Halocynthiibacter halioticoli sp. nov., isolated from the abalone Haliotis discus hannai

Dan-Dan Zhang
Shandong University
Yu-Yan Yue
Shandong University
Meng-Di Zhang
Shandong University
Zong-Jun Du (duzongjun@sdu.edu.cn)
Shandong University

Research Article

Keywords: Halocynthiibacter halioticoli, 16S rRNA gene, phylogenetic analysis, genome

Posted Date: November 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2229936/v1

Additional Declarations: No competing interests reported.

Abstract

A Gram-stain-negative, rod-shaped, glide, non-flagellated, and facultatively aerobic bacterial strain, designated as Z654^T, was isolated from the gut of abalone *Haliotis discus hannai* from Rongcheng, Shandong province, China. Cells are 0.2–0.8 μm in width and 0.7–3.4 μm in length. Cells grew best at 30°C (range, 15–37°C), pH 7 (range, 6.0–8.5) and NaCl concentration of 2.0% (w/v) (range, 1–10%). According to the phylogenetic analysis of 16S rRNA gene sequence, the strain belongs to the genus *Halocynthiibacter* and the most close strain is *Halocynthiibacter arcticus* KCTC 42129^T (97.12%). The genome size of strain Z654^T was 3296250 bp and the DNA G + C content was 54.2 mol%. The average nucleotide identity (ANI) scores and digital DNA–DNA hybridization (dDDH) scores with *H. arcticus* KCTC 42129^T was 70% and 14.6–18.2% respectively. The predominant quinone was Q-10 and the major fatty acids were $C_{18:0}$, $C_{18:1}$ *ω7c* 11-methyl and summed future 8. The polar lipids were consisted of phosphatidylcholine, phosphatidylglycerol, unidentified aminolipid and unidentifed lipids. Based on the phenotypic, phylogenetic and chemotaxonomic data, strain Z654^T was considered to represent a novel species of the genus *Halocynthiibacter*, for which the name *Halocynthiibacte halioticoli sp. nov.*, is proposed. The type strain is Z654^T (= MCCC 1H00503^T = KCTC 92003^T).

Introduction

The genus *Halocynthiibacter* belongs to the family *Rhodobacteraceae*. Currently, the genus *Halocynthiibacter* has three species, *Halocynthiibacter* was originally identified by Kim *et al*. (2014) with the type species *Halocynthiibacter namhaensis* KCTC 32362^T, followed by the effective publication of the strain of *Halocynthiibacter arcticus* KCTC 42129^T (Baek *et al*., 2015). In addition, '*Candidatus Halocynthiibacter alkanivorans'*, which has not been effectively published, was studied and its microbial communities were reported to have biodegradation potential (Campeão *et al*., 2019). The taxonomic position of the new strain Z654^T was reported in this study, based on phenotypic, chemotaxonomic and phylogenetic characters strain Z654^T is considered a new species of the genus *Halocynthiibacter* for which *Halocynthiibacter halioticoli sp. nov* is proposed.

Materials And Methods

Isolation and cultivation

The viscera samples of abalone (*Haliotis discus hannai*) from Rongcheng (122°14'E, 36°54'N), Shandong province, China, were taken and dissected with a sterile scalpel to expose the viscera, which were then placed in a glass mortar and obtained by thoroughly grinding. Take 1 g of organisms, serially dilute the sample to 10^{-4} with sterile seawater, and take 100 μl of each gradient dilution and spread it evenly onto marine agar 2216 medium (MA; Becton Dickinson). After incubation at 30°C for 5 d, a beige colony, designated as Z654^T, was obtained and stored at -80°C in 20.0% (v/v) sterile glycerol plus 3.0% (v/v) NaCl. The type strains *H. arcticus* KCTC 42129^T and *H. namhaensis* KCTC 32362^T were purchased from

the Korean Collection for Type Cultures (KCTC). They are the most closely related type strains to strain Z654^T and were used to compare physiological and chemometric characteristics.

16s Rrna Gene Sequence And Phylogenetic Analysis

Universal primers 27F and 1492R were employed for PCR amplification of strain Z654^T (Liu *et al.*, 2014) and the nearly complete 16S rRNA gene fragment was obtained. The PCR products were purified and ligated into a pMD18-T vector (Takara), then cloned according to the manufacturer's instructions and sent to BGI Co. Ltd (Qingdao, China) for screening and sequencing. The almost complete 16S rRNA gene sequence of strain Z654^T was obtained by traditional Sanger sequencing and submitted to the GenBank database. The phylogenetic tree was constructed using the neighbor-joining (NJ) (Saitou *et al.*, 1987), the maximum-likelihood (ML) (Yang, 2007) and maximum-parsimony (MP) using MEGA X software (Molecular Evolutionary Genetics Analysis) (Kumer *et al.*, 2011).

Genome Sequence Analysis

The genomic DNA of strain Z654^T was extracted according to the instructions of the genomic DNA extraction kit (Takara). The genomic draft sequence of strain Z654^T was sequenced by Beijing Bioinformatics Co., Ltd. (Beijing, PR China) using the Illumina PE150 platform. The G + C content of the DNA of strain Z654^T was obtained by submitting the sequence to the GenBank database. The NCBI Prokaryotic Genome Annotation Pipeline (PAGP) was used to obtain annotated genome content and the genes included in the metabolic pathways were analyzed by KEGG Database. The average nucleotide identity (ANI) was calculated by the OrthoANI algorithm (Yoon *et al.*, 2017) between strain Z654^T and *H. arcticus* KCTC 42129^T. Digital DNA-DNA hybridization (dDDH) values were calculated by Genome to Genome Distance Calculator (GGDC) (http://ggdc.dsmz.de/ggdc.php/). The presence of gene clusters expressing secondary metabolites was predicted in strain Z654^T using the antiSMASH 6.0 database. Carbohydrate-active enzymes of strain Z654^T were annotated using the dbCAN2 meta server (Zhang *et al.*, 2018).

Phenotypic Characterization

Strain Z654^T was placed on MA and incubated at 30°C for 3 days for morphological and physiological characteristics detection. Gram staining was performed according to the method described previously (Smibert *et al.*, 1994). The surface morphology of the colonies was observed using a light microscope (E600; Nikon) and a scanning electron microscope (FEI Nanonova SEM450). The gliding motion of the bacteria was detected as described by Bowman (Bowman, 2000). Petri dishes were placed at different temperatures (0, 4, 15, 20, 25, 28, 30, 33, 35, 37, 40 and 45°C) and colonies were observed every 12 hours for more than 1 week to obtain the temperature range at which the strains could grow. The effect of NaCl concentration on growth of strain Z654^T was examined on MA, which was prepared with artificial

seawater (0.23% $MgCl_2$, 0.32% $MgSO_4$, 0.12% $CaCl_2$, 0.07% KCl, and 0.02% $NaHCO_3$, all w/v) and supplemented with different concentrations of NaCl (0%, 0.5%, and 1–10% in 0.5% increments, w/v) at 30°C. Colony development was monitored every 12 hours. The pH range of growth was tested at pH 5.5–9.5 (interval 0.5 pH unit), and a series of buffers were added to marine broth 2216 (MB, Becton Dickinson) for adjustment. After two weeks of incubation in an anaerobic tank containing 0.1% (w/v) KNO_3 or not, the growth status was determined under anaerobic conditions. The nitrate reduction experiment was done as previously described (Cowan *et al*., 1974). The generation of bubbles in 3% (v/v) hydrogen peroxide aqueous solution was measured to detect catalase activity. According to manufacturer instructions, a bioMérieux oxidase kit was used to determine oxidase activity. Based on the previous methods (Dong *et al*., 2001), the hydrolysis of starch, cellulose, agar, alginate, casein and Tweens (20, 40, 60 and 80) were detected. DNase agar (HopeBio) was used to detect DNA enzyme activity. Susceptibility to antibiotics was investigated on MA at 30°C for 7 days using filter-paper discs containing various antibiotics as described previously (Du *et al*., 2014) and according to procedures outlined by the Clinical and Laboratory Standards Institute (CLSI 2018). Other physiological and biochemical properties were assessed using API 20NE, API ZYM and API 50CHB strips (bioMérieux, Marcy-l'É toile, France) and Biolog GEN III microplates according to the manufacturer's recommendations.

Chemotaxonomic Analysis

The cells of Strain Z654^T were harvested at exponential period and freeze-dried. It was used for the extraction of fatty acids, polar lipids, and respiratory quinones at a later stage (Kuykendall *et al*., 1988). The sherlock microbial identification system (MIDI) was used to determine cellular fatty acid composition. According to the description (Tindall, 1990), polar lipids were extracted, experiments were performed using two-dimensional thin-layer chromatography (TLC), and appropriate detection reagents were sprayed for dyeing (Kamekura *et al*., 1993). The respiratory quinone was extracted according to the researchers (Collins, 1994), and analyzed by high-performance liquid chromatography (HPLC) (Kroppenstedt, 1982).

Results And Discussion

16S rRNA gene sequence and phylogenetic analysis

The almost complete 16S rRNA gene sequence of Z654^T (1488bp) was obtained by PCR and cloning. Phylogenetic analysis of 16S rRNA gene sequences showed that the 16S rRNA gene sequence of strain Z654^T was closed with *H. arcticus* KCTC 42129^T (97.1%), and *H. namhaensis* KCTC 32362^T (96.3%) (Kim *et al*., 2014). The phylogenetic trees constructed with different tree generation algorithms (Fig. 1) showed that strain Z654^T was located in a phylogenetic tree composed of *H. arcticus* KCTC 42129^T, and *H. namhaensis* KCTC 32362^T. Strain Z654^T was placed on a node that was more distantly related to the other strains. Similar tree topologies were also shown in the 16S rRNA genes based phylogenetic trees

with the maximum-likelihood and maximum-parsimony algorithms. (Fig. S1 and Fig. S2, available with the online Supplementary Information). The above results support that strain Z654^T was a unique new species of the genus *Halocynthiibacter.*

Draft Genome Sequence Analysis

The draft genome of strain Z654^T was 3296250 bp and the N50 length was 1154162 bp. The longest scaffold was 1576913 bp. An average genome depth of 467× was achieved. The output report of the genomic information annotated by PGAP showed that there were 3227 genes, 3168 protein-coding genes, 39 tRNA, 3 rRNA, 3 ncRNAs, and 1 full sequence of 16S rRNA genes in the inclusions. The ANI and dDDH values for strain Z654^T and its closest relative species *H. arcticus* KCTC 42129^T were 70% and 14.6–18.2%, respectively. 95–96% of the ANI and 70% of the dDDH values are generally considered as species boundaries (Chun *et al*., 2018). After these calculations, strain Z654^T can be classified as a new species within the genus *Halocynthiibacter*.

Due to the emission of large amounts of organic or inorganic pollutants from human activities [Gao *et al*., 2017; Luo *et al*., 2010], mollusks such as abalone living in sediments may accumulate many pollutants from long-term exposure to fairly high levels of chemical stress (Xing *et al*., 1997). In particular, cadmium, arsenic, lead and mercury are heavy metals with known biological toxicity (Jiang *et al*., 2015). We identified certain key genes: *ACR3* and *ArsH* associated with arsenic resistance in strain Z654^T. The cluster of gene *ACR3* previously was shown to confer arsenical resistance in *Saccharomyces cerevisiae*. The overexpression of *ACR3* induced high level arsenite resistance (Wysocki *et al*., 1997). ArsH contributes to the mitigation of toxicity in As by mediating the reduction of ROS generated in vivo upon exposure to oxygen anions, for example by generating FMNH2 to facilitate ROS quenching activity (Páez-Espino *et al*., 2020).

Four probable biosynthetic gene clusters were discovered in the genome of strain Z654^T based on secondary metabolite analyses predicted by antiSMASH. These clusters include one RiPP-like cluster, one hserlactone cluster, one ectoine and one terpene cluster region. These synthesized secondary metabolites are considered a very promising source of novel pharmaceutical compounds (Chiriac *et al*., 2018).

According to reports, seaweed-associated species including marine invertebrates and bacteria are the primary hosts of enzymes that could catabolize seaweed polysaccharides. The most significant manufacturers of these enzymes are phytophagous marine gastropods, which include sea hares and abalone (Ojima *et al*., 2018). Different polysaccharide-degrading enzymes have so far been identified from these animals' hepatopancreas and digestive fluids, such as alginate lyase, 1,3-glucanase, mannanase, and cellulase (Ojima *et al*., 2018). Subsequently, we analyzed the carbohydrate activity of strain Z654^T. Strain Z654^T contained 108 carbohydrate-active enzymes (Fig. S3). Among these carbohydrate-active enzymes, glycosyltransferase (GTs) were the most numerous enzymes, with 65 in total. This is followed by the glycoside hydrolases (GHs) family, with 30 in total, which proves that the

strain has a strong ability for polysaccharide degradation. Besides, there were two polysaccharide lyases (PLs) in strain Z654^T, which were not annotated in *H. arcticus* KCTC 42129^T and *H. namhaensis* KCTC 32362^T. Considering that the abalone *Haliotis discus hannai* feeds mainly on brown algae, therefore its gut microorganisms may be involved in polysaccharide degradation processes (Nam *et al*., 2018). The strong polysaccharide degradation ability of this strain helps abalone to extract energy from brown algae polysaccharides and survive better.

Phenotypic Characterization

Cells of strains Z654^T were Gram-stain-negative, rod-shaped and facultatively aerobic. Colonies grown on MA were round, convex, flaxen with smooth, moist edges, besides the SEM (Fig. S4) showed Z654^T cells were approximately 0.2–0.8 μm in width and 0.7–3.4 μm in length. The cells were gliding on the semisolid agar. Optimal growth occurred at 30°C (range 15–37°C) and pH 7.0 (range 6.0–8.5) with 2% (w/v) NaCl (range 1.0–10.0%). No growth was observed under anaerobic conditions. The cells were positive for nitrate reduction and negative for catalase and oxidase activity. The hydrolysis of Tween 40 and starch was detected, but casein, cellulose, alginate and Tweens 20, 60, 80 were not hydrolyzed. Despite strain Z654^T showed many common traits with *H. arcticus* KCTC 42129^T and *H. namhaensis* KCTC 32362^T including the positive reaction of esterase (C4), naphthol-AS-BI-phosphohydrolase, negative for trypsin, *α*-galactosidase, *β*-glucuronidase, *α*-mannosidase, gelatinase, glucose, mannitol. However, there were some differences between the strains, such as that Z654^T showed positive for the esterase lipase (C8) and lipase (C14), while *H. arcticus* KCTC 42129^T and *H. namhaensis* KCTC 32362^T were negative for those. Further detailed characteristics determined by the API ZYM, API 20NE, API 50CHB identification systems (BioMérieux) and Biolog GEN III MicroPlates of strains are given in Table S1. Strain Z654^T showed resistance to neomycin (30 μg), streptomycin (10 μg), gentamycin (10 μg), carbenicillin (100 μg), tetracycline (30 μg), kanamycin (30 μg), polymyxin B, ampicillin (10 μg), tobramycin (10 μg), penicillin (30 μg) and norfloxacin (10 μg).

Chemotaxonomic Analysis

The only isoprenoid quinone detected in strain Z654^T is ubiquitin-10 (Q-10), which is the same as the description of all members of the genus *Halocynthiibacter*. The major cellular fatty acid of strain Z654^T were $C_{18:0}$ (17.0%), $C_{18:1}$ *ω*7c 11-methyl (13.3%) and summed future 8 ($C_{18:1}$ *ω*7c and/or C18:1 *ω*7c) (58.0%) (Table 1). The summed future 8 was also the main fatty acid for the other two reference strains. The main polar lipids of strain Z654^T were found to include phosphatidylcholine (PC), phosphatidylglycerol (PG), two unidentified aminolipid (AL1, AL2) and five unidentified lipids (L1, L2, L3, L4, L5). PC and PG were also present in the type strains *H. arcticus* KCTC 42129^T and *H. namhaensis* KCTC 32362^T, and these strains both contained AL. Three phospholipids (PL1, PL2, PL3) and

aminophospholipid (APL) were identified in *H. namhaensis* KCTC 32362^T. Z654^T contained a higher amount of unidentified lipids. The further detailed polar lipids images were shown in Fig. S2.

Table 1

Fatty acid compositions of strain Z654^T and related strains. Strains: 1, Z654^T; 2, *H. arcticus* KCTC 42129^T; 3, *H. namhaensis* KCTC 32362^T.

Fatty acid	**1**	**2**	**3**
Straight-chain:			
$C_{16:0}$	4.4	4.3	2.1
$C_{17:0}$	1.2	1.3	0.3
$C_{18:0}$	**17.0**	9.1	8.0
Unsaturated:			
$C_{18:1}$ $\omega 9c$	-	2.3	0.6
$C_{18:1}$ $\omega 7c$ 11-methyl	**13.3**	2.1	-
Branched:			
anteiso-$C_{17:0}$	-	1.3	-
Hydroxy:			
$C_{10:0}$ 3-OH	1.7	2.6	7.2
$C_{18:0}$ 3-OH	0.2	1.2	**15.9**
Unidentified			
Sum In Feature 3	1.6	0.8	0.5
Summed Feature 8	**58.0**	**73.5**	**63.1**

*Summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete equivalent chain-lengths (ECLs) as well as those where the ECLs are not reported separately. Summed feature 3 was listed as $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; Summed feature 8 was listed as $C_{18:1}$ $\omega 7c$ and $C_{18:1}$ $\omega 6c$.

Table 2

Diferential characteristics between strain Z654^T and the reference strains. Strains: 1, Z654^T; 2, *H. arcticus* KCTC 42129^T; 3, *H. namhaensis* KCTC 32362^T. All datas are from this study. +, Positive; –, negative. NA, no data.

Characteristic	**1**	**2**	**3**
Temperature (°C)	30	25[a]	21[b]
Nitrate reduction	+	-[a]	-[b]
Catalase activity	-	+[a]	+[b]
oxidase activity	-	+[a]	+[b]
DNA G + C content (mol%)	54.2	53.2[a]	52.9[b]
Enzyme activities(API ZYM and 20NE)			
alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, a-chymotrypsin, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, cystine arylamidase, valine arylamidase, β-fucosidase, o-nitrophenyl-β-d-galactopyranoside, Voges–Proskauer reaction	+	-	-
leucine arylamidase	+	+	-
acid phosphatase	+	-	+
Hydrolysis of			
Tween 60	-	+[a]	NA
Tween 80	-	+[a]	-[b]
starch	+	-[a]	-[b]
gliding motion	+	-[a]	NA
Data from: [a]Kim et al (2015); [b]Baek et al (2014).			

According to all these results of phenotypic, biochemical and physiological analyses, together with the phylogenetic differences, strain Z654^T can be assigned to the genus *Halocynthiibacter* within the family *Rhodobacteraceae*, as representing a novel species, for which the name *Halocynthiibacte halioticoli* sp. nov. is proposed.

Description of Halocynthiibacte halioticoli sp. nov.

Halocynthiibacte halioticoli (ha.li.o.ti.co.li. N.L. fem. n. Haliotis, the genus name for abalone, a rock-climbing gastropod mollusk; L. neut. n. colon, colum, great gut; N.L. gen. neut. n. halioticoli, of the gut of Haliotis).

Cells are Gram-stain-negative, rod-shaped and facultatively aerobic. Colonies grown on MA were round, convex, and flaxen with smooth, moist edges. Cells are 0.2–0.8 μm in width and 0.7–3.4 μm in length. The cells can grow in 15–37°C (optimium at 30°C), pH 6.0–8.5 (optimium at pH 7.0), and NaCl concentration of 1–10% (w/v)(optimium at 2%). Starch and Tween 40 are hydrolyzed, but agar, DNA, CM-cellulose, alginate and Tweens 20, 60 and 80 are not. Cells are positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, *a*-chymotrypsin, acid phosphatase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, N-acetyl-*β*-glucosaminidase, *β*-fucosidase, Voges–Proskauer reaction. Acids are produced from d-arabinose, l-arabinose, d-xylose, potassium 5-keto-gluconate. The predominant quinone was Q-10 and the major fatty acids were $C_{18:0}$, $C_{18:1}$ ω7c 11-methyl and summed future 8. The polar lipids were consisted of PC, PG, three unidentified aminolipids and five unidentified lipids. The DNA G + C content of strain Z654^T is 54.2 mol%.

The type strain, Z654^T (= MCCC 1H00503^T = KCTC 92003^T), was obtained from the entrail of abalone in Rongcheng, Shandong province, China. The Whole Genome Shotgun project has been deposited at GenBank under the accession JAOYFC000000000. The accession number of the 16S rRNA gene sequence is OP218735.

Abbreviations

dDDH: digital DNA-DNA hybridization, GGDC: genome-to-genome distance calculator, ANI: average nucleotide identity, APL: aminophospholipid, PC:phosphatidylcholine, PG: phosphatidylglycerol,

Declarations

Five supplementary figures and one supplementary table are available with the online version of this article.

†These authors contributed equally to this work and should be considered co-first authors

Acknowledgements

The implementation of scanning electron microscope was supported by the Physical-Chemical Materials Analytical and Testing Center of Shandong University at Weihai.

Funding information

This work was supported by Science & Technology Fundamental Resources Investigation Program (Grant No. 2022FY101100, 2019FY100700) and the National Natural Science Foundation of China (32070002).

Conflicts of interests and ethical statements

The authors declare that they have conflict of interest.

This article does not contain any studies with animals performed by any of the authors.

Informed consent was obtained from all individual participants included in the study.

References

1. Baek K, Lee YM, Shin SC, Hwang K, Hwang CY, Hong SG, Lee HK (2015) Halocynthiibacter arcticus sp. nov., isolated from Arctic marine sediment. Int J Syst Evol Microbiol 65(11):3861-3865. https://doi.org/10.1099/ijsem.0.000507
2. Bowman, J.P. (2000) Description of Cellulophaga algicola sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of Cytophaga uliginosa (ZoBell and Upham 1944) Reichenbach 1989 as Cellulophaga uliginosa comb. nov. Int. J. Syst. Evol. Microbiol. 50, 1861–1868. https://doi.org/10.1099/00207713-50-5-1861
3. Campeão ME, Swings J, Silva BS, Otsuki K, Thompson FL, Thompson CC (2019) "Candidatus Colwellia aromaticivorans" sp. nov., "Candidatus Halocyntiibacter alkanivorans" sp. nov., and "Candidatus Ulvibacter alkanivorans" sp. nov. Genome Sequences. Microbiol Resour Announc 8(15):e00086-19. https://doi.org/10.1128/MRA.00086-19
4. Chiriac C, Baricz A, Coman C (2018) Draft Genome Sequence of Janthinobacterium sp. Strain ROICE36, a Putative Secondary Metabolite-Synthesizing Bacterium Isolated from Antarctic Snow. Genome Announc 6(15):e01553-17. https://doi.org/10.1128/genomeA.01553-17
5. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68(1):461-466. https://doi.org/10.1099/ijsem.0.002516
6. Collins MD (1994) Isoprenoid quinones. In: Goodfellow M, O'Donnell AG (eds) Chemical methods in prokaryotic systematics. Wiley, Chichester, pp 345–401.
7. Cowan ST, Steel KJ (1974) Bacterial characters and characterization, 2nd edn. Cambridge University Press, Cambridge.
8. Dong XZ, Cai MY. Determination of biochemical characteristics. Manual for the Systematic Identification of General Bacteria, 398. Beijing: Science Press, 2001. p. 370.
9. Du ZJ, Wang Y, Dunlap C, Rooney AP, Chen G- J (2014) Draconibacterium orientale gen. nov., sp. nov., isolated from two distinct marine environments, and proposal of Draconibacteriaceae fam. nov. Int J Syst Evol Microbiol 64:1690–1696. https://doi.org/10.1099/ijs.0.056812-0
10. Gao X, Zhou F, Chen CTA (2014) Pollution status of the Bohai Sea: an overview of the environmental quality assessment related trace metals. Environ Int 62:12–30. https://doi.org/10.1016/j.envint.2013.09.019
11. Jiang H, Tang S, Qin D, Chen Z, Wang J, Bai S, Mou Z (2015) Heavy metals in sea cucumber juveniles from coastal areas of Bohai and Yellow seas, north China. Bull Environ Contam Toxicol 94(5):577-82. https://doi.org/10.1007/s00128-014-1432-1

12. Kamekura M (1993) Lipids of extreme halophiles. In: Vreeland RH, Hochstein LI (eds) The Biology of Halophilic Bacteria. CRC Press, Boca Raton, pp 135–161.
13. Kim M, Oh HS, Park SC, 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(Pt 2):346-351. https://doi.org/10.1099/ijs.0.059774-0
14. Kim YO, Park S, Kim H, Park DS, Nam BH, Kim DG, Yoon JH (2014) Halocynthiibacter namhaensis gen. nov., sp. nov., a novel alphaproteobacterium isolated from sea squirt Halocynthia roretzi. Antonie Van Leeuwenhoek. 105(5):881-9. https://doi.org/10.1007/s10482-014-0142-3
15. Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. J Liq Chrom 5:2359–2367. https://doi.org/10.1080/01483918208067640
16. Kumar S, Stecher G, Li M, Knyaz C, and Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *MOL BIOL EVOL* 35:1547-1549. https://doi.org/10.1093/molbev/msy096
17. Kuykendall LD, Roy MA, Oneill JJ, Devine TE (1988) Fattyacids, antibiotic-resistance, and deoxyribonucleic-acid homology groups of Bradyrhizobium-japonicum. Int J Syst Bacteriol 38:358–361. https://doi.org/10.1099/00207713-38-4-358
18. Liu QQ, Wang Y, Li J, Du ZJ, Chen GJ (2014) Saccharicrinis carchari sp. nov., isolated from a shark, and emended descriptions of the genus Saccharicrinis and Saccharicrinis fermentans. Int J Syst Evol Microbiol 64:2204–2209. https://doi.org/10.1099/ijs.0.061986-0
19. Luo W, Lu Y, Wang T, Hu W, Jiao W, Naile JE, Giesy JP (2010) Ecological risk assessment of arsenic and metals in sediments of coastal areas of northern Bohai and Yellow Seas, China. Ambio 39(5–6):367–375. https://doi.org/10.1007/s13280-010-0077-5
20. Nam BH, Jang J, Caetano-Anolles K, Kim YO, Park JY, Sohn H, Yoon SH, Kim H, Kwak W (2018) Microbial community and functions associated with digestion of algal polysaccharides in the visceral tract of Haliotis discus hannai: Insights from metagenome and metatranscriptome analysis. PLoS One 13(10):e0205594. https://doi.org/10.1371/journal.pone.0205594
21. Ojima T, Rahman MM, Kumagai Y, Nishiyama R, Narsico J, Inoue A (2018) Polysaccharide-Degrading Enzymes From Marine Gastropods. Methods Enzymol 605:457-497. https://doi.org/10.1016/bs.mie.2018.01.032
22. Páez-Espino AD, Nikel PI, Chavarría M, de Lorenzo V (2020) ArsH protects Pseudomonas putida from oxidative damage caused by exposure to arsenic. Environ Microbiol 22(6):2230-2242. https://doi.org/10.1111/1462-2920.14991
23. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406-25. https://doi.org/10.1093/oxfordjournals.molbev.a040454
24. Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Methods for general and molecular bacteriology. American Society For Microbiology 611–651.

25. Tindall BJ (1990) Lipid composition of Halobacterium lacusprofundi. FEMS Microbiol Lett 66:199–202.
26. Wysocki R, Bobrowicz P, Ułaszewski S (1997) The Saccharomyces cerevisiae ACR3 gene encodes a putative membrane protein involved in arsenite transport. J Biol Chem 28,272(48):30061-6. https://doi.org/10.1074/jbc.272.48.30061
27. Xing J, Chia FS (1997) Heavy metal accumulation in tissue/organs of a sea cucumber, Holothuria leucospilota. In Asia-Pacific Conference on Science and Management of Coastal Environment: 17–23.
28. Yang Z (2007) PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol 24(8):1586-91. https://doi.org/10.1093/molbev/msm088
29. 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(10):1281-1286. https://doi.org/10.1007/s10482-017-0844-4
30. Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y (2018) dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res 46(W1):W95-W101. https://doi.org/10.1093/nar/gky418

Figures

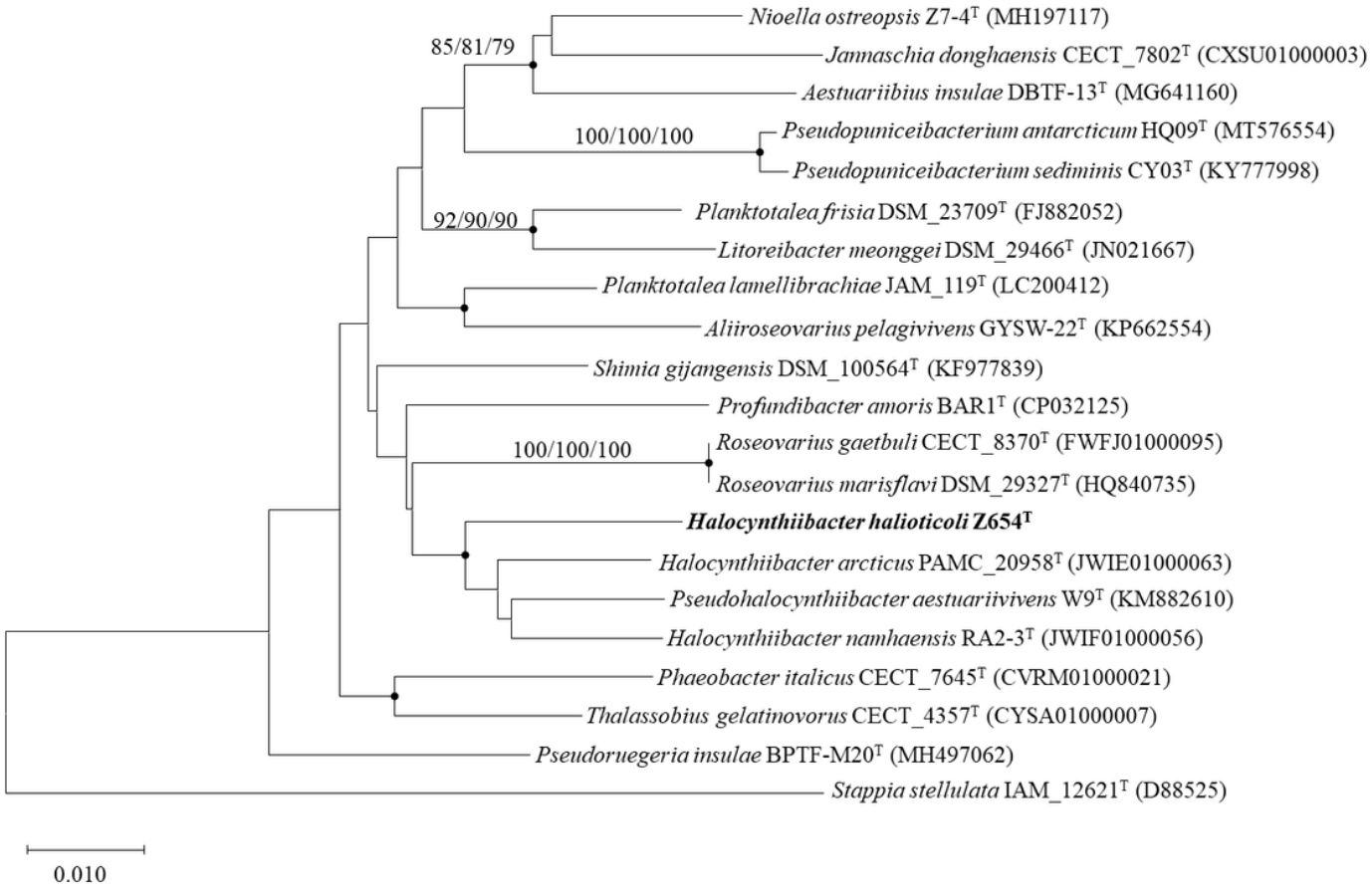

Figure 1

The Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strains Z654^T, and other closely related species is shown. Filled circles indicate branches that were also recovered in maximum-parsimony and with the maximum-likelihood algorithm. Percentage bootstrap values expressed as percentages of 1000 replications above 70% are shown at branch nodes. *Stappia stellulata* IAM_12621^T was used as the out group. Bar, 0.01 substitutions per nucleotide position.

Supplementary Files

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

- Supplementarymaterial.docx