



OPEN ACCESS

EDITED BY
Xue-Wei Xu,
Ministry of Natural Resources, China

REVIEWED BY
Satya P. Singh,
Saurashtra University, India
Rafael Montalvo,
University of Puerto Rico at Mayagüez,
Puerto Rico

*CORRESPONDENCE
Heng-Lin Cui
✉ cuihenglin@ujs.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to
Aquatic Microbiology,
a section of the journal
Frontiers in Marine Science

RECEIVED 23 November 2022
ACCEPTED 02 January 2023
PUBLISHED 17 January 2023

CITATION
Sun Y-P, Wang B-B, Wu Z-P, Zheng X-W,
Hou J and Cui H-L (2023) *Halorarius*
litoreus gen. nov., sp. nov., *Halorarius*
halobius sp. nov., *Haloglomus*
halophilum sp. nov., *Haloglomus*
salinum sp. nov., and
Natronomonas marina sp. nov., extremely
halophilic archaea isolated from tidal flat
and marine solar salt.
Front. Mar. Sci. 10:1105929.
doi: 10.3389/fmars.2023.1105929

COPYRIGHT
© 2023 Sun, Wang, Wu, Zheng, Hou and
Cui. This is an open-access article distributed
under the terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other
forums is permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication in
this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Halorarius litoreus gen. nov., sp. nov., *Halorarius halobius* sp. nov., *Haloglomus halophilum* sp. nov., *Haloglomus salinum* sp. nov., and *Natronomonas marina* sp. nov., extremely halophilic archaea isolated from tidal flat and marine solar salt

Ya-Ping Sun[†], Bei-Bei Wang[†], Zhang-Ping Wu, Xi-Wen Zheng,
Jing Hou and Heng-Lin Cui*

School of Food and Biological Engineering, Jiangsu University, Zhenjiang, China

Five novel halophilic archaeal strains, named BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T, were isolated from the coastal saline sediment of the intertidal zone located in Qingdao and the natural sea salt produced from Huanghua marine solar saltern, PRChina. These five strains demonstrated the typical morphology and growth characteristics of haloarchaea. The comparison of 16S rRNA gene revealed that strain BDN22^T was associated with *Salinirubellus salinus* ZS-35-S2^T (95.2% similarity), strain ZY10^T was related to *Halosegnis rubeus* F17-44^T (95.1% similarity), both strains ZY41^T and ZY58^T were closely related to *Haloglomus irregulare* F16-60^T (98.1% and 98.2% similarities, respectively), and strain ZY43^T was close to *Natronomonas salina* YPL13^T (98.0% similarity). In addition to the 16S rRNA gene, the *rpoB'* gene is undoubtedly another important molecular marker for the identification of halophilic archaea, and concatenated-conserved-protein phylogeny was widely used in archaeal classification in recent years. Phylogenetic and phylogenomic analyses based on *rpoB'* genes and 122 concatenated archaeal protein genes showed that these haloarchaea construct three different clades and gathered together with the current members of *Haloarculaceae*. Strains BND22^T and ZY10^T formed two distinct clades separated from *Salinirubellus salinus* ZS-35-S2^T and *Halosegnis* members, strains ZY41^T and ZY58^T clustered with *Haloglomus irregulare* F16-60^T, and strain ZY43^T gathered with the current members of *Natronomonas*. The ANI, DDH, and AAI values of these five strains against phylogenetic neighbours were no more than 91%, 45%, and 92%, respectively, far below the cut-off values for species delineation, supporting their placements in new taxa. Based on the phenotypic, chemotaxonomic, phylogenetic, and phylogenomic properties, these five strains represent five novel taxa of the family *Haloarculaceae*, *Halorarius litoreus* gen. nov., sp. nov. (type strain BND22^T = CGMCC 1.18780^T = JCM 34966^T), *Halorarius halobius* sp. nov. (type strain ZY10^T = CGMCC 1.17475^T = JCM 34319^T), *Haloglomus halophilum* sp. nov. (type strain ZY41^T = CGMCC 1.17030^T = JCM

34161^T), *Haloglomerus salinum* sp. nov. (type strain ZY58^T = CGMCC 1.17216^T = JCM 34163^T), and *Natronomonas marina* sp. nov. (type strain ZY43^T = CGMCC 1.17202^T = JCM 34162^T). This is the first report of description of a novel haloarchaeon isolated from a marine intertidal zone.

KEYWORDS

Halorarius, Haloglomerus, Natronomonas, halophilic archaea, tidal flat, marine solar salt

Introduction

Halophilic archaea of class *Halobacteria*, belonging to phylum *Euryarchaeota* of domain *Archaea*, flourish in different hypersaline habitats, including salt lakes, marine solar salterns, salted brown algae, and coarse sea salt (Cui and Dyll-Smith, 2021; Han and Cui, 2020; Wang et al., 2022). Currently, there are 73 genera and 318 species with validly published names assigned in the class *Halobacteria* (List of Prokaryotic names with Standing in Nomenclature, <https://lpsn.dsmz.de/class/halobacteria>). None of these *Halobacteria* members was isolated from the marine intertidal zone. In the previous surveys on the halophilic archaeal diversity of coastal beaches located in Shandong and coarse sea salt produced from the coastal solar saltern of Hebei, five novel halophilic archaeal strains were isolated and purified. Strains BND22^T and ZY10^T were related to each other, then to *Salinirubellus salinus* ZS-35-S2^T and *Halosegnis* members, strains ZY41^T and ZY58^T were close to *Haloglomerus irregulare* F16-60^T, and strain ZY43^T was found to be a member of genus *Natronomonas*, according to 16S rRNA gene comparison.

The genera *Haloglomerus*, *Halosegnis*, *Natronomonas*, and *Salinirubellus* are four phylogenetically-related groups within the order *Halobacteriales*. The genus *Haloglomerus* was established by Durán-Viseras in 2020, and only contained one species *Haloglomerus irregulare* F16-60^T which was cultivated from the brine of Isla Cristina saltern, Huelva, Spain (Durán-Viseras et al., 2020a). And based on the strains *Halosegnis longus* F12-1^T and *Halosegnis rubeus* F17-44^T, the genus *Halosegnis* was described in 2021 (Durán-Viseras et al., 2021). As one of the oldest and most famous halophilic archaeal genera, *Natronomonas* was established in 1997 and at present comprises six species: *Natronomonas pharaonis* (Kamekura et al., 1997), *Natronomonas moolapensis* (Burns et al., 2010), *Natronomonas gomsonensis* (Kim et al., 2013), *Natronomonas salsuginis* (Durán-Viseras et al., 2020b), *Natronomonas halophila* and *Natronomonas salina* (Yin et al., 2020). The genus *Salinirubellus* related to the above three genera was proposed based on a strain isolated from the sediment sample of a marine solar saltern in Zhejiang, PRChina (Hou et al., 2018). Most of these halophilic archaea cannot hydrolyze starch, gelatin, casein, and Tween 80, but can utilize simple sugars for aerobic growth. The polar lipid profiles of them were diverse, the members of *Halosegnis*, *Haloglomerus*, and *Natronomonas* contained phosphatidylglycerol sulphate (PGS) but that of *Salinirubellus* did not, and *Halosegnis* members contained S-TGD-1 (sulfated galactosyl mannosyl glucosyl diether) in addition to S-DGD-1 (sulfated mannosyl glucosyl diether), the common glycolipid of these four

genera. In this study, two novel species of a new genus, and three novel species of the genera *Haloglomerus* and *Natronomonas* within the family *Haloarculaceae* are proposed to accommodate strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T, respectively.

Materials and methods

Cultivation and isolation of pure haloarchaeal cultures

Strain BND22^T was cultivated from the coastal saline soil sample of the intertidal zone located in Qingdao, PRChina (36°12'36" N, 120° 22'10" E; elevation, 0 m) in 2019. The pH, salt concentration, and organic matter content of the saline soil were 7.9, 5.3 g/kg, and 1.3 g/kg, respectively. Strains ZY10^T, ZY41^T, ZY58^T, and ZY43^T were isolated from the natural sea salt produced from Huanghua marine solar saltern in Hebei, PRChina, in 2018. These samples were diluted with liquid NHM, spread on NHM agar plates, and incubated aerobically at 37°C for 30 days (Sun et al., 2022). Single colonies with a shade of red were selected and streaked on fresh NHM agar slants, and the pure colonies were formed after three-time repeated. For further use, the purified strains were preserved in liquid NHM with 15% (w/v) glycerol at -20°C. *Halobacterium salinarum* CGMCC 1.2367, *Haloglomerus irregulare* JCM 33318^T, *Halosegnis longus* JCM 33319^T, *Natronomonas gomsonensis* KCTC 4088^T, *Natronomonas halophila* C90^T, *Natronomonas salina* YPL13^T, and *Salinirubellus salinus* ZS-35-S2^T were used as reference strains and routinely grown aerobically in NHM liquid medium.

Phylogenetic analysis

The genomic DNA of strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T was prepared using a DNA extraction kit for bacteria (Jiangsu Cowin Biotech Co., Ltd.). The PCR amplification of the 16S rRNA genes and the *rpoB'* (RNA polymerase subunit B') genes were carried out by two pairs of primers, 20F (5'-ATTCCGGTTGATCCTGCCGG-3') and 1452R (5'-AGGAGGTGATCCAGCCGCAG-3'), and HrpoB2 1420F and HrpoA 153R, respectively (Cui et al., 2009; Minegishi et al., 2010). The sequencing of purified PCR amplicons was performed by SinoGenoMax (Beijing). The 16S rRNA and *rpoB'* genes of related species of the *Haloarculaceae* were downloaded from the NCBI database. Phylogenetic trees were constructed with MEGA 6 (Tamura et al., 2013) based on three different algorithms, ML

(maximum-likelihood) (Felsenstein, 1981), MP (maximum-parsimony) (Fitch, 1971) and NJ (neighbor-joining) (Saitou and Nei, 1987). The calculation of pairwise sequence similarities of 16S rRNA and *rpoB'* gene between strains BND22^T, ZY10^T, ZY41^T, ZY58^T, ZY43^T, and related species was performed by using the online pairwise sequence alignment tool of EzBioCloud (Yoon et al., 2017).

Genome assembly, annotation, and comparative genomics

The PacBio Sequel platform was used to sequence the complete genomes of these five strains. CheckM was exploited for the evaluation of the quality of assembled genome sequences (Parks et al., 2015). The 122 conserved archaeal protein marker genes were extracted from the genomes of the isolated five strains and related halophilic archaea for phylogenomic analyses according to the protocol of GTDB (Parks et al., 2018). The sequences of these core orthologous genes were aligned using GTDB-Tk and trimmed by trimAL version 1.4.1 (Capella-Gutiérrez et al., 2009; Chaumeil et al., 2019). The ML phylogenomic tree was constructed based on a standard model by IQ-TREE (Nguyen et al., 2015), and *Natronobacterium gregoryi* SP2^T was selected as the outgroup.

The annotation of these genomes was performed by means of RAST Annotation Server (Aziz et al., 2008). The metabolic pathways were figured out based on the KEGG database (Kanehisa et al., 2004). To explore the industrial applications of these halophilic archaea, the biotechnological potential of functional genes was identified following the methodology described previously (Sun et al., 2022). The online OrthoVenn was used to compare orthologous clusters (OCs) of related species and generate the Venn diagrams (Xu et al., 2019).

The whole genomic indexes, ANI (average nucleotide identity), DDH (DNA–DNA hybridization), and AAI (average amino acid identity) among strains BND22^T, ZY10^T, ZY41^T, ZY58^T, ZY43^T, and the related members were estimated by using JSpeciesWS online ANI calculator (<https://jspecies.ribohost.com/jspeciesws/>) (Richter et al., 2016), Genome-to-Genome Distance Calculator 3.0 (formula 2) (<http://ggdc.dsmz.de/ggdc.php>) (Meier-Kolthoff et al., 2013), and AAI calculator (<http://enve-omics.ce.gatech.edu/aa/>) (Luo et al., 2014), respectively.

Phenotypic characterization

For microscopical examination, these five isolates were grown in liquid NHM for 7–10 days. The cells of these haloarchaea were observed under a phase-contrast microscope (Eclipse Ci-L, Nikon). Gram staining was performed according to an improved technique developed by Dussault (Dussault, 1955). The pigmentation of the colonies was examined on NHM agar plates after aerobic incubation for 10–15 days at 37°C. The ranges of NaCl and MgCl₂ concentrations, temperature, and pH for growth were determined following the methodology described previously (Sun et al., 2022). The remaining phenotypic features of the five isolates and related members were based on the minimal criteria for the description of

halophilic archaeal new taxa (Oren et al., 1997). Each test was carried out in triplicates.

Chemotaxonomic characterization

Polar lipids of strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T were extracted with chloroform and methanol mixture, then precipitated by ten volumes of cold acetone after concentration. The polar lipid profiles of isolated halophilic archaea were analyzed by one-dimensional and two-dimensional thin-layer chromatography (TLC) (Cui et al., 2010). The phosphate stain reagent was prepared to detect phospholipids (Vaskovsky and Kostetsk, 1968). Glycolipids and phospholipids were shown with sulfuric acid-ethanol (1:2, v/v) followed by heating at 150 °C for 3 min. The polar lipids of these halophilic archaea were characterized by MALDI-TOF/MS (Xin et al., 2022).

Results and discussion

Phylogenetic analyses

Strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T all had a single copy of the 16S rRNA gene (1,467 bp, 1,467 bp, 1,472 bp, 1,472 bp, and 1,466 bp, respectively). These sequences are identical to the same genes in their genomes. Based on the 16S rRNA gene similarity, the closest relatives of strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T were *Salinirubellus salinus* ZS-35-S2^T (95.2%), *Halosegnis rubeus* F17-44^T (95.1%), *Haloglomerus irregulare* F16-60^T (98.1%), *Haloglomerus irregulare* F16-60^T (98.2%), *Natronomonas salina* YPL13^T (98.0%), respectively (Table S1). Except for 99.6% gene similarity between strains ZY41^T and ZY58^T, those among strains BND22^T, ZY10^T, and ZY43^T were 92.9–96.8%, less than 98.65%, the suggested threshold for separating different archaeal or bacterial species (Kim et al., 2014). The 16S rRNA gene phylogeny indicated that strains BND22^T and ZY10^T formed two distinct clades separated from *Salinirubellus salinus* ZS-35-S2^T and *Halosegnis* members, strains ZY41^T and ZY58^T clustered with *Haloglomerus irregulare* F16-60^T, and strain ZY43^T gathered with the current members of *Natronomonas* (Figure 1A).

The full length of the *rpoB'* genes from strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T were 1,827 bp. The gene similarities among these five strains were 84.9–95.2%, and they shared 81.1–91.7% sequence similarities with related members of the family *Haloarculaceae* (Table S2). Strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T shared maximum sequence similarities with *Sala cibi* CBA1133^T (88.3%), *Halosegnis longus* F12-1^T (89.4%), *Haloglomerus irregulare* F16-60^T (91.4%), *Haloglomerus irregulare* F16-60^T (91.6%), *Natronomonas salina* YPL13^T (91.7%), respectively. The *rpoB'* gene phylogeny revealed that strains BND22^T and ZY10^T clustered together and separated from the genus *Halosegnis*, strains ZY41^T and ZY58^T formed a monophyletic branch closely clustered with *Haloglomerus irregulare* F16-60^T, strain ZY43^T was a close relative of *Natronomonas salina* YPL13^T within *Natronomonas* (Figure 1B).

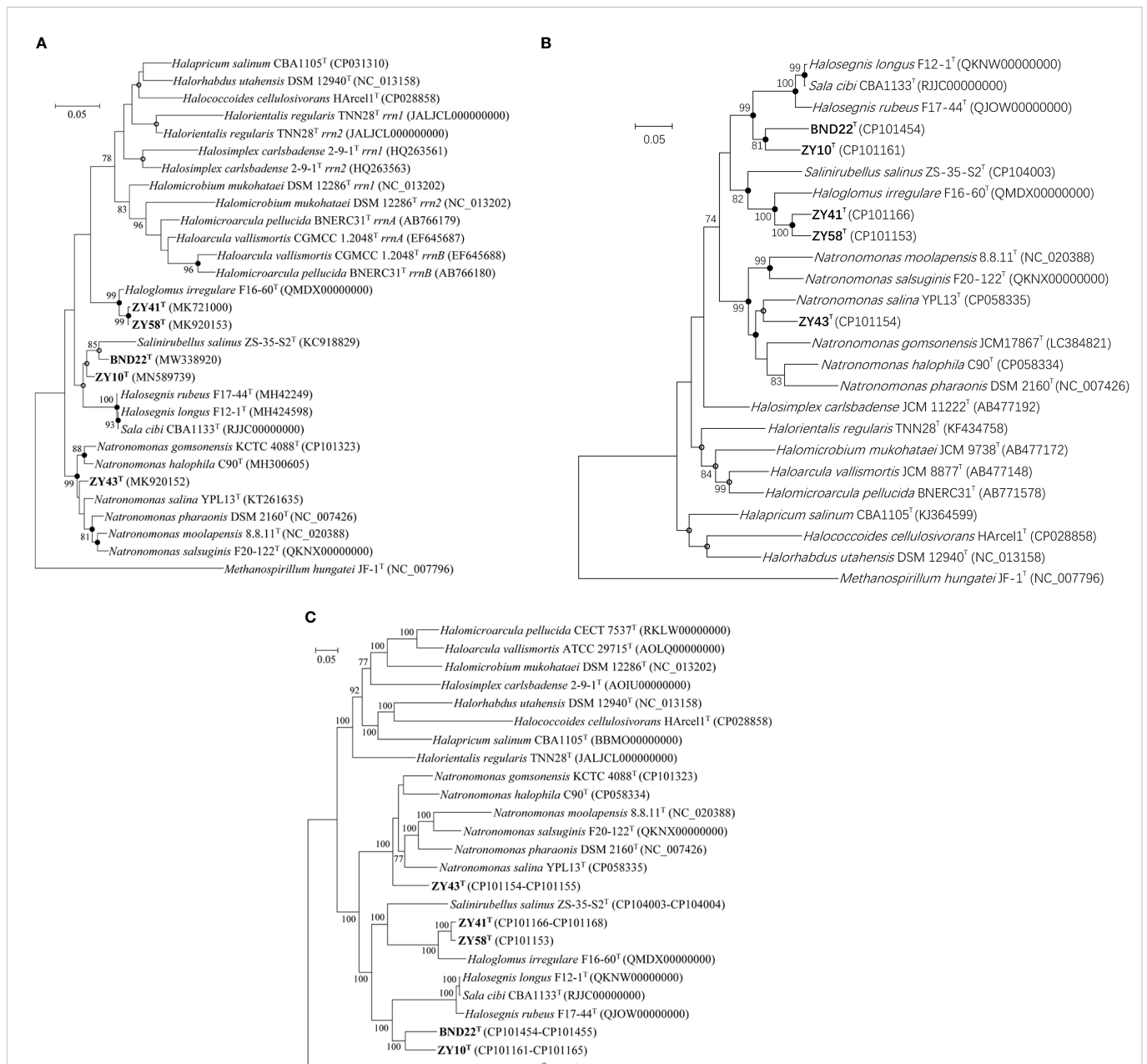


FIGURE 1

ML phylogenetic tree reconstructions based on 16S rRNA gene sequence (A), *rpoB'* gene sequence (B) and 122 conserved archaeal protein marker genes (C), showing the relationship among strains BND22^T, ZY10^T, ZY41^T, ZY58^T, ZY43^T and related species within *Haloarculaceae*. The numbers at the nodes represent bootstrap values of 1,000 replicates and support $\geq 70\%$ bootstrap. Filled circles highlight branches that were supported by the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms. Empty circles highlight branches that were supported by the maximum-likelihood and maximum-parsimony algorithms or the maximum-likelihood and neighbour-joining algorithms. The bar represents expected substitutions per nucleotide position.

Genomic features and biotechnological potential

The complete genomes of strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T were *de novo* assembled in a total of 2, 5, 3, 1, and 2 contigs, respectively. The complete genome size of new strains ranged from 4.0 to 4.4 Mb, within those of the current *Haloarculaceae* members (2.6–5.1 Mb). These five strains had one ribosomal RNA operon in their genomes and had different numbers (47, 47, 45, 43, and 48, respectively) of tRNA genes. The DNA G+C content of strains

BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T are 66.6%, 66.8%, 67.9%, 68.5%, and 66.9%, respectively (Table S3). These values are higher than those of *Halosegnis* members (64.7–66.0 mol%) and *Haloglossum* (68.0 mol%), within the range of the genus *Natronomonas* (63.2–67.4 mol%), and less than that of *Salinirubellus salinus* ZS-35-S2^T (69.0 mol%). Other genomic features were detailed in Table S3.

The ML phylogenomic tree based on 122 concatenated archaeal protein genes indicated that strain BND22^T gathered with ZY10^T and distinctly separated from *Halosegnis* and *Sala*, strains ZY41^T and ZY58^T formed a tight clade with *Haloglossum irregulare* F16-60^T, and

strain ZY43^T tightly clustered with the current *Natronomonas* members (Figure 1C).

The ANI, AAI, and DDH values among these five strains and *Haloarculaceae* members were 70–91%, 56–92%, and 18–45% (Tables S4–S6), significantly below the cut-off values, 95–96% (ANI), 95% (AAI), and 70% (DDH), for species delineation (Goris et al., 2007; Richter and Rosselló-Móra, 2009; Luo et al., 2014). This suggested that strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T represent five different species. The AAI values were proved to be useful for genera demarcation and a cut-off value ($\leq 76\%$) was proposed to differentiate genera within the family *Natrialbaeaceae* (de la Haba et al., 2021); but the value was inappropriate to be used in grouping the current *Haloarculaceae* members. If the cut-off value was used to separate the genera within the family *Haloarculaceae*, the current six *Natronomonas* species should represent six different genera (Table S6). A cut-off value of $< 69\%$ is appropriate to distinguish genera within *Haloarculaceae* according to the AAI values and evolutionary relationships among the current members of this family. Thus, strains BND22^T and ZY10^T represent two novel species of a new genus, strains ZY41^T and ZY58^T two novel different species of *Haloglomerus*, and strain ZY43^T a new species of *Natronomonas* based on the AAI cut-off ($< 69\%$).

It must be noted that the ANI, AAI, and DDH values between *Halosegnis longus* F12-1^T and *Sala cibi* CBA1133^T described recently are 99%, 94%, and 98%, much more than the threshold values of the species boundary. This close relatedness was supported by the 16S rRNA, *rpoB*, and 122 conserved archaeal protein marker genes -based phylogenies between *Halosegnis longus* F12-1^T and *Sala cibi* CBA1133^T (Figure 1). According to these results *Sala cibi* (Song et al., 2022) should be proposed as a later heterotypic synonym of *Halosegnis longus* (Durán-Viseras et al., 2021).

The subsystem category distribution of novel isolates was annotated by the RAST server (Figure S1). The amino acids and derivatives, and carbohydrates and protein metabolism were the richest RAST subsystems of these five isolates, indicating their possibility to metabolize diverse proteins and carbohydrates. Based on the KEGG database, strains BND22^T and ZY58^T, ZY41^T and ZY43^T contained the genes involved in both glycolysis and pyruvate oxidation pathways, respectively, corresponding to their capability to utilize glucose and pyruvate for growth. In contrast, the genes related to fructose utilization, casein, starch, or Tween 80 hydrolysis were not found in all these strains, which was confirmed by the results of phenotypic tests. Additionally, none of the four key enzymes involved in complete denitrification, NarG (nitrate reductase), NirK (copper-containing nitrite reductase), Nor (nitric oxide reductase), and Nos (nitrous-oxide reductase), was found in the genomes of these five strains, this confirmed by their inability to produce nitrogen growing with nitrate anaerobically.

Total of 3,788 orthologous clusters, 566 core clusters, 3,077 accessory clusters, and 145 unique clusters were found in the genomes of strains BND22^T, ZY10^T, *Salinirubellus salinus* ZS-35-S2^T, *Halosegnis longus* F12-1^T, and *Halosegnis rubeus* F17-44^T. The isolates BND22^T and ZY10^T contained 62 and 61 unique clusters, while the related members only contained 9, 9, and 4 unique clusters, respectively (Figure S2A). Strains ZY41^T, ZY58^T, and *Haloglomerus irregulare* F16-60^T had 3,850 OCs, including 2,427 core OCs, 1,320 accessory OCs and 103 unique clusters, and contained 22, 13, and 68 unique clusters, respectively (Figure S2B). Strain ZY43^T,

Natronomonas moolapensis 8.8.11^T, *Natronomonas pharaonis* DSM 2160^T, *Natronomonas salina* YPL13^T and *Natronomonas salsuginis* F20-122^T had 3,472 clusters, including 1,427 core OCs, 1,851 accessory OCs and 194 unique clusters. These strains contained 63, 24, 16, 70, and 21 unique clusters, respectively (Figure S2C). The patterns of shared and unique OCs differentiated these novel strains from their relatives.

Many previous studies reported that haloarchaea could produce valuable products of biotechnological aspects, such as haloenzymes, bacteriorhodopsin, biodegradable polyhydroxyalkanoates (PHAs), and carotenoids (Han et al., 2010; Amoozegar et al., 2017; Ashwini et al., 2017; Serrano et al., 2022). Strains ZY10^T, ZY41^T, ZY58^T, and ZY43^T possessed bacteriorhodopsin protein genes, indicating their capacity for light-mediated ATP synthesis. Strain BND22^T contained the gene cluster encoding for PHA synthesis regulatory protein (PhaR), PHA granule binding protein (PhaP), and type IIIA PHA synthase (PhaE and PhaC subunits), and strain ZY41^T contained the gene cluster encoding for acetoacetyl CoA reductase (PhaB), PhaC, PhaE and PhaR (Figure S3), indicating the probable synthesis of PHAs by the new isolates. Additionally, the genes encoded the enzymes involved in the production of β -carotene, phytoene synthase (CrtB), phytoene desaturase (CrtI), and lycopene cyclase (CrtY), were detected in strains ZY41^T and ZY58^T (Serrano et al., 2022). Moreover, one β -carotene monooxygenase (Brp) was detected in the vicinity of the *crtY* gene, suggesting the possibility of strain ZY41^T to produce retinal from β -carotene (Figure S4). These results indicate that these novel halophilic archaeal strains have much potential in biotechnological applications.

Phenotypic characteristics

Cells of strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T were motile and Gram-stain-negative. Except for strains BND22^T and ZY58^T were pleomorphic, while other strains were coccoid (Figure S5). The colonies of all strains were red and their cells lysed in distilled water. The growth of strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T occurred in NHM with NaCl concentrations of 1.7–4.8 M, 3.1–5.1 M, 2.1–4.8 M, 2.6–4.3 M, and 2.1–4.8 M, respectively; the five strains grew best at 3.1–4.8 M NaCl. The optimum magnesium concentration varied between 0.05 and 0.7 M, even if magnesium was not necessary for their growth. The growth temperature ranges for these five strains were 20–55 °C, 30–50 °C, 25–60 °C, 25–55 °C, and 30–60 °C, respectively, with optima between 35 and 40 °C. These five strains are mainly neutrophilic with pH optima at 6.5–7.5. These strains could not grow with arginine or DMSO anaerobically. Reduction of nitrate to nitrite was found in strains BND22^T, ZY10^T, ZY41^T, and ZY43^T but not in strain ZY58^T. Indole and H₂S were not produced by these strains, and none of the casein, gelatin, starch, or Tween 80 was found to be hydrolyzed. Of the 19 antibiotics tested, only bacitracin (0.04 IU per disc), nitrofurantoin (300 μ g), novobiocin (30 μ g per disc), nystatin (100 μ g), rifampin (5 μ g), and trimethoprim (5) could inhibit growth. The remained antibiotics, ampicillin (10 μ g), chloramphenicol (30 μ g), ciprofloxacin (5 μ g), erythromycin (15 μ g), gentamicin (10 μ g), kanamycin (30 μ g), nalidixic acid (30 μ g), neomycin (30 μ g), norfloxacin (10 μ g), penicillin G (10 IU), streptomycin (10 μ g), tetracycline (30 μ g), or vancomycin (30 μ g), were not found to be effective. The distinct phenotypic

characteristics differentiating isolates BND22^T and ZY10^T from the related species of the family *Haloarculaceae* (*Salinirubellus salinus* ZS-35-S2^T, *Halosegnis longus* JCM 33317^T, *Halosegnis rubeus* JCM 33319^T, and *Halogloimus irregulare* JCM 33318^T) were optimum NaCl, utilization of specific carbon sources, and presence of PGS. Strains ZY41^T and ZY58^T could be differentiated from *Halogloimus irregulare* JCM 33318^T based on cell motility, gelatin hydrolysis and presence of PGS. The difference between strain ZY43^T and the current members of the *Natronomonas* included H₂S formation, cell morphology, catalase, and oxidase activity (Tables 1, 2) (Kamekura et al., 1997; Burns et al., 2010; Kim et al., 2013; Durán-Viseras et al., 2020a; Durán-Viseras et al., 2020b; Yin et al., 2020; Durán-Viseras et al., 2021).

Chemotaxonomic characteristics

The common phospholipids, phosphatidylglycerol (PG) and phosphatidylglycerol phosphate methyl ester (PGP-Me), were detected in strains BND22^T, ZY10^T, ZY41^T, and ZY58^T. It was remarkable that the absence of phosphatidylglycerol sulfate (PGS) in these four strains compared with the *Halosegnis* and *Halogloimus* members. The phospholipid profile of strain ZY43^T was PG, PGP-Me, and PGS, similar to that of the *Natronomonas* members (Figure S6). Diverse glycolipids were detected in these five strains, three glycolipids (GL1, GL-PL1, and GL-PL2) in strain BND22^T, two glycolipids (GL1, GL2) in strain ZY10^T, one glycolipid (GL1) in strains ZY41^T and ZY58^T, and two different glycolipids (GL01, GL02) in strain ZY43^T. The TLC mobilities of GL1, GL2 (GL-PL1), and GL02 were similar to those of sulfated mannosyl glucosyl diether (S-DGD-1), mannosyl glucosyl diether (DGD-1), and sulfated galactosyl

mannosyl glucosyl diether (S-TGD-1), respectively, based on one-dimensional TLC (Figure S6).

The MALDI-TOF/MS patterns of the polar lipids of strains BND22^T, ZY10^T, ZY41^T, ZY58^T, and ZY43^T were obtained based on the mode of negative ion (Figure S7). The signals at *m/z* 731.6, 805.7, 875.7, 885.6, 899.6, 955.7, and 969.6 represented PA, PG, PG (C₂₀C₂₅), PGS, PGP-Me, PGS (C₂₀C₂₅), and PGP-Me (C₂₀C₂₅), respectively. Except for strains ZY41^T and ZY58^T, the ion of *m/z* 991.7 appearing in others represented DGD-1. Strains BND22^T, ZY10^T, ZY41^T, and ZY58^T showed S-DGD-1 signals at *m/z* 1055.7, 1071.7, and 1125.7. The ion at *m/z* 1217.7 detected in strain ZY43^T corresponded to S-TGD-1. In addition, several glycosyl-cardiolipins, DGD-PA, S-DGD-PA, and S-DGD-PA (+Na) were detected and judged by the signals of *m/z* 1690.4, 1770.3, and 1792.3, respectively (Lobasso et al., 2015). These results were mostly in accordance with those detected by TLC.

Taxonomic conclusions

The polyphasic and genome-based classification indicated that strains BND22^T (= CGMCC 1.18780^T = JCM 34966^T) and ZY10^T (= CGMCC 1.17475^T = JCM 34319^T), represent two different new species of a novel genus within *Haloarculaceae*, *Halorarius litoreus* gen. nov., sp. nov. *Halorarius halobius* sp. nov.; strains ZY41^T (= CGMCC 1.17030^T = JCM 34161^T) and ZY58^T (= CGMCC 1.17216^T = JCM 34163^T) represent two different new species of *Halogloimus*, *Halogloimus halophilum* sp. nov. and *Halogloimus salinum* sp. nov.; and strain ZY43^T (= CGMCC 1.17202^T = JCM 34162^T) represents a novel species of *Natronomonas*, *Natronomonas marina* sp. nov.

TABLE 1 Differential characteristics of strains BND22^T, ZY10^T, ZY41^T, ZY58^T and their closely related species within the family *Haloarculaceae*.

Characteristics	1	2	3	4	5	6	7	8
Cell morphology	pleomorphic	coccoid	coccoid	pleomorphic	pleomorphic	rod	rod	pleomorphic
Motility	+	+	+	+	+	-	-	-
NaCl growth range (M)	1.7–4.8	3.1–5.1	2.1–4.8	2.6–4.3	1.4–4.8	2.6–4.3	2.6–5.1	3.4–6.0
Optimum growth NaCl (M)	3.4	4.8	3.1	3.1	2.1	4.3	4.3	5.1
Temperature growth range (°C)	20–55	30–50	25–60	25–55	25–50	25–45	30–50	30–45
Optimum growth temperature (°C)	35	37	40	37	37	37	37	37
pH range for growth	6.5–8.0	7.0–8.5	6.0–8.5	5.5–8.0	5.0–9.5	6.0–8.5	6.5–8.5	6.5–9.0
Optimum growth pH	7.5	7.5	7.5	7.0	7.5	7.5	7.5	7.5
Gelatin hydrolysis	-	-	-	-	-	-	-	+
Reduction of nitrate to nitrite	+	+	+	-	+	-	-	+
Oxidase	-	+	-	-	-	-	-	-
Utilization as sole carbon source:								
Fructose	-	-	-	-	-	+	-	-
Glycerol	-	+	+	-	+	-	+	-
Presence of PGS	-	-	-	-	-	+	+	+
Taxa: 1, strain BND22 ^T ; 2, strain ZY10 ^T ; 3, strain ZY41 ^T ; 4, strain ZY58 ^T ; 5, <i>Salinirubellus salinus</i> ZS-35-S2 ^T ; 6, <i>Halosegnis longus</i> JCM 33317 ^T ; 7, <i>Halosegnis rubeus</i> JCM 33319 ^T ; 8, <i>Halogloimus irregulare</i> JCM 33318 ^T . +, Positive; -, negative.								

TABLE 2 Differential characteristics of strain ZY43^T and *Natronomonas* species.

Characteristics	1	2	3	4	5	6	7
Cell morphology	coccoid	pleomorphic	pleomorphic	rod	pleomorphic	coccoid	coccoid
NaCl range (M) for growth	2.1–4.8	0.9–4.8	0.9–4.8	2.1–5.1	2.4–6.2	3.1–5.1	1.7–5.1
Optimum growth NaCl (M)	3.1	4.3	3.4	3.5	3.1–3.4	4.1	4.3
Temperature range (°C) for growth	30–60	30–60	25–50	20–55	25–45	20–45	25–50
Optimum growth temperature (°C)	40	40	37	45	45	40	37
pH range for growth	5.5–9.0	6.5–9.5	5.0–8.0	8.0–11.0	5.5–8.5	5.5–8.0	6.0–9.5
Optimum growth pH	6.5	8.0	6.5	8.5–9.0	7.0–7.5	7.0	8.0
Gelatin hydrolysis	–	–	–	+	–	–	–
Reduction of nitrate to nitrite	+	+	+	–	+	–	+
Catalase	+	–	–	+	–	+	–
Oxidase	–	–	+	+	–	+	–
Utilization as sole carbon source:							
Fructose	–	–	–	–	–	+	–
Glycerol	+	–	–	+	+	+	–
L-lysine	+	–	–	+	–	–	+
Pyruvate	+	+	+	+	+	+	–
H ₂ S formation	–	–	–	+	–	–	–
Taxa: 1, ZY43 ^T ; 2, <i>Natronomonas halophila</i> C90 ^T ; 3, <i>Natronomonas salina</i> YPL13 ^T ; 4, <i>Natronomonas pharaonis</i> CGMCC 1.1965 ^T ; 5, <i>Natronomonas moolapensis</i> JCM 14361 ^T ; 6, <i>Natronomonas gomsonensis</i> KCTC 4088 ^T ; 7, <i>Natronomonas salsuginis</i> JCM 33320 ^T . +, Positive; –, negative.							

We propose *Sala cibi* Song et al., 2022 as a later heterotypic synonym of *Halosegnis longus* (Durán-Viseras et al., 2021). (type strain F12-1^T = CECT 9685^T = JCM 33317^T; another reference strain of this species, CBA1133 = KACC 22148 = JCM 34265).

Description of *Halorarius* gen. nov.

Halorarius (Hal.o.ra'ri.us. Gr. masc. n. *hals halos*, salt; L. masc. adj. *orarius*, belonging to the seashore; N.L. masc. n. *Halorarius* salt microorganism isolated from the seashore).

Cells are Gram-stain-negative, motile, pleomorphic and coccoid under optimal growth conditions. Cells are found to be lysed in distilled water. Colonies on NHM agar plate are red, moist and round. Growth was found at 20–55°C, 1.7–5.1 M NaCl, 0–1.0 M MgCl₂ and pH 6.5–8.5. The activity of catalase was positive while that of oxidase was negative. Some sugars can be utilized for growth and with the production of acids. The cultures contain PA, PG, PGP-Me, S-DGD-1, and DGD-1. The type species is *Halorarius litoreus*. Recommended three-letter abbreviation: *Hor*.

Description of *Halorarius litoreus* sp. nov.

Halorarius litoreus (li.to're.us. L. masc. adj. *litoreus*, of or belonging to the seashore).

The cells of type strain are pleomorphic (1.0–1.2×1.0–3.0 μm), motile, and Gram-negative. Colonies are red and circular, about 5 mm in diameter following incubation for 14 days at 37°C. The type strain could grow in NHM with NaCl concentrations of 1.7–4.8 M and grew best at 3.4 M NaCl. The optimum magnesium concentration was 0.03 M, even if magnesium was not necessary for growth. The growth temperature range for the type strain was 20–55 °C, with optimum at 35°C. The type strain is neutrophilic with pH optima at 7.5. The type strain can grow anaerobically with nitrate but not with arginine or DMSO. Reduction of nitrate to nitrite was found but gas was not produced. Diverse substrates, *D*-glucose, sucrose, *D*-mannitol, *D*-sorbitol, acetate, succinate, *L*-aspartate, *L*-glutamate, *L*-lysine and *L*-ornithine, can be utilized for growth, while *D*-fructose, *D*-galactose, lactose, maltose, *D*-mannose, *D*-ribose, *L*-sorbose, *D*-xylose, glycerol, citrate, fumarate, *D,L*-lactate, *L*-malate, pyruvate, *L*-arginine, glycine or *L*-alanine do not support growth. The catalase test is positive while the oxidase is negative. Indole and H₂S formation are not detected. None of casein, gelatin, starch or Tween 80 was found to be hydrolyzed. The type strain contains PA, PG, PGP-Me, DGD-1 (DGD-PA), and S-DGD-1 (S-DGD-PA).

The type strain, BND22^T (= CGMCC 1.18780^T = JCM 34966^T), was isolated from the coastal saline soil sample of the intertidal zone located in Qingdao, PRChina. The DNA G+C content of the type strain is 66.6% (genome). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and genome of strain BND22^T are MW338920 and CP101454–CP101455, respectively.

Description of *Halorarius halobius* sp. nov.

Halorarius halobius (ha.lo'bi.us. Gr. masc. n. *hals*, halos, salt; Gr. masc. n. *bios*, life; N.L. masc. adj. *halobius*, growing in salt).

The cells of type strain are coccoid (0.5–2.0 µm), motile, and Gram-negative. Colonies are red and circular, about 0.5 mm in diameter following incubation for 14 days at 37°C. The type strain could grow in NHM with NaCl concentrations of 3.1–5.1 M and grew best at 4.8 M NaCl. The optimum magnesium concentration was 0.5 M, even if magnesium was not necessary for growth. The growth temperature range for the type strain was 30–50 °C, with optimum at 37°C. The type strain is neutrophilic with pH optima at 7.5. The type strain can grow anaerobically with nitrate but not with arginine or DMSO. Reduction of nitrate to nitrite was found but gas was not produced. Diverse substrates, *D*.mannose, sucrose, glycerol, *D*.mannitol, *D*.sorbitol, fumarate, *DL*.lactate, *L*.malate, pyruvate, succinate, glycine, *L*.aspartate, *L*.glutamate and *L*.lysine, can be utilized for growth, while *D*.fructose, *D*.galactose, *D*.glucose, lactose, maltose, *D*.ribose, *L*.sorbitol, *D*.xylose, acetate, citrate, *L*.arginine, *L*.alanine or *L*.ornithine do not support growth. Positive for catalase and oxidase activity. Indole and H₂S formation are not detected. None of casein, gelatin, starch or Tween 80 was found to be hydrolyzed. The type strain contains PG, PGP-Me, DGD-1 and S-DGD-1.

The type strain, ZY10^T (= CGMCC 1.17475^T = JCM 34319^T), was isolated from the natural sea salt produced from Huanghua marine solar saltern in Hebei, PRChina. The DNA G+C content of the type strain is 66.8% (genome). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and whole genome of strain ZY10^T are MN589739 and CP101161–CP101165, respectively.

Description of *Haloglomerus halophilum* sp. nov.

Haloglomerus halophilum (ha.lo'phi.lum. Gr. masc. n. *hals*, halos salt; Gr. masc. adj. *philos* loving; N.L. neut. adj. *halophilum* salt-loving, concerning the requirement for salt).

The cells of type strain are coccoid (0.5–2.0 µm), motile, and Gram-negative. Colonies are red and circular, about 0.5 mm in diameter following incubation for 14 days at 37°C. The type strain could grow in NHM with NaCl concentrations of 2.1–4.8 M and grew best at 3.1 M NaCl. The optimum magnesium concentration was 0.7 M, even if magnesium was not necessary for growth. The growth temperature range for the type strain was 25–60 °C, with optimum at 40°C. The type strain is neutrophilic with pH optima at 7.5. The type strain cannot grow anaerobically with arginine, DMSO, or nitrate. Reduction of nitrate to nitrite was found but gas was not produced. Diverse substrates, glycerol, *D*.mannitol, *D*.sorbitol, acetate, *DL*.lactate, pyruvate, succinate, *L*.ornithine and *L*.lysine, can be utilized for growth, while *D*.fructose, *D*.galactose, *D*.glucose, lactose, maltose, *D*.mannose, *D*.ribose, *L*.sorbitol, sucrose, *D*.xylose, citrate, fumarate, *L*.alanine, *L*.arginine, *L*.aspartate, *L*.glutamate, glycine, or *L*.malate do not support growth. Catalase positive and oxidase negative. Indole and H₂S are not produced. None of casein, gelatin, starch or Tween 80 was found to be hydrolyzed. The type strain contains PG, PGP-Me, and S-DGD-1.

The type strain, ZY41^T (= CGMCC 1.17030^T = JCM 34161^T), was isolated from the natural sea salt produced from Huanghua marine solar saltern in Hebei, PRChina. The DNA G+C content of the type

strain is 67.9% (genome). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and whole genome of strain ZY41^T are MK721000 and CP101166–CP101168, respectively.

Description of *Haloglomerus salinum* sp. nov.

Haloglomerus salinum (sa.li'num. N.L. neut. adj. *salinum* belonging to salt works)

The cells of type strain are pleomorphic (0.8–1.0×1.0–2.0 µm), motile, and Gram-negative. Colonies are red and circular, about 0.5 mm in diameter following incubation for 14 days at 37°C. The type strain could grow in NHM with NaCl concentrations of 2.6–4.3 M and grew best at 3.1 M NaCl. The optimum magnesium concentration was 0.03 M, even if magnesium was not necessary for growth. The growth temperature range for the type strain was 25–55 °C, with optimum at 37°C. The type strain is neutrophilic with pH optima at 7.0. The type strain cannot grow anaerobically with arginine, DMSO, or nitrate. Reduction of nitrate to nitrite was not found and gas was not produced. Diverse substrates, *D*.galactose, *D*.glucose, *D*.mannose, sucrose, acetate, pyruvate, *DL*.lactate, can be utilized for growth, while *D*.fructose, lactose, maltose, *D*.ribose, *L*.sorbitol, *D*.xylose, glycerol, *D*.mannitol, *D*.sorbitol, citrate, fumarate, *L*.malate, succinate, *L*.aspartate, *L*.alanine, *L*.arginine, *L*.glutamate, glycine, *L*.lysine, or *L*.ornithine do not support growth. Catalase positive and oxidase negative. Indole and H₂S production are not detected. None of casein, gelatin, starch or Tween 80 was found to be hydrolyzed. The type strain contains PG, PGP-Me, and S-DGD-1.

The type strain, ZY58^T (= CGMCC 1.17216^T = JCM 34163^T), was isolated from the natural sea salt produced from Huanghua marine solar saltern in Hebei, PRChina. The DNA G+C content of the type strain is 68.5% (genome). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and whole genome of strain ZY58^T are MK920153 and CP101153, respectively.

Description of *Natronomonas marina* sp. nov.

Natronomonas marina (ma.ri'na. L. fem. adj. *marina* of the marine).

The cells of type strain are coccoid (0.5–2.0 µm), motile, and Gram-negative. Colonies are red and circular, about 0.5 mm in diameter following incubation for 14 days at 37°C. The type strain could grow in NHM with NaCl concentrations of 2.1–4.8 M and grew best at 3.1 M NaCl. The optimum magnesium concentration was 0.05 M, even if magnesium was not necessary for growth. The growth temperature range for the type strain was 30–60 °C, with optimum at 40°C. The type strain is neutrophilic with pH optima at 6.5. The type strain cannot grow anaerobically with arginine, DMSO, or nitrate. Reduction of nitrate to nitrite was found but gas was not produced. Diverse substrates, glycerol, *D*.sorbitol, acetate, *DL*.lactate, pyruvate, succinate, *L*.aspartate, *L*.alanine, *L*.lysine, and *L*.ornithine can be utilized for growth, while *D*.fructose, *D*.galactose, *D*.glucose, lactose, maltose, *D*.mannose, *D*.ribose, *L*.sorbitol, sucrose, *D*.xylose, *D*.mannitol, acetate, citrate, fumarate, *L*.arginine, *L*.glutamate glycine or *L*.malate do not support growth. The catalase test is positive while the oxidase is

negative. Indole and H₂S production are not detected. None of casein, gelatin, starch or Tween 80 was found to be hydrolyzed. The type strain contains PG, PGP-Me, PGS, DGD-1, and S-TGD-1.

The type strain, ZY43^T (= CGMCC 1.17202^T = JCM 34162^T), was isolated from the natural sea salt produced from Huanghua marine solar saltern in Hebei, PRChina. The DNA G+C content of the type strain is 66.9% (genome). The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene and whole genome of strain ZY43^T are MK920152 and CP101154–CP101155, respectively.

Data availability statement

The sequence data of the study are deposited in the NCBI database with accession numbers MW338920, MN589739, MK721000, MK920153, MK920152, CP101454–CP101455, CP101161–CP101165, CP101166–CP101168, CP101153, and CP101154–CP101155.

Author contributions

Y-PS, B-BW, Z-PW, X-WZ performed the experiments and Y-PS drafted the manuscript. JH and H-LC designed and supervised the study. JH and H-LC revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was financially supported by Science & Technology Fundamental Resources Investigation Program (Grant No.

2019FY100700; No. 2021FY100900), and the National Natural Science Foundation of China (No. 32070003).

Acknowledgments

We are grateful to Prof. Aharon Oren (The Hebrew University of Jerusalem) for his generous and meaningful comments.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1105929/full#supplementary-material>

References

- Amoozegar, M. A., Siroosi, M., Atashgahi, S., Smidt, H., and Ventosa, A. (2017). Systematics of haloarchaea and biotechnological potential of their hydrolytic enzymes. *Microbiology* 163, 623–645. doi: 10.1099/mic.0.000463
- Ashwini, R., Vijayanand, S., and Hemapriya, J. (2017). Photonic potential of haloarchaeal pigment bacteriorhodopsin for future electronics: A review. *Curr. Microbiol.* 74, 996–1002. doi: 10.1007/s00284-017-1271-5
- Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., et al. (2008). The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9, 75. doi: 10.1186/1471-2164-9-75
- Burns, D. G., Janssen, P. H., Itoh, T., Minegishi, H., Usami, R., Kamekura, M., et al. (2010). *Natronomonas moolapensis* sp. nov., non-alkaliphilic isolates recovered from a solar saltern crystallizer pond, and emended description of the genus *Natronomonas*. *Int. J. Syst. Evol. Microbiol.* 60, 1173–1176. doi: 10.1099/ijs.0.010132-0
- Capella-Gutiérrez, S., Silla-Martínez, J. M., and Gabaldón, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25, 1972–1973. doi: 10.1093/bioinformatics/btp348
- Chaumeil, P. A., Mussig, A. J., Hugenholtz, P., and Parks, D. H. (2019). GTDB-tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 36, 1925–1927. doi: 10.1093/bioinformatics/btz848
- Cui, H. L., and Dyal-Smith, M. L. (2021). Cultivation of halophilic archaea (class *Halobacteria*) from thalassohaline and athalassohaline environments. *Mar. Life Sci. Technol.* 3, 243–251. doi: 10.1007/s42995-020-00087-3
- Cui, H. L., Gao, X., Yang, X., and Xu, X. W. (2010). *Halorussus rarus* gen. nov., sp. nov., a new member of the family *Halobacteriaceae* isolated from a marine solar saltern. *Extremophiles* 14, 493–499. doi: 10.1007/s00792-010-0329-0
- Cui, H. L., Zhou, P. J., Oren, A., and Liu, S. J. (2009). Intraspecific polymorphism of 16S rRNA genes in two halophilic archaeal genera, *Haloarcula* and *Halomicrobium*. *Extremophiles* 13, 31–37. doi: 10.1007/s00792-008-0194-2
- de la Haba, R. R., Minegishi, H., Kamekura, M., Shimane, Y., and Ventosa, A. (2021). Phylogenomics of haloarchaea: The controversy of the genera *Natrinema*-*Haloterrigena*. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.740909
- Durán-Viseras, A., Andrei, A.Ş., Vera-Gargallo, B., Ghai, R., Sánchez-Porro, C., and Ventosa, A. (2021). Culturomics-based genomics sheds light on the ecology of the new haloarchaeal genus *Halosegnis*. *Environ. Microbiology* 23, 3418–3434. doi: 10.1111/1462-2920.15082
- Durán-Viseras, A., Sánchez-Porro, C., and Ventosa, A. (2020a). *Haloglomerum irregulare* gen. nov., sp. nov., a new halophilic archaeon isolated from a marine saltern. *Microorganisms* 8, 206. doi: 10.3390/microorganisms8020206
- Durán-Viseras, A., Sánchez-Porro, C., and Ventosa, A. (2020b). *Natronomonas salsuginis* sp. nov., a new inhabitant of a marine solar saltern. *Microorganisms* 8, 605. doi: 10.3390/microorganisms8040605
- Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J. Bacteriol.* 70, 484–485. doi: 10.1128/jb.70.4.484-485.1955
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* 17, 368–376. doi: 10.1007/BF01734359
- Fitch, W. M. (1971). Toward defining the course of evolution: Minimum change for a specific tree topology. *Syst. Biol.* 20, 406–416. doi: 10.2307/2412116
- Goris, J., Konstantinidis, K. T., Klappenbach, J. A., Coenye, T., Vandamme, P., and Tiedje, J. M. (2007). DNA-DNA Hybridization values and their relationship to whole-genome sequence similarities. *Int. J. Syst. Evol. Microbiol.* 57, 81–91. doi: 10.1099/ijs.0.64483-0
- Han, D., and Cui, H. L. (2020). *Halostella pelagica* sp. nov. and *Halostella litorea* sp. nov., isolated from salted brown alga *Laminaria*. *Int. J. Syst. Evol. Microbiol.* 70, 1969–1976. doi: 10.1099/ijsem.0.004003
- Han, J., Hou, J., Liu, H., Cai, S., Feng, B., Zhou, J., et al. (2010). Wide distribution among halophilic archaea of a novel polyhydroxyalkanoate synthase subtype with

- homology to bacterial type III synthases. *Appl. Environ. Microbiol.* 76, 7811–7819. doi: 10.1128/AEM.01117-10
- Hou, J., Zhao, Y. J., Zhu, L., and Cui, H. L. (2018). *Salinirubellus salinus* gen. nov., sp. nov., isolated from a marine solar saltern. *Int. J. Syst. Evol. Microbiol.* 68, 1874–1878. doi: 10.1099/ijsem.0.002757
- Kamekura, M., Dyal-Smith, M. L., Upasani, V., Ventosa, A., and Kates, M. (1997). Diversity of alkaliphilic halobacteria: Proposals for transfer of *Natronobacterium vacuolatum*, *Natronobacterium magadii*, and *Natronobacterium pharaonis* to *Halorubrum*, *Natrialba*, and *Natronomonas* gen. nov., respectively, as *Halorubrum vacuolatum* comb. nov., *Natrialba magadii* comb. nov., and *Natronomonas pharaonis* comb. nov., respectively. *Int. J. Syst. Bacteriol.* 47, 853–857. doi: 10.1099/00207713-47-3-853
- Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., and Hattori, M. (2004). The KEGG resource for deciphering the genome. *Nucleic Acids Res.* 32, D277–D280. doi: 10.1093/nar/gkh063
- Kim, T. Y., Kim, S. J., Park, S. J., Kim, J. G., Cha, I. T., Jung, M. Y., et al. (2013). *Natronomonas gomsonensis* sp. nov., isolated from a solar saltern. *Antonie van Leeuwenhoek* 104, 627–635. doi: 10.1007/s10482-013-9970-9
- Kim, M., Oh, H. S., Park, S. C., and Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int. J. Syst. Evol. Microbiol.* 64, 346–351. doi: 10.1099/ijse.0.059774-0
- Lobasso, S., Pérez-Davó, A., Vitale, R., Sánchez, M. M., and Corcelli, A. (2015). Deciphering archaeal glycolipids of an extremely halophilic archaeon of the genus *Halobellus* by MALDI-TOF/MS. *Chem. Phys. Lipids* 186, 1–8. doi: 10.1016/j.chemphyslip.2014.11.002
- Luo, C., Rodriguez-R, L. M., and Konstantinidis, K. T. (2014). MyTaxa: An advanced taxonomic classifier for genomic and metagenomic sequences. *Nucleic Acids Res.* 42, e73. doi: 10.1093/nar/gku169
- Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., and Göker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinf.* 14, 60. doi: 10.1186/1471-2105-14-60
- Minegishi, H., Kamekura, M., Itoh, T., Echigo, A., Usami, R., and Hashimoto, T. (2010). Further refinement of the phylogeny of the *Halobacteriaceae* based on the full-length RNA polymerase subunit b' (*rpoB'*) gene. *Int. J. Syst. Evol. Microbiol.* 60, 2398–2408. doi: 10.1099/ijse.0.017160-0
- Nguyen, L. T., Schmidt, H. A., von Haeseler, A., and Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32, 268–274. doi: 10.1093/molbev/msu300
- Oren, A., Ventosa, A., and Grant, W. D. (1997). Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int. J. Syst. Evol. Microbiol.* 47, 233–238. doi: 10.1099/00207713-47-1-233
- Parks, D. H., Chuvochina, M., Waite, D. W., Rinke, C., Skarshewski, A., Chaumeil, P. A., et al. (2018). A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat. Biotechnol.* 36, 996–1004. doi: 10.1038/nbt.4229
- Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., and Tyson, G. W. (2015). CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* 25, 1043–1055. doi: 10.1101/gr.186072.114
- Richter, M., and Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19126–19131. doi: 10.1073/pnas.0906412106
- Richter, M., Rosselló-Móra, R., Glöckner, F. O., and Peplies, J. (2016). JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32, 929–931. doi: 10.1093/bioinformatics/btv681
- Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425. doi: 10.1093/oxfordjournals.molbev.a040454
- Serrano, S., Mendoza, S., and Caetano, T. (2022). Haloarchaea have a high genomic diversity for the biosynthesis of carotenoids of biotechnological interest. *Res. Microbiology* 173, 103919. doi: 10.1016/j.resmic.2021.103919
- Song, H. S., Kim, J., Kim, Y. B., Lee, S. H., Whon, T. W., and Roh, S. W. (2022). Sala cibi gen. nov., sp. nov., an extremely halophilic archaeon isolated from solar salt. *J. Microbiol.* 60, 899–904. doi: 10.1007/s12275-022-2137-5
- Sun, Y. P., Wang, B. B., Zheng, X. W., Wu, Z. P., Hou, J., and Cui, H. L. (2022). Description of *Halosolutus amylolyticus* gen. nov., sp. nov., *Halosolutus halophilus* sp. nov. and *Halosolutus gelatinilyticus* sp. nov., and genome-based taxonomy of genera *Natribaculum* and *Halovarius*. *Int. J. Syst. Evol. Microbiol.* 72, 5598. doi: 10.1099/ijsem.0.005598
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. doi: 10.1093/molbev/mst197
- Vaskovsky, V. E., and Kostetsky, E. Y. (1968). Modified spray for the detection of phospholipids on thin-layer chromatograms. *J. Lipid Res.* 9, 396. doi: 10.1016/S0022-2275(20)43111-6
- Wang, B. B., Sun, Y. P., Wu, Z. P., Zheng, X. W., Hou, J., and Cui, H. L. (2022). *Halorientalis salina* sp. nov., *Halorientalis marina* sp. nov., *Halorientalis litorea* sp. nov.: Three extremely halophilic archaea isolated from a salt lake and coarse sea salt. *Extremophiles* 26, 26. doi: 10.1007/s00792-022-01275-y
- Xin, Y. J., Bao, C. X., Li, S. Y., Hu, X. Y., Zhu, L., Wei, W., et al. (2022). Genome-based taxonomy of genera *Halomicrobium* and *Halosiccatus*, and description of *Halomicrobium salinisoli* sp. nov. *Syst. Appl. Microbiol.* 45, 126308. doi: 10.1016/j.syapm.2022.126308
- Xu, L., Dong, Z., Fang, L., Luo, Y., Wei, Z., Guo, H., et al. (2019). OrthoVenn2: A web server for whole-genome comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Res.* 47, W52–W58. doi: 10.1093/nar/gkz333
- Yin, X. M., Yang, X. Y., Hou, J., Zhu, L., and Cui, H. L. (2020). *Natronomonas halophila* sp. nov. and *Natronomonas salina* sp. nov., two novel halophilic archaea. *Int. J. Syst. Evol. Microbiol.* 70, 5686–5692. doi: 10.1099/ijsem.0.004463
- Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., et al. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. *Int. J. Syst. Evol. Microbiol.* 67, 1613–1617. doi: 10.1099/ijsem.0.001755