by growth on M372 medium adjusted to pH 5.0–11.0 at intervals of 1.0 by addition of 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. The requirement for Mg^{2+} for growth was determined using M372 medium made without $MgSO_4{\cdot}7H_2O$, which was then supplemented with 10 mM bis–Tris propane and $MgSO_4{\cdot}7H_2O$ at molar concentrations of 0, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 or 0.5 M.

The cell morphology and size were determined by observation with a phase-contrast microscope (Eclipse 80*i*; Nikon) and motility was observed by the hanging-drop and semi-solid agar methods with 0.5 % M372 agar (Agarwal et al. 1997; Bernardet et al. 2002). Gram staining was performed according to the method of Dussault (1955). Salt concentrations required to prevent cell lysis were determined using saline solutions containing 0–14 % NaCl (w/v) at 2 % intervals and the cell lysis was measured by light microscopic examination (Cui et al. 2011). The tests for catalase, oxidase and urease activity, nitrate to nitrite or nitrogen reduction under aerobic conditions, indole production and hydrolysis of casein and starch were examined as described by Benson (2002) using M372 medium as the basal medium. The hydrolysis of Tweens 40 and 80 was determined as described by Gonzalez et al. (1978) and the hydrolysis of gelatine was examined as described by Smibert and Krieg (1994). The production of H_2S was tested according to Cui et al. (2007). Acid production was tested in M372 medium supplemented with 1 % (w/v) D-fructose, D-galactose, D-glucose, D-mannose and L-sorbose. Methyl red or Voges–Proskauer test was determined using MR-VP Broth (BD). The production of arginine, lysine and ornithine decarboxylase and *β*-galactosidase were performed as described by Oren et al. (1997). To test growth under anaerobic conditions, the strains were cultivated using the M372 as the basal medium in the presence of nitrate (30 mM), L-arginine (5 g l^{-1}), trimethylamine *N*-oxide (TMAO) (5 g l^{-1}) and dimethyl sulfoxide (DMSO) (5 g l^{-1}) at 37 °C for 1 month in a Coy anaerobic chamber with an atmosphere of $N_2{\cdot}CO_2{\cdot}H_2$ (90:5:5, v/v/v). To test for the utilization of sole carbon and energy sources, M372 medium was modified by replacing the casamino acid, yeast extract, sodium glutamate and trisodium citrate with 0.1 g l^{-1} yeast extract and 10 mM bis–Tris propane, and 1 % of the following substrates were added to the medium to test individually: 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. To test antibiotic sensitivity, the strains were cultivated on the M372 agar for 1 month at 37 °C with the following antibiotic discs (μg/disc, unless otherwise indicated): ampicillin (20), bacitracin (0.1 IU), chloramphenicol (50), ciprofloxacin (10), erythromycin (25), gentamicin (50), kanamycin (50), nalidixic acid (50), neomycin (50), nitrofurantoin (50), norfloxacin (20), novobiocin (50), nystatin (2), penicillin G (20 IU), rifampicin (10), streptomycin (50), tetracycline (50), trimethoprim (20) and vancomycin (50).

Determination of the DNA G+C content, DNA–DNA hybridisation (DDH)

The DNA G+C content was determined as described by Gonzalez and Saiz-Jimenez (2002). DDH experiments were performed by the fluorometric method

using photobiotin-labelled DNA probes and microwell plates (MaxiSorp, FluoroNunc) as described by Ezaki et al. (1989).

Polar lipid analysis

Polar lipids were extracted and detected with specific reagents (Dittmer and Lester 1964; Xin et al. 2000) on a Merck silica gel 60 F_{254} plate as described by Oren et al. (1996) with the following reagents: sulfuric acid–ethanol (1:2, v/v) for total lipids, ninhydrin for amino-containing lipids, molybdenum blue for phospholipids, and α-naphthol-sulphuric acid for glycolipids. The results of the polar lipid spot tests were compared with the three reference species, *H. pelagica* JCM 16809^T, *H. salina* JCM 18549^T and *H. salifodinae* JCM 18548^T (Cui et al. 2011; Zhang et al. 2013).

Results and discussion

Nearly complete sequences were obtained from the strains CBA1107^T and CBA1108 for the 16S rRNA (1,473 bp for each) and *rpoB′* (1,833 and 1,833 bp, respectively) genes. The sequence similarity between strains CBA1107^T and CBA1108 was 99.8 % for the 16S rRNA gene and 99.0 % for *rpoB′*. The sequence similarities between strain CBA1107^T and *H. pelagica* TBN21^T, *H. salina* WSY15-H3^T and *H. salifodinae* WSY15-H1^T were 98.3, 97.6 and 97.3 % for the 16S rRNA and 96.0, 95.3 and 94.6 % for the *rpoB′* gene sequences, respectively. The sequence similarity values of the 16S rRNA and *rpoB′* gene sequences indicated that strains CBA1107^T and CBA1108 are affiliated with members of the genus *Halolamina* in the family *Halobacteriaceae*. In the phylogenetic trees based on the 16S rRNA and *rpoB′* gene sequences, strains CBA1107^T and CBA1108 clustered with the type strains in the genus *Halolamina*, with 100 % bootstrap values, regardless of the used tree-reconstruction algorithm (Fig. 1). Thus, phylogenetic analyses of the 16S rRNA and *rpoB′* gene sequences with other haloarchaea showed that strains CBA1107^T and CBA1108 clearly belong to the genus *Halolamina*, forming a tight phyletic lineage with the following members of the three validly named species in the genus *Halolamina*: *H. pelagica*, *H. salifodinae* and *H. salina*, which was supported by the high gene sequence similarities and high bootstrap values of the phylogenetic trees.

The cells of strains CBA1107^T and CBA1108 were observed to be red pigmented, Gram-stain negative, rod-shaped and 0.3–0.5 μm wide by 1.5–2.5 μm long (Supplementary Fig. S1). Strain CBA1107^T grew in 15–30 % NaCl (optimum 20–25 %), at 20–50 °C (optimum 37 °C), pH 6.0–9.0 (optimum 7.0) and with 0.005–0.5 M Mg^{2+} (optimum 0.1–0.2 M). Strain CBA1108 was able to grow in 15–30 % NaCl (optimum 20 %), at 20–50 °C (optimum 37 °C), pH 6.0–8.0 (optimum 7.0) and with 0–0.5 M Mg^{2+} (optimum 0.1–0.2 M). The cells of strains CBA1107^T and CBA1108 lysed in distilled water. The minimal NaCl concentration to prevent cell lysis was found to be 12 % (w/v). Both strains were determined to be motile and positive for activity of catalase and oxidase, but they were negative for activity of urease, *β*-galactosidase, arginine, lysine and ornithine decarboxylase, nitrate reduction to nitrite or nitrogen, and H_2S and indole production. Strains CBA1107^T and CBA1108 produced acid from D-galactose, D-glucose and D-mannose. The two strains were negative for Methyl red-Voges-Proskauer test. Neither strain grew in M372 medium supplemented with DMSO, TMAO, L-arginine and nitrate under anaerobic conditions, nor were they able to hydrolyse starch, Tween 40, Tween 80, casein or gelatine. Strain CBA1107^T was not able to grow without Mg^{2+}, but strain CBA1108 was found to be able to grow. Strain CBA1107^T was found to utilize L-arginine, D-galactose, D-glucose, L-glutamate, glycine, L-lysine and D-mannose and pyruvate whereas strain CBA1108 was able to utilize D-galactose, D-glucose, L-glutamate, L-lysine D-mannose and pyruvate as sole carbon and energy source. Strains CBA1107^T and CBA1108 were observed to be sensitive to bacitracin, chloramphenicol, erythromycin, neomycin, nitrofuranotoin, novobiocin and rifampicin but resistant to ampicillin, ciprofloxacin, gentamicin, kanamycin, nalidxic acid, norfloxacin, nystatin, penicillin G, streptomycin, tetracycline, trimethoprim and vancomycin. The detailed characteristics of the strains are presented in the species description and are compared to related members of the genus *Halolamina* in Table 1. Strains CBA1107^T and CBA1108 differ from the three validly named species in the genus *Halolamina* with regard to distinct phenotypic properties, such as minimal NaCl concentration for growth, minimal NaCl concentration to prevent cell lysis and acid production from D-mannose, as well as the utilization pattern of various

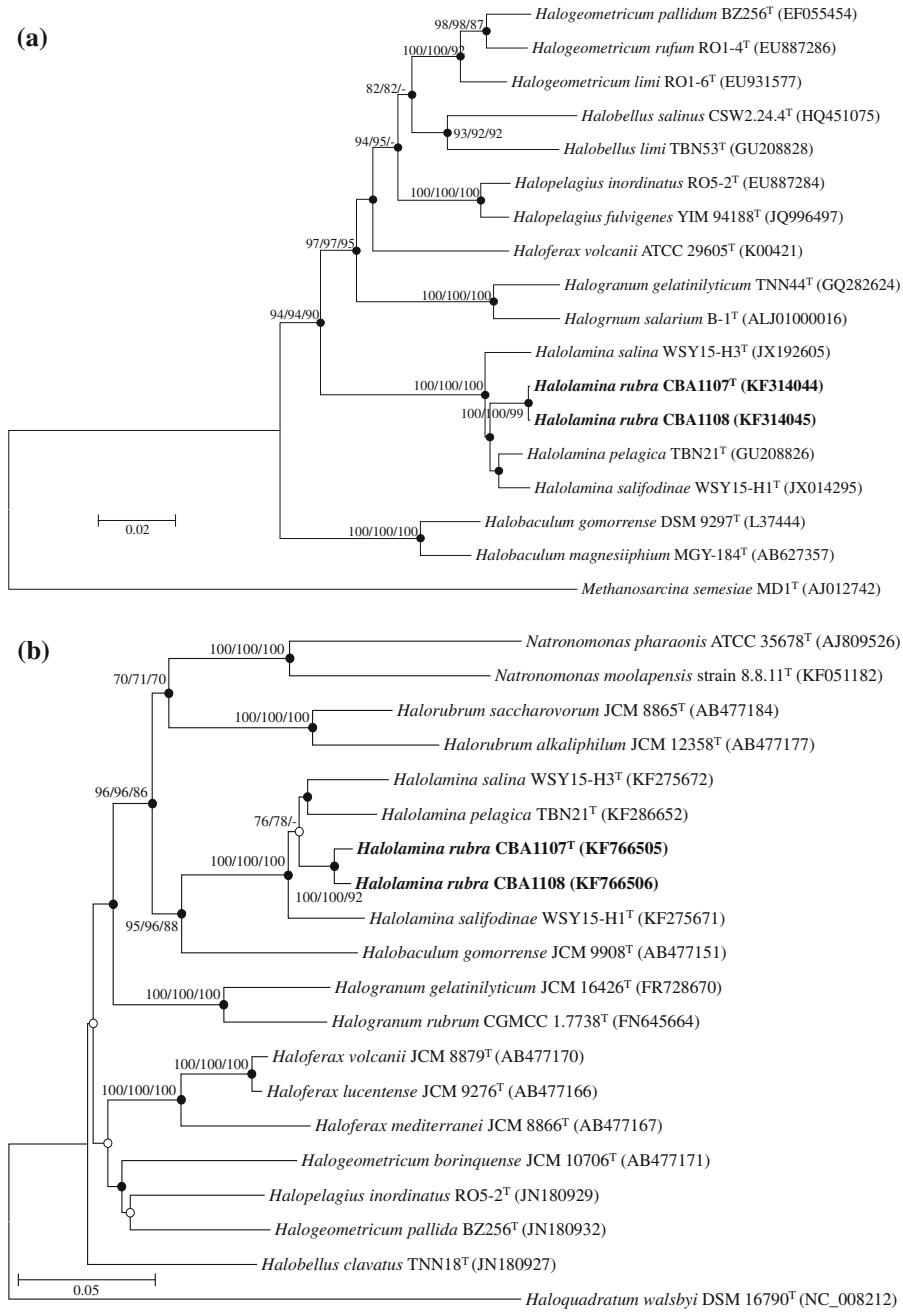

Fig. 1 Phylogenetic trees based on the NJ algorithm for the 16S rRNA (**a**) and *rpoB′* (**b**) gene sequences of strains CBA1107^T, CBA1108 and closely related taxa. The numbers on the nodes indicate the bootstrap values (>70 %) calculated using the NJ/ME/ML probabilities. The closed circles represent nodes also recovered by both the ME and ML methods, while the open circles indicate nodes recovered by either the ME or ML method. *Methanosarcina semesiae* MD1^T and *Haloquadratum walsbyi* DSM 16790^T served as the outgroups in the phylogenetic trees of the 16S rRNA and *rpoB′* genes, respectively. *Bars* represent 0.02 and 0.05 accumulated changes per nucleotide for the 16S rRNA and *rpoB′* genes, respectively

carbon sources. Interestingly, the motility of the two isolates is the first report of this feature in the genus *Halolamina* (Cui et al. 2011; Zhang et al. 2013).

The DDH values for the hybridizations between strains CBA1107^T and CBA1108, *H. pelagica* JCM 16809^T, *H. salina* JCM 18549^T and *H. salifodinae*

Table 1 Differential characteristics of strains CBA1107^T, CBA1108 and closely related species in the genus *Halolamina*

Characteristic	1	2	3	4	5
Motility	+	+	–	–	–
NaCl range for growth (%, w/v)	15–30	15–30	10–30	10–30	10–30
Optimum NaCl (%, w/v)	20–25	20	20–23	20	20–23
Mg^{2+} range for growth (M)	0.005–0.5	0–0.5	0–0.7	0–2.5	0–2.0
Optimum Mg^{2+} (M)	0.1–0.2	0.1–0.2	0.01–0.05	0.02–2.5	0.1–0.5
Temperature range for growth (°C)	20–50	20–50	25–50	20–50	20–45
Optimum temperature (°C)	37	37	37	37–42	37
pH range for growth	6.0–9.0	6.0–8.0	5.5–9.5	6.0–7.5	6.0–9.0
Optimum pH	7.0	7.0	7.0–7.5	7.0	7.0–7.5
Minimal NaCl concentration to prevent cell lysis (%, w/v)	12	12	8	8	9
Starch hydrolysis	–	–	+	–	–
Nitrate reduction	–	–	+	–	–
Acid production from D-mannose[a]	+	+	–	+	–
Utilization of[a]					
L-Alanine	–	–	+	–	–
L-Arginine	+	–	+	+	+
L-Aspartate	–	–	–	–	+
D-Galactose	+	+	+	+	–
Glycine	+	–	–	–	–
DL-Lactate	–	–	+	–	+
L-Lysine	+	+	–	–	+
L-Malate	–	–	–	–	+
D-Mannose	+	+	+	–	–
L-Ornithine	–	–	+	–	+
Starch	–	–	+	–	–
Sucrose	–	–	–	+	–
DNA G + C content (mol%)	65.1	64.3	64.8	66.2	65.4

Data were obtained from this study, Cui et al. (2011) and Zhang et al. (2013), unless otherwise indicated. All of the strains lysed in distilled water and growth were not obtained from anaerobic conditions; positive for activity of catalase and oxidase and utilisation of D-glucose, L-glutamate and pyruvate and acid production of D-galactose and D-glucose; negative for H_2S production, urase activity, arginine, lysine, ornithin decarboxylase, β-galactosidase, acid production from D-fructose and L-sorbose, MR-VP test, hydrolysis of gelatine, casein and Tween 80, indole production and utilisation of acetate, citrate, D-fructose, fumarate, glycerol, lactose, maltose, mannitol, D-ribose, sorbitol L-sorbose, succinate and D-xylose

Taxa: 1 Halolamina rubra CBA1107^T sp. nov.; *2 H. rubra* CBA1108 sp. nov.; *3 H. pelagica* JCM 16809^T; *4 H. salina* JCM 18549^T; *5 H. salifodinae* JCM 18548^T. + positive; − negative

[a] Data from this study

JCM 18548^T were 97 ± 3, 49 ± 4, 42 ± 6 and 25 ± 2 %, respectively. Current prokaryotic systematics defines DDH values of <70 % as an indicator of a distinct species (Wayne et al. 1987; Stackebrandt and Goebel 1994). Thus the DDH values indicate that strains CBA1107^T and CBA1108 are members of the same species and can be distinguished from other species in the genus *Halolamina*. The genomic DNA G+C content of strains CBA1107^T and CBA1108 were determined to be 65.1 and 64.3 mol%, respectively, which are similar to values previously reported for the strains *H. pelagica* JCM 16809^T (64.8 mol%), *H. salina* JCM 18549^T (66.2 mol%) and *H. salifodinae* JCM 18548^T (65.4 mol%) (Cui et al. 2011; Zhang et al. 2013).

The polar lipids of strains CBA1107^T and CBA1108 comprised PG, PGP-Me, PGS, an unidentified phospholipid (PL) and five glycolipids (GLs);

two of these glycolipids (GL3 and GL4) are chromatographically identical to sulfated mannosyl glucosyl diether (S-DGD-1) and mannosyl glucosyl diether (DGD-1), respectively (Fig. S2). The major polar lipid profiles of the strains were similar with the profiles of the reference strains, supporting inclusion of the strains in the genus *Halolamina*.

In conclusion, strains CBA1107^T and CBA1108 are closely related to members of the genus *Halolamina* in the family *Halobacteriaceae*, but could be differentiated from the other type strains of the genus *Halolamina* (*H. pelagica*, *H. salina* and *H. salifodinae*). Based on the polyphasic taxonomic analyses, the extremely halophilic archaeal strains CBA1107^T and CBA1108 are considered to represent a novel species of the genus *Halolamina*, for which the name *Halolamina rubra* sp. nov. is proposed.

Description of *Halolamina rubra* sp. nov

Halolamina rubra (ru′bra. L. fem. adj. *rubra*, red)

Cells are Gram-stain negative, motile and rod shaped under optimal growth conditions. When colonies are grown on agar plates containing 20 % NaCl (w/v), they are red pigmented and round. The cells are 0.3–0.5 μm in width and 1.5–2.5 μm in length. Growth occurs at 20–50 °C (optimum 37 °C), in the presence of 15–30 % NaCl (w/v; optimum 20–25 %, with 0–0.5 M $MgSO_4 \cdot 7H_2O$ (optimum 0.1–0.2 M) and at pH 6.0–9.0 (optimum pH 7.0). Does not grow under anaerobic conditions on medium containing nitrate, L-arginine, DMSO or TMAO. Lyses in distilled water and the minimal NaCl concentration to prevent cell lysis is 12 % (w/v). Positive for catalase and oxidase; negative for urease activity, reduction of nitrate to nitrite or nitrogen, production of H_2S, Methyl red-Voges-Proskauer test, arginine, lysine and ornithine decarboxylase, β-galactosidase, indole production and hydrolysis of starch, gelatine, Tween 40, Tween 80 or casein. Acid is produced from D-galactose, D-glucose and D-mannose, but not from D-fructose or L-sorbose. D-Galactose, D-glucose, L-glutamate D-mannose, pyruvate and L-lysine are utilized as a single carbon and energy source for growth. L-arginine or glycine utilisation is variable. No growth occurs on the following substrates when offered as single carbon and energy sources: acetate, L-alanine, L-aspartate, citrate, D-fructose, fumarate, glycerol, DL-lactate, lactose, L-malate, maltose, mannitol, L-ornithine, D-ribose, sorbitol, L-sorbose, starch, succinate, sucrose and D-xylose. The polar lipids are PG, PGP-Me, PGS, phospholipids and GLs including S-DGD-1 and DGD-1. The genomic DNA G+C content of the species is 64.3–65.1 mol%.

The type strain CBA1107^T (=CECT 8421^T =JCM 19436^T) and an additional strain CBA1108 (=CECT 8420 =JCM 19437) were isolated from non-purified solar salt in the Republic of Korea. The GenBank/EMBL/DDBJ accession numbers for strains CBA1107^T and CBA1108 of the 16S rRNA gene sequences are KF314044 and KF314045, respectively, and the *rpoB′* gene sequences are KF766505 and KF766506, respectively.

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References

Agarwal S, Hunnicutt DW, McBride MJ (1997) Cloning and characterization of the *Flavobacterium johnsoniae* (*Cytophaga johnsonae*) gliding motility gene, *gldA*. Proc Natl Acad Sci USA 94(22):12139–12144

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3): 403–410

Antunes A, Taborda M, Huber R, Moissl C, Nobre MF, da Costa MS (2008) *Halorhabdus tiamatea* sp. nov., a non-pigmented, extremely halophilic archaeon from a deep-sea, hypersaline anoxic basin of the Red Sea, and emended description of the genus *Halorhabdus*. Int J Syst Evol Microbiol 58(1):215–220

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

Bernardet JF, Nakagawa Y, Holmes B (2002) Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. Int J Syst Evol Microbiol 52(3):1049–1070

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(Pt 10):2204–2206

Cui HL, Zhou PJ, Oren A, Liu SJ (2009) Intraspecific polymorphism of 16S rRNA genes in two halophilic archaeal genera, *Haloarcula* and *Halomicrobium*. Extremophiles 13(1):31–37

Cui HL, Gao X, Yang X, Xu XW (2011) *Halolamina pelagica* gen. nov., sp. nov., a new member of the family *Halobacteriaceae*. Int J Syst Evol Microbiol 61(7):1617–1621

Dittmer JC, Lester RL (1964) A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. J Lipid Res 15:126–127

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

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

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

Gonzalez 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

Gonzalez C, Gutierrez 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 Biol Sci 359:1249–1267

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

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

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, Duker S, Ritter S (1996) The polar lipid composition of Walsby's square bacterium. FEMS Microbiol Lett 138(2–3):135–140

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

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 59:2359–2363

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

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt PRGEM, Wood WA. Wood, 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

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

Xin H, Itoh T, Zhou P, Suzuki K, Kamekura M, Nakase T (2000) *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. Int J Syst Evol Microbiol 50:1297–1303

Zhang WY Jr, Huo YY, Zhang XQ, Zhu XF, Wu M (2013) *Halolamina salifodinae* sp. nov. and *Halolamina salina* sp. nov., two extremely halophilic archaea isolated from a salt mine. Int J Syst Evol Microbiol 63(12):4380–4385