Haloterrigena Gelatinilytica sp. nov., A New Extremely Halophilic Archaeon Isolated From Salt-Lake

Bing-Bing Liu
Nanyang Institute of Technology https://orcid.org/0000-0002-2161-8585
Nimaichand Salam
Sun Yat-Sen University
Manik Prabhu Narsing Rao
Sun Yat-Sen University
Shuang Cheng
Nanyang Institute of Technology
Yuan-Guo Xie
Sun Yat-Sen University
Lai-You Wang
Nanyang Institute of Technology
Yuan-Ru Zhang
Nanyang Institute of Technology
Xin-Yuan Yu
Nanyang Institute of Technology
Shu-Xian Guo
Nanyang Institute of Technology
Wen-Jun Li (liwenjun3@mail.sysu.edu.cn)
Sun Yat-Sen University

Research Article

Keywords: Haloterrigena gelatinilytica sp. nov., Natrialbaceae, Aiding salt lake, Saline sediment, Polyphasic taxonomy

Posted Date: January 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1043332/v1

Version of Record: A version of this preprint was published at Archives of Microbiology on February 15th, 2022. See the published version at https://doi.org/10.1007/s00203-022-02783-3.

Abstract

Two extremely halophilic strains, designated SYSU A558-1^T and SYSU A121-1, were isolated from a saline sediment sample collected from Aiding salt lake, China. Cells of strains SYSU A558-1^T and SYSU A121-1 were Gram-stain-negative, coccoid, and non-motile. The isolates were aerobic and grew at NaCl concentration of 10-30% (optimum, 20-22%), at 20-55ºC (optimum, 37-42ºC) and at pH 6.5-8.5 (optimum, 7.0-8.0). Cells lysed in distilled water. Major polar lipids were phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, disulphated diglycosyl diether-1 and one unidentified glycolipid. Phylogenetic analyses based on the 16S rRNA gene sequence revealed that the two strains SYSU A558-1^T and SYSU A121-1 were closely related to the membranes of the genus *Haloterrigena.* Phylogenetic trees based on strains SYSU A558-1^T and SYSU A121-1 16S rRNA gene sequence, *rpoB'* gene sequence and concatenation of 87 protein markers demonstrated a robust clade with *Haloterrigena turkmenica, Haloterrigena salifodinae* and *Haloterrigena salina*. The genomic DNA G+C contents of strains SYSU A558-1^T and SYSU A121-1 were 65.8 and 65.0%, respectively. Phenotypic, chemotaxonomic characteristics and phylogenetic properties suggested that the two strains SYSU A558-1^T and SYSU A121-1 represent a novel species of the genus *Haloterrigena*, for which the name *Haloterrigena gelatinilytica* sp. nov. is proposed. The type strain is SYSU A558-1^T (= KCTC 4259^T = CGMCC 1.15953^T).

Introduction

Extremophiles include organisms from all three domains of life, but archaea are the most common to live in extreme conditions. The genus *Haloterrigena* which includes a group of halophiles was introduced by Ventosa et al., (1999) and currently classified under the family *Natrialbaceae* of the order *Natrialbales* (Gupta et al. 2015). At the time of writing, the genus *Haloterrigena* consists of eleven species as described on https://lpsn.dsmz.de/genus/haloterrigena.

Members of the genus *Haloterrigena* (*Htg*) are chemo-organotrophs, and are mainly found in alkaline high-salt environments such as solar saltern, salt lakes, fermented food, etc (Ventosa et al. 2015; Ding et al. 2017; Chen et al. 2019). Their shape ranges from rod, cocci to pleomorphic. They are extremely halophilic and require 1.7-5.5 M NaCl for growth. The optimal pH for growth was observed between pH 7.0-7.5 and for most species the presence of disulphated diglycosyl ether (S2-DGD-1) was reported (Ventosa et al. 2015; Ding et al. 2017; Chen et al. 2019).

During a survey of the diversity of halophilic archaea in salt lakes of China, two strains SYSU A558-1^T and SYSU A121-1 were isolated and could grow at high salt concentrations. The 16S rRNA sequence analysis showed low similarity to the members of the genus *Htg.* Owing to the potential applications of halophilic microorganisms, the present study evaluates the taxonomic status of strains SYSU A558-1^T and SYSU A121-1 and elucidates their mechanisms for overcoming salt stress.

Materials And Methods

Isolation and preservation

Strains SYSU A558-1^T and SYSU A121-1 were isolated from saline sediment samples collected from Aiding salt lake in Xinjiang province (42°686'816" N and 89°330'891" E) by standard dilution-plating technique on a modified Gause medium (abbreviated as mG) containing the following composition ($g \cdot L^{-1}$): soluble starch, 20; lotus root starch, 5; KNO_3, 1; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 0.5; NaCl, 150 or 200; trace solution (2% $FeSO_4 \cdot 7H_2O$; 1% $MnCl_2 \cdot 4H_2O$; 1% $ZnSO_4 \cdot 7H_2O$; 1% $CuSO_4 \cdot 5H_2O$) 1 mL (pH adjusted to 7.2). The isolation plates were incubated at 37ºC for at least 4 weeks in sealed plastic bags. The isolates SYSU A558-1^T and SYSU A121-1 were recovered by successive re-streaking on mG medium (containing 20% and 15% NaCl, respectively). The purified strains were preserved at -80ºC as glycerol suspension (20%, v/v) supplemented with 15% (w/v) NaCl. The reference type strain *Htg. salina* CGMCC 1.6203^T and *Htg. turkmenica* CGMCC 1.2364^T were cultivated in the same medium using similar conditions.

Phenotypic, microscopic, physiological and biochemical characterization

The phenotypic characters were examined according to the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren et al. 1997). Cells morphologies were examined using scanning electron microscopy (S3400N) and transmission electron microscopy (JEM-2100). The minimal salt concentration preventing cell lysis was tested by suspending washed cells in sterile saline solutions (up to 10% NaCl (w/v) and the stability of the cells detected by light microscopy (Cui et al. 2010a). Gram reaction was performed following the method outlined by Dussault (1955). Optimal conditions for growth were determined using mG medium as the basal growth medium unless otherwise mentioned. Salt tolerance was observed by supplementing mG broth (without NaCl) with 5, 8, 10, 13, 15, 18, 20, 22, 25, 27, 30, 32 and 35 (%, w/v) NaCl. Growth at 4, 10, 20, 28, 37, 42, 45, 50, 55 and 60°C was observed by culturing in mG medium prepared with optimal NaCl salt. Mg^{2+} requirement for growth was examined by adding 0.1, 0.3, 0.5, 0.8, 1.0, 1.2 or 1.5 M $MgSO_4.7H_2O$ in the mG medium containing optimal NaCl concentration and incubating at the optimal temperature. The pH range for growth (from pH 4.0 to 10.0 at intervals of 0.5 pH unit) was observed by culturing in mG broth (supplemented with 15% NaCl (w/v); the pH of the medium was maintained by using the following buffer systems: pH 4.0-5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0-8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0-10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3.

Growth and gas formation with nitrate as the electron acceptor was tested as described by Cui et al (2010b). Anaerobic growth was tested on agar plates in the presence of L-arginine and DMSO ($5\ g \cdot L^{-1}$) using a Whitley A35 anaerobic workstation (Don Whitley Scientific). Hydrolysis of casein, gelatin, starch and Tweens (20, 40, 60, 80) was tested according to the methods of Cui et al (2007). Catalase activity was detected by the production of bubbles on the addition of a drop of 3% (v/v) H_2O_2. Oxidase activity was determined using an oxidase reagent (bioMérieux). H_2S and indole formation was carried out as described by Cui et al (2007). Utilization of sole carbon and energy sources was tested in mG broth by

replacing starch with the compound to be tested at a concentration of 5 g·L^{-1}. Growth rates were determined by monitoring the increase in OD_{600} compared to a control (mG broth without any energy source). Acid production was tested in mG broth (without $K_2HPO_4 \cdot 3H_2O$) supplemented with different sole carbon sources. The changes in pH of the medium due to the production of acid were monitored after one month of incubation using phenolsulfonphthalein as a pH indicator. The culture was considered positive for acid production if the color changes from red to yellow with respect to control. Susceptibility to antimicrobial agents was tested as described by Gutiérrez et al (2008).

Phylogeny and 16S rRNA gene analysis

The 16S rRNA gene sequences were recovered from the genomes of the two isolates using ContEst16S (Lee et al. 2017) and analyzed in EzBioCloud server (Yoon et al. 2017). Sequences of the related strains were retrieved for multiple sequence alignment and generation of phylogenetic trees. The full-length *rpoB'* gene sequences (encoding *β'* subunit of bacterial RNA polymerase) were amplified using a described procedure by Minegishi et al. (2010) and sequenced as described by Liu et al (2014). The *rpoB'* sequence similarities of the two strains were calculated by NCBI Nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/) and the sequences of related strains were retrieved accordingly. Multiple sequence alignments of the above sequences were performed using the CLUSTAL_X 2.1 program (Larkin et al. 2007). Phylogenetic trees were generated by neighbor-joining (NJ) (Saitou and Nei 1987), maximum-parsimony (MP) (Fitch 1971) and maximum-likelihood (ML) (Felsenstein 1981) algorithms using the software packages MEGA version 7 (Kumar et al. 2016). Evolutionary distances in the NJ, MP and MP trees were calculated by the Kimura two-parameter model (Kimura 1980). The topologies of the phylogenetic trees were evaluated by the bootstrap analysis with 1000 replicates (Felsenstein 1985).

Chemotaxonomy

Polar lipids were extracted from cells cultured on mG medium for three weeks as described by Cui et al. (2010b) and analyzed by two-dimensional thin-layer chromatography (TLC) (Kates 2010). The isolate *Htg. salina* CGMCC 1.6203^T was used as a reference strain for the comparison of polar lipids in the one-dimensional TLC chromatogram.

Genomic characterization

The genomic DNAs from the strains SYSU A558-1^T and SYSU A121-1 were extracted and purified using the Star Prep Gel Extraction kit. The concentration and purity of the DNA was measured using NanoDrop (Thermo Scientific NanoDrop One). Whole-genome sequencing of strains SYSU A558-1^T and SYSU A121-1 were performed using a paired-end sequencing method with the HiSeq 2000 platform (Illumina, San Diego, CA, USA). The obtained Illumina reads were assembled using the SPAdes software (Bankevich et al. 2012). The genomic G+C contents of the DNAs were calculated and gene prediction was carried out using Glimmer version 3.02 (Delcher et al. 2007). The software Infernal 1.1 (Nawrocki et al. 2013) was used to predict rRNA and other ncRNAs in the genome based on the RNA family database (Nawrocki et al. 2015). Transfer RNA in the genome was predicted using tRNAscan-SE v1.21 (Lowe et al. 1997). Functional annotation of the genome was performed with RAST (Aziz et al. 2008). The CRT software was

used for CRISPR prediction of the genome (Bland et al. 2007). BLAST (Altschul et al. 1997) was used to compare the predicted gene sequences with COG (Tatusov et al. 2000), KEGG (Kanehisa et al. 2004) and other functional databases to obtain the annotation results of gene function. Based on the comparison results of Nr database, the application software Blast2GO (Conesa et al. 2005) annotated the function of GO (Ashburner et al. 2000) database. The software hmmer (Eddy 1998) was used to annotate Pfam functions based on Pfam (Finn et al. 2016) database. BLAST was used to compare the protein sequences of the predicted genes with functional databases such as the classification database of transporters (TCDB) (Saier et al. 2006), the pathogen-host interaction factor database (PHI) (Winnenburg et al. 2006), the antibiotic resistance gene database (ARDB) (Liu and Pop 2009), and the virulence factor database (VFDB) (Chen et al. 2005) to obtain corresponding annotation results. In addition, the software hmmer was used to annotate the function of carbohydrate enzyme genes based on the carbohydrate-related enzyme database (CAZyme) (Cantarel et al. 2009). The software SignalP 4.0 (Petersen et al. 2011) was used to analyze the protein-containing signal peptides sequences of all the predicted genes. The transmembrane protein sequences of all the predicted genes were analyzed using software TMHMM (Krogh et al. 2001) to find the proteins containing transmembrane helices. The protein containing the signal peptide was removed from the protein containing the transmembrane helix, and the remaining protein was the secreted protein. The application software Circos (Krzywinski et al. 2009) was used to map the genome circle.

For generation of phylogenomic tree, sixteen protein marker genes were retrieved from the genomes of the genera *Haloterrigena, Natrinema, Natronorubrum* and other type species in the family *Natrialbaceae* using AMPHORA2 (Wu and Scott 2012). Sequence alignment, concatenation, and generation of phylogenomic tree were followed as described earlier (Asem et al. 2020).

For further investigation of phylogenetic relationships, average nucleotide identity (ANI) values between strains SYSU A558-1^T and SYSU A121-1 and those of the type strains of their three closest phylogenomic neighbors were calculated using the OrthoANIu algorithm (Yoon et al. 2017). The digital DNA-DNA hybridization (dDDH) values were calculated by Genome-to-Genome Distance Calculator (http://ggdc.dsmz.de/ggdc.php) with BLAST+ and the recommended parameter formula 2 (Meier-Kolthoff et al. 2013).

Results And Discussion

Phenotypic characteristics

Cells of strains SYSU A558-1^T and SYSU A121-1 were non-motile and coccoid with a diameter ranging between 0.9 and 1.1μm (Supplementary Fig. S1). They lyse in distilled water. Colonies were red-pigmented. The two strains grew at 20-55°C (optimum at 37-45°C) and pH 6.5-8.5 (optimum at pH 7.0-8.0). Mg^{2+} was not necessary for growth. Strains SYSU A558-1^T and SYSU A121-1optimal NaCl (w/v) concentration for growth was 20-22% which was a little above than *Htg. salina* CGMCC 1.6203^T (20%)

and *Htg. turkmenica* CGMCC 1.2364^T (15-20%). Strains SYSU A558-1^T and SYSU A121-1 were negative for oxidase while *Htg. salina* CGMCC 1.6203^T was positive. Strains SYSU A558-1^T and SYSU A121-1 were positive for nitrate reduction while *Htg. turkmenica* CGMCC 1.2364^T was negative. Strains SYSU A558-1^T and SYSU A121-1 could hydrolyze gelatin but not *Htg. salina* CGMCC 1.6203^T and *Htg. turkmenica* CGMCC 1.2364^T. Detailed differentiating features of strains SYSU A558-1^T and SYSU A121-1 and their closely related species are listed in Table 1. All the negative results of strain SYSU A558-1^T are listed in Supplementary Table S1.

Table 1

Differentiating characteristics of strains SYSU A558-1^T and SYSU A121-1 from the closely related members of the genus *Haloterrigena*. Note: 1, SYSU A558-1^T; 2, SYSU A121-1; 3, *Htg. salina* CGMCC 1.6203^T; 4, *Htg. turkmenica* CGMCC 1.2364^T. +, positive; -, negative; w, weakly positive. All data are from this study.

Characteristics	**1**	**2**	**3**	**4**
Growth conditions				
NaCl range (%, w/v)	10-30	10-30	13-30	11-35
NaCl optimum (%, w/v)	20-22	20-22	20	15-20
Temperature range (ºC)	25-55	20-55	25-50	30-55
Temperature optimum	37-45	37-45	37-42	37-45
pH range	6.5 -8.5	6.5-8.5	6.0-9.0	6.0-8.5
Nitrate reduction	+	+	+	-
Oxidase	-	-	+	-
Hydrolysis of:				
Starch	+	+	-	+
Gelatin	+	+	-	-
Tween 20	+	+	+	-
Tween 80	+	+	+	-
Utilization of sole carbon and energy source:				
$_D$-Arabinose	-	-	+	-
$_L$-Arginine	+	+	-	+
$_D$-Fructose	-	-	+	+
$_D$-Galactose	-	-	+	-
$_D$-Glucose	-	-	+	+
$_D$-Lactose	-	-	+	-
$_L$-Lysine	w	+	-	+
$_D$-Maltose	-	-	+	-
$_D$-Mannose	-	-	+	w

Characteristics	1	2	3	4
D-Raffinose	+	+	-	+
D-Rhamnose	-	-	w	+
D-Ribose	-	-	+	-
D-Sucrose	+	+	-	+
L-Threonine	-	-	+	+
D-Trehalose	w	+	-	w
D-Xylose	-	-	+	-
Succinate	-	-	-	+
Citrate	-	-	-	+
Fumarate	-	-	w	+

Strains SYSU A558-1^T and SYSU A121-1 were susceptible to the following antibiotics (μg per disc, unless otherwise indicated): aphidicolin (20), novobiocin (30) and rifampicin (5), but resistant to ampicillin (10), anisomycin (20), bacitracin (0.04 IU), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), neomycin (30), norfloxacin (10), penicillin (10 IU), and vancomycin (30). They could utilize a wide range of compounds as the sole carbon and energy sources and were described in the species description.

The 16S rRNA gene sequences and phylogenetic analysis

Three heterogeneous 16S rRNA gene sequences were determined in the genomes of strains SYSU A558-1^T and SYSU A121-1. Strain SYSU A558-1^T shared the highest 16S rRNA gene sequences identities to the type strain of *Htg. turkmenica* (98.0 – 98.6%), *Htg. salifodinae* (98.1-98.5%) and *Htg. salina* (97.5 – 98.2%). Correspondingly, strain SYSU A121-1 also shared the highest 16S rRNA gene sequence identities to the type strain of *Htg. turkmenica* (98.4-98.9%), *Htg. salifodinae* (98.2-98.5%) and *Htg. salina* (98.0 – 98.2%). The 16S rRNA gene sequence similarities between the strains SYSU A558-1^T and SYSU A121-1 range between 97.7-98.9%.

The *rpoB'* gene sequence similarities of strains SYSU A558-1^T and SYSU A121-1 with all known genera in the class *Halobacteria* were less than 98% while sharing the highest sequence identities to the type strains of *Htg. salina* (97.9 and 97.8%, respectively), followed by *Htg. salifodinae* (97.7 and 97.4%) and *Htg. turkmenica* (96.4 and 96.2%).

ML phylogenetic trees based on 16S rRNA (Fig. 1) and *rpoB'* (Fig. 2) gene sequence showed that strains SYSU A558-1^T and SYSU A121-1 clade with the type strains of *Htg. salina*, *Htg. salifodinae* and *Htg.*

turkmenica. Phylogenetic trees constructed using NJ and MP also found similar clade (Supplementary Fig. S2 and S3). The trees were polyphyletic which was consistence with earlier study (Romano et al. 2007; Chen et al. 2019).

Chemotaxonomic features

The major polar lipids of the two strains SYSU A558-1^T and SYSU A121-1 were phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), disulphated diglycosyl diether-1 (S_2-DGD-1) and one unidentified glycolipid (Supplementary Fig. S4). Phosphatidylglycerol sulphate, which was the common characteristic of the genus *Haloterrigena*, was absent in both SYSU A558-1^T and SYSU A121-1 (Supplementary Fig. S4). The unidentified glycolipid present in both the strains was not identified in *Htg. salina* CGMCC 1.6203^T (Supplementary Fig. S4).

Phylogenomic analysis and genomic relatedness values

In phylogenomic tree strains SYSU A558-1^T and SYSU A121-1 (Fig. 3) clustered with the type strains of *Htg. salina*, *Htg. salifodinae* and *Htg. turkmenica*. The ANI and dDDH values of strain SYSU A558-1^T with its closest members (Table 2) were far below the standard cut-off values for species boundary for ANI (95-96%) (Goris et al. 2007) and dDDH (70%) (Meier-Kolthoff et al. 2013).

Table 2
Overall genome relatedness indices (%) of strains SYSU A558-1^T and SYSU A121-1 with closely related species

Strains	ANI (%)		dDDH (%)	
	SYSU A558-1^T	SYSU A121-1	SYSU A558-1^T	SYSU A121-1
SYSU A558-1^T	100	98.9	100	95.3
SYSU A121-1	98.9	100	95.3	100
Htg. salina JCM 13891^T	93.9	93.9	66.5	68.7
Htg. salifodinae ZY 19^T	93.6	93.6	63.0	64.5
Htg. turkmenica DSM 5511^T	91.0	91.0	68.1	70.9

Genomic features

The complete genome sequence of strain SYSU A558-1^T consisted of one circular chromosome and two circular plasmids, with lengths of 4,294,945 bp (chromosome), 179,538 bp (plasmid 1) and 77,522 bp (plasmid 2), respectively with N_{50} length was 4,294,945 bp. The genomic DNA G+C contents of the chromosome and the two plasmids of strain SYSU A558-1^T were 65.8, 65.2 and 62.0%, respectively. The complete genome sequence of isolate SYSU A121-1 consisted of one circular chromosome and three circular plasmids, with lengths of 4,142,763 bp (chromosome), 357.995 bp (plasmid 1), 315414 bp

(plasmid 2) and 100260 bp (plasmid 3), respectively. N_{50} length was 4,142,763 bp. The genomic DNA G+C contents of the chromosome and three plasmids were 66.1, 63.0, 61.9 and 65.4%, respectively. Circos map representing the genome of two strains was represented in Fig. S5. Strain SYSU A558-1^T contained 9 rRNA (three 16S rRNA, three 5S rRNA and three 23S rRNA genes), 49 tRNA genes and 4 ncRNA (Table 3). A total of 4542 genes were recovered, of which 2228 genes were annotated to COG, 2181 to GO, 1432 to KEGG, and 2909 genes to Pfam databases. A total of 90 signaling peptide-containing proteins, 1069 transmembrane proteins, and 34 secreted proteins were predicted. The genome of strain SYSU A121-1 contained 12 rRNA genes (three 16S rRNA, five 5S rRNA and four 23S rRNA genes), 49 tRNA genes and 3 ncRNA. A total of 4733 genes were predicted, of which 2508 genes were annotated to COG, 2310 to GO, 1640 to KEGG, and 3278 genes to Pfam. A total of 87 signaling peptide-containing proteins, 1123 transmembrane proteins, and 37 secreted proteins were predicted. A comparative analysis of the genome data between the two isolates and that of the closely related type strains of the genus *Haloterrigena* is presented in Table 3.

Table 3
Genome attributes

Name	Accession number	Size (Mb)	G+C %	rRNA	tRNA	Other RNA	Gene
SYSU A558-1^T	JABUQZ000000000	4.29	65.8	9	48	4	4542
SYSU A121-1	JABURA000000000	4.12	66.1	12	49	3	4733
Htg. salina JCM 13891^T	AOIS00000000	4.84	65.2	8	53	2	4643
Htg. salifodinae ZY19^T	RQWN00000000	4.96	64.5	5	49	2	4817
Htg. turkmenica DSM 5511^T	CP001860	3.89	65.8	10	50	2	3749

Stress-related genes

Microorganisms overcome the osmotic stress through various mechanisms such as reinforcement of cell walls, accumulation of various osmolytes and adjusting their cell turgor for altered growth conditions (Shabala et al. 2009; Han et al. 2018). Potassium ion homeostasis is regulated by three major K^+ transporters: high and low-affinity transporters (Kdp and Kup) and potassium uptake protein Trk (Liu et al. 2013). RAST annotation results suggest that strain SYSU A558-1^T encode genes for potassium uptake protein Trk. Further genes related to potassium channels (are involved in the transport and release of potassium) and oxidative stress were also noticed. The above results suggest the mechanism of strain SYSU A558-1^T to overcome salt stress.

Taxonomic conclusion

Based on the phenotypic, chemotaxonomic and phylogenomic analysis, strains SYSU A558-1^T and SYSU A121-1 are considered to represent a novel species within the genus *Haloterrigena*, for which the name *Haloterrigena gelatinilytica* sp. nov. is proposed.

Description of Haloterrigena gelatinilytica **sp. nov.**

Haloterrigena gelatinilytica (ge.la.ti.ni.ly'ti.ca. N.L. neut. n. *gelatinum*, gelatin; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lytikos*), able to dissolve; N.L. fem. adj. *Gelatinilytica*, gelatin-dissolving).

Cells are Gram-stain-negative, non-motile and coccoid (0.9-1.1 μm mean cell diameter). Colonies are red-pigmented, elevated and round. Cells require 10-30% (w/v) NaCl and grow at 20-55 ºC, pH 6.5-8.5, and Mg^{2+} (0-0.8 M). Optimum growth occurs with 20-22% (w/v) NaCl, pH 7.0-8.0, temperature 37-42 ºC and 0.03 M $MgSO_4 \cdot 7H_2O$. Cells lyse in distilled water. Anaerobic growth does not occur even in the presence of arginine and DMSO. Positive for catalase and nitrate reduction. Gelatin, starch, and Tweens (20, 40, 60, 80) are hydrolyzed but not casein. Acid is produced from $_D$-sucrose. Utilizes glycerol, $_D$-raffinose, sodium pyruvate, starch, $_D$-sucrose, $_D$-trehalose, $_L$-glysine, $_L$-glutamate, $_L$-alanine, $_L$-arginine, $_L$-lysine, and $_L$-serine as sole carbon and energy source. Polar lipids include phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, disulphated diglycosyl diether-1 and one unidentified glycolipid. The genomic DNA G+C content of the type strain is 65.8%.

The type strain, SYSU A558-1^T (=KCTC 4259^T=CGMCC 1.15953^T), was isolated from saline soil of Aiding salt lake in Xinjiang province, China. The GenBank accession numbers for the 16S rRNA and *rpoB'* gene sequences of strain SYSU A558-1^T are MT809061 (rrnA), MT809062 (rrnB), MT809063 (rrnC) and MT572483 (*rpoB'*). The accession number for the genome sequence is JABUQZ000000000.

Declarations

Availability of data and material The draft genome sequences of isolates SYSU A558-1^T and SYSU A121-1 are available at the NCBI genome database under the accessions JABUQZ000000000 and JABURA000000000, respectively. The three copies of 16S rRNA gene sequences of the isolate SYSU A558-1^T are available under the accessions MT809061 (rrnA), MT809062 (rrnB) and MT809063 (rrnC), and that of isolate SYSU A121-1 under the accessions MT808217 (rrnA), MT808218 (rrnB) and MT808219 (rrnC). The *rpoB'* gene sequences of isolates SYSU A558-1^T and SYSU A121-1 have been deposited in GenBank/EMBL/DDBJ under the accession numbers MT572483 and MT572484, respectively.

Acknowledgements

The authors are grateful to Prof. Yu-Guang Zhou (CGMCC, China) for providing the reference type strains.

Author contributions S-X G and W-J L conceived the study; B-B L, N S, S C, Y-G X, Y-R Z and X-Y Y performed the experiments; B-B L, MP NR, L-Y W and N S analyzed wrote the draft manuscript; S-X G and

W-J L finalized the manuscript.

Funding This research was supported by National Natural Science Foundation of China (31800001, 31850410475), Science and Technology Development Special Fund Program of Guangdong Province (2017A030310206), Key Scientific Research Project of Colleges and Universities in Henan Province (20A180019), Henan Provience University Youth Researcher Support Project (2021GGJS153), Key Technologies R&D Program of Henan Province (212102310232) and Key Technologies R&D Program of Nanyang city (KJGG079). WJ Li was also supported by Introduction project of high-level talents in Xinjiang Uygur Autonomous Region.

Ethical statement: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: The authors declare that there are no conflicts of interest.

References

1. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-3402. https://doi.org/10.1093/nar/25.17.3389
2. Asem MD, Salam N, Idris H, Zhang XT, Bull AT et al (2020) *Nocardiopsis deserti* sp. nov., isolated from a high altitude Atacama Desert soil. Int J Syst Evol Microbiol 70: 3210-3218. https://doi.org/10.1099/ijsem.0.004158
3. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T et al (2008) The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9: 75. https://doi.org/10.1186/1471-2164-9-75
4. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H et al (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25: 25-29. https://doi.org/10.1038/75556
5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19: 455-477. https://doi.org/10.1089/cmb.2012.0021
6. Bland C, Ramsey TL, Sabree F, Lowe M, Brown K et al (2007) CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. BMC Bioinformatics 8: 209. https://doi.org/10.1186/1471-2105-8-209 Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V et al (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res 37: D233-238. https://doi.org/10.1093/nar/gkn663
7. Chen L, Yang J, Yu J, Yao Z, Sun L et al (2005) VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res 33: D325-328. https://doi.org/10.1093/nar/gki008
8. Chen S, Xu Y, Sun S, Chen F (2019) *Haloterrigena salifodinae* sp. nov., an extremely halophilic archaeon isolated from a subterranean rock salt. Antonie Van Leeuwenhoek 112: 1317-1329.

https://doi.org/10.1007/s10482-019-01264-w

9. Conesa A, Götz S, García-Gómez JM, Terol J, Talón M et al (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21: 3674-3676. https://doi.org/10.1093/bioinformatics/bti610
10. Cui HL, Li XY, Gao X, Xu XW, Zhou YG, et al (2010a) *Halopelagius inordinatus* gen. nov., sp. nov., a new member of the family *Halobacteriaceae* isolated from a marine solar saltern. Int J Syst Evol Microbiol 60: 2089-2093. https://doi.org/10.1099/ijs.0.018598-0
11. 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: 2204-2206. https://doi.org/10.1099/ijs.0.65268-0
12. Cui HL, Gao X, Yang X, Xu XW (2010b) *Halorussus rarus* gen. nov., sp. nov., a new member of the family *Halobacteriaceae* isolated from a marine solar saltern. Extremophiles 14: 493-499. https://doi.org/10.1007/s00792-010-0329-0
13. Delcher AL, Bratke KA, Powers EC, Salzberg SL (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23: 673-679. https://doi.org/10.1093/bioinformatics/btm009
14. Dussaul HP (1955) An improved technique for staining red halophilic bacteria. J Bacteriol 70: 484-485. https://doi.org/10.1007/s10482-019-01264-w
15. Eddy SR (1998) Profile hidden Markov models. Bioinformatics 14: 755-763. https://doi.org/10.1093/bioinformatics/14.9.755
16. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17: 368-376. https://doi.org/10.1007/bf01734359
17. Felsenstein J (1985) Confidence Limits On Phylogenies: An Approach Using The Bootstrap. Evolution 39: 783-791. https://doi.org/10.2307/2408678
18. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J et al (2016) The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res 44: D279-285. https://doi.org/10.1093/nar/gkv1344
19. Fitch WM (1971) Toward Defining the Course of Evolution: Minimum Change for a Specific Tree Topology. Systematic Zoology 20: 406-416. https://doi.org/10.1093/sysbio/20.4.406
20. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57: 81-91. https://doi.org/10.1099/ijs.0.64483-0
21. Gupta RS, Naushad S, Baker S (2015) Phylogenomic analyses and molecular signatures for the class *Halobacteria* and its two major clades: a proposal for division of the class *Halobacteria* into an emended order *Halobacteriales* and two new orders, *Haloferacales* ord. nov. and *Natrialbales* ord. nov., containing the novel families *Haloferacaceae* fam. nov. and *Natrialbaceae* fam. nov. Int J Syst Evol Microbiol 65: 1050-1069. https://doi.org/doi:10.1099/ijs.0.070136-0
22. Han J, Gao QX, Zhang YG, Li L, Mohamad OAA et al (2018) Transcriptomic and Ectoine Analysis of Halotolerant *Nocardiopsis gilva* YIM 90087(T) Under Salt Stress. Front Microbiol 9: 618.

https://doi.org/10.3389/fmicb.2018.00618

23. Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M (2004) The KEGG resource for deciphering the genome. Nucleic Acids Res 32: D277-280. https://doi.org/10.1093/nar/gkh063
24. Kates M (1972) Techniques of lipidology; isolation, analysis and identification of lipids. Laboratory techniques in biochemistry and molecular biology 3: 347-353. https://doi.org/10.1016/0305-0491(74)90118-7
25. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120. https://doi.org/10.1007/bf01731581
26. Krogh A, Larsson B, Von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305: 567-580. https://doi.org/10.1006/jmbi.2000.4315
27. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R et al (2009) Circos: an information aesthetic for comparative genomics. Genome Res 19: 1639-1645. https://doi.org/10.1101/gr.092759.109
28. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33: 1870-1874. https://doi.org/10.1093/molbev/msw054
29. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948. https://doi.org/10.1093/bioinformatics/btm404
30. Lee I, Chalita M, Ha SM, Na SI, Yoon SH et al (2017) ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. Int J Syst Evol Microbiol 67: 2053-2057. https://doi.org/10.1099/ijsem.0.001872
31. Liu B, Pop M (2009) ARDB--Antibiotic Resistance Genes Database. Nucleic Acids Res 37: D443-447. https://doi.org/doi:10.1093/nar/gkn656
32. Liu Y, Ho KK, Su J, Gong H, Chang AC et al (2013) Potassium transport of Salmonella is important for type III secretion and pathogenesis. Microbiology (Reading) 159: 1705-1719. https://doi.org/10.1099/mic.0.068700-0
33. Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25: 955-964. https://doi.org/10.1093/nar/25.5.0955
34. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14: 60. https://doi.org/10.1186/1471-2105-14-60
35. Minegishi H, Kamekura M, Itoh T, Echigo A, Usami R, et al (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: 2398-2408. https://doi.org/10.1099/ijs.0.017160-0
36. Nawrocki EP, Burge SW, Bateman A, Daub J, Eberhardt RY et al (2015) Rfam 12.0: updates to the RNA families database. Nucleic Acids Res 43: D130-137. https://doi.org/10.1093/nar/gkn766

37. Nawrocki EP, Eddy SR (2013) Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 29: 2933-2935. https://doi.org/10.1093/bioinformatics/btt509
38. Oren A, Ventosa A, Grant WD (1997) Proposed Minimal Standards for Description of New Taxa in the Order *Halobacteriales*. International Journal of Systematic and Evolutionary Microbiology 47: 233-238. https://doi.org/10.1099/00207713-47-1-233
39. Petersen TN, Brunak S, von Heijne, G.; Nielsen, H. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods* 2011, 8, 785-786, doi:10.1038/nmeth.1701. https://doi.org/10.1038/nmeth.1701
40. Saier Jr MH, Tran CV, Barabote RD (2006) TCDB: the Transporter Classification Database for membrane transport protein analyses and information. Nucleic Acids Res 34: D181-186. https://doi.org/10.1093/nar/gkj001
41. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
42. Shabala L, Bowman J, Brown J, Ross T, McMeekin T et al (2009) Ion transport and osmotic adjustment in Escherichia coli in response to ionic and non-ionic osmotica. Environ Microbiol 11: 137-148. https://doi.org/10.1111/j.1462-2920.2008.01748.x
43. Tatusov RL, Galperin MY, Natale DA, Koonin EV (2000) The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 28: 33-36. https://doi.org/10.1093/nar/28.1.33
44. Tatusov RL, Galperin MY, Natale DA, Koonin EV (2000) The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 28: 33-36. https://doi.org/10.1093/nar/28.1.33
45. Ventosa A, Gutiérrez MC, Kamekura M, Dyall-Smith ML (1999) Proposal to transfer *Halococcus turkmenicus*, *Halobacterium trapanicum* JCM 9743 and strain GSL-11 to *Haloterrigena turkmenica* gen. nov., comb. nov. Int J Syst Bacteriol 49: 131-136. https://doi.org/10.1099/00207713-49-1-131
46. Winnenburg R, Baldwin TK, Urban M, Rawlings C, Köhler J et al (2006) PHI-base: a new database for pathogen host interactions. Nucleic Acids Res 34: D459-464. https://doi.org/10.1093/nar/gkj047
47. Wu M, Scott AJ (2012) Phylogenomic analysis of bacterial and archaeal sequences with AMPHORA2. Bioinformatics 28: 1033-1034. https://doi.org/10.1093/bioinformatics/bts079
48. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67: 1613-1617. https://doi.org/10.1099/ijsem.0.001755
49. Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110: 1281-1286. https://doi.org/10.1007/s10482-017-0844-4

Figures

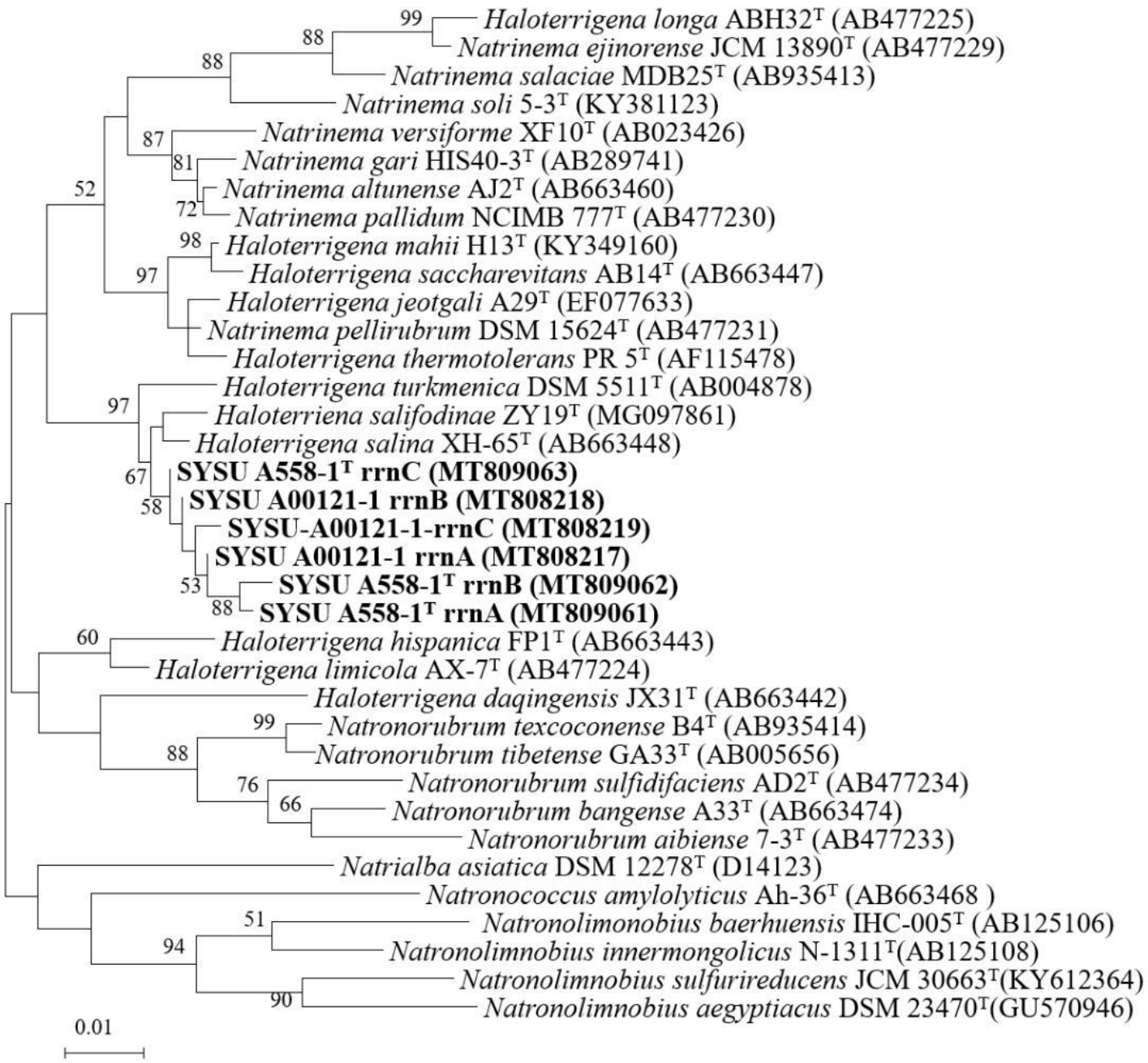

Figure 1

Maximum-likelihood phylogenetic tree based on nearly complete 16S rRNA gene sequences of the two strains SYSU A558-1^T and SYSU A121-1 with related members of the family *Natrialbaceae*. Percentage bootstrap values (based on 1000 resampling's) are given at branch points. *Halobacterium salinarum* NRC-1^T (AE004437) was used as outgroup. Bar, 0.01 nucleotide substitutions per site.

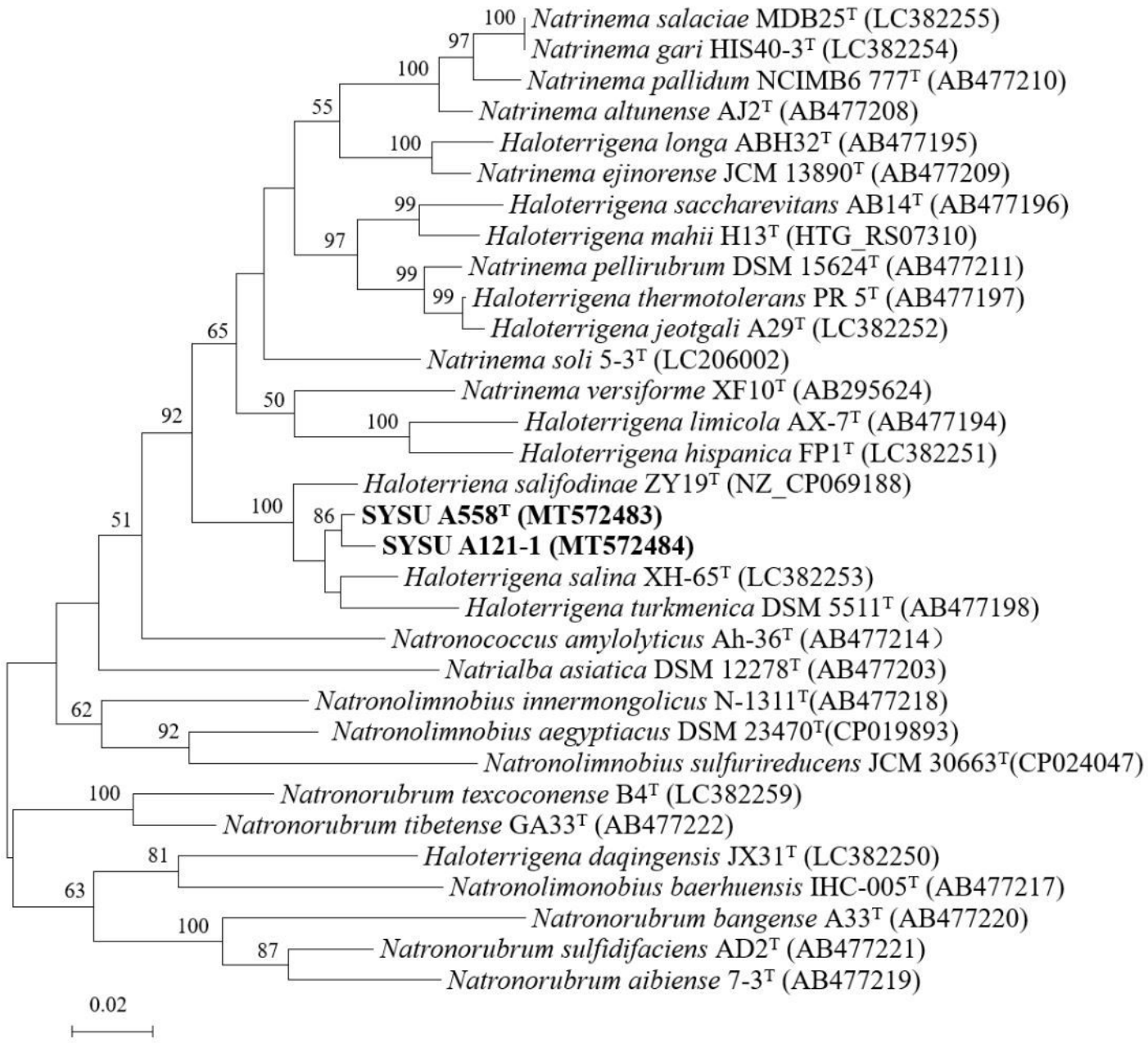

Figure 2

Maximum-likelihood phylogenetic tree based on complete *rpoB'* gene sequences of the two strains SYSU A558-1^T and SYSU A121-1 with related members of the family *Natrialbaceae*. Percentage bootstrap values (based on 1000 resampling's) are given at branch points. Filled circles are nodes that are also recovered in the neighbor-joining and maximum parsimony phylogenetic trees. *Halobacterium salinarum* JCM 8978^T (AB477150) was used as outgroup. Bar, 0.02 nucleotide substitutions per site.

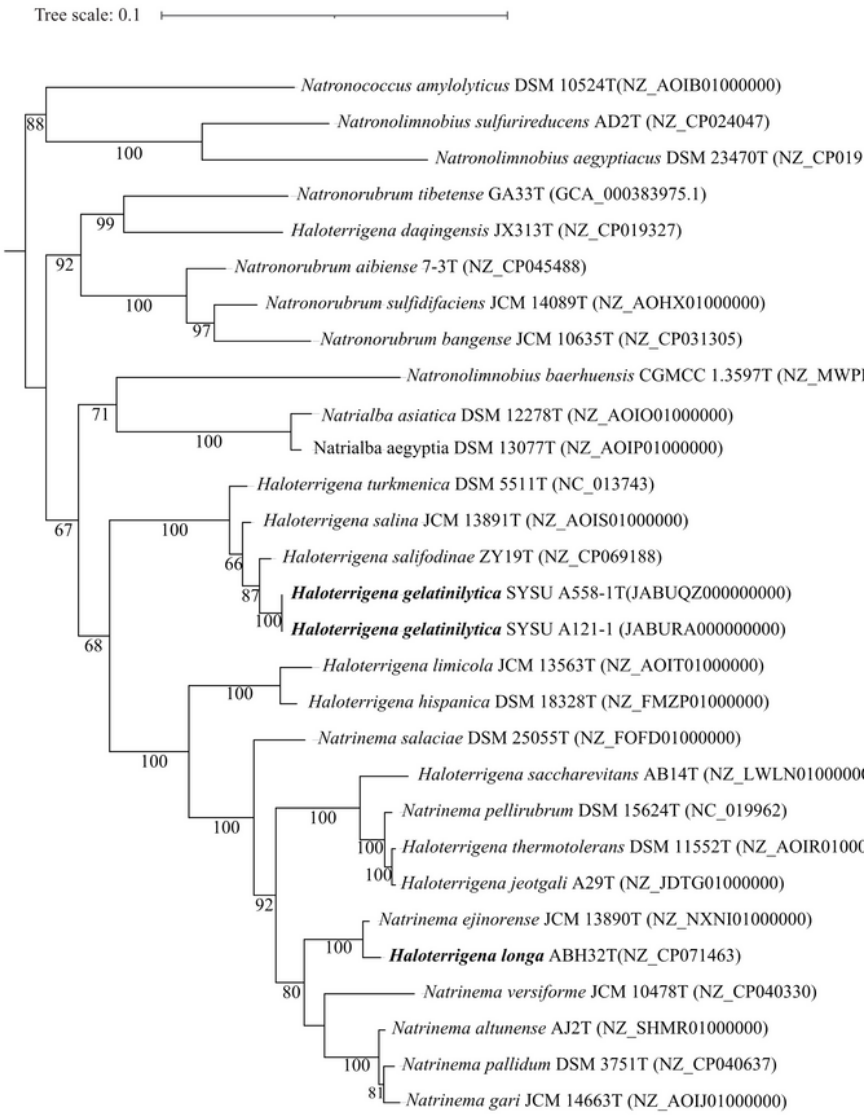

Figure 3

RAxML phylogenomic tree based on concatenation of 16 protein markers (*rpl*14, *rpl*15, *rpl*16, *rpl*18, *rpl*2, *rpl*22, *rpl*24, *rpl*3, *rpl*4, *rpl*5, *rpl*6, *rps*10, *rps*17, *rps*19, *rps*3, *rps*8) present in the genomes of strains SYSU A558-1^{T} and SYSU A121-1 and related strains of the family *Natrialbaceae*. *Halobacterium salinarum* 91-R6^{T} (GCA_004799605.1) was used as outgroup. Bar, 0.01 substitutions per site.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.pdf