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A report of sixteen unrecorded haloarchaea species in Korea, isolated from a solar saltern

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In July 2018, solar saltern samples were collected from Siheung, Gyeonggi-do Province to obtain unrecorded haloarchaea in Korea. The samples were suspended in a 20% NaCl (w/v) solution, and serial dilution was performed in fresh DB Characterization media No. 2. The strains isolated in this study showed at least 98.7% sequence similarity or more compared to the previously reported. Finally, 16 haloarchaeal strains, which were not reported in Korea but validly published under the International Code of Nomenclature of Prokaryotes (ICNP), were obtained from a solar saltern in Siheung. These 16 isolates were allocated to the orders *Halobacteriales* and *Haloferacales*. The 10 *Halobacteriales* strains were classified into the family *Halobacteriaceae* and *Haloarculaceae*. Each family belonged to three genera, respectively. The other six *Haloferacales* belonged to the families *Haloferacaceae* and *Halorubraceae*. Each family belonged to genus genus, respectively. Collectively, the unrecorded haloarchaeal strains belonged to two orders, four families, and eight genera. During the research, the possibility of discovering previously unknown species in domestic solar saltern was established. Gram-staining, cell morphology, physiological and basic biochemical parameters, and phylogenetic analysis were all performed in this study and are described in detail for each strain.

Keywords: 16S rRNA gene sequences, domestic solar saltern, haloarchaea, unrecorded haloarchaea species

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Introduction

Extremophiles are microbes that can grow, adapt, and live in severe environments such as high salinity, high or low temperature, and acidic or alkaline conditions (Rampelotto, 2013). Among them, haloarchaea flourish in hypersaline habitats such as natural brine, the Dead Sea, alkaline salt lakes, marine solar salterns, and rock salt deposits (Kato *et al*., 1995; Stan-Lotter and Fendrihan, 2006). In addition to high salinity, haloarchaea are subjected to extreme circumstances such as high temperature, UV radiation, severe ionic stresses, and alkaline pH. These microorganisms create compounds such as carotenoids, which aid in adaptability, survival, and growth in harsh environmental conditions (Oren, 2014). Carotenoids are abundant in haloarchaea and serve as antioxidants, sun protection pigments, and membrane stabilizers (Squillaci *et al*., 2017). Bacterioruberin, a haloarchaeal carotenoid, is utilized in a variety of food and cosmetic items and is said to have higher free radical scavenging action than others. These metabolites and proteins have properties that make them appropriate for a variety of industrial and research uses. Proteins and enzymes from this haloarchaea group function at salt concentrations where bacterial counterparts fail to function (Singh and Singh, 2017). According to these qualities, haloarchaeal enzymes are appropriate for salt-based applications and use under dehydrating circumstances.

There are limited studies describing microbial biodiversity in the overall solar salterns. Therefore, we have been working on the isolation of extreme haloarchaea strains from solar salterns to study the diversity. The current study was part of an effort to determine domestic biodiversity, and it focused mostly on unrecorded haloarchaea species that have not been reported in Korea before. In the current study, we briefly describe 16 haloarchaea species in the orders *Halobacteriales* and *Haloferacales* belonging to eight genera (*Haloarcula*, *Halobacterium*, *Haloferax*, *Halomicroarcula*, *Haloplanus*, *Halorientalis*, *Halorubrum*, and *Salarchaeum*) of four families (*Haloarculaceae*, *Halobacteriaceae*, *Haloferacaceae*, and *Halorubraceae*) based on the 16S rRNA gene sequences. Phenotypic characteristics were reported, which were identified as unrecorded haloarchaea species in Korea.

Materials and Methods

Samples were collected from a solar saltern in Siheung in July 2018. The obtained solar saltern samples (1.0 g) were suspended in 10 mL of 20% NaCl (v/v) and vortexed. The sample solution was serially diluted and an aliquot (100 μL) of the solar saltern samples was directly spread onto DB characterization medium No. 2 (DBCM2). The medium contained the following components (l^{-1}): 833 mL MDS salt water (7 g of KCl, 35 g of $MgSO_4 \cdot 7H_2O$, 30 g of $MgCl_2 \cdot 6H_2O$, 240 g of NaCl, and 5 mL of 1 M $CaCl_2$ solution), 1 mL of $FeCl_2$ solution, 0.25 g of peptone (Oxoid), 0.05 g of yeast extract (Difco), 5 mL of 1 M NH_4Cl, 2 mL of potassium phosphate buffer, 1 mL of trace element solution (2.0 mg of $FeSO_4 \cdot 7H_2O$, 141.5 mg of $ZnSO_4 \cdot 7H_2O$, 57.6 mg of $MnSO_4 \cdot H_2O$, 6.0 mg of H_3BO_3, 190.0 mg of $CoCl_2 \cdot 6H_2O$, 2.0 mg of $CuCl_2 \cdot 2H_2O$, 24.0 mg of $NiCl_2 \cdot 6H_2O$, and 36.0 mg of $Na_2MoO_4 \cdot 2H_2O$ per liter), 3 mL of vitamin solution (3.0 mg of biotin, 4.0 mg of folic acid, 50.0 mg of pyridoxine·HCl, 33.0 mg of thiamine · HCl, 10.0 mg of riboflavin, 33.0 mg of nicotinic acid, 17.0 mg of DL-calcium pantothenate, 17.0 mg of vitamin B_{12}, 13.0 mg of *p*-aminobenzoic acid, and 10 mg of lipoic acid per liter), and 10 mL of 1 M sodium pyruvate solution with 2% (w/v) agar adjusted to pH 7.0 using 1 M Tris-base buffer solution. Each plate was incubated at 37°C for 14 days, and different colonies were selected and repeatedly subcultured to the new culture media at the interval of 14 days. After more than thrice time, the pure colonies were obtained and stored at −80°C in a 20% (w/v) glycerol with 15% NaCl (w/v) stock solution for preservation.

Phenotypic experiments were performed on cultures grown at 37°C in the DBCM2 agar plate. The cell morphology of isolated strains was examined using light microscopy (model CX 23; Olympus, Tokyo, Japan) and transmission electron microscopy (LIBRA 120; Carl Zeiss, Oberkochen, Germany). Gram-staining of isolated strain was performed using a Gram stain kit (BioWORLD, Dublin, OH) according to the manufacturer's instructions. Each strain was cultured in DBCM2 liquid media with NaCl ranging from 5% to 30% (w/v) at increments of 5% for 14 days at 37°C and 200 rpm to evaluate the cell growth at varied salinities. The pH range for cell growth of isolated strains was assessed by growing the cells for 14 days with increments of 1.0 pH unit from pH 4.0 to 12.0 at 30°C and 200 rpm using the following buffer solutions: 100 mM of CH_3COOH/CH_3COONa buffer (pH 4.0−6.0), 100 mM of NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0−8.0), 100 mM of $NaHCO_3/Na_2CO_3$ buffer (pH 9.0−10.0), and 100 of mM Na_2CO_3/NaOH buffer (pH 11.0−12.0). To ascertain the temperature range for growth, each strain was cultivated on solid culture and incubated at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45, and 55°C. Catalase and oxidase tests were performed using 3% (v/v) H_2O_2 and 1% tetramethyl-p-phenylenediamine. Energy sources used in assimilation tests were D-glucose, D-mannose, D-galactose, maltose, sucrose, lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, D-xylose, trehalose, raffinose, glycerol, L-sorbose, pyruvate, D-mannitol, succinate, DL-lactate, L-malate, fumarate, citrate, acetate, L-glycine, L-histidine, L-alanine, L-glutamate, L-arginine, L-lysine, and L-ornithine at 0.1% (w/v) based on DBCM2 broth culture.

Genomic DNA was extracted using the HiYield™ Genomic DNA Mini Kit (RBC Bioscience, Taiwan). The partial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using archaeal-specific primers (Cui *et al*., 2009). The obtained PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). The purified PCR products were sent to Macrogen Co., Ltd. (Seoul, Korea) for sequence analysis. The products were assembled using the SeqMan™ II expert sequence analysis software (Thombre *et al*., 2016). Assembled sequences were identified based on the EzBioCloud 16S-based ID (https://www.ezbiocloud.net/identify). The closely related taxa were obtained by the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon) (Yoon *et al*., 2017). Multiple sequence alignments were conducted using the ClustalW multiple sequence alignment program in the BioEdit 7.2.6.1 software (Thompson *et al*., 1994; Hall, 1999). Phylogenetic trees were constructed by phylogeny to build the application in MEGA 7.0 (Kumar *et al*., 2016), based on the 16S rRNA gene sequence. Using the neighbor-joining (NJ) algorithm, the sequence relatedness was calculated (Saitou and Nei, 1987). The phylogenetic tree was conducted using the bootstrap method, and the bootstrap values were set to 1000 replications. The Kimura two-parameter model was applied to calculate evolutionary distances (Kimura, 1980).

Results and Discussion

The 16S rRNA gene sequence analyses obtained herein revealed that a total of 16 strains belonged to previously unreported species in Korea. The strain information, identification, taxonomic assignment from species to classes, isolation source, and sequence accession numbers including the NIBR and GenBank are listed in Table 1. The 16 strains are distributed in two orders of *Halobacteriales* and *Haloferacales*, 10 strains in the order *Halobacteriales*, and six strains in the order *Haloferacales*. In this study, isolated unrecorded haloarchaea strains were various morphology such as coccus, rod, pleomorphic coccus, and rod shaped (Fig. 1). All these strains were Gram-staining negative and lysed in distilled water.

The 10 strains in the order *Halobacteriales* belong to the two families *Halobacteriaceae* and *Haloarculaceae*. The family *Halobacteriaceae* included the genera *Halobacterium* (3 species), *Haloplanus* (1 species), and *Salarchaeum* (1 species), and the family *Haloarculacea* included the genera *Haloarcula* (2 species), *Halomicroarcula* (1 species), and *Halorientalis* (2 species). The six strains in the order *Haloferacales* belong to the two families *Haloferacaceae* and *Halorubraceae*. The family *Haloferacaceae* included the genus *Haloferax* (2 species) and the family *Halorubraceae* included the genus *Halorubrum* (4 species). The identification of the 16 strains corresponding to 2 different orders based on sequence similarity is supported by the phylogenetic tree using the NJ algorithm, respectively (Figs. 2 and 3). Detailed morphological, physiological, and basic biochemical characteristics of the 16 unrecorded haloarchaea species are elucidated in the following strain descriptions.

Table 1. Summary of all strains isolated in this study and their taxonomic affiliations.

Order	Family	Strain ID	Accession/ NIBRARC number	Closest species	Similarity (%)	Isolation source	Medium
Halobacteriales	*Halobacteriaceae*	MBLA0001	OP077334 NIBRARC000509741	*Halobacterium salinarum*	100	Solar saltern	DBCM2 at 37°C, 14 days
		MBLA0010	OP077335 NIBRARC000509742	*Halobacterium hubeiense*	99.8		
		MBLA0034	OP077337 NIBRARC000509744	*Halobacterium rubrum*	99.4		
		MBLA0145	OP077347 NIBRARC000509752	*Haloplanus natans*	98.7		
		MBLA0217	OP077349 NIBRARC000509756	*Salarchaeum japonicum*	99.8		
	Haloarculaceae	MBLA0131	OP077342 NIBRARC000509749	*Haloarcula marismortui*	99.4		
		MBLA0133	OP077343 NIBRARC000509750	*Haloarcula rubripromontorii*	99.1		
		MBLA0135	OP077344 NIBRARC000509753	*Halomicroarcula salina*	99.3		
		MBLA0140	OP077346 NIBRARC000509754	*Halorientalis persicus*	99.7		
		MBLA0170	OP077348 NIBRARC000509755	*Halorientalis pallida*	99.4		
Haloferacales	*Haloferacaceae*	MBLA0123	OP077340 NIBRARC000509747	*Haloferax denitrificans*	99.9		
		MBLA0129	OP077341 NIBRARC000509748	*Haloferax prahovense*	100		
	Halorubraceae	MBLA0028	OP077336 NIBRARC000509743	*Halorubrum xinjiangense*	99		
		MBLA0042	OP077338 NIBRARC000509745	*Halorubrum coriense*	99		
		MBLA0071	OP077339 NIBRARC000509746	*Halorubrum trapanicum*	98.8		
		MBLA0137	OP077345 NIBRARC000509751	*Halorubrum sodomense*	99.2		

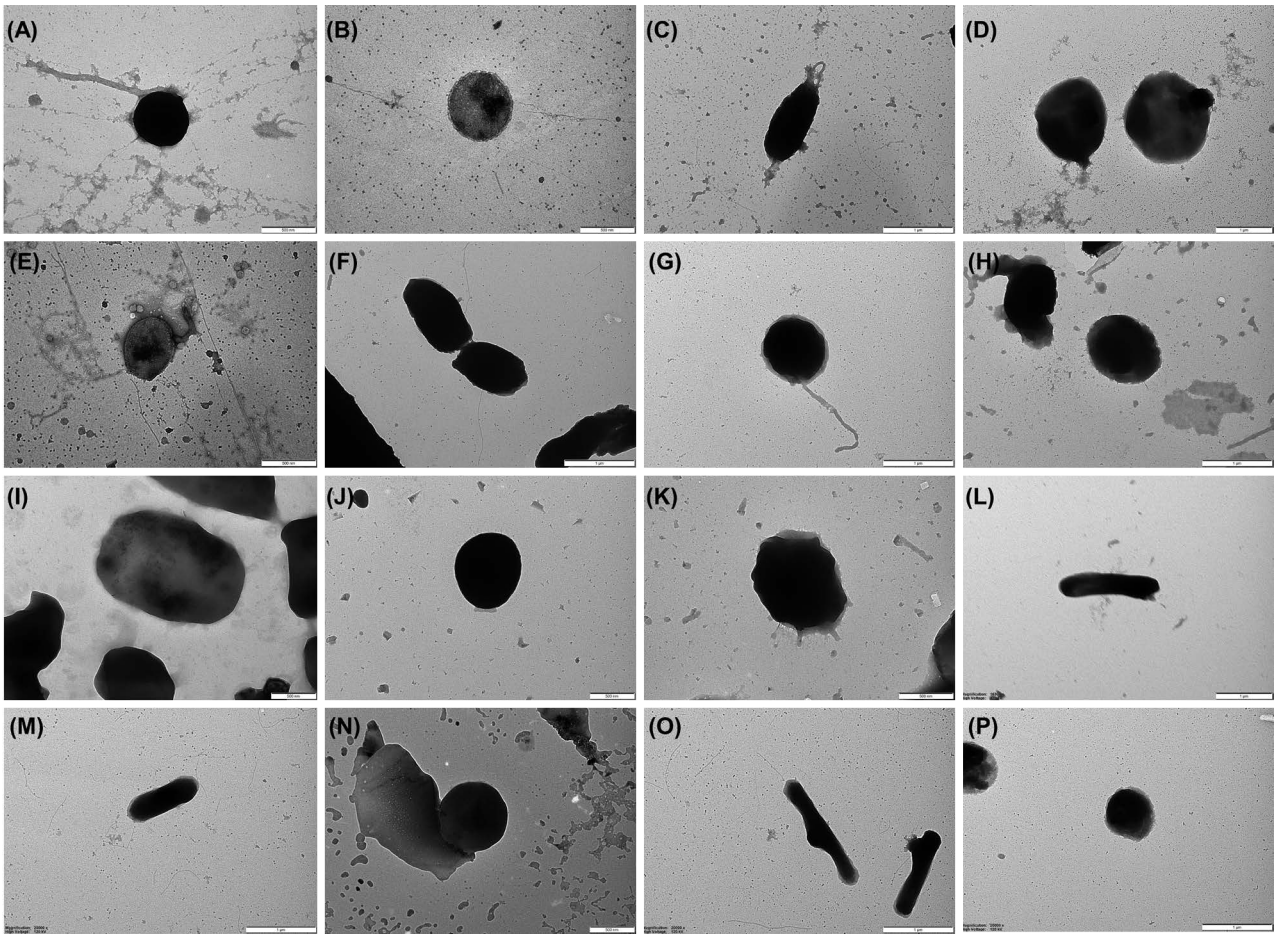

Fig. 1. Transmission electron micrographs of the strains isolated in this study. Strains: A, MBLA0001; B, MBLA0010; C, MBLA0028; D, MBLA0034; E, MBLA0042; F, MBLA0071; G, MBLA0123; H, MBLA0129; I, MBLA0131; J, MBLA0133; K, MBLA0135; L, MBLA0137; M, MBLA0140; N, MBLA0145; O, MBLA0170; P, MBLA0217.

Description of *Halobacterium salinarum* MBLA0001

Cells are Gram-stain-negative and coccus-shaped cells with 0.4–0.5 μm in diameter. Colonies are red, irregular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 25–50°C (optimum, 37°C), 10–25% NaCl (optimum, 20%), and pH 6.0–8.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, glycerol, DL-lactate, pyruvate, L-alanine, L-arginine, and L-lysine are utilized as sole energy sources for growth; but D-mannose, D-galactose, maltose, sucrose, lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, D-xylose, trehalose, raffinose, L-sorbose, D-mannitol, succinate, L-malate, fumarate, citrate, acetate, L-glycine, L-glutamate, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077334. Strain MBLA0001 (=NIBRARC000509741) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Halobacterium hubeiense* MBLA0010

Cells are Gram-stain-negative and coccus-shaped cells with 0.6–0.7 μm in diameter. Colonies are red, irregular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 25–50°C (optimum, 37°C), 10–25% NaCl (optimum, 20%), and pH 6.0–8.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-mannose, D-xylose, glycerol, DL-lactate, succinate, pyruvate, L-malate, fumarate, citrate, acetate, and L-alanine are utilized as sole energy sources for growth; but D-mannose, D-galactose, maltose, sucrose, lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, trehalose, raffinose, L-sorbose, D-mannitol, L-malate, fumarate, citrate, acetate, L-glycine, L-glutamate, L-arginine, L-lysine and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077335. Strain MBLA0010 (=NIBRARC000509742) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea

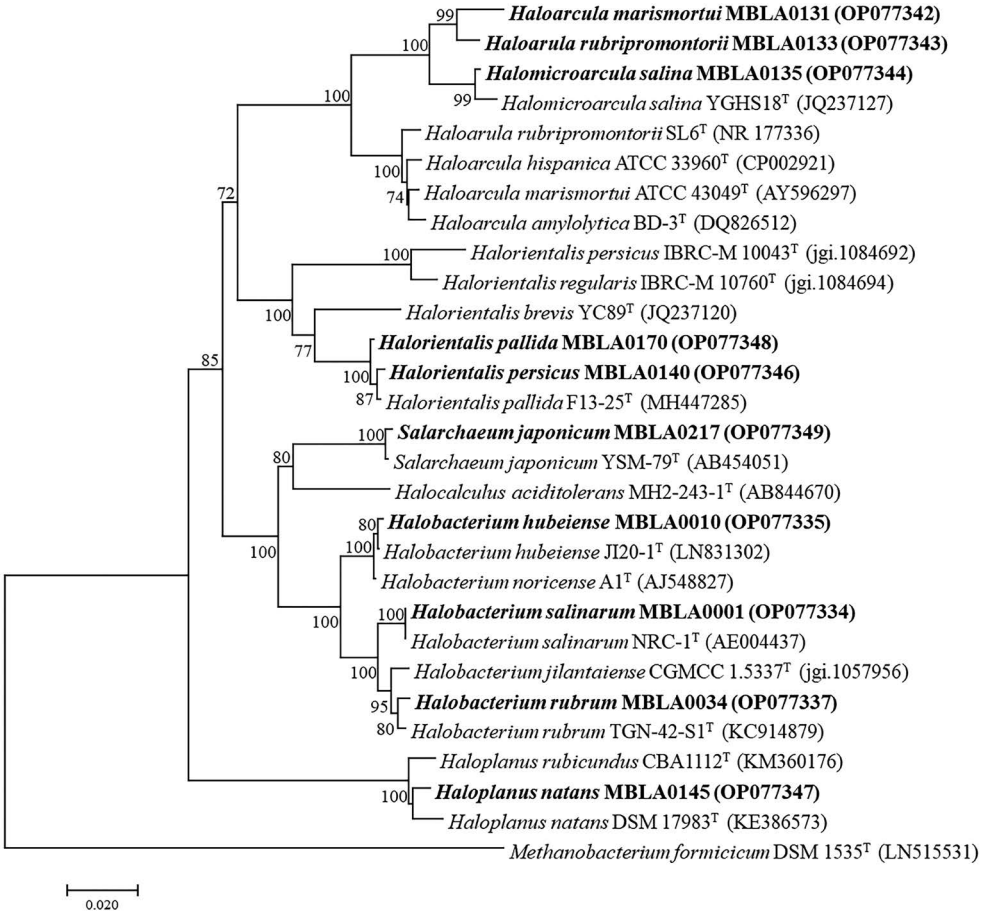

Fig. 2. Neighbor-joining (NJ) phylogenetic tree based on the 16S rRNA gene sequences between the strains isolated in this study belonging to the order *Halobacteriales*. A phylogenetic tree was constructed with their relatives of the genera *Halobacterium*, *Haloplanus*, *Salarchaeum*, *Haloarcula*, *Halomicroarcula*, and *Halorientalis*. The numbers on the nodes indicate the bootstrap values (>70%). Bar, 0.02 accumulated changes per nucleotide, respectively.

(37°24′08.6″N, 126°45′22.7″E).

Description of *Halorubrum xinjiangense* MBLA0028

Cells are Gram-stain-negative and rod-shaped cells with 0.6−0.7 μm in width by 1.3−1.5 μm in length. Colonies are red, irregular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 25−50°C (optimum, 37°C), 10−25% NaCl (optimum, 20%), and pH 6.0−10.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-mannose, D-arabinose, D-xylose, pyruvate, trehalose, D-mannitol, succinate, and L-alanine are utilized as sole energy sources for growth; but D-galactose, maltose, sucrose, lactose, D-fructose, D-ribose, L-rhamnose, raffinose, glycerol, L-sorbose, DL-lactate, L-malate, fumarate, citrate, acetate, L-glycine, L-histidine, L-glutamate, L-arginine, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077335. Strain MBLA0028 (=NIBRARC000509743) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Halobacterium rubrum* MBLA0034

Cells are Gram-stain-negative and coccus-shaped cells with 1.5−1.6 μm in diameter. Colonies are red, irregular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase and oxidase activities are negative. Growth occurs at 20−50°C (optimum, 37°C), 10−25% NaCl (optimum, 20%), and pH 5.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-galactose, D-arabinose, D-xylose, trehalose, pyruvate, acetate, and L-glutamate are utilized as sole energy sources for growth; but D-mannose, maltose, sucrose, lactose, D-fructose, D-ribose, L-rhamnose, glycerol, L-sorbose, D-mannitol, succinate, DL-lactate, malate, fumarate, citrate, L-glycine, L-histidine, L-alanine, L-arginine, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077337. Strain MBLA0034 (=NIBR

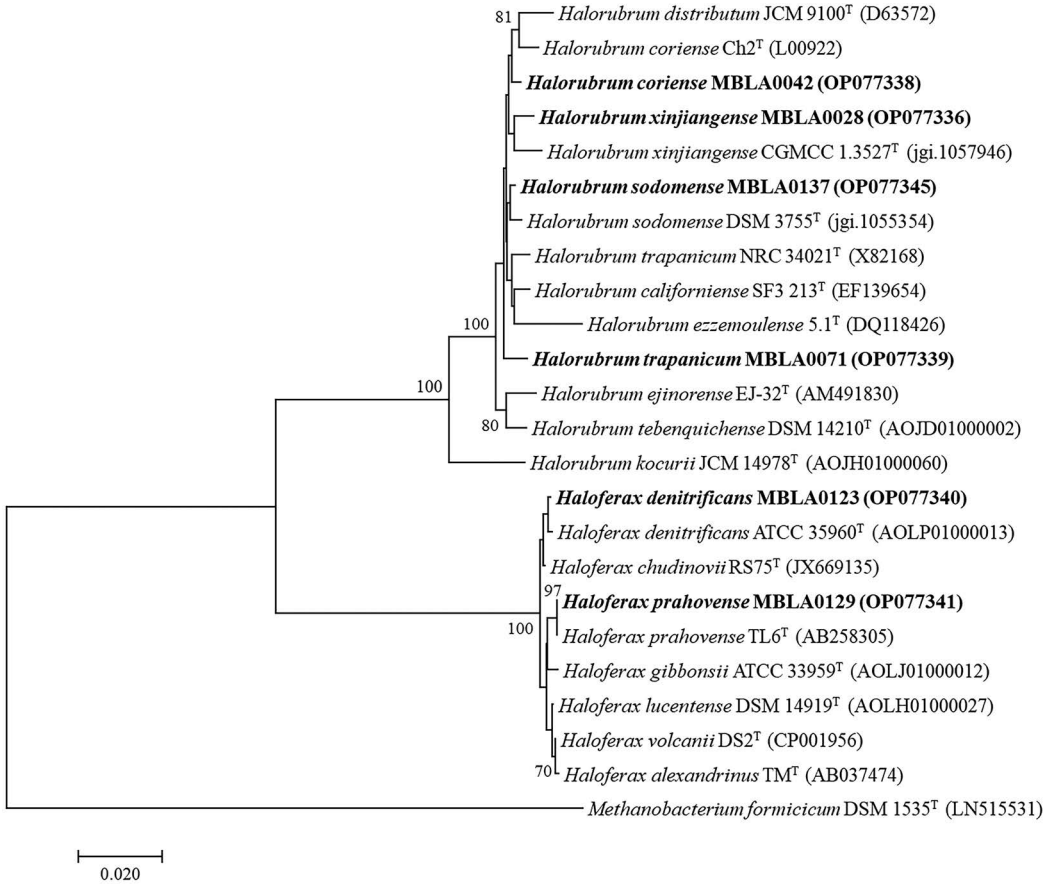

Fig. 3. Neighbor-joining (NJ) phylogenetic tree based on the 16S rRNA gene sequences between the strains isolated in this study belonging to the order *Haloferacales*. A phylogenetic tree was constructed with their relatives of the genera *Haloferax* and *Halorubrum*. The numbers on the nodes indicate the bootstrap values (>70%). Bar, 0.02 accumulated changes per nucleotide, respectively.

ARC000509744) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Halorubrum coriense* MBLA0042

Cells are Gram-stain-negative and coccus-shaped cells with 0.4−0.5 μm in diameter. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 20−40°C (optimum, 37°C), 10−25% NaCl (optimum, 15%), and pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-galactose, maltose, sucrose, glycerol, pyruvate, DL-lactate, and L-alanine are utilized as sole energy sources for growth; but D-mannose, lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, D-xylose, trehalose, raffinose, L-sorbose, D-mannitol, succinate, malate, fumarate, citrate, acetate, L-glycine, L-histidine, L-glutamate, L-arginine, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077338. Strain MBLA0042 (=NIBRARC000509745) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Halorubrum trapanicum* MBLA0071

Cells are Gram-stain-negative and pleomorphic rod-shaped cells with 0.6−0.9 μm in width by 1.2−1.5 μm in length. Colonies are red, irregular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 20−40°C (optimum, 37°C), 10−25% NaCl (optimum, 15%), and pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-galactose, maltose, D-xylose, glycerol, pyruvate, DL-lactate, acetate, L-glutamate, and L-arginine are utilized as sole energy sources for growth; but D-mannose, sucrose, lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, trehalose, raffinose, L-sorbose, D-mannitol, succinate, malate, fumarate, citrate, L-glycine, L-histidine, L-alanine, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077339. Strain MBLA0071 (=NIBR ARC000509746) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Haloferax denitrificans* MBLA0123

Cells are Gram-stain-negative and coccus-shaped cells with 1.0−1.2 μm in diameter. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 30−50°C (optimum, 37°C), 10−30% NaCl (optimum, 20%), and pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-galactose, maltose, sucrose, D-fructose, D-ribose, D-xylose, trehalose, glycerol, pyruvate, DL-lactate, malate, fumarate, L-histidine, L-alanine, L-glutamate, L-arginine, and L-lysine are utilized as sole energy sources for growth; but D-mannose, lactose, D-arabinose, L-rhamnose, raffinose, L-sorbose, D-mannitol, succinate, citrate, acetate, L-glycine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077340. Strain MBLA0123 (=NIBRARC000509747) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Haloferax prahovense* MBLA0129

Cells are Gram-stain-negative and pleomorphic coccus-shaped cells with 1.2−1.3 μm in diameter. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 30−50°C (optimum, 37°C), 10−30% NaCl (optimum, 20%), and pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-mannose, D-galactose, maltose, sucrose, lactose, D-ribose, D-arabinose, L-rhamnose, D-xylose, trehalose, raffinose, glycerol, L-sorbose, pyruvate, DL-lactate, malate, fumarate, citrate, acetate, L-glycine, L-glutamate, and L-arginine are utilized as sole energy sources for growth; but D-fructose, D-mannitol, succinate, L-histidine, L-alanine, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP07 7341. Strain MBLA0129 (=NIBRARC000509748) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Haloarcula marismortui* MBLA0131

Cells are Gram-stain-negative and pleomorphic rod-shaped cells with 1.5−1.9 μm in width by 2.2−2.7 μm in length. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 30−45°C (optimum, 37°C), 10−30% NaCl (optimum, 20%), and pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-galactose, maltose, sucrose, D-fructose, D-ribose, D-arabinose, L-rhamnose, trehalose, L-sorbose, D-mannitol, pyruvate, succinate, malate, fumarate, acetate, L-glycine, L-glutamate, and L-arginine are utilized as sole energy sources for growth; but D-mannose, lactose, D-xylose, raffinose, glycerol, DL-lactate, citrate, L-histidine, L-alanine, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077342. Strain MBLA0131 (=NIBR ARC000509749) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126° 45′22.7″E).

Description of *Haloarcula rubripromontorii* MBLA0133

Cells are Gram-stain-negative and coccus-shaped cells with 1.1−1.3 μm in diameter. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 30−45°C (optimum, 37°C), 10−30% NaCl (optimum, 20%), and pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-mannose, D-galactose, maltose, sucrose, D-fructose, D-arabinose, L-rhamnose, trehalose, glycerol, L-sorbose, D-mannitol, pyruvate, succinate, DL-lactate, malate, fumarate, citrate, acetate, L-histidine, L-alanine, and L-glutamate are utilized as sole energy sources for growth; but lactose, D-ribose, raffinose, L-glycine, L-arginine, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077343. Strain MBLA0133 (=NIBRARC000509750) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Halomicroarcula salina* MBLA0135

Cells are Gram-stain-negative and pleomorphic coccus-shaped cells with 0.8−1.0 μm in diameter. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 30−45°C (optimum, 37°C), 10−30% NaCl (optimum, 20%), and pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-mannose, D-galactose, maltose, sucrose, D-fructose, D-ribose, D-arabinose, D-xylose, trehalose, glycerol, L-sorbose, D-mannitol, pyruvate, succinate, malate, fumarate, citrate, acetate, L-histidine, and L-lysine are utilized as sole energy sources for growth; but L-rhamnose, raffinose, DL-lactate, L-glycine, L-alanine, L-glutamate, L-arginine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077344. Strain MBLA0135 (=NIBRARC000509753) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Halorubrum sodomense* MBLA0137

Cells are Gram-stain-negative and rod-shaped cells with

0.3−0.4 μm in width by 1.6−1.9 μm in length. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 20−40°C (optimum, 37°C), 10−25% NaCl (optimum, 15%), and pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-galactose, maltose, trehalose, glycerol, succinate, pyruvate, DL-lactate, malate, fumarate, citrate, acetate, L-glycine, L-histidine, L-alanine, L-glutamate, and L-lysine are utilized as sole energy sources for growth; but D-mannose, sucrose, lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, D-xylose, raffinose, L-sorbose, D-mannitol, L-arginine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077345. Strain MBLA0137 (=NIBRARC000509751) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Halorientalis persicus* MBLA0140

Cells are Gram-stain-negative and rod-shaped cells with 0.3−0.4 μm in width by 1.2−1.4 μm in length. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 25−45°C (optimum, 37°C), 15−25% NaCl (optimum, 20%), and pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-mannose, D-galactose, maltose, sucrose, D-fructose, glycerol, pyruvate, L-alanine, L-glutamate, L-arginine, L-lysine, and L-ornithine are utilized as sole energy sources for growth; but lactose, D-ribose, D-arabinose, L-rhamnose, D-xylose, raffinose, L-sorbose, D-mannitol, succinate, DL-lactate, malate, fumarate, citrate, acetate, L-glycine, and L-histidine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077346. Strain MBLA0140 (=NIBRARC000509754) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Haloplanus natans* MBLA0145

Cells are Gram-stain-negative and coccus-shaped cells with 0.5−0.6 μm in diameter. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 30−50°C (optimum, 37°C), 15−25% NaCl (optimum, 20%), and pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, pyruvate, succinate, lactate, fumarate, citrate, acetate, L-glutamate, and L-lysine are utilized as sole energy sources for growth; but D-mannose, D-arabinose, L-rhamnose, D-xylose, trehalose, raffinose, L-sorbose, D-mannitol, L-glycine, L-histidine, L-alanine, L-arginine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077347. Strain MBLA0145 (=NIBRARC 000509752) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′ 22.7″E).

Description of *Halorientalis pallida* MBLA0170

Cells are Gram-stain-negative and rod-shaped cells with 0.3−0.4 μm in width by 1.9−2.2 μm in length. Colonies are red, irregular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 25−45°C (optimum, 37°C), 15−25% NaCl (optimum, 20%), and pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, D-mannose, D-galactose, maltose, sucrose, D-fructose, glycerol, pyruvate, L-alanine, L-glutamate, L-arginine, L-lysine, and L-ornithine are utilized as sole energy sources for growth; but lactose, D-ribose, D-arabinose, L-rhamnose, D-xylose, raffinose, L-sorbose, D-mannitol, succinate, DL-lactate, malate, fumarate, citrate, acetate, L-glycine, and L-histidine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077348. Strain MBLA0170 (=NIBRARC000509755) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

Description of *Salarchaeum japonicum* MBLA0217

Cells are Gram-stain-negative and rod-shaped cells with 0.8−1.0 μm in diameter. Colonies are red, circular, and flat after 14 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 20−50°C (optimum, 37°C), 10−25% NaCl (optimum, 20%), and pH 5.0−9.0 (optimum, pH 7.0) and lysed in distilled water. D-Glucose, maltose, L-rhamnose, pyruvate, D-mannitol, succinate, DL-lactate, malate, fumarate, acetate, L-glycine, L-alanine, L-glutamate, L-arginine, and L-lysine are utilized as sole energy sources for growth; but D-mannose, D-galactose, sucrose, lactose, D-fructose, D-ribose, D-arabinose, D-xylose, trehalose, raffinose, glycerol, L-sorbose, citrate, L-histidine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OP077349. Strain MBLA0217 (=NIBRARC000509756) was isolated from a solar saltern in Siheung, Gyeonggi-do Province, Korea (37°24′08.6″N, 126°45′22.7″E).

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References

Cui, H.L., P.J. Zhou, A. Oren and S.J. Liu. 2009. Intraspecific polymorphism of 16S rRNA genes in two halophilic archaeal genera, *Haloarcula* and *Halomicrobium*. Extremophiles 13:31-37.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Serries 41:95-98.

Kato, C., T. Sato and K. Horikoshi. 1995. Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples. Biodiversity and Conservation 4:1-9.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular and Evolution 16:111-120.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

Oren, A. 2014. Halophilic archaea on Earth and in space: growth and survival under extreme conditions. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 372(2030):20140194.

Rampelotto, P.H. 2013. Extremophiles and Extreme Environments. Life 3(3):482-485.

Saitou, N. and M. Nei. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Singh, A. and A.K. Singh. 2017. Haloarchaea: worth exploring for their biotechnological potential. Biotechnology Letters 39:1793-1800.

Squillaci, G., R. Parrella, V. Carbone, P. Minasi, F.L. Cara and A. Morana. 2017. Carotenoids from the extreme halophilic archaeon Haloterrigena turkmenica: identification and antioxidant activity. Extremophiles 21:933-945.

Stan-Lotter, H. and S. Fendrihan. 2015. Halophilic Archaea: Life with Desiccation, Radiation and Oligotrophy over Geological Times. Life 5(3):1487-1496.

Thombre, R.S., V.D. Shinde, R.S. Oke, S.K. Dhar and Y.S. 2016. Shouche. Biology and survival of extremely halophilic archaeon *Haloarcula marismortui* RR12 isolated from Mumbai salterns, India in response to salinity stress. Scientific Report 6:25642.

Thompson, J.D., D.G. Higgins and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673-4680.

Yoon, S.H., S.M. Ha, S. Kwon, J. Lim, Y. Kim, H. Seo and J. Chun. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International Journal of Systematic and Evolutionary Microbiology 67:1613-1617.

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