Halomonas Maris Sp. Nov., a Moderately Halophilic Bacterium Isolated From Marine Sediment in the Southwest Indian Ocean

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Research Article

Keywords: Halomonas maris sp. nov., Novel species, Genomic analysis, Salt tolerance

DOI: https://doi.org/10.21203/rs.3.rs-207875/v1

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Abstract

A halophilic, Gram-staining-negative, rod-shaped, flagellated and motile bacterium, strain QX-1\(^T\), was isolated from deep-sea sediment at a depth of 3332 m in the southwestern Indian Ocean. Strain QX-1\(^T\) growth was observed at 4–50 °C (optimum 37 °C), pH 5.0–11.0 (optimum pH 7.0), 3%–25% NaCl (w/v; optimum 7%), and it did not grow without NaCl. A phylogenetic analysis based on the 16S rRNA gene placed strain QX-1\(^T\) in the genus _Halomonas_ and most closely related to _Halomonas sulfidaeris_ (97.90%), _Halomonas zhaodongensis_ (97.80%), _Halomonas songnenensis_ (97.59%), _Halomonas hydrothermalis_ (97.37%), _Halomonas subterranea_ (97.25%), _Halomonas salicampi_ (97.09%), and _Halomonas arcis_ (97.01%). DNA–DNA hybridization (< 26.50%) and average nucleotide identity values (< 83.54%) between strain QX-1\(^T\) and the related type strains meet the accepted criteria for a new species. The principal fatty acids (> 10%) of strain QX-1\(^T\) are C\(_{16:0}\) (25.50%), C\(_{17:0}\) cyclo (14.02%), C\(_{19:0}\) cyclo \(\omega_8\)c (18.72%), and summed feature 8 (C\(_{18:1}\) \(\omega_7\)c and/or C\(_{18:1}\) \(\omega_6\)c, 18.08%). The polar lipids of strain QX-1\(^T\) are mainly diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, unidentified phospholipid, unidentified aminophospholipid, and five unidentified lipids. The main respiratory quinone is Q-9. The G+C content of its chromosomal DNA is 54.4 mol%. Its fatty acid profile, respiratory quinones, and G+C content also support the placement of QX-1\(^T\) in the genus _Halomonas_. These phylogenetic, phenotypic, and chemotaxonomic analyses indicate that QX-1\(^T\) is a novel species, for which the name _Halomonas maris_ is proposed. The type strain is QX-1\(^T\) (=MCCC 1A17875\(^T\) = KCTC 82198\(^T\) = NBRC 114670\(^T\)).

Introduction

In recent years, extensive studies of high-salinity environments in different geographic locations have led to the isolation and characterization of a large number of halophilic microbial species. The family Halomonadaceae belongs to the Gammaproteobacteria (Arahal et al. 2002; Jiang et al. 2014b), and contains 14 genera: _Aidingimonas_, _Carnimonas_, _Chromohalobacter_, _Cobetia_, _Halomonas_, _Halotalea_, _Halovibrio_, _Kushneria_, _Larsenimonas_, _Modicisalibacter_, _Pistricoccus_, _Salinicola_, _Terasakiispira_, and _Zymobacter_. The genus _Halomonas_ is one of 14 genera occurring in the family Halomonadaceae, and was originally described by Vreeland _et al._ in 1980 (Vreeland et al. 1980). At the time of writing, 109 species of _Halomonas_ have been reported (https://lpsn.dsmz.de/genus/halomonas), including the newly described _H. umiana_ (Khan _et al._ 2020), _H. piezotolerans_ (Yan _et al._ 2020), and _H. lactosivorans_ (Ming _et al._ 2020).

_Halomonas_ is described as containing halophilic or salt-tolerant Gram-staining-negative bacilli. Most species of _Halomonas_ have been isolated from salt lakes, marine environments, or saline soils (Guan _et al._ 2010; Ming _et al._ 2020; Poli _et al._ 2013). _Halomonas_ has a unique structure and special physiological mechanisms, and has high research and utilization value. Some scholars have studied _Halomonas_ strains isolated from marine sediments, finding that _Halomonas_ is strongly adapted to the extreme environment of the deep sea. This is mainly reflected in its tolerance of heavy metal stress and its strong adaptability to changes in temperature, salinity, pressure, and oxygen concentration.
In this study, we report the characterization of a novel bacterium of the genus *Halomonas* that was isolated from deep-sea sediment in the southwestern Indian Ocean.

**Materials And Methods**

**Isolation and Culture**

Strain QX-1\textsuperscript{T} was isolated from deep-sea sediment at a depth of 3332 m in the southwestern Indian Ocean (China Ocean 40 voyages, leg III, 55.15°W, 34.32°S, TV grab sampling). To isolate this strain, we added the sediment to marine broth (MB; BD Difco) containing 10% NaCl (w/v). After 7 days in shaking culture at 10 °C, the culture was serially 10-fold diluted ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$), and the diluted cultures were plated onto marine agar (MA; BD Difco) containing 10% NaCl (w/v) and placed at 10 °C. After culture for about 30 days, a large number of colonies were observed, and single colonies were picked and streaked repeatedly to obtain pure cultures. The pure bacterial liquid cultures were stored in 15% glycerol solution at −80 °C.

**Morphological, Physiological, and Biochemical Analyses**

A Gram-staining kit (Hangzhou Tianhe Microbial Reagent Co., Ltd) was used to test the bacterium, according to the manufacturer’s instructions. The morphology of the cells was observed with a transmission electron microscope (JEM-1230, JEOL) (Fig. S1, available in the online Supplementary Material). Cell movement was observed with the hanging drop method (Skerman 1960).

The temperature range of QX-1\textsuperscript{T} growth was determined in MB by incubating cultures at 0, 4, 10, 15, 20, 25, 30, 37, 45, 50, 55, and 60 °C. The pH range for growth was determined in MB at pHs 3.0–12.0 in intervals of 1 pH unit, established with citric acid/phosphate (pH 3.0–7.0), Tris/HCl (pH 8.0–9.0), or sodium carbonate/sodium bicarbonate buffers (pH 10.0–12.0). The formula of MB was adjusted so that the salinity of the medium was 0, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, or 30% (w/v), and was used to determine the salinity growth range of QX-1\textsuperscript{T}.

To investigate whether strain QX-1\textsuperscript{T} can grow under anaerobic conditions, we created an anaerobic environment by placing 10 ml of MB medium into a 60 ml anaerobic flask and adding 10 mg/l azurol solution in the ratio of 1000:1 as the oxygen indicator. The pH was adjusted to 7.2 at room temperature. The anaerobic bottle was pumped with N$_2$ gas to remove the oxygen in the bottle. During this process, 4 ml 0.5 M Na$_2$S.9H$_2$O was added to the bottle, and the solution turned weakly red. After the end of ventilation, the anaerobic bottle was immediately sealed with an anaerobic bottle stopper and an aluminum cap, and then sterilized with high-pressure steam. Sterile l-cysteine hydrochloride solution (20 g/l; Hopebiol, China) was added to the sterilized anaerobic MB medium in a ratio of 1%, and the solution turned colorless, indicating that the oxygen in the bottle had been exhausted. The medium was inoculated with strain QX-1\textsuperscript{T} in the ratio of 1:100, and incubated at 37 °C.
API ZYM, API 20NE, API 20E, and API 50CH reagent strips (BioMérieux) and a Gen III MicroPlate (Biolog Inc.) were used to detect the enzyme production, hydrolysis, and substrate utilization of the strain, respectively, according to the manufacturers’ instructions, with the single modification of adjusting the NaCl concentration to 3.0% for all tests. Seven related type strains were tested at the same time. In the Gen III MicroPlate experiment, IF-A, Gen III Inoculating Fluid was used for the matching test. The turbidity meter was calibrated with the standard turbid tube (85% turbidity), and the IF-A inoculum was initially adjusted to 100% turbidity. Fresh strain QX-1\textsuperscript{T} was scraped from the MA plate, IF-A inoculum was added to form a bacterial suspension, well-mixed, and the optical density was controlled at 95% turbidity. The prepared bacterial suspension (100 \mu l) was added to each well of the Gen III plate, which was placed at 37 \degree C.

To observe the hydrolysis of starch, cellulose, and Tween 20, 40, 60, and 80 by strain QX-1\textsuperscript{T}, 0.2% (w/v) soluble starch, 0.8% (w/v) cellulose, or 0.5% (v/v) Tween 20, 40, 60, or 80 was added to MA, respectively (Dong and Cai 2001). Oxidase activity was determined with tetramethyl p-phenylenediamine. If the reaction turned purple immediately, it was oxidase positive; otherwise, it was negative. Catalase activity was determined by adding 3% H\textsubscript{2}O\textsubscript{2} to the colony. If a large number of bubbles were generated immediately, the colony was positive for catalase activity; if a small number of bubbles was generated within 1 min, it was weakly positive; if no bubbles were generated, it was catalase negative.

**Molecular Analysis**

A bacterial genome extraction kit (SBS) was used according to the manufacturer’s instructions to extract the genomic DNA of QX-1\textsuperscript{T}. The 16S rRNA gene was amplified with the universal bacterial primers 27F (5\textsuperscript{c}-AGAGTTTGATCCTGGCTCAG-3\textsuperscript{c}) and 1492R (5\textsuperscript{c}-TACGGTTACCTTGTTACGACTT-3\textsuperscript{c}) (Lane 1991) and Ex Taq DNA Polymerase in a 50 \mu l amplification system (Sangon Biotech, China).

The draft genome of QX-1\textsuperscript{T} was determined by Shanghai Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China), using the Illumina paired-end (500 bp library) sequencing technique. The clean data were assembled with SPAdes v 3.8.1 with the default settings (Bankevich et al. 2012). Contigs longer than 1 kb and with similar read coverage were retained for further analysis. The G+C content of the chromosomal DNA of strain QX-1\textsuperscript{T} was determined from the draft genomic sequence. The RAST website ([https://rast.nmpdr.org/](https://rast.nmpdr.org/)) was used to annotate the genomic data of strain QX-1\textsuperscript{T}.

The 16S rRNA and \textit{gyrB} and \textit{rpoD} gene sequences were extracted from the draft genomic data of strain QX-1\textsuperscript{T}. We used the EzBioCloud program ([https://www.ezbiocloud.net](https://www.ezbiocloud.net)) to compare the 16S rRNA gene sequences (Kim et al. 2012; Maidak et al. 2000) and analyzed the \textit{gyrB} and \textit{rpoD} gene sequences in the GenBank database with BLAST.

A phylogenetic analysis was performed with MEGA version X (Kumar et al. 2016). The distance option was used according to the Kimura two-parameter model, and the neighbor-joining (NJ) (Saitou and Nei...
1987), maximum-likelihood (ML) (Felsenstein 1981), and minimum evolution (ME) clustering methods were applied (Rzhetsky and Nei 1992). Bootstrap values were calculated based on 1000 replications. The sequences of related taxa were obtained from the GenBank database and EzBioCloud (Yoon et al. 2017).

DNA–DNA hybridization (DDH) and average nucleotide identity (ANI) are considered the gold standard techniques for the delineation of bacterial species (Chun et al. 2018). To compare strain QX-1^T with other strains, we calculated DDH using the web-based Genome-to-Genome Calculator (GGDC 2.1) (http://ggdc.dsmz.de/ggdc.php) (Oguntoyinbo et al. 2018), and used the EZGenome website to calculate the ANI between two genomes (Goris et al. 2007).

Chemotaxonomic Characterization

The fatty acids of QX-1^T were extracted with the standard Sherlock™ Microbial Identification System, version 6.0B (MIDI). Strain QX-1^T and related type strains were cultured on MA at 37 °C for 48 h, and the fatty acids were saponified, methylated, and extracted from the whole cells. The fatty acids were analyzed with gas chromatography (Agilent Technologies 6850) and identified with the TSBA6.0 database of the Microbial Identification System (Sasser 1990).

The polar lipids of strain QX-1^T were extracted with the chloroform–methanol system and analyzed with one-dimensional and two-dimensional thin layer chromatography (TLC) on a Merck silica gel 60 F254 aluminum-backed thin layer plate (Kates 1986). The two-dimensional development of the dot sample plate was performed with chloroform–methanol–water in a volumetric ratio of 65:25:4 as the first solvent and chloroform–methanol–acetic acid–water in a volumetric ratio of 85:12:15:4 as the second solvent. The total lipid substances were then detected with molybdenum phosphoric acid, and the specific functional groups were detected with spray reagents for specific functional groups.

Quinones were extracted with silica gel TLC, divided into different categories, and analyzed with HPLC (Tindall 1990a; Tindall 1990b).

Results And Discussion

Strain QX-1^T is a Gram-staining-negative, rod-shaped (0.7–1 μm in width and 1.8–3 μm in length), motile bacterium. The results of anaerobic culture showed that strain QX-1^T did not grow in anaerobic culture after 15 days. Strain QX-1^T growth was observed at 4–50 °C (optimum 37 °C), pH 5.0–11.0 (optimum pH 7.0), and 3%–25% NaCl (optimum 7%). Strain QX-1^T grew best in 7% NaCl (w/v) and tolerated up to 25% (w/v) NaCl. It did not grow without NaCl, so it can be considered a moderately halophilic bacterium (Ventosa et al. 1998).

Biochemical analyses showed that strain QX-1^T produces catalase and oxidase, but does not hydrolyze Tween 20, 40, 60, or 80, starch, or cellulase. It uses d-trehalose, sucrose, d-mannose and other
compounds as carbon sources. The phenotypic differences between strain QX-1\textsuperscript{T} and related type strains are shown in Table 1. The complete biochemical characteristics of strain QX-1\textsuperscript{T} are given in the species description.

Strain QX-1\textsuperscript{T} shared highest sequence similarity with *H. sulfaeferis* (97.90%), *H. zhaodongensis* (97.80%), *H. songnenensis* (97.59%), *H. hydrothermalis* (97.37%), *H. subterranea* (97.25%), *H. salicampi* (97.09%), and *H. arcis* (97.01%). The related type strains *H. sulfaeferis* ATCC BAA-803\textsuperscript{T}, *H. songnenensis* CGMCC 1.12152\textsuperscript{T}, *H. hydrothermalis* CGMCC 1.6325\textsuperscript{T}, *H. subterranea* CGMCC 1.6495\textsuperscript{T}, and *H. arcis* CGMCC 1.6494\textsuperscript{T} were obtained from the China General Microbiological Culture Collection Center. *Halomonas zhaodongensis* DSM 25869\textsuperscript{T} was obtained from the German Collection of Microorganisms and Cell Cultures. *H. salicampi* NBRC 109914\textsuperscript{T} was obtained from the Biological Resource Center, NITE.

In determining the QX-1\textsuperscript{T} genome sequence, 1 Gbp of clean data was generated, achieving a ~200-fold depth of coverage, with the Illumina HiSeq 2000 platform. The genome size of strain QX-1\textsuperscript{T} is 4.52 Mb, and included 82 contigs. The N50 value of the genomic sequence of QX-1\textsuperscript{T} was 179332, and the L50 value was 10. The accession number for strain QX-1\textsuperscript{T} is JABWCV000000000 at DDBJ/ENA/GenBank. We also submitted the genomic sequences of *H. zhaodongensis* and *H. salicampi*, determined in this study, under accession numbers JACCDD000000000 and JACCDF000000000, respectively. The genomic sequences of *H. sulfaeferis* ATCC BAA-803\textsuperscript{T}, *H. songnenensis* NEAU-ST10-39\textsuperscript{T}, *H. hydrothermalis* Slthf2\textsuperscript{T}, *H. subterranea* CGMCC 1.6495\textsuperscript{T}, and *H. arcis* CGMCC 1.6494\textsuperscript{T} were from the National Center for Biotechnology Information (accession numbers AP019514, PVTK00000000, AP022843, FOGS00000000, and FNII00000000, respectively). The G+C content of the genomic DNA of strain QX-1\textsuperscript{T} is 54.4 mol%, which is consistent with the range described for the genus *Halomonas*, 52–74.3 mol% (Franzmann et al. 1988; Martinez-Canovas et al. 2004).

The almost full-length 16S rRNA (1451 nt) and the *gyrB* (2421 nt) and *rpoD* (1851 nt) gene sequences were obtained from the draft genome of strain QX-1\textsuperscript{T}. Sequence alignment showed that there was only difference in sequencing coverage between the 16S rRNA sequences isolated from the genome and those determined by PCR.

On a phylogenetic tree constructed with the NJ method, strain QX-1\textsuperscript{T} formed a clade with *H. sulfaeferis* ATCC BAA-803\textsuperscript{T} (Fig. S1). The maximum-likelihood and minimum-evolution trees of the 16S rRNA gene sequences presented similar topologies to that of the NJ tree, so they were condensed into the NJ tree. Phylogenetic trees were also constructed from the *gyrB* and *rpoD* genes (Fig. S3 and S4), and the 16S rRNA, *gyrB*, and *rpoD* genes all indicated that the novel strain QX-1\textsuperscript{T} is closely related to members of the genus *Halomonas*.

The DDH estimates (GGDC) for QX-1\textsuperscript{T} were 26.50% with *H. sulfaeferis* ATCC BAA-803\textsuperscript{T}, 19.40% with *H. zhaodongensis* DSM 25869\textsuperscript{T}, 19.80% with *H. songnenensis* CGMCC 1.12152\textsuperscript{T}, 20.90% with *H. hydrothermalis* CGMCC 1.6325\textsuperscript{T}, 20.60% with *H. subterranea* CGMCC 1.6495\textsuperscript{T}, 20.00% with *H. salicampi*
NBRC 109914T, and 20.20% with *H. arcis* CGMCC 1.6494T (Table S2). These DDH values were significantly lower than the recommended value of 70% that is considered to define a new species (Wayne et al. 1987). The ANI values for QX-1T were 83.54% with *H. suldaeris* ATCC BAA-803T, 75.54% with *H. zhaodongensis* DSM 25869T, 76.32% with *H. songnenensis* CGMCC 1.12152T, 76.47% with *H. hydrothermalis* CGMCC 1.6325T, 76.68% with *H. subterranea* CGMCC 1.6495T, 74.94% with *H. salicampi* NBRC 109914T, and 76.40% with *H. arcis* CGMCC 1.6494T (Table S3), all of which met the standard ANI criterion for novel species identity (< 95%–96%) (Richter and Rossello-Mora 2009). These results indicate that QX-1T is affiliated with the genus *Halomonas* and may represent a new species.

The principal fatty acids (> 10%) of strain QX-1T are C\textsubscript{16:0} (25.50%), C\textsubscript{17:0} cyclo (14.02%), C\textsubscript{19:0} cyclo ω8c (18.72%), and summed feature 8 (C\textsubscript{18:1} ω7c and/or C\textsubscript{18:1} ω6c, 18.08%). Thus, its principal fatty acids meet the description of *Halomonas* (Arahal et al. 2007), but the percentages of C\textsubscript{17:0} cyclo and C\textsubscript{19:0} cyclo ω8c differ from those of the related type strains. The whole-cell fatty acids are shown in Table S1. The polar lipids of strain QX-1T are mainly diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, unidentified phospholipid, unidentified aminophospholipid, and five unidentified lipids) (Fig. S2). The results of the respiratory quinone test showed that the main component of strain QX-1T is Q-9. This is consistent with the main respiratory quinone of *Halomonas* (Dobson and Franzmann 1996).

According to the annotation of the draft genome, strain QX-1T encodes 60 RNAs, and contains 4473 protein-coding genes. Fifty-nine protein-coding genes in the QX-1T genome are related to Na\textsuperscript{+} transport (eight genes), K\textsuperscript{+} transport (six genes), trehalose synthesis/metabolism (six genes), ectoine synthesis/metabolism (10 genes), or betaine synthesis/metabolism (29 genes), which may be key elements in the adaptation of QX-1T to high-salinity environments (Table S4). Strain QX-1T is a halophilic bacterium, and its normal growth depends on a high concentration of Na\textsuperscript{+}. The genes *nhaC* and *nahD* encode Na\textsuperscript{+}/H\textsuperscript{+} antiporters (Ventosa et al. 1998; Yang et al. 2006), and the gene *sstT* encodes a serine/threonine–Na\textsuperscript{+} symporter, which maintains the stability of the intracellular Na\textsuperscript{+} concentration and prevents the toxic effects of high intracellular Na\textsuperscript{+}. Similarly, there is a K\textsuperscript{+}-regulatory mechanism in halophilic bacteria, which balances the osmotic pressure inside and outside the cell by accumulating high concentrations of K\textsuperscript{+} in the cell. The genes *trkA* and *trkH* in the QX-1T genome are related to the Trk-like K\textsuperscript{+} transport system, which has been reported in *H. elongate* DSM 2581T (Kraegeloh et al. 2005). As mentioned above, there are also genes related to trehalose, ectoine, and betaine biosynthesis/metabolism in the strain QX-1T genome. Under high-salinity conditions, halophilic microorganisms can improve their intracellular water activity by the uptake, synthesis, and accumulation of compatible substances, such as sugars, amino acids, ectoine, betaine, and trehalose (Ben-Amotz and Avron 1983; Oren 2008). The genes *doeC*, *doeX*, *teaA*, *teaB*, and *teaC* may be related to the anabolism of ectoine (Grammann et al. 2002; Schwibbert et al. 2011); the gene *betT* encodes and controls the transformation of choline to betaine under high osmotic pressure (Csonka 1989); and the genes *otaB* and *otaC* encode betaine transporters. There are also other protein-coding genes related to trehalose, ectoine, and betaine synthesis,
metabolism, and transport in the strain QX-1\textsuperscript{T} genome, which may guarantee its adaptation to a high-salt environment.

**Conclusion**

Phenotypic, phylogenetic, and chemotaxonomic analyses have shown that strain QX-1\textsuperscript{T} belongs to the genus *Halomonas*. However, it differ in some respects from related type strains. QX-1\textsuperscript{T} also showed low DDH and ANI values when compared with related type strains. These results confirm that strain QX-1\textsuperscript{T} is a novel species of the genus *Halomonas*, for which the name *Halomonas maris* sp. nov. is proposed.

**Taxonomic and Nomenclatural Proposals**

**Description of *Halomonas maris* sp. nov.**

*Halomonas maris* (ma'ris L. gen. neut. n. *maris*, of the sea, isolated from sediment of the southwestern Indian Ocean)

Cells are Gram-staining-negative, aerobic, halophilic, motile, rod-shaped, about 0.7–1 μm wide and 1.8–3.0 μm long. Colonies on MA are beige-yellow, convex, glossy, smooth, circular with an entire margin, and 1 mm in diameter after 1 day of incubation at 37 °C. Growth was observed at 4–50 °C (optimum 37 °C), pH 5.0–11.0 (optimum pH 7.0), 3%–25% NaCl (w/v; optimum 7%), and it cannot grow without NaCl. Strain QX-1\textsuperscript{T} cannot hydrolyze Tween 20, 40, 60, or 80, starch, cellulose, urea, or gelatin. Indole, acetoin (Voges-Proskauer test), and H\textsubscript{2}S are not produced. Nitrate is reduced to nitrite, and the bacterium is catalase and oxidase positive. Enzyme activities were observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, arginine hydrolase, and phenylalanine deaminase, but not for lipase (C14), trypsin, chymotrypsin, acid phosphatase, lysine decarboxylase, ornithine decarboxylase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, α-mannosidase, or α-fucosidase. Acid is produced from l-arabinose, aesculin, and 2-ketogluconate, but not from glycerol, d-arabinose, d-ribose, d-glucose, d-fructose, d-mannose, melibiose, d-xylene, l-xylene, raffinose, lactose, sucrose, trehalose, d-galactose, l-sorbose, l-rhamnose, inositol, l-arabitol, l-arabitol, l-mannitol, d-sorbitol, d-adenitol, d-fucose, l-fucose or 5-ketogluconate. It utilizes d-maltose, d-trehalose, d-cellobiose, gentiobiose, sucrose, d-turanose, d-lactose, d-melibiose, d-glucose, d-mannose, d-fructose, d-galactose, d-fucose, l-fucose, l-rhamnose, d-sorbitol, d-mannitol, d-arabitol, myo-inositol, d-aspartic acid, l-aspartic acid, l-glutamic acid, and l-malic acid, but not arabinose, d-raffinose, glycerol, d-serine, l-alanine, l-arginine, l-histidine, d-malic acid, l-serine, l-lactic acid, or citrate. Its principal fatty acids are C\textsubscript{16:0} (25.5%), C\textsubscript{17:0} cyclo (14.02%), C\textsubscript{19:0} cyclo ω8c (18.72%), and summed feature 8 (C\textsubscript{18:1} ω7c and/or C\textsubscript{18:1} ω6c, 18.08%). Its polar lipids are diphasphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, unidentified phospholipid, unidentified aminophospholipid, and five unidentified lipids. The predominant ubiquinone is Q-9. The DNA G+C
content of the type strain is 54.4 mol%. The GenBank accession numbers for the 16S rRNA gene sequence and the whole genome sequence of QX-1<sup>T</sup> are MT372903 and <code>JABWCV000000000</code>, respectively.

The type strain, QX-1<sup>T</sup> (=MCCC 1A17875<sup>T</sup> = KCTC 82198<sup>T</sup> = NBRC 114670<sup>T</sup>), was isolated from a deep-sea sediment sample at 3332 m in the southwestern Indian Ocean.

**Declarations**

**Author Contributions** Xu Qiu performed the technical characterization on strain QX-1 and drafted the manuscript. Xiaorong Cao, Guangxin Xu and Huangming Wu conceived the study and aided to draft the manuscript. Xixiang Tang conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

**Funding information** This work was supported by the COMRA Project of China (DY135-B2-16) and National Basic Research Program of China (973 Program) (No.2015CB755901).

**Conflicts of interest**

The authors declare that there is no conflict of interest.

**Footnotes**

The GenBank accession numbers for the 16S rRNA, gyrB, and rpoD gene sequences of *Halomonas maris* QX-1<sup>T</sup> are MT372903, MT672349, and MT672348, respectively. The Whole Genome Shotgun project for strain QX-1<sup>T</sup> has been deposited at DDBJ/ENA/GenBank under accession number <code>JABWCV000000000</code>. Transmission electron micrographs of cells of strain QX-1<sup>T</sup> and the polar lipids of strain QX-1<sup>T</sup> are available as supplementary figures in the online Supplementary Materials, together with a table listing the cellular fatty acid profiles of strain QX-1<sup>T</sup> and related type strains.

**References**

1. Arahal DR, Ludwig W, Schleifer KH, Ventosa A (2002) Phylogeny of the family Halomonadaceae based on 23S and 16S rDNA sequence analyses Int J Syst Evol Microbiol 52:241-249 doi:10.1099/00207713-52-1-241
2. Arahal DR et al. (2007) Recommended minimal standards for describing new taxa of the family Halomonadaceae Int J Syst Evol Microbiol 57:2436-2446 doi:10.1099/ijs.0.65430-0
3. Bankevich A et al. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing J Comput Biol 19:455-477 doi:10.1089/cmb.2012.0021
4. Ben-Amotz A, Avron M (1983) Accumulation of metabolites by halotolerant algae and its industrial potential Annu Rev Microbiol 37:95-119 doi:10.1146/annurev.mi.37.100183.000523
5. Chun J et al. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes Int J Syst Evol Microbiol 68:461-466 doi:10.1099/ijsem.0.002516

6. Csonka LN (1989) Physiological and genetic responses of bacteria to osmotic stress Microbiol Rev 53:121-147

7. Dobson SJ, Franzmann PD (1996) Unification of the Genera Deleya (Baumann et al. 1983), *Halomonas* (Vreeland et al. 1980), and *Halovibrio* (Fendrich 1988) and the Species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a Single Genus, *Halomonas*, and Placement of the Genus *Zymobacter* in the Family Halomonadaceae Int J Syst Evol Microbiol 46:550-558 doi:10.1099/00207713-46-2-550

8. Dong X-Z, Cai M-Y (2001) Determinative Manual for Routine Bacteriology. Beijing: Scientific Press (English translation),

9. Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. J MOL EVOL 17:368-376

10. Franzmann PD, Wehmeyer U, Stackebrandt E (1988) Halomonadaceae fam. nov., a New Family of the Class Proteobacteria to Accommodate the Genera *Halomonas* and *Deleya* SYST APPL MICROBIOL 11:16-19 doi:doi.org/10.1016/S0723-2020(88)80043-2

11. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (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

12. Grammann K, Volke A, Kunte HJ (2002) New type of osmoregulated solute transporter identified in halophilic members of the bacteria domain: TRAP transporter TeaABC mediates uptake of ectoine and hydroxyectoine in Halomonas elongata DSM 2581(T) J Bacteriol 184:3078-3085 doi:10.1128/jb.184.11.3078-3085.2002

13. Guan TW, Xiao J, Zhao K, Luo XX, Zhang XP, Zhang LL (2010) Halomonas xinjiangensis sp. nov., a halotolerant bacterium isolated from a salt lake Int J Syst Evol Microbiol 60:349-352 doi:10.1099/ijs.0.011593-0

14. Jiang J et al. (2014a) *Halomonas songnenensis* sp. nov., a moderately halophilic bacterium isolated from saline and alkaline soils Int J Syst Evol Microbiol 64:1662-1669 doi:10.1099/ijs.0.056499-0

15. Jiang J et al. (2013) Halomonas zhaodongensis sp. nov., a slightly halophilic bacterium isolated from saline-alkaline soils in Zhaodong, China Antonie Van Leeuwenhoek 104:685-694 doi:10.1007/s10482-013-9976-3

16. Jiang W et al. (2014b) Halomonas shantousis sp. nov., a novel biogenic amines degrading bacterium isolated from Chinese fermented fish sauce Antonie Van Leeuwenhoek 106:1073-1080 doi:10.1007/s10482-014-0275-4

17. Kates M (1986) Lipid extraction procedures Techniques of lipidology Elsevier, Amsterdam:100-111

18. Kaye JZ, Marquez MC, Ventosa A, Baross JA (2004) *Halomonas neptunia* sp. nov., *Halomonas sulfidaeis* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.:
halophilic bacteria isolated from deep-sea hydrothermal-vent environments Int J Syst Evol Microbiol 54:499-511 doi:10.1099/ijs.0.02799-0

19. Khan SA et al. (2020) Halomonas urmiana sp. nov., a moderately halophilic bacterium isolated from Urmia Lake in Iran Int J Syst Evol Microbiol 70:2254-2260 doi:10.1099/ijs.0.004005

20. Kim OS et al. (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species Int J Syst Evol Microbiol 62:716-721 doi:10.1099/ijs.0.038075-0

21. Kraegeloh A, Amendt B, Kunte HJ (2005) Potassium transport in a halophilic member of the bacteria domain: identification and characterization of the K+ uptake systems TrkH and TrkI from Halomonas elongata DSM 2581T J Bacteriol 187:1036-1043 doi:10.1128/JB.187.3.1036-1043.2005

22. Kumar S, Stecher G, Tamura KJMB, Evolution (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets 33:1870

23. Lane DJ (1991) 16S/23S rRNA Sequencing. Nucleic Acid Techniques in Bacterial Systematics 463:115-175

24. Lee JC, Kim YS, Yun BS, Whang KS (2015) Halomonas salicampi sp. nov., a halotolerant and alkalitolerant bacterium isolated from a saltern soil Int J Syst Evol Microbiol 65:4792-4799 doi:10.1099/ijsem.0.000650

25. Maidak BL et al. (2000) The RDP (Ribosomal Database Project) continues Nucleic Acids Res 28:173-174 doi:10.1093/nar/28.1.173

26. Martinez-Canovas MJ, Quesada E, Llamas I, Bejar V (2004) Halomonas ventosae sp. nov., a moderately halophilic, denitrifying, exopolysaccharide-producing bacterium Int J Syst Evol Microbiol 54:733-737 doi:10.1099/ijs.0.02942-0

27. Ming H et al. (2020) Halomonas lactosivorans sp. nov., isolated from salt-lake sediment Int J Syst Evol Microbiol 70:3504-3512 doi:10.1099/ijsem.0.004209

28. Oguntoyinbo FA et al. (2018) Halomonas nigricans sp. nov., isolated from cheese Int J Syst Evol Microbiol 68:371-376 doi:10.1099/ijsem.0.002515

29. Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity Saline Syst 4:2 doi:10.1186/1746-1448-4-2

30. Poli A, Nicolaus B, Denizci AA, Yavuzturk B, Kazan D (2013) Halomonas smyrnensis sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium Int J Syst Evol Microbiol 63:10-18 doi:10.1099/ijs.0.037036-0

31. Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proceedings of the National Academy of Sciences of the United States of America 106:19126-19131 doi:10.1073/pnas.0906412106

32. Rzhetsky A, Nei M (1992) Statistical properties of the ordinary least-squares, generalized least-squares, and minimum-evolution methods of phylogenetic inference. J Mol Evol 35:367-375
33. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution 4:406-425 doi:10.1093/oxfordjournals.molbev.a040454
34. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids USFCC News 20:1-6
35. Schwibbert K et al. (2011) A blueprint of ectoine metabolism from the genome of the industrial producer Halomonas elongata DSM 2581 T Environ Microbiol 13:1973-1994 doi:10.1111/j.1462-2920.2010.02336.x
36. Skerman VBD (1960) A guide to the identification of the genera of Bacteria Q Rev Biol 36:870
37. Tindall BJ (1990a) A Comparative Study of the Lipid Composition of Halobacterium saccharovorum from Various Sources. Syst Appl Microbiol 13:128-130 doi:10.1016/S0723-2020(11)80158-X
38. Tindall BJ (1990b) Lipid composition of Halobacterium lacusprofundi. FEMS Microbiol Lett 66:199-202
39. Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria Microbiol Mol Biol Rev 62:504-544
40. Vreeland RH, Litchfield CD, Martin EL, Elliot E (1980) Halomonas elongata, a New Genus and Species of Extremely Salt-Tolerant Bacteria Int J Syst Evol Microbiol 30:485-495 doi:doi.org/10.1099/00207713-30-2-485
41. Wayne LG et al. (1987) Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics Int J Syst Evol Microbiol 37:463-464 doi:doi.org/10.1099/ijsem.0.001755
42. Xu XW et al. (2007) Halomonas saccharovitans sp. nov., Halomonas arcis sp. nov. and Halomonas subterranea sp. nov., halophilic bacteria isolated from hypersaline environments of China Int J Syst Evol Microbiol 57:1619-1624 doi:10.1099/ijs.0.65022-0
43. Yan F, Fang J, Cao J, Wei Y, Liu R, Wang L, Xie Z (2020) Halomonas piezotolerans sp. nov., a multiple-stress-tolerant bacterium isolated from a deep-sea sediment sample of the New Britain Trench Int J Syst Evol Microbiol 70:2553-2561 doi:10.1099/ijsem.0.004069
44. Yang LF et al. (2006) A Na+/H+ antiporter gene of the moderately halophilic bacterium Halobacillus dabanensis D-8T: cloning and molecular characterization FEMS Microbiol Lett 255:89-95 doi:10.1111/j.1574-6968.2005.00055.x
45. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613-1617 doi:10.1099/ijsem.0.001755

Tables

Table 1. Phenotypic characteristics differentiating strain QX-1T from the type strains of related Halomonas species.
Strains: 1, QX-1$^T$; 2, *H. sulfidaeris* ATCC BAA-803$^T$; 3, *H. zhaodongensis* DSM 25869$^T$; 4, *H. songnenensis* CGMCC 1.12152$^T$; 5, *H. hydrothermalis* CGMCC 1.6325$^T$; 6, *H. subterranea* CGMCC 1.6495$^T$; 7, *H. salicampi* NBRC 109914$^T$; 8, *H. arcis* CGMCC 1.6494$^T$. All strains are Gram-staining-negative, aerobic, halophilic, and rod-shaped. The data obtained with API 20NE, API 20E, API ZYM, API 50CH, and GEN III for strain QX-1$^T$ and five related type strains were examined in this study. Characteristics are scored as: +, positive; −, negative; w, weakly positive. All data were obtained in this study, unless otherwise indicated.
| Characteristic                  | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|--------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| **Growth properties**          |     |     |     |     |     |     |     |     |
| Optimal temperature (°C)       | 37  | 20-35<sup>a</sup> | 35<sup>b</sup> | 35<sup>c</sup> | 30<sup>a</sup> | 30<sup>d</sup> | 28<sup>e</sup> | 30<sup>d</sup> |
| Optimal salts (%)              | 5   | 2-3<sup>a</sup> | 3<sup>b</sup> | 4<sup>c</sup> | 4-7<sup>a</sup> | 1-5<sup>d</sup> | 14<sup>e</sup> | 1-5<sup>d</sup> |
| Optimal pH                     | 7.0 | ND<sup>a</sup> | 9.0<sup>b</sup> | 7.0<sup>c</sup> | ND<sup>a</sup> | 7.0-8.0<sup>d</sup> | 8.5<sup>e</sup> | 7.0-8.0<sup>d</sup> |
| **Activity of**                |     |     |     |     |     |     |     |     |
| Cystine arylamidase            | +   | +   | -   | +   | -   | +   | +   | w   |
| Acid phosphatase               | -   | -   | +   | +   | -   | w   | +   | -   |
| Arginine dihydrolase           | +   | -   | -   | +   | -   | -   | -   | -   |
| Phenylalanine deaminase        | +   | -   | -   | +   | -   | +   | -   | +   |
| Gelatinases                    | -   | -   | -   | +   | +   | -   | +   | -   |
| **Acid production from**       |     |     |     |     |     |     |     |     |
| L-arabinose                    | w   | -   | -   | -   | -   | +   | -   | +   |
| D-ribose                       | -   | +   | +   | -   | +   | -   | +   | -   |
| D-xylose                       | -   | +   | +   | -   | -   | -   | -   | -   |
| D-glucose                      | -   | +   | +   | -   | +   | +   | +   | +   |
| D-sucrose                      | -   | -   | +   | -   | -   | +   | +   | +   |
| D-trehalose                    | -   | -   | +   | -   | -   | +   | +   | +   |
| **Utilization of**             |     |     |     |     |     |     |     |     |
| Arabinose                      | -   | +   | -   | -   | +   | +   | -   | -   |
| D-maltose                      | w   | -   | +   | -   | +   | +   | -   | +   |
| D-lactose                      | w   | -   | -   | -   | -   | -   | +   | -   |
| D-galactose                    | +   | -   | -   | -   | +   | -   | -   | -   |
| D-sorbitol                     | w   | -   | +   | -   | -   | -   | -   | -   |
| Glycerol                       | -   | -   | +   | +   | +   | +   | +   | +   |
| Citrate                        | -   | -   | +   | +   | +   | +   | -   | +   |
Data from: a(Kaye et al. 2004); b(Jiang et al. 2013); c(Jiang et al. 2014a); d(Xu et al. 2007); e(Lee et al. 2015)