Original Paper

Haloterrigena gelatinilytica sp. nov., a new extremely halophilic archaeon isolated from salt-lake

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Abstract
Two extremely halophilic strains, designated SYSU A558-1T and SYSU A121-1, were isolated from a saline sediment sample collected from Aiding salt-lake, China. Cells of strains SYSU A558-1T and SYSU A121-1 were Gram-stain-negative, coccoid, and non-motile. The strains were aerobic and grew at NaCl concentration of 10–30% (optimum, 20–22%), at 20–55 °C (optimum, 37–42 °C) and at pH 6.5–8.5 (optimum, 7.0–8.0). Cells lysed in distilled water. The polar lipids were phosphatidyl choline, phosphatidylglycerol phosphate methyl ester, disulfated diglycosyl diether-1 and unidentified glycolipid. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that the two strains SYSU A558-1T and SYSU A121-1 were closely related to the membranes of the genus Haloterrigena. Phylogenetic and phylogenomic trees of strains SYSU A558-1T and SYSU A121-1 demonstrated a robust clade with Haloterrigena turkmenica, Haloterrigena salifodinae and Haloterrigena salina. The genomic DNA G+C content of strains SYSU A558-1T and SYSU A121-1 were 65.8 and 65.0%, respectively. Phenotypic, phylogenetic, chemotaxonomic and genome analysis suggested that the two strains SYSU A558-1T 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 A558-1T (= KCTC 4259T = CGMCC 1.15953T).

Keywords Haloterrigena gelatinilytica sp. nov. · Natrialbaceae · Aiding salt-lake · Saline sediment · Polyphasic taxonomy

Introduction
Extremophiles include organisms from all three domains of life, but archaea are the most common to live in extreme conditions (Liu et al. 2019). Among archaea, halophilic archaea are regarded as a significant source of enzymes (Liu et al. 2019). 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 11 species (https://lpsn.dsmz.de/genus/haloterrigena).

Members of the genus Haloterrigena (Htg) are chemooorganotrophs, and are mainly found in alkaline high-salt environments such as solar saltern, salt lakes, fermented food, etc. (Ventosa and Kamekura 2015; Ding et al. 2017; Chen et al. 2019). Their shape ranges from rod, coccii 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 disphalted diglycosyl ether (S2-DGD-1) was...
reported (Ventosa and Kamekura 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 A558-1T and SYSU A121-1 were isolated and could grow at high salt concentrations. The 16S rRNA gene sequence analysis showed low similarity to the members of the genus Haloterrigena. Owing to the potential applications of halophilic microorganisms, the present study evaluates the taxonomic status of strains SYSU A558-1T and SYSU A121-1 and elucidates their mechanisms for overcoming salt stress.

Materials and methods

Isolation and preservation

Strains SYSU A558-1T 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 L−1): soluble starch, 20; lotus root starch, 5; KNO3, 1; MgSO4·7H2O, 0.5; K2HPO4, 0.5; NaCl, 150 or 200; trace solution (2% FeSO4·7H2O; 1% MnCl2·4H2O; 1% ZnSO4·7H2O; 1% CuSO4·5H2O); 1 mL (pH adjusted to 7.2). The isolation plates were incubated at 37 °C for at least 4 weeks in sealed plastic bags. The isolates SYSU A558-1T 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 −80 °C as glycerol suspension (20%, v/v) supplemented with 15% (w/v) NaCl. The reference type strains Htg. salina CGMCC 1.2364T and Htg. turkmenica CGMCC 1.2364T were cultivated on 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). Cell 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. Mg2+ requirement for growth was examined by adding 0.1, 0.3, 0.5, 0.8, 1.0, 1.2 or 1.5 M MgSO4·7H2O 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 KH2PO4/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO3/0.1 M Na2CO3.

Growth and gas formation with nitrate as the electron acceptor was tested as described by Cui et al. (2010c). Anaerobic growth was tested on agar plates in the presence of l-arginine and DMSO (5 g L−1) using a Whitley A35 anaerobic workstation (Don Whitley Scientific). Hydrolysis of casein, gelatin, starch and Tween (20, 40, 60 and 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) H2O2. Oxidase activity was determined using an oxidase reagent (bioMérieux). H2S 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 OD600 compared to a control (mG broth without any energy source). Acid production was tested in mG broth (without K2HPO4·3H2O) supplemented with different sole carbon sources. The changes in pH of the medium due to the production of acid were monitored after 1 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 sequencing

The 16S rRNA gene sequences were recovered from the genomes using ContEst16S (Lee et al. 2017) and analyzed in EzBioCloud server (Yoon et al. 2017a). 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′ gene 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 package 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 3 weeks as described by Cui et al. (2010b) and analyzed by two-dimensional thin-layer chromatography (TLC) (Kates 1972). Htg. salina CGMCC 1.6203T was used as a reference strain for the comparison of polar lipids in the one-dimensional TLC chromatogram.

Genomic characterization

The genomic DNA from the strains SYSU A558-1T 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 the DNA was measured using NanoDrop (Thermo Scientific NanoDrop One). Whole-genome sequencing of strains SYSU A558-1T 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 content of the DNA was calculated and gene prediction was carried out using Glimmer version 3.02 (Delcher et al. 2007). The software Infernal 1.1 (Nawrocki and Eddy 2013) was used to predict rRNA and other ncRNAs in the genome based on the RNA families database (Nawrocki et al. 2015). Transfer RNA in the genome was predicted using tRNAscan-SE v1.21 (Lowe and Eddy 1997). Functional annotation of the genome was performed with RAST (Aziz et al. 2008). The Crt software was used for CRISPR prediction (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 GenMark (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 TMM (Rogh 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 (Krzynowski et al. 2009) was used to map the genome circle.

For generation of phylogenomic tree, 87 protein marker genes were retrieved from the genomes of the genera Haloterrigena, Natrinema, Natronorubrum and other type species in the family Natrathalaceae using AMPHORA2 (Wu and Scott 2012). Sequence alignment, concatenation, and generation of phylogenomic tree were followed as described by Asem et al. (2020).

Average nucleotide identity (ANI) values between strains SYSU A558-1T and SYSU A121-1 and those of the type strains of their closest phylogenomic neighbors were calculated using the OrthoAnNu algorithm (Yoon et al. 2017b). 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 A558-1T and SYSU A121-1 were non-motile and coccoid with a diameter ranging between 0.9 and 1.1 μm (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). Mg2+ was not necessary for growth. Strains SYSU A558-1T and SYSU A121-1 optimal NaCl (w/v) concentration for growth was 20–22% which was a little above 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 package 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).

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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.

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.

Table 1 Differentiating characteristics of strains SYSU A558-1^T and SYSU A121-1 from the closely related members of the genus Haloterrigena

| 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 (°C)              | 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     |
| d-Raffinose                         | +     | +     | −     | +     |
| d-Rhamnose                          | −     | −     | +     | w     |
| d-Ribose                            | −     | −     | −     | −     |
| d-Sucrose                           | +     | +     | −     | +     |
| L-Threonine                         | −     | −     | +     | +     |
| d-Trehalose                         | w     | +     | −     | w     |
| d-Xylose                            | −     | −     | +     | −     |
| Succinate                           | −     | −     | −     | +     |
| Citrate                             | −     | −     | +     | −     |
| Fumarate                            | −     | −     | w     | +     |

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.

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 sequence 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 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 Figs. S2 and S3). The trees were polyphyletic which was consistence with earlier study (Romano et al. 2007; Chen et al. 2019).

Chemotaxonomic features

The polar lipids of the two strains SYSU A558-1^T and SYSU A121-1 were phosphatidyl choline, phosphatidylglycerol phosphate methyl ester (PGP-Me), disulphated diglycosyl diether-1 (S2-DGD-1) and one unidentified glycolipid (Supplementary Fig. S4). Phosphatidylglycerol sulphate, which was the common characteristic of the genus Haloterrigena, was absent in strains SYSU A558-1 T and SYSU A121-1 (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).

Genomic features

The complete genome sequence of strain SYSU A558-1T 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 N50 length was 4,294,945 bp. The genomic DNA G+C contents of the chromosome and the two plasmids of strain SYSU A558-1T 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), 315,414 bp (plasmid 2) and 100,260 bp (plasmid 3), respectively. N50 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 Supplementary Fig. S5. Strain SYSU A558-1T 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.

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.
RAST annotation results suggest that strain SYSU A558-1T 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 A558-1T to overcome salt stress.

**Taxonomic conclusion**

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

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

*Haloterrigena gelatinilytica* (g.e.l.a.t.i.n.i.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 °C, pH 6.5–8.5, and Mĝ²⁺ (0–0.8 M). Optimum growth occurs with 20–22% (w/v) NaCl, pH 7.0–8.0, temperature 37–42 °C and 0.03 M MgSO₄·7H₂O. 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 and 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, disulfated diglycosyl diether-1 and one unidentified glycolipid. The genomic DNA G + C content of the type strain is 65.8%. The type strain, SYSU A558-1T (＝KCTC 4259T = CGMCC 1.15953T), was isolated from saline soil of Aiding salt-lake in Xinjiang province, China.
**Fig. 3** RAxML phylogenomic tree based on concatenation of 87 protein markers (adk, cca, dph2, dph5, fbpA, ghsX, ghsY, rpl1, hisS, infA, ksgA, ligT, miaB, monA, monC, mth, muk, nueG, pdcT, pelA, phiS, phiT, pitT, prmA, prh, pus, pyrB, pyrG_ archaea, pyrL, rdgB, rplA, rpl10e, rpl11p, rpl13p, rpl14p, rpl15e, rpl18e, rpl18p, rpl19e, rpl1p, rpl21e, rpl22p, rpl24p, rpl26p, rpl30p, rpl32e, rpl3p, rpl4lp, rpl5p, rpl6p, rpl7ae, rplP0, rpoA1, rpoB_ archaea1, rpoB_ archaea2, rpoD, rpoE1, rpp, rps11p, rps12p, rps13p, rps15p, rps17p, rps19e, rps19p, rps28e, rps2p, rps3ae, rps3p, rps4e, rps4p, rps5p, rps7p, rps8e, rps8p, rps9p, sbds, tRNAmeth, tRNAmod, tef2, tif2a, tif2g, tif5a, tif6, trm, trn1, trnH) present in the genomes of strains SYSU A558-1T and SYSU A121-1 and related strains of the family Natrialbaceae. Halobacterium salinarum 91-R6T was used as an outgroup. Bar, 0.01 substitutions per site.

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

| Strains            | ANI (%) | dDDH (%) |
|--------------------|---------|----------|
| **SYSU A558-1T**   | 100     | 98.9     |
| **SYSU A121-1**    | 98.9    | 100      |
| *Htg. salina* JCM 13891T | 93.9   | 93.9     |
| *Htg. salifodinae* ZY 19T | 93.6 | 93.6   |
| *Htg. turkmenica* DSM 5511T | 91.0 | 91.0   |

The relatedness indices were calculated using the ANI and dDDH methods. The bar indicates the occurrence of 0.01 substitutions per site.
Table 3 Genome attributes

| Name                | Accession number          | Size (Mb) | G+C % | rRNA | tRNA | Other RNA | Gene   |
|---------------------|---------------------------|-----------|-------|------|------|-----------|--------|
| SYSU A558-1<sup>T</sup> | JABUQZ0000000000         | 4.29      | 65.8  | 9    | 48   | 4         | 4542   |
| SYSU A121-1         | JABURA0000000000         | 4.12      | 66.1  | 12   | 49   | 3         | 4733   |
| *Htg. salina* ICN 13891<sup>T</sup> | A0I5000000000          | 4.84      | 65.2  | 8    | 53   | 2         | 4643   |
| *Htg. salifodinae* ZY19<sup>T</sup> | RQWN0000000000       | 4.96      | 64.5  | 5    | 49   | 2         | 4817   |
| *Htg. turkmenica* DSM 5511<sup>T</sup> | CP001860                  | 3.89      | 65.8  | 10   | 50   | 2         | 3749   |

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Author contributions S-XG and W-JL conceived the study; B-BL, NS, SC, Y-GX, Y-RZ and X-YY performed the experiments; B-BL, M-PNR, L-YW and NS wrote the draft manuscript; S-XG and W-JL finalized the manuscript.

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Availability of data and materials The draft genome sequences of isolates SYSU A558-1<sup>T</sup> and SYSU A121-1 are available at the NCBI genome database under the accessions JABUQZ0000000000 and JABURA0000000000, respectively. The three copies of 16S rRNA gene sequences of the isolate SYSU A558-1<sup>T</sup> are available under the accession numbers JABUQZ0000000000, respectively. The three copies of 16S rRNA gene sequences of isolates SYSU A558-1T and SYSU A121-1 are available at the NCBI GenBank/EMBL/DDBJ database search programs. Nucleic Acids Res 25:3389–3402.

Declarations

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

Conflicts of interest The authors declare that there are no conflicts of interest.

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