



Halomonas sedimenti sp. nov., a Halotolerant Bacterium Isolated from Deep-Sea Sediment of the Southwest Indian Ocean

Xu Qiu^{1,2} · Libo Yu¹ · Xiaorong Cao¹ · Huangming Wu¹ · Guangxin Xu¹ · Xixiang Tang¹

Received: 17 August 2020 / Accepted: 10 February 2021 / Published online: 2 March 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract

A Gram-staining-negative, aerobic, flagellated, motile, rod-shaped, halophilic bacterium QX-2^T was isolated from the deep-sea sediment of the Southwest Indian Ocean at a depth of 2699 m. Growth of the QX-2^T bacteria was observed at 4–50 °C (optimum 30 °C), pH 5.0–12.0 (optimum pH 6.0) and 0%–30% NaCl (w/v) [optimum 4% (w/v)]. 16S rRNA gene sequencing revealed that strain QX-2^T has the closest relationship with *Halomonas titanicae* DSM 22872^T (98.2%). Phylogeny analysis classified the strain QX-2^T into the genus *Halomonas*. The average nucleotide identity and DNA–DNA hybridization values between strain QX-2^T and related type strains were lower than the currently accepted new species definition standards. Principal fatty acids (> 10%) determined were C_{16:0} (12.41%), C_{12:0-3OH} (25.15%), summed feature 3 (C_{16:1 ω7c} and/or C_{16:1 ω6c}, 11.55%) and summed feature 8 (C_{18:1 ω7c} and/or C_{18:1 ω6c}, 16.06%). Identified polar lipids in strain QX-2^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, unidentified phospholipid, unidentified aminophospholipid and five unidentified lipids (L1–L5). The main respiratory quinone was Q-9. The content of DNA G+C was determined to be 54.34 mol%. The results of phylogenetic analysis, phenotypic analysis and chemotaxonomic studies showed that strain QX-2^T represents a novel species within the genus *Halomonas*, for which the name *Halomonas sedimenti* sp. nov. is proposed, with the type strain QX-2^T (MCCC 1A17876^T = KCTC 82199^T).

Abbreviations

MCCC	Marine Culture Collection of China
CGMCC	China General Microbiological Culture Collection Center
KCTC	Korean Collection for Type Cultures
DSM	Leibniz Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures)
ANI	Average nucleotide identity
DDH	DNA–DNA hybridization

Introduction

The genus *Halomonas* belongs to the family *Halomonadaceae*, which was described originally by Vreeland et al. [1]. Currently, the number of validly published *Halomonas* species is 105 (<https://lpsn.dsmz.de/genus/halomonas>). The genus *Halomonas* is usually described as Gram-staining-negative bacilli that tolerate or like salt, and species are isolated from a variety of habitats, e.g., saline-alkali land, saline lakes and marine habitats [2–8]. *Halomonas* adopt unique structure and physiological mechanism, displays strong adaptation to the environment and has high research and utilization value.

Among the published species, there are few studies on *Halomonas* from a deep-sea environment. Results have revealed that *Halomonas* has a unique adaptive mechanism to survive in the extreme environment of the deep-sea and can tolerate changes in temperature and salinity and heavy metal stress [3]. Heavy metals are stable and cannot be degraded or eliminated easily [9]. Therefore, the mechanism of *Halomonas* strain resistances to heavy metal stress may provide new concepts to prevent and control heavy metal pollution. In addition, it has been found that many halotolerant and halophilic bacteria can synthesize ectoine,

Xu Qiu and Libo Yu have contributed equally to this work.

✉ Xixiang Tang
tangxixiang@tio.org.cn

¹ Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, China

² School of Ocean Sciences, China University of Geosciences (Beijing), Beijing 100083, China

such as *Spiribacter salinus* [10], *Paenibacillus lautus* [11], *Halomonas elongate* [12] and *Halomonas boliviensis* [13]. Ectoine is an important osmotic pressure regulator, which may explain the tolerance of *Halomonas* strains to high salt environments. On the basis of its moisturizing and radiation protection properties, ectoine has been used in high-grade cosmetic preparations. There is no chemical synthesis method for ectoine. Thus, exploring the biosynthetic pathway and developing a mass fermentation method of ectoine are important research topics.

During an investigation into the biodiversity of the deep-sea sediment of the Southwest Indian Ocean, Strain QX-2^T, which is closely related to the genus *Halomonas*, was isolated and the exact taxonomic position of this novel strain was determined based on polyphasic taxonomic data. In addition, genes associated with resistance to heavy metal stress and ectoine synthesis in strain QX-2^T were analyzed.

Materials and Methods

Isolation and Cultivation

Strain QX-2^T was isolated from deep-sea sediment samples collected between May and June 2016 at the SWIR (Southwest Mid-Indian Ridge) area at a depth of 2699 m in the Southwest Indian Ocean (47.43° W, 38.76° S) by the TV grab method. The sample was enriched in marine broth (MB; BD Difco, Franklin Lakes, NJ, USA) containing 10% NaCl (w/v) at 10 °C for 7 days. Cultures were then diluted according to set ratios (i.e., 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵) and plated onto marine agar (MA, BD Difco) containing 10% NaCl (w/v). Pure cultures were obtained after cultivating for ~30 days. Pure bacterial liquid cultures in a 15% glycerol solution were stored at -80 °C.

Morphological, Physiological and Biochemical

Gram staining was carried out according to the instructions of the Gram Staining Kit (Hangzhou Tianhe microbial reagent). The size and morphology of the cells were observed by transmission electron microscopy (JEM-1230, JEOL). Cell movement was monitored by the hanging drop method [14].

Growth of strain QX-2^T was examined at 0, 4, 10, 15, 20, 25, 30, 37, 45, 50, 55 and 60 °C in MB. The pH range for growth was determined in MB at intervals of 1 pH unit with citrate/phosphate (pH 3.0–7.0), Tris/HCl (pH 8.0–9.0) or sodium carbonate/sodium bicarbonate (pH 10.0–12.0) buffers. Growth of strain QX-2^T under different salt concentrations was examined by setting the salinity of MB to 0, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30% or 35% (w/v).

API ZYM, API 20NE, API 20E, API 50CH reagent strips (BioMérieux) and the GEN III microplate (Biology) were used to detect enzyme production, hydrolysis and substrate utilization by the QX-2^T strain and according to the manufacturer's instructions, with the single modification of adjusting the NaCl concentration to 3.0% in all tests. The IF A solution was used as the inoculum in GEN III microplate experiments. The turbidimeter was calibrated with a standard turbidimetric tube (85% T). The blank of the turbidimeter was adjusted by inoculating the IF A solution in the tube and adjusting the indicator of the turbidimeter to 100%. The bacterial suspension was prepared by scraping bacteria into the IF A inoculum. The light transmittance was controlled to 95% T. One hundred microliters of the bacterial suspension was added to all wells of the GEN III microplate. The microplate was incubated at the optimal growth temperature of QX-2^T for 36 h and the results were observed and recorded. Oxidase activity and catalase activity were determined by adding tetramethyl *p*-phenylenediamine and 3% H₂O₂ to growing colonies, respectively. The strain was scraped from the fresh plate and smeared onto the oxidase reagent. An immediate change in color to purple or within a short period (i.e., within a minute) showed that the bacteria strain tested positive for oxidase activity. Strains that produced strong bubbles immediately after exposure to hydrogen peroxide indicated that oxidase activity was positive, whereas the presence of weak bubbles indicated a weak positive result. The absence of bubbles indicated that no oxidase activity was present. In addition, 0.2% (w/v) soluble starch, 0.8% (w/v) cellulose or 0.5% (v/v) Tween 20, 40, 60 or 80 were added to MA, respectively. The hydrolysis of these substrates by strain QX-2^T was examined [15].

Chemotaxonomic Characterization

After culturing QX-2^T on MA at 30 °C for 48 h, fatty acids in the cells were saponified, methylated and extracted according to the standard MIDI (Sherlock microbial identification system, version 6.0B) [16]. The extracted fatty acids were analyzed by gas chromatography (Agilent Technologies 6850), and the TSBA 6.0 database of the microbial identification system was used to identify the test results [17]. The polar lipids of strain QX-2^T were extracted by a chloroform/methanol system and analyzed by one-dimensional and two-dimensional thin-layer chromatography (TLC), as described previously [18]. Merck silica gel 60 F254 aluminum back thin-layer plates aluminum-backed thin-layer plates were used in TLC analysis. Two-dimensional development of the dot-shaped sample plates used chloroform–methanol–water (65:25:4, by vol.) as the first solvent and chloroform–methanol–acetic acid–water (85:12:15:4, by vol.) as the second solvent. Subsequently, total lipid amounts were detected with molybdophosphoric acid and specific functional groups

were detected using spray reagents for specific functional groups. The quinones were extracted and separated into their different classes by thin-layer chromatography on silica gel, then further analyzed by HPLC (SPD-10AV; Shimadzu), as described by Collins and Jones [19].

Molecular Analysis

The taxonomic position of strain QX-2^T was determined by 16S rRNA gene sequencing [20]. The 16S rRNA gene sequence of strain QX-2^T was obtained by PCR using bacterial 16S rRNA universal primers to amplify genomic DNA. Genomic DNA was extracted using the bacterial Genomic Extraction Kit (SBS) following the manufacturer's instructions. In a 50 µL amplification system (SangonBiotech), bacterial universal primers 27F (5'-AGAGTTTGATCCTGG CTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACG ACTT-3') [21] and ExTaq were used to amplify the QX-2^T 16S rRNA gene, and gene sequencing was carried out to obtain the 16S rRNA sequence. The GenBank database and EzTaxon (<https://www.ezbiocloud.net>) software were used to calculate the nucleotide sequence similarity among the 16S rRNA genes from the QX-2^T strain and related type strains [22].

According to the results from the GenBank database and EzTaxon web software analysis, type strains with the highest similarity to the QX-2^T 16S rRNA gene sequence were selected [23] and used for MEGA Version X phylogenetic analysis [24]. The distance option used was according to the Kimura two-parameter model, and the neighbor-joining (NJ) [25], maximum-likelihood (ML) [26] and minimum evolution (ME) [27] methods were used for clustering. Bootstrap values were calculated based on 1000 repeats.

The reference strains *Halomonas titanicae* (*H. titanicae*) DSM 22872^T, *H. glaciei* CGMCC 1.7263^T, *H. variabilis* DSM3051^T and *H. salicampi* NBRC 109914^T were obtained from the culture collections and used as controls in phenotypic tests. The draft genome of QX-2^T, *H. glaciei* CGMCC 1.7263^T and *H. salicampi* NBRC 109914^T were obtained by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). The Illumina paired end (500 bp library) sequencing technique was used. The clean data were assembled by SPAdes v3.8.1 using default settings [28]. The contigs longer than 1 kb and similar read coverage were kept for further analysis. The genome sequence data of *H. titanicae* DSM 22872^T and *H. variabilis* DSM3051^T were taken from NCBI with accession numbers APO000000000 and BJXV000000000, respectively. *gyrB* and *rpoD* gene sequences were extracted from the draft genome of strain QX-2^T. BLAST analysis was then performed on *gyrB* and *rpoD* gene sequences using the GenBank database. The G+C content of chromosome DNA of the QX-2^T strain was determined from the genomic draft sequence. The

DNA–DNA hybridization (DDH) calculation was performed using the genome-to-genome calculation tool of the web version of GGDC 2.1 (<http://ggdc.dsmz.de/ggdc.php>) and the BLAST method [29, 30]. The average nucleotide identity (ANI) value between two genomes was calculated by the EZGenome's web service [31]. The genome annotation tool RAST (<https://rast.nmpdr.org/>) was used to annotated and analyzed the draft genome of strain QX-2^T. Rastk was selected as a comment scheme.

Results and Discussion

Strain QX-2^T was observed to be Gram-staining-negative, rod-shaped, flagellated and motile (Supplementary Fig. S1, available in the online Electronic Supplementary Material). Colonies that formed after 1 day incubation on MA at 30 °C were observed to be creamy, circular and 1 mm in diameter. Cells grew between 4 and 50 °C (optimum 30 °C), at pH values of 5.0–12.0 (optimum pH 6.0) and at NaCl concentrations of 0%–30% (w/v) (optimum 4% (w/v)). Catalase and oxidase activities were measured as positive. Starch, cellulose, Tween 20, 40, 60 and 80 were not hydrolyzed. Phenotypic traits that differentiate QX-2^T from related type strains of *Halomonas* species are given in Table 1.

The principal fatty acids (> 10% of the total fatty acids) of strain QX-2^T were determined to be C_{16:0} (12.41%), C_{12:0}-3OH (25.15%), summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c, 11.55%) and summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c, 16.06%). The percentage of C_{12:0}-3OH differs to that found in other related type strains. Whole-cell fatty acids are presented in Supplementary Table S1. The primary polar lipids of strain QX-2^T were identified to be diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), unidentified phospholipid (PL), unidentified aminophospholipid (APL) and five unidentified lipids (L1–L5) (Supplementary Fig. S2). The main component of respiratory quinone for strain QX-2^T was Q-9, which is also the main component for species in the genus *Halomonas* [36].

In this study, a near full-length 16S rRNA gene sequence of QX-2^T (1446 nt) was obtained and this sequence was submitted to the NCBI under accession number MT372904. This sequence was consistent with the 16S rRNA gene sequence we extracted from the strain QX-2^T draft genome data. Pairwise comparison of the 16S rRNA gene sequences using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Ezbiocloud website (<https://www.ezbiocloud.net/identify>) for the 16S rRNA gene sequence strains QX-2^T and *H. titanicae* DSM 22872^T, *H. glaciei* CGMCC 1.7263^T, *H. variabilis* DSM 3051^T and *H. salicampi* NBRC 109914^T gave values of 98.2%, 98.1%, 97.8% and 97.5%, respectively.

Table 1 Phenotypic traits that differentiate *H. sedimenti* sp. nov. from the type strains of closely related *Halomonas* species

Characteristic	1	2	3	4	5
Habitat	Deep-sea sediment	Fast ice ^a	Saltern soil ^b	RMS titanic wreck ^c	Hyper-saline lake ^d
Growth properties					
Optimal temperature (°C)	30	22 ^a	28 ^b	30-37 ^c	33 ^d
Optimal salts (%)	4	2 ^a	14 ^b	2-8 ^c	10 ^d
Optimal pH	6	7 ^a	8.5 ^b	ND ^c	ND ^d
Activity of					
Alkaline phosphatase	+	-	-	+	+
Esterase lipase (C8)	+	+	-	+	-
Lipase (C14)	-	-	+	-	+
Cystine arylamidase	+	-	+	+	-
β -galactosidase	-	-	-	+	+
Arginine dihydrolase	+	-	-	+	-
Phenylalanine deaminase	+	+	-	-	+
Acid production from					
L-Arabinose	+	-	-	-	-
D-Ribose	-	+	+	+	-
D-Glucose	-	-	+	+	-
D-Maltose	-	+	+	-	-
Sucrose	-	+	+	-	-
Utilization of					
L-Arabinose	-	+	-	-	+
D-Maltose	+	-	-	-	+
D-Melibiose	w	-	-	-	+
D-Mannose	w	-	-	-	+
D-Sorbitol	+	-	-	+	-
D-Mannitol	+	-	-	+	-
Citrate	+	-	-	+	+

Strains: 1, QX-2^T; 2, *H. titanicae* DSM 22872^T; 3, *H. glaciei* CGMCC 1.7263^T; 4, *H. variabilis* DSM 3051^T; 5, *H. salicampi* NBRC 109914^T. All strains are Gram-staining-negative, aerobic, halophilic and rod-shaped. The data of API 20NE, API 20E, API ZYM, API 50CH and GEN III for strain QX-2^T and four related type strains were examined in this study. Characteristics are scored as: + positive; - negative; w, weak positive. Data were obtained in this study unless indicated

^aData from [32]

^bData from [33]

^cData from [34]

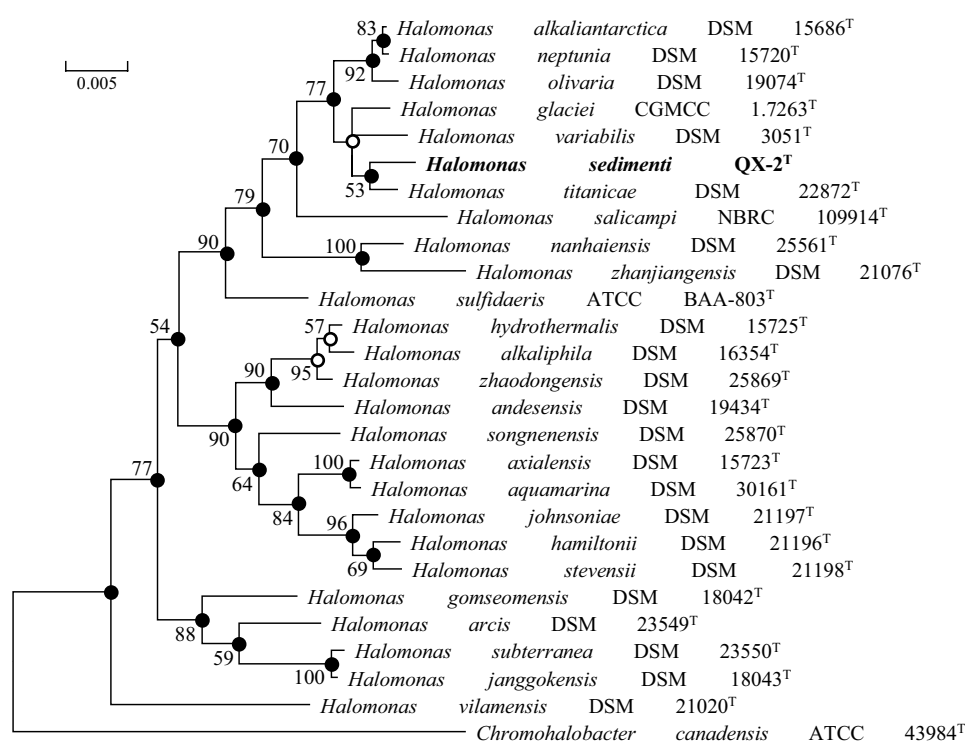
^dData from [35]

The phylogenetic tree based on the NJ method showed that strain QX-2^T formed a clade with *H. titanicae* DSM 22872^T. We used the *gyrB* (2421 nt, MT672351) and *rpoD* (1851 nt, MT672350) genes of strain QX-2^T to perform phylogenetic tree analysis with other species in the family *Halomonadaceae*, and the results showed that strain QX-2^T belongs to the genus *Halomonas* (Figs. S3 and S4). The NJ tree based on 16S rRNA gene sequences is shown in Fig. 1. *Chromohalobacter canadensis* ATCC 43984^T (AJ295143) was used as out-group which belongs to the family *Halomonadaceae*. The ML and ME trees of 16S rRNA gene sequences presented a similar topology to that

of the NJ-derived phylogenetic tree. Therefore, these trees were condensed into the NJ phylogenetic tree. The results of the phylogenetic analysis indicated that QX-2^T is affiliated to the genus *Halomonas*.

A total of 1 Gbp clean data was generated to reach about 200-fold depth of coverage using the Illumina HiSeq 2000 platform. The genome size of strain QX-2^T was 4.94 Mb, including 61 contigs. The N50 value of the genome sequence of QX-2^T was 214,892, and the L50 value was 9 (Table S2). The accession number for strain QX-2^T was JACCGK000000000 at the DDBJ/ENA/GenBank. We also obtained the draft genome of *H. glaciei* and *H. salicampi*,

Fig. 1 Neighbor-Joining tree showing the phylogenetic positions of strain QX-2^T and related species of the genus *Halomonas* based on 16S rRNA gene sequences. Bootstrap values (> 50%) based on 1000 replication are shown at branch points. *Chromohalobacter canadensis* ATCC 43984^T (AJ295143) was used as out-group. Filled and open circles at nodes indicate generic branches that were also recovered using the Maximum-Likelihood and Minimum Evolution algorithms, respectively. Bar, 0.005 substitutions per nucleotide position



under accession numbers JACCDE000000000 and JAC-CDF000000000, respectively. The G+C content of the genomic DNA of strain QX-2^T was 54.34 mol%, which is consistent with the description range of the genus *Halomonas* [37, 38].

The DDH estimate values of QX-2^T were 58.90% with *H. titanicae* DSM 22872^T, 30.10% with *H. glaciei* CGMCC 1.7263^T, 22.90% with *H. variabilis* DSM 3051^T and 21.30% with *H. salicampi* NBRC 109914^T (Table S3). The ANI values of QX-1^T were 94.11% with *H. titanicae* DSM 22872^T, 85.96% with *H. glaciei* CGMCC 1.7263^T, 67.26% with *H. variabilis* DSM 3051^T and 75.65% with *H. salicampi* NBRC 109914^T (Table S4). The DDH similarity and ANI values between strain QX-2^T and the reference bacteria are within the range of QX-2^T being identified as a new species, which requires a DDH similarity lower than the recommended value of $\pm 70\%$ [39] and ANI values below the standard criteria for species identity (95%–96%) [40].

Annotation results of the draft genome showed that there were 4890 coding genes in the genome of strain QX-2^T, including 65 RNA coding genes (Table S2). We found 35 genes encoding for proteins involved in heavy metal resistance for strain QX-2^T (Table S5). These genes provided heavy metal resistance to mercury, arsenic, manganese, cobalt, zinc, cadmium, lead and copper. This resistance most likely arises from adaptation of strain QX-2^T to the extreme environment of the southwest Indian Ocean hydrothermal region; this is a deep-sea hydrothermal region that is a typical heavy metal rich area [41]. Modern industry continues

to produce large quantities of waste-water containing heavy metals that are a major hidden danger to human safety and the environment. In recent years, biosorbents have been used for treating environmental pollution because these products are readily available, economically viable and display fast adsorption properties [42]. Given that strain QX-2^T produces proteins that afford heavy metal resistance, this strain has potential use in the development of biosorbents and offers insights into the effective control of heavy metal pollutants.

Microorganisms in extreme environments accumulate compounds to survive in high osmotic environments. These compounds mainly include amino acids and their derivatives, polyols, sugars, betaine and ectoine [43, 44]. Ectoine is the most common compatible solute synthesized by moderately halophilic bacteria. Ectoine is compatible with intracellular metabolism, balances osmotic pressure inside and outside the cell, protects against osmotic stress caused by high salt concentrations and is compatible with the intracellular system without affecting the function of other biological macromolecules [45]. Thus, ectoine protects cells and biomacromolecules under adverse environmental conditions [12, 46]. Therefore, ectoine has important application value in pharmaceutical preparations [47], cell protectants [48], cosmetics and other fields.

Genes associated with the synthesis of ectoine were identified in the genome of strain QX-2^T. Previous studies have shown that the biosynthesis of ectoine involves a series of enzymatic reactions. The genes *ectA*, *ectB* and *ectC* of strain QX-2^T may encode L-diaminobutyric acid transaminase,

L-diaminobutyric acid acetyltransferase and ectoine synthase, respectively, and using aspartate semialdehyde as the precursor substrate these three enzymes can catalyze the synthesis of ectoine in three steps [12]. The four genes *doeA*, *doeB*, *doeC* and *doeD* of strain QX-2^T may encode proteases that degrade ectoine [12]. The TeaABC system belongs to the TRAP-T transporter family, which may participate in the absorption and excretion of ectoine and regulate the biosynthesis of ectoine [49]. In addition, we also found the *rpoS* regulatory factor in strain QX-2^T, which is similar to that found in strain *Chromohalobacter salexigens*. This gene product may also participate in the biosynthesis of ectoine [50]. However, although there were genes related to the synthesis of ectoine in the genome of strain QX-2^T, the specific biosynthetic pathway for producing ectoine requires further studies and evaluation.

Conclusion

Phylogenetic analysis, phenotypic analysis and chemotaxonomic studies confirmed that strain QX-2^T represents a novel species within the genus *Halomonas*, for which the name *Halomonas sedimenti* sp. nov. is proposed. The genes encoding heavy metal resistance proteins and ectoine synthesis were found in the draft genome of strain QX-2^T. Thus, strain *Halomonas sedimenti* sp. nov. QX-2^T offers great potential in various commercial applications.

Taxonomic and Nomenclatural Proposals

Description of *Halomonas sedimenti* sp. nov.

Halomonas sedimenti (se.di.men'ti L. gen. n. *sedimenti*, of sediment, referring to the sediment of the Southwest Indian Ocean, where the type strain was isolated).

Cells are Gram-staining-negative, aerobic, halophilic, motile and rod-shaped with dimensions of 0.7–0.9 μm wide and 2.3–3.0 μm long. Colonies on MA are creamy, convex, glossy, smooth, circular with an entire margin and 1 mm in diameter after 1 day of incubation at 30 °C. Growth occurs in MB with 0%–30% NaCl (optimum 4%), at 4–50 °C (optimum 30 °C) and over the pH range of 5.0–12.0 (optimum pH 6.0). Starch, cellulose, gelatin, Tween 20, 40, 60 and 80 were not hydrolyzed. Catalase and oxidase activities were positive. Nitrate was reduced to nitrite. Indole and H₂S were not produced. Enzyme activity was observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase,

naphthol-AS-BI-phosphohydrolase, α-glucosidase, urease, arginine hydrolase and phenylalanine deaminase, but not for lipase (C14), trypsin, chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, α-mannosidase, α-fucosidase lysine decarboxylase or ornithine decarboxylase. Acid was produced from L-arabinose, D-fructose and aesculin, but not from D-ribose, D-xylose, L-xylose, D-galactose, D-glucose, L-rhamnose, cellobiose, D-maltose, D-lactose, melibiose, sucrose, trehalose, raffinose, D-mannitol, D-sorbitol, inositol, 2-ketogluconate or 5-ketogluconate. Strain QX-2^T utilized D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, D-lactose, D-melibiose, D-salicin, D-glucose, D-mannose, D-fructose, D-galactose, D-fucose, L-fucose, L-rhamnose, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, D-fructose-6-PO₄, D-aspartic acid, L-alanine, L-aspartic acid, L-glutamic acid, D-malic acid, L-malic acid, dextrin, inosine, glycerol, gelatin and citrate, but not arabinose, stachyose, D-raffinose, d-Glucose-6-PO₄, D-serine, L-serine, L-arginine, L-histidine or D-saccharic. Principal fatty acids (> 10%) determined were C_{16:0} (12.41%), C_{12:0-3OH} (25.15%), summed feature 3 (C_{16:1 ω7c} and/or C_{16:1 ω6c}, 11.55%) and summed feature 8 (C_{18:1 ω7c} and/or C_{18:1 ω6c}, 16.06%). The polar lipids were DPG, PG, PE, PL, APL and L1–L5. The main respiratory ubiquinone was Q-9. The DNA G+C content of the type strain was 54.34 mol%.

The type strain, QX-2^T (= MCCC 1A17876^T = KCTC 82199^T), was isolated from deep-sea sediment of the Southwest Indian Ocean at 2699 m. The GenBank accession number of the 16S rRNA gene sequence of strain QX-2^T is MT372904, and the draft genome sequence accession number of strain QX-2^T is JACCGK000000000.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00284-021-02425-9>.

Author Contributions XQ performed the technical characterization on strain QX-2 and drafted the manuscript. LY, XC, HW and GX conceived the study and aided to draft the manuscript. XT conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the COMRA Project of China (DY135-B2-16), COMRA Project of China (DY135-B2-08) and National Basic Research Program of China (973 Program) (No.2015CB755901).

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals.

References

- 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(3):485–495. <https://doi.org/10.1099/00207713-30-2-485>
- 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(1):10–18. <https://doi.org/10.1099/ijs.0.037036-0>
- Kaye JZ, Marquez MC, Ventosa A, Baross JA (2004) *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* 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(Pt 2):499–511. <https://doi.org/10.1099/ijs.0.02799-0>
- Jiang J, Pan Y, Hu S, Zhang X, Hu B, Huang H, Hong S, Meng J, Li C, Wang K (2014) *Halomonas songnenensis* sp. nov., a moderately halophilic bacterium isolated from saline and alkaline soils. *Int J Syst Evol Microbiol* 64(Pt 5):1662–1669. <https://doi.org/10.1099/ijs.0.056499-0>
- Gan L, Long X, Zhang H, Hou Y, Tian J, Zhang Y, Tian Y (2018) *Halomonas saliphila* sp. nov., a moderately halophilic bacterium isolated from a saline soil. *Int J Syst Evol Microbiol* 68(4):1153–1159. <https://doi.org/10.1099/ijsem.0.002644>
- Wang T, Wei X, Xin Y, Zhuang J, Shan S, Zhang J (2016) *Halomonas lutescens* sp. nov., a halophilic bacterium isolated from a lake sediment. *Int J Syst Evol Microbiol* 66(11):4697–4704. <https://doi.org/10.1099/ijsem.0.001413>
- Ming H, Ji WL, Li M, Zhao ZL, Cheng LJ, Niu MM, Zhang LY, Wang Y, Nie GX (2020) *Halomonas lactosivorans* sp. nov., isolated from salt-lake sediment. *Int J Syst Evol Microbiol* 70(5):3504–3512. <https://doi.org/10.1099/ijsem.0.004209>
- Xu L, Xu XW, Meng FX, Huo YY, Oren A, Yang JY, Wang CS (2013) *Halomonas zincidurans* sp. nov., a heavy-metal-tolerant bacterium isolated from the deep-sea environment. *Int J Syst Evol Microbiol* 63(Pt 11):4230–4236. <https://doi.org/10.1099/ijs.0.051656-0>
- Twardowska I (2004) Ecotoxicology, environmental safety, and sustainable development—challenges of the third millennium. *Ecotoxicol Environ Saf* 58(1):3–6. <https://doi.org/10.1016/j.ecoen.2004.03.008>
- Leon MJ, Hoffmann T, Sanchez-Porro C, Heider J, Ventosa A, Bremer E (2018) Compatible solute synthesis and import by the moderate halophile *Spiribacter salinus*: physiology and GENOMICS. *Front Microbiol* 9:108. <https://doi.org/10.3389/fmicb.2018.00108>
- Richter AA, Mais CN, Czech L, Geyer K, Hoepfner A, Smits SHJ, Erb TJ, Bange G, Bremer E (2019) Biosynthesis of the stress-protectant and chemical chaperon ectoine: biochemistry of the transaminase EctB. *Front Microbiol* 10:2811. <https://doi.org/10.3389/fmicb.2019.02811>
- Schwibbert K, Marin-Sanguino A, Bagyan I, Heidrich G, Lentzen G, Seitz H, Rampp M, Schuster SC, Klenk HP, Pfeiffer F, Oesterhelt D, Kunte HJ (2011) A blueprint of ectoine metabolism from the genome of the industrial producer *Halomonas elongata* DSM 2581 T. *Environ Microbiol* 13(8):1973–1994. <https://doi.org/10.1111/j.1462-2920.2010.02336.x>
- Van-Thuoc D, Guzman H, Quillaguaman J, Hatti-Kaul R (2010) High productivity of ectoines by *Halomonas boliviensis* using a combined two-step fed-batch culture and milking process. *J Biotechnol* 147(1):46–51. <https://doi.org/10.1016/j.jbiotec.2010.03.003>
- Skerman VBD (1960) A guide to the identification of the genera of Bacteria. *Q Rev Biol* 36(2):870
- Dong X-Z, Cai M-Y (2001) Determinative manual for routine bacteriology. Scientific Press, Beijing (**English translation**)
- Fykse EM, Tjarnhage T, Humpi T, Eggen VS, Ingebretsen A, Skogan G, Olofsson G, Wasterby P, Gradmark PA, Larsson A, Dybwad M, Blatny JM (2015) Identification of airborne bacteria by 16S rDNA sequencing, MALDI-TOF MS and the MIDI microbial identification system. *Aerobiologia* 31(3):271–281. <https://doi.org/10.1007/s10453-015-9363-9>
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC News* 20:1–6
- Kates M (1986) Lipid extraction procedures. *Techniques of lipidology*. Elsevier, Amsterdam, pp 100–111
- Collins MD, Jones D (1981) Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* 45(2):316–354
- Olsen GJ, Woese CR (1993) Ribosomal RNA: a key to phylogeny. *FASEB J* 7:113–123. <https://doi.org/10.2307/2420341>
- Lane DJ (1991) 16S/23S rRNA sequencing. *Nucleic Acid Tech. Bacteriol Syst.* 463:115–175
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62(Pt 3):716–721. <https://doi.org/10.1099/ijs.0.038075-0>
- 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(5):1613–1617. <https://doi.org/10.1099/ijsem.0.001755>
- Kumar S, Stecher G, Tamura KJMB (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 bigger dataset. *Evolution* 33(7):1870
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4(4):406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17(6):368–376
- 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(4):367–375
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19(5):455–477. <https://doi.org/10.1089/cmb.2012.0021>
- Oguntoyinbo FA, Cnockaert M, Cho GS, Kabisch J, Neve H, Bockelmann W, Wenning M, Franz C, Vandamme P (2018) *Halomonas nigrificans* sp. nov., isolated from cheese. *Int J Syst Evol Microbiol* 68(1):371–376. <https://doi.org/10.1099/ijsem.0.002515>
- Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinform.* 14:60. <https://doi.org/10.1186/1471-2105-14-60>
- Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–NA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57(Pt 1):81–91. <https://doi.org/10.1099/ijs.0.064483-0>
- Sanchez-Porro C, Kaur B, Mann H, Ventosa A (2010) *Halomonas titanicae* sp. nov., a halophilic bacterium isolated from the RMS Titanic. *Int J Syst Evol Microbiol* 60(Pt 12):2768–2774. <https://doi.org/10.1099/ijs.0.020628-0>

33. Reddy GS, Raghavan PU, Sarita NB, Prakash JS, Nagesh N, Delille D, Shivaji S (2003) *Halomonas glaciei* sp. nov. isolated from fast ice of Adelie Land, Antarctica. *Extremophiles* 7(1):55–61. <https://doi.org/10.1007/s00792-002-0295-2>
34. Sorokin DY, Tindall BJ (2006) The status of the genus name *Halovibrio Fendrich* 1989 and the identity of the strains *Pseudomonas halophila* DSM 3050 and *Halomonas variabilis* DSM 3051. Request for an opinion. *Int J Syst Evol Microbiol* 56(Pt 2):487–489. <https://doi.org/10.1099/ijs.0.63965-0>
35. 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(12):4792–4799. <https://doi.org/10.1099/ijsem.0.000650>
36. 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(2):550–558. <https://doi.org/10.1099/00207713-46-2-550>
37. 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(1):16–19. [https://doi.org/10.1016/S0723-2020\(88\)80043-2](https://doi.org/10.1016/S0723-2020(88)80043-2)
38. 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(Pt 3):733–737. <https://doi.org/10.1099/ijs.0.02942-0>
39. 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>
40. Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 106(45):19126–19131. <https://doi.org/10.1073/pnas.0906412106>
41. Edgcomb VP, Molyneux SJ, Saito MA, Lloyd K, Boer S, Wirsen CO, Atkins MS, Teske A (2004) Sulfide ameliorates metal toxicity for deep-sea hydrothermal vent archaea. *Appl Environ Microbiol* 70(4):2551–2555. <https://doi.org/10.1128/aem.70.4.2551-2555.2004>
42. Fu F, Wang Q (2011) Removal of heavy metal ions from wastewaters: a review. *J Environ Manage* 92(3):407–418. <https://doi.org/10.1016/j.jenvman.2010.11.011>
43. Ben-Amotz A, Avron M (1983) Accumulation of metabolites by halotolerant algae and its industrial potential. *Annu Rev Microbiol* 37:95–119. <https://doi.org/10.1146/annurev.mi.37.100183.000523>
44. Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. *Saline Syst* 4:2. <https://doi.org/10.1186/1746-1448-4-2>
45. Satyanarayana T (2012) Microorganisms in environmental management. Springer, Dordrecht. <https://doi.org/10.1007/978-94-007-2229-3>
46. Zaccai G, Bagyan I, Combet J, Cuello GJ, Deme B, Fichou Y, Gallat FX, Galvan Josa VM, von Gronau S, Haertlein M, Martel A, Moulin M, Neumann M, Weik M, Oesterhelt D (2016) Neutrons describe ectoine effects on water H-bonding and hydration around a soluble protein and a cell membrane. *Sci Rep* 6:31434. <https://doi.org/10.1038/srep31434>
47. Salvador M, Argandona M, Naranjo E, Piubeli F, Nieto JJ, Csonka LN, Vargas C (2018) Quantitative RNA-seq analysis unveils osmotic and thermal adaptation mechanisms relevant for ectoine production in *Chromohalobacter salexigens*. *Front Microbiol* 9:1845. <https://doi.org/10.3389/fmicb.2018.01845>
48. Czech L, Hoppner A, Kobus S, Seubert A, Riclea R, Dickschat JS, Heider J, Smits SHJ, Bremer E (2019) Illuminating the catalytic core of ectoine synthase through structural and biochemical analysis. *Sci Rep* 9(1):364. <https://doi.org/10.1038/s41598-018-36247-w>
49. 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(11):3078–3085. <https://doi.org/10.1128/jb.184.11.3078-3085.2002>
50. Salvador M, Argandoña M, Pastor JM (2015) Contribution of RpoS to metabolic efficiency and ectoines synthesis during the osmoand heat-stress response in the halophilic bacterium *Chromohalobacter salexigens*. *Environmental Microbiology Reports* 7(2):301–311

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.