Halorussus halobius sp. nov., *Halorussus marinus* sp. nov. and *Halorussus pelagicus* sp. nov., isolated from salted brown alga *Laminaria*

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Research Article

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Abstract

Four halophilic archaeal strains, designated HD8-83^T, LYG-36^T, DLLS-82 and RC-68^T, were isolated from the salted brown alga *Laminaria* of three different origins (Dalian, Lianyungang, Dalian and Rongcheng) in China. All strains had pleomorphic rod cells that were motile, lysed in distilled water, stained Gram-negative and formed red-pigmented colonies on agar plate, except DLLS-82 showing white colonies. Based on phylogenetic analyses of the 16S rRNA genes, strain HD8-83^T was closely related to *Halorussus litoreus* HD8-51^T (97.9 % similarity), strain LYG-36^T and DLLS-82 were closely related to *Halorussus rarus* TBN4^T (94.4 % and 94.7 % similarities, respectively), and strain RC-68^T exhibit close to *Halorussus salinus* YJ-37-H^T (96.9 % similarity). Phylogenetic analyses based on *rpoB'* gene and 728 concatenated single-copy orthologous clusters also show these strains formed three different branches and clustered tightly with the *Halorussus* members. The ANI, AAI and *is*DDH values between strain LYG-36^T and DLLS-82 were 98.9 %, 98 % and 92.4 %, showing they were different strains of same species. While those values between isolates and other *Halorussus* members with values below 84.7 %, 82.9 % and 28.9 %. Based on the phenotypic, chemotaxonomic and phylogenetic properties, the strains HD8-83^T, LYG-36^T, DLLS-82 and RC-68^T represent three novel species of the genus *Halorussus* for which the names *Halorussus halobius* sp. nov. (type strain HD8-83^T = CGMCC 1.15334^T = JCM 31110^T), *Halorussus marinus* sp. nov. (type strain LYG-36^T = CGMCC 1.13606^T = JCM 32952^T, reference strain DLLS-82 = CGMCC 1.13604 = JCM 32951) and *Halorussus pelagicus* sp. nov. (type strain RC-68^T = CGMCC 1.13609^T = JCM 32953^T) are proposed.

Introduction

In China, the brown alga *Laminaria* is the most important artificial mariculture seaweed and most of these were used for making food (www.seaweed.ie). For annual kelp production was huge, salting, one of the oldest methods for food preservation, was mainly selected for processing and preserving *Laminaria*, which can extend shelf life by preventing spoilage and growth of microorganisms (Taormina 2010). Meanwhile high concentration of sodium chloride becomes a nice habitat for halophilic archaea and bacteria, once they were taken from raw kelp or processing salt, then grow and multiply. According to our previous results, salted *Laminaria* inhabited by a dense population of haloarchaea (Wei et al. 2021). Members of haloarchaea require high salt concentrations to grow, most species like more than 2.0 M NaCl (Yadav et al. 2015), heterotrophic aerobically or anaerobically.

In the previous focusing on the halophilic archaeal diversity in salted kelp produced in different coastal regions of China, four halophilic archaeal strains were isolated and found to be most closely related to the members of *Halorussus* according 16S rRNA gene analysis. The genus *Halorussus* (*Hrs.*) was established based on two strains of *Hrs. rarus* (Cui et al. 2010), and now it comprises six validly published species, which were isolated from Taibei, Yangjiang and Zhoushan marine solar saltern (*Hrs. rarus*, *Hrs. salinus* and *Hrs. halophilus*), Yuncheng salt lake (*Hrs. amylolyticus* and *Hrs. ruber*), salted *Laminaria* (*Hrs. litoreus*) in China (Cui et al. 2010; Ding et al. 2020; Han et al. 2019; Xu et al. 2016; Yuan et al. 2015a; Yuan et al. 2015b). In this study, the isolated four halophilic archaeal strains were

characterized by describing phenotypic and phylogenetic properties then assigned into three novel species, *Halorussus halobius* sp. nov., *Halorussus marinus* sp. nov. and *Halorussus pelagicus* sp. nov.

Materials And Methods

Strains isolation and cultivation

The novel halophilic archaeal strains were isolated from brine samples of salted *Laminaria* produced in different coastal regions of China. Strain HD8-83^T and DLLS-82 were isolated from salted *Laminaria* produced in Daliang, Liaoning Province (pH 6.9 and salinity 293 g l^{-1}), strain LYG-36^T from that produced in Lianyungang, Jiangsu Province, China (pH 6.3 and salinity 208 g l^{-1}) and strain RC-68^T from that produced in Rongcheng, Shandong Province, China (pH 7.5 and salinity 285 g l^{-1}). The serially diluted the brine was inoculated onto the neutral haloarchaeal medium (NHM) which contained (l^{-1}): yeast extract (Angel Yeast Co., Ltd.) 0.05 g, fish peptone (Sinopharm Chemical Reagent Co., Ltd.) 0.25 g, sodium pyruvate 1.0 g, KCl 5.4 g, K_2HPO_4 0.3 g, $CaCl_2$ 0.29 g, NH_4Cl 0.27 g, $MgSO_4 \cdot 7H_2O$ 26.8 g, $MgCl_2 \cdot 6H_2O$ 23.0 g, NaCl 184.0 g, agar 15.0 g, pH 7.0 (Han and Cui 2020) . The agar plates were incubated at 37 °C for one month under aerobic condition. The single colonies were transferred and were successively re-streaked on NHM agar plates at least three times to obtain pure colonies. The isolated strains were preserved at −20 °C as a suspension in NHM broth supplemented with glycerol (150 g l^{-1}). The isolated strains together with reference strains were routinely grown aerobically at 37 °C in NHM medium.

Whole-genome sequencing and phylogenetic analysis

The fresh haloarchaeal cultures grown in NHM liquid medium at 37 °C were used for genomic DNA extraction and purification using a genomic DNA extraction kit (CW0552, Beijing ComWin Biotech Co., Ltd.). The draft genomes of strain HD8-83^T, LYG-36^T, DLLS-82 were sequenced using an Illumina HiSeq PE150 platform (Novogene Co., Ltd, Beijing). The library construction included A-tailed, ligated to paired-end adaptors and PCR amplified with a 350 bp insert. The reads were *de novo* assembled using SOAP *de novo* software (Luo et al. 2012) . The complete genome of strain RC-68^T was sequenced using a PacBio RS II platform and Illumina HiSeq 4000 platform at the Beijing Genomics Institute (BGI, Wuhan, China). The reads were assembled using the Celera Assembler, then improved the accuracy of the genome sequences with the GATK (https://www.broadinstitute.org/gatk/) and SOAP tool packages (SOAP2, SOAPsnp, SOAPindel). The quality of final contigs was assessed by the bioinformatics tool CheckM (Parks et al. 2015) .

The 16S rRNA gene sequences obtained from the genome of new isolates using the RNAmmer 1.2 server (Lagesen et al. 2007) were analyzed for phylogeny. The 16S rRNA gene sequences were assessed by PCR amplifing (primer 20F 5'-ATTCCGGTTGATCCTGCCGG-3' and 1452R 5'-AGGAGGTGATCCAGCCGCAG-3'), cloning and sanger sequencing as described previously (Cui et al. 2009) . The *rpoB′* (RNA polymerase subunit B’) gene was amplified using the primer pair HrpoB2 1420F and HrpoA 153R (Minegishi et al. 2010) and the PCR product was sequenced using the following primers: HrpoB2 1420F, HrpoA 153R and B1-628F (5′-CCNGCNGSVCAGAACTTC-3′). The sequences were aligned using the ClustalW program integrated in the MEGA 6 software (Tamura et al. 2013) and the phylogenetic trees were reconstructed using maximum-likelihood (ML) (Felsenstein 1981) , maximum-parsimony (MP) (Fitch 1971) and neighbour-joining (NJ) (Saitou and Nei 1987) algorithms in the MEGA 6 software. Sequence similarity was analyzed by comparing the 16S rRNA gene sequence of strains with those available from the EzBioCloud tool (Yoon et al. 2016) . In multilocus sequence analysis (MLSA), OrthoMCL version 2.0 (Li et al. 2003) and in-house Perl scripts were used to detect single-copy shared orthologous clusters (OCs). The maximum-likelihood

phylogenetic tree was performed with 728 concatenated single-copy OCs aligned by MUSCLE (Edgar 2004) and refined by trimAL version 1.4.1 (Capella-Gutiérrez et al. 2009) , using IQ-TREE 1.6.1 software (Nguyen et al. 2015) . The bootstraps analysis set to 1000 replicates, with the best amino acid substitutional model set as LG + F + R4 as proposed by IQ-TREE 1.6.1 software. *In silico* DNA-DNA hybridization (DDH), average nucleotide identity (ANI), and average amino acid identity (AAI) values of the isolates and related type strains were calculated using Genome-to-Genome Distance Calculator 2.1 (GGDC), ChunLab's online Average Nucleotide Identity calculator and AAI calculator , respectively (Lee et al. 2015; Luo et al. 2014; Meier-kolthoff et al. 2013) .

Genomic analyses

The genomes were annotated through RAST server (Overbeek et al. 2014) . The OrthoVenn2 online tool (Xu et al. 2019) was used to compare the OCs among the novel strains and their closely related species.

Morphological, physiological and chemotaxonomic characterization

The cellular morphological features were observed by Nikon microscopy (Ci-L) equipped with phase-contrast optics (model: E400) using cells grown in NHM broth at 37 °C for exponential and stationary phases of growth. The minimum salt concentration preventing cell lysis was determined by suspending washed cells in serial solutions containing 0−2.6 M NaCl examined by light microscopic examination. The range of NaCl concentrations for growth was determined with modified NHM medium containing NaCl of 0.9, 1.4, 1.7, 2.1, 2.6, 3.1, 3.4, 3.9, 4.3, 4.8 and 5.1 M. The range of $MgCl_2$ concentrations

for growth was studied in modified NHM medium with $MgCl_2$ of 0, 0.005, 0.01, 0.03, 0.05, 0.1, 0.3, 0.5, 0.7 and 1.0 M. The temperature range for growth was examined at 10, 15, 20, 25, 30, 37, 40, 42, 45, 50, 55 and 60 °C. The pH range for growth was determined in modified NHM medium at pH 5.0−10.0 (with an interval of 0.5 pH units), using the following buffers: CPBS (pH 5.0–6.0), PBS (pH 6.0–7.5), Tris-HCl (pH 7.5–9.0) at a concentration of 100 and 50 mM CHES-NaOH (pH 9.0–10.0). Other phenotypic tests were performed according to the proposed minimal standards for description of new taxa in the order *Halobacteriales* (Oren et al. 1997) .

Halophilic archaeal polar lipids were extracted using a chloroform-methanol system and analyzed by one- and two-dimensional TLC according to Cui et al (Cui et al. 2010) . The general detection reagent, sulfuric acid–ethanol (1:2, by vol.), was used to detect total polar lipids. Isoprenoid quinones were extracted, purified by thin-layer chromatography. The isoprenoid quinones were confirmed by HPLC-MS analysis (Wainø et al. 2000) .

Results And Discussion

Phylogenetic and phylogenomic analyses

The 16S rRNA gene sequence obtained both by conventional cloning Sanger sequencing and extracted from genome, showing that strain RC-68^T had three 16S rRNA genes, two of them were completely consistent, anther had 5 bp miss match (total 1472 bp). Strain HD8-83^T, LYG-36^T, DLLS-82 had one kind of 16S rRNA gene (1472 bp, 1472 bp, and 1471 bp, respectively). The highest 16S rRNA gene sequence similarities between each novel strain and its related *Halorussus* species are as following, 97.9 % similarity between strain HD8-83^T and *Hrs. litoreus* HD8-51^T, 94.4% and 94.7 % similarities between strains LYG-36^T, DLLS-82 and *Hrs. rarus* TBN4^T, 96.9 % similarity between strain RC-68^T and *Hrs. salinus* YJ-37-H^T, respectively. These 16S rRNA gene similarities are lower than the recommended thresholds (98.65 %) to separate two prokaryotic species (Kim et al. 2014).

A phylogenetic tree based on the 16S rRNA gene sequences of isolated strains and their closest neighbours is shown in Fig. 1a. Four strains grouped together with members of genus *Halorussus*, strain HD8-83^T was located in a separated branch closely related to *Hrs. litoreus* HD8-51^T; strain LYG-36^T and DLLS-82 clustered together in one branch with similarity of 99.5% and was close to *Hrs. rarus*

TBN4^T and *Hrs. ruber* YC25^T; strain RC-68^T exhibit close phylogenetic relatedness to *Hrs. salinus* YJ-37-H^T. The phylogenetic position was also confirmed in other trees generated using the MP and NJ algorithms. The 16S rRNA sequence similarity data and phylogenetic tree of the isolated strains support the establishment of three novel species placement in the genus *Halorussus.* Phylogenetic tree reconstruction based on *rpoB'* gene indicate the same taxonomic affiliation of the new strains (Fig. 1b). Strain HD8-83^T, LYG-36^T, DLLS-82 and RC-68^T tightly clustered with the members of *Halorussus* but formed independent branchs, clearly separated from the other genus. Strain HD8-83^T was phylogenetically relatedness to *Hrs. rarus* TBN4^T (92% similarity), strain LYG-36^T clustered with DLLS-82 and formed a separated branch, strain RC-68^T eas close to the clade consisted by *Hrs. ruber* YC25^T and *Hrs. salinus* YJ-37-H^T (92.8% and 93.8% similarity, respectively). The phylogenetic position was also confirmed in trees generated using the maximum-parsimony (MP) and neighbour-joining (NJ) algorithms.

The phylogenomic tree reconstruction based on concatenated aligned single-copy OCs (Fig. 1c) showed that strain LYG-36^T together with DLLS-82 formed a distinct clade, which is closest to *Hrs. amylolyticus* YC93^T, strain HD8-83^T and RC-68^T form two separate branch closest to *Hrs. litoreus* HD8-51^T and *Hrs. salinus* YJ-37-H^T, respectively. This result support that the four strains belong to three novel species within the genus *Halorussus*.

OrthoANI, AAI and *in silico* DDH values between strains LYG-36^T and DLLS-82 were 98.9 %, 98 % and 92.4% (Table 1), showing they belong to same species. While ANI, AAI and DDH values between each strain and *Halorussus* members ranged 76.6–84.7 %, 69.4–82.9 % and 21–28.9 % (Table 1), strikingly lower than the threshold values of the species boundary (95–96% of ANI, 85% of AAI and 70 % of *is*DDH) (Goris et al. 2007; Meier-kolthoff et al. 2013; Richter and Rossellomora 2009). These suggested that strains HD8-83^T, LYG-36^T, DLLS-82 and RC-68^T represent three different genomic species.

Morphological, physiological, and biochemical analysis

Cells of four strains were Gram-stain negative, motile pleomorphic rods (Fig. S1) when grown in NHM liquid medium. The three strains were red-pigmented on agar plate colonies while strain DLLS-82 showed white. Their cells lysed in distilled water and the minimal NaCl concentration to prevent cell lysis was 5% (w/v). All strains were able to grow in the presence of 0–1.0 M $MgCl_2$ with an optimum at pH 7.0–7.5, and could not hydrolyze starch or Tween 80. All strains were able to utilize following substrates: D-glucose, D-galactose, sucrose, glycerol, D-mannitol, D-sorbitol, pyruvate, DL-lactate, succinate, L-malate, citrate, L-arginine, L-aspartate, L-glutamate and L-ornithine. But none of them can use D-fructose, L-sorbose, D-ribose, D-xylose or starch. They were sensitive to the following antimicrobial compounds (µg per disc, unless otherwise indicated): novobiocin (30), rifampin (5), nitrofurantoin (300), and resistant to

the following antimicrobial compounds: erythromycin (15), ampicillin (10), chloramphenicol (30), neomycin (30), norfloxacin (10), streptomycin (10), kanamycin (30), tetracycline (30), vancomycin (30), gentamicin (10) and nalidixic acid (30). The main phenotypic characteristics differentiating strain HD8-83^T, LYG-36^T, DLLS-82 and RC-68^T from the closest members of *Halorussus* were shown in Table 2. Strain RC-68^T together with the closest strain YJ-37-H^T required higher concentration of Mg^{2+} (0.3 M) for optimum growth than the others wanted (0.005-0.1 M). The abilities of anaerobic growth, indole formation, H_2S formation, gelatin hydrolysis, casein hydrolysis and oxidase were strain-dependent. More detailed results of phenotypic features are given in the species description.

The major phospholipids of four strains were similar to those of *Halorussus* members, containing phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), and phosphatidylglycerol sulfate (PGS) (Fig. S2). The glycolipid profiles of these strains were diverse, strain LYG-36^T, DLLS-82, RC-68^T had three kinds of glycolipids, sulfated mannosyl glucosyl diether (S-DGD-1), galactosyl mannosyl glucosyl diether (TGD-1) and diglycosyl diether (DGD-2), while strain HD8-83^T had six glycolipids, sulfated mannosyl glucosyl diether (S-DGD-1), galactosyl mannosyl glucosyl diether (TGD-1), mannosyl glucosyl diether (DGD-1), diglycosyl diether (DGD-2) and two unknown glycolipids (GL0 and GL1b). All strains have the same composition of major respiratory quinones, which are menaquinone MK-8 and MK-8(H_2).

Genome characteristics

Three draft genomes and one complete genome of new strains ranged from 3.5–3.9 Mbp (more genomic features in Table S1), slightly lower than the most genome size of *Halorussus* type strains (3.8–4.4 Mbp). The sequenced genomes were quality enough, with completeness ≥98.23 % and contamination ≥2.55 % (using CheckM software estimated according several marker genes (Parks et al. 2015)). The DNA G+C content of four isolates based on genomes was in the range 63.8–67.3 mol%. These percentages were close to the range reported for members of the genus *Halorussus*, 61.5–68.1 mol% based on genomes (Cui et al. 2010; Ding et al. 2020; Han et al. 2019; Xu et al. 2016; Yuan et al. 2015a; Yuan et al. 2015b).

The genomes of strains HD8-83^T, LYG-36^T and DLLS-82 had a single rRNA operon (one 5S rRNA, one 16S rRNA and one 23S rRNA gene), tRNA genes 51, 72, 49 and sRNA genes 3, 5, 5, respectively. The genome of strain RC-68^T had three rRNA operon (three 5S rRNA, three 16S rRNA and three 23S rRNA gene, located on the chromosome), tRNA genes 50 and sRNA genes 9 (Table S1). The heatmap based on gene counts of subsystem feature annotated by RAST server revealed that amino acids and derivatives, protein

metabolism were the most abundant RAST subsystems for *Halorussus* members (Fig. S3), which suggest diverse metabolic functions in *Halorussus*. The clustering relationship of the number of annotation genes indicates the similarity of functional genes and the similarity of strain functions. For example, strains LYG-36^T and DLLS-82 are the same species, and their physiological and biochemical characteristics are also the most similar with each other. Strains LYG-36^T and DLLS-82 have the largest number of amino acids and derivatives functional genes (223 and 224), and they can use a variety of amino acids (glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine and L-ornithine), while strain RC-68^T has only 184 amino acids and derivatives functional genes, it also has the ability to use almost the same amino acid except glycine as the sole carbon source or nitrogen source for growth, which may be due to insufficient gene function annotation caused by imperfect metabolic pathway analysis of haloarchaea.

Comparison of the OCs among the novel type strains and *Halorussus* members revealed that all the species had 5322 clusters, including 3688 accessory OCs (shared with more than two but not all strains), 1716 core OCs (shared with all strains) and 104 unique (strain-specific) clusters. Strain HD8-83^T contained 9 clusters, 2 of them annotated by GO datebase showing the function of mitotic DNA replication checkpoint and ATPase activity, strain LYG-36^T contained 20 unique clusters (4 had GO annotation: nicotine catabolic process, nucleotide binding, RNA-directed DNA polymerase activity, mitotic DNA replication checkpoint), strain RC-68^T contained 4 unique clusters (2 had GO annotation: structural molecule activity and DNA restriction-modification system), other strains had various number of unique clusters (Fig. S4). These results indicated that strains HD8-83^T, LYG-36^T and RC-68^T were different from each other and their closely related species.

Conclusions

The low level of 16S rRNA gene sequence similarity, ANI, AAI and DDH values, the independent phylogenetic position, and the differences in phenotypic properties and chemotaxonomic characteristics between strain HD8-83^T, LYG-36^T, DLLS-82, RC-68^T and its closest phylogenetic described species of *Halorussus* indicated that these strains diverged from current members. Therefore, we suggest that the strains HD8-83^T, LYG-36^T, DLLS-82, and RC-68^T represents three novel species of the genus *Halorussus* for which the names *Halorussus halobius* sp. nov. (type strain HD8-83^T = CGMCC 1.15334^T = JCM 31110^T), *Halorussus marinus* sp. nov. (type strain LYG-36^T = CGMCC 1.13606^T = JCM 32952^T, reference strain DLLS-82 = CGMCC 1.13604 = JCM 32951) and *Halorussus pelagicus* sp. nov. (type strain RC-68^T = CGMCC 1.13609^T = JCM 32953^T) are proposed.

Description of Halorussus halobius **sp. nov.**

Halorussus halobius (ha.lo'bi.us. Gr. n. *hals, halos* salt; Gr. n. *bius* life; M.L. masc. adj. *halobius* living on salt).

Cells are Gram-stain-negative, motile, rod (cell size 0.8–1.0×1.0–3.5 μm) under optimal growth conditions. Colonies on agar plates are red, elevated and round. Optimal growth is obtained at 4.3 M NaCl (range: 0.9–4.8 M), 0.05 M $MgCl_2$ (range: 0–1.0 M), 35°C (range: 25–50°C) and pH 7.5 (range: 6.0–9.5). Cells lyse in distilled water and the minimal NaCl concentration to prevent cell lysis is 0.85 M. The catalase activity and oxidase activity are positive. Anaerobic growth is observed in the presence of nitrate, with nitrate reduction to nitrite but no gas formation from nitrate. Anaerobic growth with DMSO and L-arginine is not detected. Indole formation and H_2S formation are negative. The type strain can not hydrolyze gelatin, hydrolyze casein, starch or Tween 80. The following substrates are utilized as single carbon and energy sources for growth: D-glucose, D-galactose, maltose, sucrose, glycerol, D-mannitol, D-sorbitol, pyruvate, DL-lactate, succinate, L-malate and citrate. The following substrates are utilized as single carbon, nitrogen or energy sources for growth: glycine, L-arginine, L-aspartate, L-glutamate and L-ornithine. D-mannose, D-fructose, L-sorbose, D-ribose, D-xylose, lactose, starch, acetate or fumarate cannot be utilized as single carbon and energy sources for growth. L-alanine and L-lysine cannot be utilized as single carbon, nitrogen or energy sources for growth.

The major polar lipids are phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulfate (PGS) and six glycolipids, sulfated mannosyl glucosyl diether (S-DGD-1), galactosyl mannosyl glucosyl diether (TGD-1), mannosyl glucosyl diether (DGD-1), diglycosyl diether (DGD-2) and two unknown glycolipids (GL0 and GL1b). The major respiratory quinones were menaquinone MK-8 and MK-8(H_2).

The DNA G + C content of strain HD8-83^T is 67.3 mol%. The type strain is HD8-83^T (= CGMCC 1.15334^T = JCM 31110^T) and was isolated from salted brown alga *Laminaria* produced at Dalian, Liaoning Province, China.

Description of Halorussus marinus **sp. nov.**

Halorussus marinus (ma.ri'nus. L. masc. adj. *marinus*, of the sea, marine).

Cells are Gram-stain-negative, motile, pleomorphic rod (cell size 1.0–1.5×1.2–3.6 μm) under optimal growth conditions. Colonies on agar plates are white or red, elevated and round. Optimal growth is obtained at 3.1 M NaCl (range: 0.9–4.8 M), 0.05–0.1 M $MgCl_2$ (range: 0–1.0 M), 30–40°C (range: 20–55°C) and pH 7.0-7.5 (range: 5.0–9.5). Cells lyse in distilled water and the minimal NaCl concentration to prevent cell lysis is 0.85 M. The catalase activity is positive and the oxidase activity is negative. Nitrate reduction to nitrite is observed but gas formation from nitrate is not detected. Anaerobic growth is observed in the presence of nitrate, DMSO and L-arginine. Indole formation and H_2S formation are positive. The type strain cannot hydrolyze gelatin, hydrolyze casein, starch or Tween 80. The following substrates are utilized as single carbon and energy sources for growth: D-glucose, D-mannose, D-

galactose, maltose, sucrose, lactose, glycerol, D-mannitol, D-sorbitol, acetate, pyruvate, DL-lactate, succinate, L-malate, fumarate and citrate. The following substrates are utilized as single carbon, nitrogen or energy sources for growth: glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine and L-ornithine. D-fructose, L-sorbose, D-ribose, D-xylose or starch cannot be utilized as single carbon and energy sources for growth.

The major polar lipids are phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulfate (PGS) and three glycolipids, sulfated mannosyl glucosyl diether (S-DGD-1), galactosyl mannosyl glucosyl diether (TGD-1) and diglycosyl diether (DGD-2). The DNA G + C content of strain LYG-36^T is 66.9 mol%. The major respiratory quinones were menaquinone MK-8 and MK-8(H_2).

The type strain is LYG-36^T (= CGMCC 1.13606^T = JCM 32952^T), reference strain is DLLS-82 (= CGMCC 1.13604 = JCM 32951), and they were isolated from salted brown alga *Laminaria* produced at Lianyungang, Jiangsu Province, China and Dalian, Liaoning Province, China, respectively.

Description of Halorussus pelagicus **sp. nov.**

Halorussus pelagicus (pe.la'gi.cus. L. masc. adj. *pelagicus*, belonging to the sea).

Cells are Gram-stain-negative, motile, pleomorphic rod (cell size 0.8–1.0×1.0–5.0 μm) under optimal growth conditions. Colonies on agar plates are red, elevated and round. Optimal growth is obtained at 2.6 M NaCl (range: 2.0–4.8 M), 0.3 M $MgCl_2$ (range: 0–1.0 M), 35°C (range: 20–50°C) and pH 7.0 (range: 5.0–9.5). Cells lyse in distilled water and the minimal NaCl concentration to prevent cell lysis is 0.85 M. The catalase activity is positive and the oxidase activity is negative. Anaerobic growth is not observed in the presence of nitrate, DMSO and L-arginine, and not detected nitrate reduction to nitrite or gas formation from nitrate. Indole formation and H_2S formation are positive. The type strain can hydrolyze gelatin and casein, but not hydrolyze starch or Tween 80. The following substrates are utilized as single carbon and energy sources for growth: D-glucose, D-mannose, D-galactose, sucrose, lactose, glycerol, D-mannitol, D-sorbitol, acetate, pyruvate, DL-lactate, succinate, L-malate, fumarate and citrate. The following substrates are utilized as single carbon, nitrogen or energy sources for growth: L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine and L-ornithine. D-fructose, L-sorbose, D-ribose, D-xylose,maltose or starch cannot be utilized as single carbon and energy sources for growth. Glycine cannot be utilized as single carbon, nitrogen or energy sources for growth.

The major polar lipids are phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulfate (PGS) and three glycolipids, sulfated mannosyl glucosyl diether (S-DGD-1), galactosyl mannosyl glucosyl diether (TGD-1) and diglycosyl diether (DGD-2). The DNA G + C content of strain RC-68^T is 63.8 mol%. The major respiratory quinones were menaquinone MK-8 and MK-8(H_2).

The type strain is RC-68^T (= CGMCC 1.13609^T = JCM 32953^T) and was isolated from salted brown alga *Laminaria* produced at Rongcheng, Shandong Province, China.

Declarations

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Author contributions

HLC conceived the study. DH performed the microbiological, genomic analyses.

DH wrote the first draft and all authors revised and approved the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical statement

The article does not contain any studies related to human participants or animals.

Consent to participate

Both authors gave their consent to participate in this study.

Consent for publication

Both authors gave their consent to publish results from this study and to be listed as a co-author.

Data availability

Both authors have declared that all data are availability.

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Figures

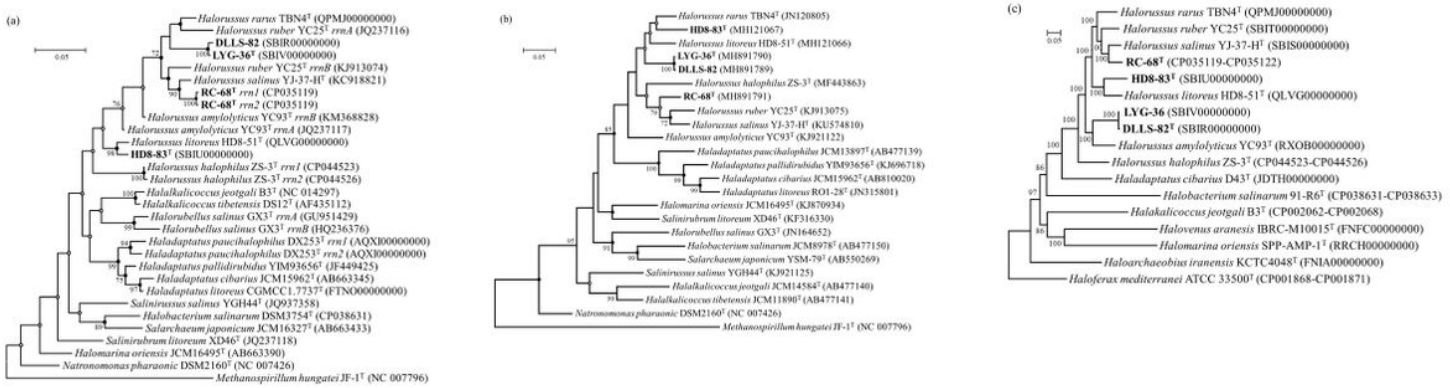

Figure 1

Maximum-Likelihood phylogenetic tree reconstructions based on 16S rRNA gene (a), rpoB' gene (b) sequences and 728 conserved single-copy protein sequences (c), showing the relationships between strains HD8-83T, LYG-36T, DLLS-82, RC-68T and related members within the family Halobacteriaceae. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 70 % of bootstrap support. Filled circles indicate branches that were recovered in the neighbour-joining, maximum-likelihood and maximum-parsimony phylogenetic trees. Empty circles indicate branches that were recovered in the maximum-likelihood and maximum-parsimony phylogenetic trees. Bar represents expected substitutions per nucleotide position.