Halomonas Jincaotanensis sp. nov., Isolated From the Pamir Plateau Degrading Polycyclic Aromatic Hydrocarbon

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Abstract

A Gram-stain-negative, rod-shaped, aerobic bacterium, designated strain TRM 85114^T, was isolated from the Jincaotan wetland in the Pamir Plateau of China. We identified this isolate as a novel species with an ability to degrade 1-naphthylamine. Strain grew optimally at 30 °C and pH 6.0 in the presence of 3% (w/v) NaCl. Phylogenetic analysis of 16S rRNA gene sequences revealed that strain TRM 85114^T was affiliated with the genus *Halomonas*, and shared high sequence similarity (97.3%) with *Halomonas korlensis* XK1^T. Strain TRM 85114^T contained C_{16:0} and C_{19:0} cyclo ω 8c as primary cellular fatty acids, Q-9 as predominate respiratory quinone, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phospholipids of unknown structure containing glucosamine, unidentified aminophospholipids, unidentified lipids and three unidentified phospholipids as the major polar lipids. The complete genome of TRM 85114^T comprises 3,902 putative genes with a total of 4,126,476 bp and a G + C content of 61.6 mol%. The average nucleotide identity and digital DNA–DNA hybridization values between strain TRM 85114^T and related type *Halomonas* strains was 78.6–88.9% and 25.7–39.2%, respectively. Based on phenotypic, chemotaxonomic, and molecular features, strain TRM 85114^T represents a novel species of the genus *Halomonas*, due to which the name *Halomonas jincaotanensis* sp. nov. is proposed. The type strain is TRM 85114^T (CCTCC AB 2021006^T = LMG 32311^T). Degradation rate of 1-naphthylamine by strain TRM 85114^T reached up to 32.0 mg/L in 14 days.

Introduction

Halophilic microorganisms, particularly *Halomonas* spp., usually accumulate polyphosphate (Nguyen et al. 2012), produce biodegradable polyhydroxyalkanoates (Jiang et al. 2018; Tuma et al. 2020) and various metabolic chemicals (Du et al. 2020; Jiang et al. 2021). Based on advantages of energy-saving, water-saving, time-saving, less investment in equipment, high concentration of final product, and simplification of separation process, *Halomonas* spp. have been previously used as platform strains to produce multiple products in Next Generation Industrial Biotechnology (NGIB) (Yu et al. 2019). The genus *Halomonas* initially proposed by Vreeland et al. (1980) belongs to the family *Halomonadaceae* of the phylum Proteobacteria. At present, this genus contains more than 160 validly named species. Members of the genus *Halomonas* have been isolated from diverse terrestrial and aquatic habitats, such as lake water (Kazemi et al. 2021), saline-alkali land (Dou et al. 2015), tidal flat (Koh et al. 2017), Arctic marine (Williamson et al. 2016), deep-sea sediment (Xu et al. 2013), and hypersaline wetlands (Ramezani et al. 2020). The Jincaotan wetland in Pamir Plateau is an environment characterised with saline and high altitude, in which extremophiles could be abundant.

Polycyclic aromatic hydrocarbon (PAH) is one of the most common pollutants in the soil and/or water environment and have become a matter of urgency due to their negative impacts on human health (Premnath et al. 2021). 1-Naphthylamine is a derivative of PAHs, as one of the top priority contaminants and carcinogens (Hu et al. 2011), significant accumulation in the soil and subsequent migration into the aquatic system have led to chronic exposure, which is associated with cancerous diseases in humans

and aquatic animals and enhanced mutagenicity of soil and/or sediments. The genus of *Halomonas* has shown an eminent capacity to degrade polycyclic aromatic hydrocarbons (Al Farraj et al. 2020; Govarthanan et al. 2020), such as anthracene, phenanthrene, pyrene, naphthalene, and benzo [a]pyrene, and therefore have tremendous potential for environmental remediation. In this study, we obtained a novel *Halomonas*-like strain isolated from the Pamir Plateau, designated TRM 85114^T, explored its taxonomic characterisation and ability to degrade 1-naphthylamine, which has yet to be reported.

Materials And Methods

Sample collection, isolation, and preservation

A soil sample was collected from the Jincaotan wetland (37°47'N, 75°16'E) in the Pamir Plateau, 3100 m above sea level. During the isolation process, 100 µL of mixed water and soil sample was spread onto 12% modified growth medium (MGM) agar plates (Dyall-Smith 2015), and incubated at 15 °C for 2 weeks. Colonies showing different morphologies were picked and purified, among which strain TRM 85114^T was routinely grown on 12% MGM agar. Colonies were preserved in 12% MGM containing 50% (v/v) glycerol at -80 °C. The reference strains *H. korlensis* CGMCC 1.6981^T, *Halomonas urumqiensis* CGMCC 1.12917^T, and *Halomonas daqiaonensis* CGMCC 1.9150^T were obtained from the China General Microbiological Culture Collection Center (CGMCC), while *Halomonas tibetensis* KCTC 52660^T was obtained from the Korean Collection for Type Cultures (KCTC). All strains were cultured under the same conditions, as specified, for comparative purposes.

Phenotypic and physiological identification

Cellular morphology was observed under light microscopy (Leica DM1000) and scanning electron microscopy (SEM) (Quanta; FEI) using cells grown on 12% MGM agar plates at 30 °C for 4 days. Anaerobic growth was carried out using a MGC AnaeroPack Series (MGC) on 12% MGM agar plates, which replaced O₂ with CO₂, and motility was tested on 0.5% 12% MGM agar. The temperature range (4, 8, 16, 24, 28, 30, 32, 35, 37, 40, and 45 °C), pH range (5.0-11.0, at 1.0 pH unit intervals) [50 mM MES (pH 5; 6), HEPES (pH 7), TAPS (pH 8), CAPSO (pH 9; 10), and CAPS (pH 11)], and NaCl tolerance (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, and 30 w/v%) for growth were evaluated in 12% MGM broth at 30 °C for 3 days. Tolerance to different NaCl concentrations were tested in basic 12% MGM liquid medium without NaCl. Growth requirements of Mg²⁺, SO₄²⁻, K⁺ as well as production of exopolysaccharide and polyhydroxyalkanoate (PHA) were determined as described previously (Poli et al. 2013). Physiological tests of oxidation/fermentation of D-glucose, respiration of nitrate and nitrite, production of H₂S from L-cysteine, reduction of nitrate to nitrite, hydrolysis of gelatin, casein, starch, Tween (20, 40, 60, and 80), aesculin, and DNA, production of indole, test of methyl red and Voges-Proskauer, activity of catalase, oxidase, urease, phenylalanine deaminase, lysine and ornithine decarboxylases, and *o*-nitrophenyl-β-D-galactopyranosidase were performed according to the ways proposed previously (Mata et al. 2002; Arahal et al. 2007). Rapid identification systems, including API 20E, API 20NE, and API 50CH (bioMérieux), were applied to detect acid production and substrate utilization in three replicates at 30 °C

for 72 h, in accordance with the manufacturer's instructions. Antibiotic susceptibility was detected by filter paper discs containing the following antimicrobial compounds (content per disc): ampicillin (10 µg), vancomycin (30 µg), carbenicillin (100 µg), penicillin (1 µg), oxacillin (1 µg), piperacillin (100 µg), cefalexin (30 µg), cefradine (30 µg), ceftriaxone (30 µg), cefoperazone (75 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), tetracycline (30 µg), deoxytetracycline (30 µg), minocycline (30 µg), erythromycin (15 µg), norfloxacin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), polymyxin B (300 µg), Bactrim (25 µg), or clindamycin (2 µg) on 12% MGM agar plates with inoculated strain TRM 85114^T. The inhibition zones were measured as described previously by Zhong et al. (2016), after incubation at 30 °C for 2 days. The test was conducted in triplicate and included only results exhibiting the same resistant pattern in all the plates.

Chemotaxonomic characterization

The respiratory quinones of strain TRM 85114^T was extracted from freeze-dried biomass (Collins et al. 1977), and subsequently confirmed using HPLC (Collins and Jones 1981). For the analysis of cellular fatty acids, strain TRM 85114^T and four reference strains were cultured on 12% MGM plates at 30 °C and the microbial cells were harvested at the 4th day. The fatty acids were extracted from fresh cells (Athalye et al. 1985), and analyzed by gas chromatography referred to the Microbial Identification System (Sherlock version 6.1; MIDI database: RTSBA6). The compositions of polar lipids were extracted, separated using two-dimensional TLC (Jain and Subrahmanyam 1978) and determined by spraying with four dyes of 10% ethanolic molybdatophosphoric acid (for total lipids), ninhydrin (for aminolipids), molybdenum blue (for phospholipids), and anisaldehyde (for glycolipids), respectively.

Sequence similarity and phylogenetic analysis

Genomic DNA of strain TRM 85114^T was extracted by using a TIANGEN (Beijing, China) bacterial DNA extraction kit. The 16S rRNA gene of strain TRM 85114^T was amplified and sequenced using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACCTTGTTACGACTT-3'). Primers *gyrB*216F (5'-GARGTBATCATGACSGTGCT-3') and *gyrB*1419R (5'-GCRTCSGTCATGATGATSAY-3'), *rpoD*88F (5'-ATGATYAACGACATGGGYAT-3') and *rpoD*1321R (5'-TTS AKCTTRTTGATGGTCTC-3') were applied to amplified and sequenced the housekeeping genes *gyrB* and *rpoD*, respectively. The 16S rRNA gene sequence was then compared with the available sequences using EzBioCloud database (<https://www.ezbiocloud.net/identify>) (Yoon et al. 2017) and the NCBI database (<https://blast.ncbi.nlm.nih.gov>) (Altschul et al. 1990). Phylogenetic trees based on neighbor-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981), and maximum-parsimony (MP) (Czelusniak et al. 1990) algorithms with bootstrap values (1,000 replications) (Felsenstein 1985), and based on Kimura two-parameter model (Kimura 1980) to calculated evolutionary distance, were constructed using MEGA version 7.0 software (Kumar et al. 2016). The 16S rRNA, *gyrB*, and *rpoD* gene sequences of strain TRM 85114^T and the most closely species were aligned using CLUSTAL_X software (Thompson et al. 1997), and made multilocus sequence analysis (MLSA) (de la Haba et al. 2012) to infer the phylogenetic relationships of strain TRM 85114^T.

Genome sequencing and analysis

The whole genome of strain TRM 85114^T was sequenced on the Illumina Hiseq platform and assembled using the ABySS (version 2.0) assembler (Jackman et al. 2017), with a depth-sequencing coverage of 456×. We used the software CheckM to evaluate the completeness and contamination of the genome (Parks et al. 2015), the online resource prodigal to predict assembled genome (<http://compbio.ornl.gov/prodigal/>) (Hyatt et al. 2010), software tRNA scan-SE to predict the tRNAs in the genome (Lowe and Eddy 1997), and the software Infernal 1.1 (Nawrocki and Eddy 2013) to predict the rRNAs in the genome based on the Rfam database (Nawrocki et al. 2015). Genome annotation was performed by Personal Biotechnology Co., Ltd (Shanghai, China). The genomic DNA G+C content was calculated based on the entire genome sequence. Prediction of the average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values were estimated using the ChunLab's online ANI Calculator ([www.ezbiocloud.net/tools/ani](http://ezbiocloud.net/tools/ani)) (Lee et al. 2016) and the Genome-to-Genome Distance Calculator (<http://ggdc.dsmz.de/distcalc2.php>) (Meier-Kolthoff et al. 2013), respectively. To investigate the details of the phylogenetic placement of TRM 85114^T, we constructed a phylogenetic tree based on single-copy orthologues identified by grouping orthologous protein sequences from strain TRM 85114^T and 19 other related species of *Halomonas*, whose genome has been acquired from the NCBI database. Based on all amino acid sequences of the selected species, software Orthofinder (Emms and Kelly 2019) was used to cluster gene family, Blastp to compare, and software Muscle (Edgar 2004) to compare multiple protein sequences in each single-copy gene family. In addition, software Trimal (Capella-Gutierrez et al. 2009) to filter the comparison results, merge the filtered comparison results and connect them into super genes. Finally, software RAxML (Stamatakis 2014) was used to construct a maximum-likelihood phylogenetic tree based on supergene.

Detection of 1-naphthylamine degradation capacity

Batch experiments were performed to study the ability of TRM 85114^T on 1-naphthylamine biodegradation (Govarthanan et al. 2020). Isolate TRM 85114^T was inoculated into 250 mL Erlenmeyer flasks containing 100 mL 12% MGM broth with 1-naphthylamine (5 mg), then incubated in 180 rpm at 30 °C for 14 days. Samples (1 mL) were collected on days 0, 2, 4, 6, 8, 10, 12 and 14, and detected the content of 1-naphthylamine using HPLC at 222 nm, based on methanol as gradient elution ranging from 10% to 100% for 40 min. Furthermore, the content of 1-naphthylamine in the samples was calculated based on a standard curve, which was constructed according to different contents of 1-naphthylamine solutions (20, 40, 60, 80, 100, and 120 mg/L). All the experiments were performed in triplicate, and mean values were reported. The isolates without 1-naphthylamine treatment were used as control.

Results And Discussion

Phenotypic and physiological characterization

The cells of strain TRM 85114^T was Gram-negative, short rod-shaped, aerobic, and non-motile bacteria (Fig. 1). Colonies were cream-white and smooth with entire margins after incubated at 30 °C on 12% MGM plates for 4 days. Strain TRM 85114^T grew occurred at 4–35 °C (optimum, 30 °C), NaCl 1–10% (w/v) (optimum, 3%), and pH 6.0–9.0 (optimum, 6.0). Physiological and biochemical characteristics of strain TRM 85114^T are additionally described in the species descriptions and compared with those of closely related *Halomonas* type strains in Table 1.

Chemotaxonomic characteristics

The predominate respiratory quinone identified in strain TRM 85114^T was Q-9 (34.8%), followed by Q-8 (15.2%), Q-6 (4.7%) and an unknown quinone (45.2%), which were coincide to members detected in other strains of the genus *Halomonas* (Qu et al. 2011; Zhang et al. 2016; Lu et al. 2018). As shown in Supplementary Table 1, the primary cellular fatty acids in strain TRM 85114^T were C_{16:0} (18.0%) and C_{19:0} cyclo ω 8c (10.3%), in that C_{10:0} (0.8%), C_{11:0} 3-OH (0.3%), and C_{20:2} ω 6,9c (0.3%) were generally similar to those detected in other closely related strains. Moreover, strain TRM 85114^T was different from the four reference strains in terms of the types of polar lipids present, including diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phospholipids of unknown structure containing glucosamine (NPG), unidentified aminophospholipids, unidentified lipids, and three unidentified phospholipids (Supplementary Fig. S1). Two components of PG and PE were detected in most *Halomonas* spp., but NPG were evidently different from other strains of the genus *Halomonas* (Zhong et al. 2016; Lu et al. 2018; Ramezani et al. 2020). Based on the information of genome annotation, a process of UDP-N-acetylglucosamine biosynthetic was searched, which suggests that the presence of NPG in strain TRM 85114^T.

Phylogenetic and phylogenomic analyses

Based on the EzBioCloud analysis, the 16S rRNA gene sequence of strain TRM 85114^T (GenBank accession no. MW584241) has highly similar with members of the genus *Halomonas*. The highest similarity was shown with the type strain of *H. korlensis* XK1^T (97.3%), followed by *H. tibetensis* pyc13^T (96.4%), *H. urumqiensis* BZ-SZ-XJ27^T (96.1%), *H. daqiaonensis* CGMCC 1.9150^T (96.0%). However, the sequence similarity was less than 96.0% with other species of genera within the family *Halomonadaceae*. Above all analysis of 16S rRNA gene sequences of strain TRM 85114^T, it was considered as a possible novel species in the genus *Halomonas*.

A NJ tree based on 16S rRNA gene (1504 bp) (Fig. 2) showed that strain TRM 85114^T clustered tightly with *H. korlensis* XK1^T with strong bootstrap support (> 99%) forming an independent sub-cluster with the members of the genus *Halomoans*, which was in good agreement with the results of the ML and MP trees (Supplementary Fig. S2 and S3). A NJ tree based on the concatenated gene sequences (16S rRNA; *gyrB* 472 bp; *rpoD* 1243 bp) phylogenetic tree (Supplementary Fig. S4) showed that strain TRM 85114^T forming an independent cluster separating from other group members. To further elucidate the

phylogenetic relationship of strain TRM 85114^T, we compared its proteome with 19 related species to investigate evolutionary specialization. A total of 8,805 orthologous gene families consisting of 71,909 genes were identified across these species, including a core set of 1140 genes shared among them. The ML tree based on these core gene sequences revealed the position of strain TRM 85114^T (Fig. 3), which was grouped into a new clade comprising *H. korlensis* XK1^T, which was consistent with the MLSA phylogenetic trees, supporting the view that strain TRM 85114^T represent a novel member of the genus *Halomonas*.

Genomic features and analysis

The obtained draft genome of TRM 85114^T (accession no. JAHCLU0000000000) was 4,126,476 bp in length with G+C content of 61.6 mol%, comprising of 60 contigs (N50 = 190,960 bp) and 52 scaffolds (N50 = 208,915 bp). The annotated genome encodes 3902 genes, including 1666 CDSs, 57 tRNAs and 3 rRNAs (one copy of 5S, one copy of 16S, and one copy of 23S rRNA gene). The general features of the genomes of strains of TRM 85114^T, including genome size, DNA G + C contents, and the numbers of rRNA and tRNA genes, were similar with those of closely related reference strains (Table 3). The ANI and dDDH values between strain TRM 85114^T and the type strains of *H. korlensis* XK1^T, *H. urumqiensis* BZ-SZ-XJ27^T, *H. daqiaonensis* CGMCC 1.9150^T were significantly lower than the the criteria for the prokaryotic species delineation thresholds for ANI (95–96%) (Kim et al. 2014) and dDDH (70%) (Wayne et al. 1987) (Table 3), suggesting that strain TRM 85114^T represents a novel species of the genus *Halomonas*.

To survive and compete in diverse environmental habitats, *Halomonas* species evolved a variety of metabolic pathways. Based on the KEGG orthology-based annotation, 3544 genes (90.8%) were annotated and assigned to putative functions, of which 1389 genes were related to metabolism associated pathways, and 249 genes belonged to environmental information processing pathways. What's more, 44 ORFs were detected for aromatic hydrocarbon degradation (Supplementary Table S2), in which contig15_2804 (*frmA*, *ADH5*, *adhC*) were comment as naphthalene degradation. The above data indicated taht TRM 85114^T is a novel strain of the genus *Halomonas* with naphthalene degradation ability.

1-Naphthylamine degradation rate

The biodegradation efficiency of 1-naphthylamine by strain TRM 85114^T was further verified and confirmed in this study. Results indicated that the retention time of 1-naphthylamine was at the 26th min, and the absorption peak area of it decreased significantly over time (Supplementary Fig. S5). According to the standard curve of 1-naphthylamine (Supplementary Fig. S6), the degradation rates of strain TRM 85114^T on day 4 and 14 was calculated to be 21.6 mg/L and 32.0 mg/L, respectively (Supplementary Fig. S7). These findings indicated that TRM 85114^T has a strong ability to degrade 1-naphthylamine, which may be applied to treat sewage containing 1-naphthylamine in printing and dyeing plants. As a result,

strain TRM 85114^T has important environmental remediation ability could contribute for bioremediation of water and/or soil systems.

Taxonomical conclusion

The results of phylogenetic analyses based on 16S rRNA gene, housekeeping gene, and core gene analyses illustrate that strain TRM 85114^T was a member of the genus *Halomonas*. Furthermore, phenotypic and chemotaxonomic traits showed that strain TRM 85114^T was distinct from several closely related species in the genus *Halomonas*. In addition, the genomic relatedness (ANI and dDDH values) also suggested that strain TRM 85114^T was distinguishable from its closest phylogenetic neighbour *H. korlensis* XK1^T. In conclusion, a new species, *Halomonas jincaotanensis* sp. nov., is proposed as the type strain.

Description of *Halomonas jincaotanensis* sp. nov.

Halomonas jincaotanensis (jin.caotan.en' sis. NL fem. adj. jincaotanensis pertaines to Jincaotan, Pamir Plateau, Xinjiang, China, from where the type strain was isolated).

Cells are Gram-strain-negative, short rod-shaped, non-motile, and aerobic (0.9–1.0 × 0.4–0.5 µm in size). Colonies are circular, cream-white, convex, and smooth. It grows between 4–35 °C (optimum, 30 °C), tolerate for 1–10% (w/v) NaCl (optimum, 3%), and pH of 6.0–9.0 (optimum, pH 6.0). This strain has the ability to hydrolyze starch Starch, aesculin, Tween 20, 40, and 60, but not Tween 80, casein, DNA, gelatin, and urea. Positive for catalase, oxidase, phenylalanine deaminase, reduce nitrate to nitrite, ferment D-glucose to produce acid, respire on nitrate and nitrite, produce H₂S from L-cysteine, produce poly-β-hydroxyalkanoate, and methyl red test, but negative for lysine and ornithine decarboxylases, α-Nitrophenyl-β-D-galactopyranosidase, produce indole, produce exopolysaccharides and Voges-Proskauer test. Growth without Mg²⁺, SO₄²⁻, and K⁺. Acid is produced from D-glucose, rhamnose, and arabinose, but not from mannitol, inositol, sorbitol, sucrose, melibiose, and amygdalin. D-Mannose and malic acid are assimilated instead of D-glucose, arabinose, mannose, mannitol, maltose, gluconate, hydroxydecanoate, citric acid, adipic acid, and phenylacetic acid. D-glucose, D-fructose, mannitol, sorbitol, glycerin, inositol, glucuronic acid, maltose, and fumaric acid can be used as sole carbon source, individually. Susceptible to most antibiotics including vancomycin, penicillin, piperacillin, cefradine, ceftriaxone, cefoperazone, gentamicin, kanamycin, neomycin, erythromycin, norfloxacin, ofloxacin, ciprofloxacin, polymyxin B, and Bactrim, but resistant to ampicillin, carbenicillin, oxacillin, cefalexin, tetracycline, minocycline, clindamycin, and deoxytetracycline. The predominant respiratory quinone is Q-9. The major fatty acids of the cells are C_{16:0} and C_{19:0} cyclo ω8c. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phospholipids of unknown structure containing glucosamine, unidentified aminophospholipids, unidentified lipids, and three unidentified phospholipids. The genomic G+C content of the type strain is 61.6 mol%. The degradation rate of 1-naphthylamine is 32.0 mg/L in 14 days.

The type strain, TRM 85114^T (= CCTCC AB 2021006^T = LMG 32311^T), was isolated from the wetland soil of Jincaotan in the Pamir Plateau. The GenBank accession numbers for the 16S rRNA gene and the draft genome sequences of strain TRM 85114^T are MW584241 and JAHCLU0000000000, respectively.

Declarations

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Author contributions

XB performed the experiments, analyzed the data, and drafted the manuscript. ZL conducted the degradation test of 1-naphthylamine. ZX, CW, and MR critically revised the manuscript. LZ contributed to the creation. All authors read and approved the manuscript. We thank HC and PX for finding and providing *Halomonas tibetensis* pyc13^T.

Conflict of interests

All authors declare no conflict of interest.

Ethical approval

No specific ethical or institutional permission was required for sampling, and our experimental studies did not involve endangered or protected species.

References

1. Al Farraj DA, Hadibarata T, Yuniarto A et al (2020) Exploring the potential of halotolerant bacteria for biodegradation of polycyclic aromatic hydrocarbon. *Bioprocess Biosyst Eng* 43:2305–2314. <https://doi.org/10.1007/s00449-020-02415-4>
2. Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
3. Arahal DR, Vreeland RH, Litchfield CD et al (2007) Recommended minimal standards for describing new taxa of the family *Halomonadaceae*. *Int J Syst Evol Microbiol* 57:2436–2446. <https://doi.org/10.1099/ijs.0.65430-0>
4. Athalye M, Noble WC, Minnikin DE (1985) Analysis of cellular fatty acids by gas chromatography as a tool in the identification of medically important coryneform bacteria. *J Appl Bacteriol* 58:507–512. <https://doi.org/10.1111/j.1365-2672.1985.tb01491.x>

5. Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972–1973.
<https://doi.org/10.1093/bioinformatics/btp348>
6. Collins MD, Jones D (1981) Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* 45:316–354. <https://doi.org/10.1128/mr.45.2.316-354.1981>
7. Collins MD, Pirouz T, Goodfellow M et al (1977) Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 100:221–230. <https://doi.org/10.1099/00221287-100-2-221>
8. Czelusniak J, Goodman M, Moncrief ND et al (1990) Maximum parsimony approach to construction of evolutionary trees from aligned homologous sequences. *Methods Enzymol* 183:601–615.
[https://doi.org/10.1016/0076-6879\(90\)83039-c](https://doi.org/10.1016/0076-6879(90)83039-c)
9. de la Haba RR, Marquez MC, Papke RT et al (2012) Multilocus sequence analysis of the family *Halomonadaceae*. *Int J Syst Evol Microbiol* 62:520–538. <https://doi.org/10.1099/ijs.0.032938-0>
10. Dou G, He W, Liu H et al (2015) *Halomonas heilongjiangensis* sp. nov., a novel moderately halophilic bacterium isolated from saline and alkaline soil. *Antonie Van Leeuwenhoek* 108:403–413.
<https://doi.org/10.1007/s10482-015-0493-4>
11. Du H, Zhao Y, Wu F et al (2020) Engineering *Halomonas bluephagenesis* for L-Threonine production. *Metab Eng* 60:119–127. <https://doi.org/10.1016/j.ymben.2020.04.004>
12. Dyll-Smith M (2015) The Halohandbook. <http://www.haloarchaea.com/resources/halohandbook/>
13. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>
14. Emms DM, Kelly S (2019) OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol* 20:238. <https://doi.org/10.1186/s13059-019-1832-y>
15. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376. <https://doi.org/10.1007/BF01734359>
16. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evol Int J Org Evol* 39:783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
17. Govarthanan M, Khalifa AY, Kamala-Kannan S et al (2020) Significance of allochthonous brackish water *Halomonas* sp. on biodegradation of low and high molecular weight polycyclic aromatic hydrocarbons. *Chemosphere* 243:125389. <https://doi.org/10.1016/j.chemosphere.2019.125389>
18. Hu J, Shao D, Chen C et al (2011) Removal of 1-naphthylamine from aqueous solution by multiwall carbon nanotubes/iron oxides/cyclodextrin composite. *J Hazard Mater* 185:463–471.
<https://doi.org/10.1016/j.jhazmat.2010.09.055>
19. Hyatt D, Chen GL, Locascio PF et al (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <https://doi.org/10.1186/1471-2105-11-119>
20. Jackman SD, Vandervalk BP, Mohamadi H et al (2017) ABySS 2.0: resource-efficient assembly of large genomes using a Bloom filter. *Genome Res* 27:768–777.
<https://doi.org/10.1101/gr.214346.116>

21. Jain SK, Subrahmanyam D (1978) Two dimensional thin-layer chromatography of polar lipids. *Ital J Biochem* 27:11–18
22. Jiang XR, Yan X, Yu LP et al (2021) Hyperproduction of 3-hydroxypropionate by *Halomonas bluephagenesis*. *Nat Commun* 12:1513. <https://doi.org/10.1038/s41467-021-21632-3>
23. Jiang XR, Yin J, Chen XB, Chen GQ (2018) *Halomonas* and Pathway Engineering for Bioplastics Production. *Methods Enzymol* 608:309–328. <https://doi.org/10.1016/bs.mie.2018.04.008>
24. Kazemi E, Tarhriz V, Amoozegar MA et al (2021) *Halomonas azerbaijanica* sp. nov., a halophilic bacterium isolated from Urmia Lake after the 2015 drought. *Int J Syst Evol Microbiol* 71. <https://doi.org/10.1099/ijsem.0.004578>
25. Kim M, Oh HS, Park SC et al (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 64:346–351. <https://doi.org/10.1099/ijms.0.059774-0>
26. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120. <https://doi.org/10.1007/bf01731581>
27. Koh HW, Rani S, Kim SJ et al (2017) *Halomonas aestuarii* sp. nov., a moderately halophilic bacterium isolated from a tidal flat. *Int J Syst Evol Microbiol* 67:4298–4303. <https://doi.org/10.1099/ijsem.0.001824>
28. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>
29. Lee I, Ouk Kim Y, Park SC et al (2016) OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 66:1100–1103. <https://doi.org/10.1099/ijsem.0.000760>
30. Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25:955–964. <https://doi.org/10.1093/nar/25.5.955>
31. Lu HB, Xing P, Zhai L et al (2018) *Halomonas tibetensis* sp. nov., isolated from saline lakes on Tibetan Plateau. *J Microbiol* 56:493–499. <https://doi.org/10.1007/s12275-018-8076-5>
32. Mata JA, Martinez-Canovas J, Quesada E et al (2002) A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Syst Appl Microbiol* 25:360–375. <https://doi.org/10.1078/0723-2020-00122>
33. Meier-Kolthoff JP, Auch AF, Klenk HP et al (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <https://doi.org/10.1186/1471-2105-14-60>
34. Nawrocki EP, Burge SW, Bateman A et al (2015) Rfam 12.0: updates to the RNA families database. *Nucleic Acids Res* 43:D130–137. <https://doi.org/10.1093/nar/gku1063>
35. Nawrocki EP, Eddy SR (2013) Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics* 29:2933–2935. <https://doi.org/10.1093/bioinformatics/btt509>

36. Nguyen HT, Nielsen JL, Nielsen PH (2012) '*Candidatus Halomonas phosphatis*', a novel polyphosphate-accumulating organism in full-scale enhanced biological phosphorus removal plants. *Environ Microbiol* 14:2826–2837. <https://doi.org/10.1111/j.1462-2920.2012.02826.x>
37. Parks DH, Imelfort M, Skennerton CT et al (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>
38. Poli A, Nicolaus B, Denizci AA et al (2013) *Halomonas smyrnensis* sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* 63:10–18. <https://doi.org/10.1099/ijms.0.037036-0>
39. Premnath N, Mohanrasu K, Guru Raj Rao R et al (2021) A crucial review on polycyclic aromatic Hydrocarbons - Environmental occurrence and strategies for microbial degradation. *Chemosphere* 280:130608. <https://doi.org/10.1016/j.chemosphere.2021.130608>
40. Qu L, Lai Q, Zhu F et al (2011) *Halomonas daqiaonensis* sp. nov., a moderately halophilic, denitrifying bacterium isolated from a littoral saltern. *Int J Syst Evol Microbiol* 61:1612–1616. <https://doi.org/10.1099/ijms.0.026344-0>
41. Ramezani M, Pourmohyadini M, Nikou MM et al (2020) *Halomonas lysinitropha* sp. nov., a novel halophilic bacterium isolated from a hypersaline wetland. *Int J Syst Evol Microbiol* 70:6098–6105. <https://doi.org/10.1099/ijsem.0.004504>
42. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
43. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
44. Thompson JD, Gibson TJ, Plewniak F et al (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882. <https://doi.org/10.1093/nar/25.24.4876>
45. Tuma S, Izaguirre JK, Bondar M et al (2020) Upgrading end-of-line residues of the red seaweed *Gelidium sesquipedale* to polyhydroxyalkanoates using *Halomonas boliviensis*. *Biotechnol Rep (Amst)* 27:e00491. <https://doi.org/10.1016/j.btre.2020.e00491>
46. Vreeland R, Litchfield C, Martin E et al (1980) *Halomonas elongata*, a new genus and species of extremely salttolerant bacteria. *Int J Syst Evol Microbiol* 30:485–495
47. Williamson A, De Santi C, Altermark B et al (2016) Complete genome sequence of *Halomonas* sp. R5-57. *Stand Genomic Sci* 11:62. <https://doi.org/10.1186/s40793-016-0192-4>
48. Xu L, Xu XW, Meng FX et al (2013) *Halomonas zincidurans* sp. nov., a heavy-metal-tolerant bacterium isolated from the deep-sea environment. *Int J Syst Evol Microbiol* 63:4230–4236. <https://doi.org/10.1099/ijms.0.051656-0>
49. Yoon SH, Ha SM, Kwon S et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617. <https://doi.org/10.1099/ijsem.0.001755>

50. Yu LP, Wu FQ, Chen GQ (2019) Next-Generation Industrial Biotechnology-Transforming the Current Industrial Biotechnology into Competitive Processes. *Biotechnol J* 14:e1800437.
<https://doi.org/10.1002/biot.201800437>
51. Zhang S, Pan J, Lu W et al (2016) *Halomonas urumqiensis* sp. nov., a moderately halophilic bacterium isolated from a saline-alkaline lake. *Int J Syst Evol Microbiol* 66:1962–1969.
<https://doi.org/10.1099/ijsem.0.000975>
52. Zhong ZP, Liu Y, Wang F et al (2016) *Lacimicrobium alkaliphilum* gen. nov., sp. nov., a member of the family Alteromonadaceae isolated from a salt lake. *Int J Syst Evol Microbiol* 66:422–429.
<https://doi.org/10.1099/ijsem.0.000735>

Tables

Table 1

Differential characteristics of strain TRM 85114^T and related species of the genus *Halomonas*.

Characteristic	1	2	3	4	5
Colony pigmentation	Cream–white	Brown	Brown	Cream–yellow	Cream–yellow
Cell morphology	Short rods	Rods or short [⊠] rods [⊠]	Rods [†]	Short rod [⊠]	Rods [‡]
Cell size (μm)	0.4–0.5×0.9–1.0	0.8–1.0×1.3–1.7 [⊠]	0.4×0.6–1.2 [†]	1.0×1.5–2.0 [⊠]	0.3–0.6×1.2–2.2 [‡]
Mobility	–	–	–	+	+
Temperature range (°C)	4–35 (30)	4–37 (30)	8–37 (30)	4–40 (37)	4–40 (37)
Salinity range (% w/v)	1–10 (3)	1–20 (7)	1–15 (6)	1–25 (8)	1–15 (8)
pH range	6–9 (6)	6–9 (7)	6–10 (8)	6–9 (8)	6–9 (8)
Hydrolysis of:					
Starch	+	+	–	–	–
Tween40	+	–	–	–	–
Tween60	+	–	–	–	–
H ₂ S production	+	+	–	–	–
Nitrate reduction	–	+	–	+	+
Voges-Proskauer test	+	+	+	+	–
Urease activity	–	+	–	–	–
Phenylalanine deaminase activity	+	–	–	–	+
Exopolysaccharides	–	+	+	–	–
Acid from (API 20E):					
D-Glucose	+	+	–	+	–

Taxa: 1, Strain TRM 85114^T; 2, *H. korlensis* CGMCC 1.6981^T; 3, *H. tibetensis* KCTC 52660^T; 4, *H. urumqiensis* CGMCC 1.12917^T; 5, *H. daqiaonensis* CGMCC 1.9150^T. Except special note parts, data are from this study. +, positive; –, negative; ND, not determined.

[⊠] Data from Li et al. (2008); [†] Data from Lu et al. (2018); [⊠]Data from Zhang et al. (2016); [‡] Data from Qu et al. (2011)

Characteristic	1	2	3	4	5
Sucrose	–	+	–	+	–
Rhamnose	+	–	–	+	–
Arabinose	+	+	–	–	–
Melibiose	–	+	–	–	–
Assimilation of (API 20NE):					
D-Glucose	–	–	–	+	–
D-Mannose	+	+	–	+	–
Mannitol	–	–	–	+	–
Maltose	–	–	–	+	–
Gluconate	–	+	–	+	–
Citric acid	–	+	–	+	–
Adipic acid	–	+	–	–	–
Malic acid	+	+	–	+	–
Utilization as sole carbon source (API 50CH):					
D-Galactose	+	+	–	–	+
D-Lactose	–	+	+	+	–
D-Mannose	+	+	–	+	–
D-Arabinose	+	+	+	+	–
D-Ribose	+	+	+	+	–
D-Xylose	–	+	–	+	–
D-Melibiose	–	+	–	+	–
D-Gentiose	–	+	–	–	–
D-Fucose	–	+	–	–	–

Taxa: 1, Strain TRM 85114^T; 2, *H. korlensis* CGMCC 1.6981^T; 3, *H. tibetensis* KCTC 52660^T; 4, *H. urumqiensis* CGMCC 1.12917^T; 5, *H. daqiaonensis* CGMCC 1.9150^T. Except special note parts, data are from this study. +, positive; –, negative; ND, not determined.

⊠ Data from Li et al. (2008); [†] Data from Lu et al. (2018); [⊐] Data from Zhang et al. (2016); [‡] Data from Qu et al. (2011)

Characteristic	1	2	3	4	5
Sensitivity to:					
Ampicillin	–	+	+	–	+
Vancomycin	+	–	–	–	+
Carbenicillin	–	–	+	–	+
Cefalexin	–	+	+	+	+
Tetracycline	–	–	–	–	+
Erythromycin	+	+	+	–	–
Neomycin	+	–	+	–	–
Oxacillin	–	–	–	–	+
Doxycycline	–	–	+	–	+
Clindamycin	–	–	–	+	–
Polymyxin B	+	+	+	–	+
Taxa: 1, Strain TRM 85114 ^T ; 2, <i>H. korlensis</i> CGMCC 1.6981 ^T ; 3, <i>H. tibetensis</i> KCTC 52660 ^T ; 4, <i>H. urumqiensis</i> CGMCC 1.12917 ^T ; 5, <i>H. daqiaonensis</i> CGMCC 1.9150 ^T . Except special note parts, data are from this study. +, positive; –, negative; ND, not determined.					
⌘ Data from Li et al. (2008); [†] Data from Lu et al. (2018); [Ⓜ] Data from Zhang et al. (2016); [‡] Data from Qu et al. (2011)					

Table 2 Genomic characteristics[†] of strain TRM 85114^T and closely related species.

Attribute	1	2	3	4
Genome size (Mb)	4.13	3.62	3.98	3.72
DNA G + C content (%)	61.6	62.6	61.7±0.8	63.7
Coding	60	108	22	49
Total genes	3,892	3,795	3,597	3,512
Protein coding genes	3,738	3,346	3,504	3,351
RNA genes	137	79	65	71
rRNA genes (5S/16S/23S)	3	7	6	13
tRNA genes	57	54	55	54
Other RNAs	77	18	4	4
GenBank accession number	JAHCLU0000000000	FPBP0000000000	PYUD0000000000	FOBC0000000000
dDDH				
ANI value (%) [‡]	1	–	39.2	25.7
	2	88.9	–	21.6
	3	78.6	77.6	–
	4	80.5	79.2	80.0

Taxa: 1, strain TRM 85114^T; 2, *H. korlensis* XK1^T; 3, *H. urumqiensis* BZ-SZ-XJ27^T; 4, *H. daqiaonensis* CGMCC 1.9150^T.

[†]The bioinformatic analysis of the genomes was carried out using the NCBI prokaryotic genome annotation pipeline (www.ncbi.nlm.nih.gov/genome/annotation_prok/).

[‡]ANI, average nucleotide identity.

Figures

Figure 1

Scanning electron microscope image of TRM 85114T cells. Strain TRM 85114T grown on 12% MGM at 30 °C for 3 days. Scale bar, 2 µm.

Figure 2

Neighbour-joining tree constructed based on 16S rRNA gene sequences showing the phylogenetic relationships between strain TRM 85114^T and related taxa. Bootstrap values over 50% are shown on the nodes as percentages of 1,000 replicates. *Pseudomonas frederiksbergensis* DSM 13022^T (NR028906) was used as an outgroup. Bars indicate 0.01 changes per nucleotide position. *, branches were also found using the maximum-parsimony method; +, branches were also found using the maximum-likelihood method; *+, branches were found using all three methods.

Figure 3

Maximum-likelihood tree constructed based on 1140 core genes of TRM 85114^T and related type strains. Bootstrap values over 50% are shown on the nodes as percentages of 1,000 replicates.

Supplementary Files

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