Halomonas jincaotanensis sp. nov., a 1-naphthylamine degrading bacterium isolated from Pamir Plateau

Xuying Bu
College of Life Science, Tarim University
Zhanfneg Xia
College of Life Science, Tarim University
Zhanwen Liu
College of Life Science, Tarim University
Min Ren (renminlure@163.com)
Tarim University https://orcid.org/0000-0003-1480-9962
Chuanxing Wan
College of Life Science, Tarim University
Lili Zhang
College of Life Science, Tarim University

Research Article

Keywords: Halomonas, Jincaotan, Polyphasic taxonomy, 1-Naphthylamine, Degradation

Posted Date: November 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1049474/v1

Halomonas jincaotanensis sp. nov., a 1-naphthylamine degrading bacterium isolated from Pamir Plateau

Xuying Bu, Zhanfeng Xia, Zhanwen Liu, Min Ren*, Chuanxing Wan and Lili Zhang

State Key Laboratory Breeding Base for The Protection and Utilization of Biological Resources in Tarim Basin Co-funded by Xinjiang Corps and The Ministry of Science and Technology, College of Life Science, Tarim University, Alar, 843300, Xinjiang, PR China

Author for Correspondence:Min Ren

Tel/Fax: +86 997 4681612

E-mail: renminlure@163.com

Running title: *Halomonas jincaotanensis* sp. nov.

Category: New Tax-Proteobacteria

The GenBank accession number for the genome and 16S rRNA gene sequence of strain TRM 85114^{T} are JAHCLU000000000 and MW584241, respectively.

Abstract

A Gram-staining-negative, aerobic, rod-shaped bacterium, designated strain TRM 85114^T, was isolated from Jincaotan wetland in Pamir Plateau, PR China. According to the study, we found it have an ability to degrade 1-naphthylamine. Strain TRM 85114^T grow at 4−35 ℃ (optimum, 30 ℃), pH of 6.0−9.0 (optimum, pH 6.0) and can tolerate 1%−10% (w/v) NaCl (optimum, 3%). Phylogenetic analysis of 16S rRNA gene sequences revealed that strain TRM 85114^T was affiliated of the genus *Halomonas*, sharing high sequence similarity (97.3%) with the type strain of *Halomonas korlensis* XK1^T. The primary cellular fatty acids of strain TRM 85114^T were $C_{16:0}$ and $C_{19:0}$ cyclo ω8*c*. The predominate respiratory quinone was Q-9. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, lyso-phospatidylglycerol, phospholipids of unknown structure containing glucosarmine, and five unidentified lipids. The genomic DNA G+C content of strain TRM 85114^T was 61.6 mol%. Calculated the average nucleotide identities and the digital DNA-DNA hybridization values between strain TRM 85114^T and the related type *Halomonas* strains further revealed that TRM 85114^T represented a novel species of the genus *Halomonas*, for which the name *Halomonas jincaotanensis* sp. nov. is proposed. The type strain is TRM 85114^T (CCTCC AB 2021006^T =LMG 32311^T).

Keywords *Halomonas*; Jincaotan; Polyphasic taxonomy; 1-Naphthylamine; Degradation

Introduction

Halophilic microorganisms, especially *Halomonas* spp., usually have ability to accumulate polyphosphate (Nguyen et al. 2012), produce biodegradable polyhydroxyalkanoates (Tuma et al. 2020; Jiang et al. 2018) and various metabolic chemicals (Du et al. 2020; Jiang et al. 2021), for which have been the platform strain for producing multiple products in various industrial fields. Vreeland et al. (1980) initially proposed *Halomonas*, belongs to the family *Halomonadaceae* of the phylum Proteobacteria. At present, the genus contains more than 150 validly named species. Members of the genus *Halomonas* have been isolated from diverse terrestrial and aquatic habitats, such as lake water (Kazemi et al. 2020), saline-alkali land (Dou et al. 2015), tidal flat (Koh et al. 2017), Arctic tundra soil (Zhou et al. 2019), deep-sea sediment (Xu. et al. 2013) and hypersaline wetland (Ramezani et al. 2020). This study obtained a *Halomonas*-like strain isolated from Pamir Plateau, designated TRM 85114^{T}. We explored the taxonomic characterization and the ability to degrade 1-naphthylamine by strain TRM 85114^{T}.

1-Naphthylamine, as one of the top priority contaminants and carcinogens (Hu et al. 2011), is a derivative of polycyclic aromatic hydrocarbon, have been used as an intermediate for synthesis industries (Yang et al. 2020). The degree of human activity in industrial applications determined the concentration and transport of these ubiquitous compounds. The genus of *Halomonas* has shown the degradation

ability of polycyclic aromatic hydrocarbon (Govarthanan et al. 2020; Farraj et al. 2020), which is a tremendous potential capability in environmental remediation.

Materials and methods

Sample collection, isolation and preservation

Soil sample was collected from a wetland named Jincaotan (37°47′N, 75°16′E) in Pamir Plateau, at 3100 m. Strain TRM 85114^T was isolated from the sample by using general dilution-plating method. During the isolation process, 100 μL of mixed water and soil sample was spread onto the 12% Modified Growth Medium (MGM) agar plate (Smith, 2009), which contained: 5 g/L peptone (Oxoid), 1 g/L yeast extract (Oxoid) and 400 mL/L salt water (30% stock) with an adjusted pH of 7.0, incubated for 2 weeks at 15 ℃. Colonies on the plates showing different morphological were picked and purified, among which strain TRM 85114^T was routinely grown in 12% MGM agar at 15 ℃. Colonies were preserved in 12% MGM containing 50% (v/v) glycerol at - 80 °C.

The reference strain *H. korlensis* CGMCC 1.6981^T, *Halomonas urumqiensis* CGMCC 1.12917^T and *Halomonas daqiaonensis* CGMCC 1.9150^T were obtained from China General Microbiological Culture Collection Center (CGMCC), *Halomonas tibetensis* KCTC 52660^T was obtained from Korean Collection for Type Cultures (KCTC), all of them cultured under the same conditions, as specified, for comparative purposes.

Phenotypic and physiological identification

Procedures for phenotypic characterization followed Mata et al. (2002) and Arahal et al. (2007). Cellular morphology was examined by light microscopy (Leica DM1000) and scanning electron microscope (SEM) (Quanta; FEI) when cells were growing on 12% MGM agar for 4 days at 30 ℃. Anaerobic growth was tested on solid 12% MGM medium by using an MGC AnaeroPack Series (MGC). Motility was observed by using 12% MGM medium with 5 g/L agar. Growth was tested at different temperatures (4, 8, 16, 24, 28, 30, 32, 35, 37, 40 and 45 ℃) by using 12% MGM liquid medium. Different NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 and 30 w/v%) tolerance were tested in basic 12% MGM liquid medium without NaCl at 30 ℃. The pH tolerance of strains was also tested at 30 ℃ by using buffered 12% MGM liquid medium (50 mM MES (pH 5; 6), HEPES (pH 7), TAPS (pH 8), CAPSO (pH 9; 10)and CAPS (pH 11)) over different pH range (5−11 at 1.0 pH unit intervals). The incubation times for temperature, NaCl, and pH ranges tests were 3 days. The tests for requirement of Mg^{2+}, $SO4^{2-}$ and K^{+} to growth, exopolysaccharide and Polyhydroxyalkanoate (PHA) production were following the method described by Poli et al. (2013). Oxidation/fermentation of D-glucose, respiration on nitrate and nitrite, H_2S production from L-cysteine, reduction of nitrate to nitrite, hydrolysis of gelatin, casein, starch, Tween (20, 40, 60, and 80), aesculin and DNA, indole production, methyl red and Voges-Proskauer test, catalase, oxidase, urease, phenylalanine deaminase, lysine and ornithine decarboxylases, and *o*-Nitrophenyl-*β*-D-galactopyranosidase activity, were tested according to the methods described by

Dong et al. (2001). Rapid identification systems, including API 20E, API 20NE, and API 50CH (bioMérieux), were used to detect acid production and substrate utilization in three replicates at 30 ℃ for 72 h in accordance with the manufacturer's instructions. Antibiotic susceptibility were tested on 12% MGM agar plates inoculated with strains by using filter paper discs containing the following 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), clindamycin (2 μg). After incubated at 30 ℃ for 2 days, inhibition zones were measured according to the method described by Zhong et al. (2016).

Chemotaxonomic characterization

Biomass used for studies was obtained by culturing in liquid 12% MGM medium for 4 days in shake flasks at 30 ℃. The cells were collected by centrifugation and washed with distilled water and then freeze-dried. Ubiquinone were extracted from freeze-dried biomass according to the method proposed by Collins et al. (1977), analyzed by high-performance liquid chromatography (HPLC) followed the method by Collins (1985). Cellular fatty acids were extracted from the fresh cells according to the method proposed by Sasser (1990), and analyzed by gas

chromatography using the Microbial Identification System (Sherlock version 6.1; MIDI database: RTSBA6). TRM 85114^T and four reference strains were cultivated on 12% MGM plates and harvested at the same time to detect the composition of cellular fatty acids. Polar lipids were detected by two-dimensional thin-layer chromatography and identified by the method proposed by Minnikin et al. (1984).

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 with primers 27F (5′-AGAGTTTGATCCT-GGCTCAG-3′) and 1492R (5′-TACCTTGTTACGACTT-3′). Housekeeping genes *gyrB* and *rpoD* were amplified by using primers *gyrB*216F (5′-GARGTBA-TCATGACSGTGCT-3′) and *gyrB*1419R (5′-GCRTCSGTCATGATGATSAY-3′), *rpoD*88F (5′-ATGATYAACGACATGGGYAT-3′) and *rpoD*1321R (5′-TTSAKC-TTRTTGATGGTCTC-3′), respectively. For determination of the almost full-length of 16S rRNA gene sequence, the PCR amplicons were ligated into the pMD18-T vector using a pMD18-T cloning kit (TaKaRa) according to the manufacturer's instructions. The plasmid DNA was sequenced by using primers M13F (5′-TGTAAAACGACGGCCAGT-3′) and M13R (5′-CAGGAAA-CAGCTATGACC-3′). The 16S rRNA gene sequence was then compared with the available sequences in the EzBioCloud database (https://www.ezbiocloud.net/identify) using Identify Analysis (Yoon et al. 2017) and the NCBI database by

using BLAST (Altschul et al. 1990). Phylogenetic trees of the 16S rRNA gene were performed by using MEGA version 7.0 (Kumar et al. 2016) with the maximum-likelihood (Felsenstein 1981), neighbor-joining (Saitou et al. 1987), and maximum-parsimony (Fitch 1971) methods. Multilocus sequence analysis (MLSA) was used to infer the phylogenetic relation ships of strain TRM 85114^T (de la Haba et al. 2012). The 16S rRNA, *gyrB*, and *rpoD* gene sequence of strain TRM 85114^T was aligned with those of the most closely related species by using CLUSTAL_X (Thompson et al. 1997). Bootstrap analysis was performed for 1000 replicates to estimate the confidence of branches in the phylogenetic trees generated (Felsenstein 1985). Evolutionary distances were calculated by using the Kimura two-parameter model (Kimura 1980).

Genome sequencing and analysis

Genomic DNA sequence was sequenced by the Illumina Hiseq platform and assembled by ABySS (version 2.0) assembler (Jackman et al. 2017). We used the software CheckM to evaluate the completeness and contamination of the genome (Parks et al. 2015), online prodigal to predict assembled genome (Hyatt et al. 2010), software tRNAscan-SE to predict the tRNA in the genome (Lowe et al. 1997), and the software Infernal 1.1 (Nawrocki et al. 2013) to predict the rRNA in the genome based on the Rfam (Nawrocki et al. 2015) database. Genome annotation obtained by Personal Biotechnology Co., Ltd (Shanghai, China). The DNA G+C content was calculated based on the whole genome sequence. Calculations of the digital DNA-DNA hybridization (dDDH) and the average

153 nucleotide identity (ANI) values was performed by using the Genome-to-Genome
154 Distance Calculator (GGDC, version 2.1; http://ggdc.dsmz.de/distcalc2.php)
155 (Meier-Kolthoff et al. 2013) and the ChunLab's online ANI Calculator
156 (www.ezbiocloud.net/tools/ani) (Lee et al. 2015), respectively. Moreover, a
157 phylogenomic tree was drawn via the Type Strain Genome Server (TYGS;
158 https://tygs.dsmz.de) (Meier-Kolthoff et al. 2019) to provide a whole
159 genome-based taxonomic analysis .

160 **Detection of 1-naphthylamine degradation capacity**

161 Degradation of 1-naphthylamine on TRM 85114^T growth was performed in batch
162 experiments (Govarthanan et al. 2020). The isolate TRM 85114^T was inoculated
163 into 250 mL Erlenmeyer flasks containing 100 mL of 12% MGM medium, and 50
164 mg/L concentration of 1-naphthylamine was added into the culture flasks at the
165 same time, the resuspension was incubated at 30 ℃ temperature in a shaking
166 incubator (180 rpm) for 14 days. Sampling after incubation for 2day, 4day, 6day,
167 8day, 10day, 12day, and 14day, the extracted samples were detected to HPLC at
168 222 nm to analysis for the content of 1-naphthylamine. All the experiments were
169 performed in triplicates and mean values were reported. Growth of the isolate
170 without 1-naphthylamine was used as a control for this experiment. The
171 1-naphthylamine standard curve was drawn by the content of 20 mg/L, 40 mg/L,
172 60 mg/L, 80 mg/L, 100 mg/L and 120 mg/L. The elution procedure of the study
173 was used methanol ranging from 10% to 100% to gradient elution for 40 minutes.
174

Results and discussion

Phenotypic, physiological characterization

Strain TRM 85114^{T} was aerobic, Gram-negative, Short rods, and non-motile bacteria (Fig. 1). After 4 days of incubation at 30 ℃, cream-white colonies grew on 12% MGM agar plates. The strain can tolerate 1−10% (w/v) NaCl (optimum, 3%), pH of 6.0–9.0 (optimum, 6.0), and grow at 4–35 ℃ (optimum, 30 ℃) . Strain have the ability to hydrolyze starch, aesculin, Tween 20, 40 and 60, but not Tween 80, casein, DNA, gelatin, and urae. The strain was positive for methyl red test, catalase, oxidase, phenylalanine deaminase, reduction of nitrate to nitrite, respiration on nitrate and nitrite, and product H_2S from L-cysteine, but negative for indole production, Voges-Proskauer production, lysine and ornithine decarboxylases, and *o*-Nitrophenyl-*β*-D- galactopyranosidase activity. It also has the ability to ferment D-glucose to produce acid. This strain could produce poly-*β*-hydroxyalkanoate, but not for exopolysaccharides. Mg^{2+}, $SO4^{2-}$ and K^{+} were not necessary elements for this strain to grow. In the API 20E system, strain can produce acid from D-glucose, rhamnose, and arabinose, not from mannitol, inositol, sorbitol, sucrose, melibiose, and amygdalin. In the API 20NE system, strain has ability to assimilation D-mannose and malic acid, not D-glucose, arabinose, mannose, mannitol, maltose, gluconate, hydroxydecanoate, citric acid, adipic acid, and phenylacetic acid. In the API 50CH system, D-glucose, D-fructose, mannitol, sorbitol, glycerin, inositol, glucuronic acid, maltose, and fumaric acid can be used as sole carbon sources to grow. The strain was be

197 sensitive to the following antimicrobial agents (content per disc): vancomycin (30
198 μg), penicillin (1 μg), piperacillin (100 μg), cefradine (30 μg), ceftriaxone (30 μg),
199 cefoperazone (75 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg),
200 erythromycin (15 μg), norfloxacin (10 μg), ofloxacin (5 μg), ciprofloxacin (5 μg),
201 polymyxin B (300 μg) and bactrim (25 μg). Resistant to ampicillin (10 μg),
202 carbenicillin (100 μg), oxacillin (1 μg), cefalexin (30 μg), tetracycline (30 μg),
203 minocycline (30 μg), clindamycin (2 μg) and deoxytetra- cycline (30 μg).
204 Numerous characteristics of the four reference strains were similar with strain
205 TRM 85114^{T}. All of the five closely related strains were aerobic, positive for
206 oxidase, catalase, poly-*β*-hydroxyalkanoate production, methyl red test, negative
207 for *o*-Nitrophenyl- *β*-D-galactopyranosidase, lysine and ornithine decarboxylases
208 and indole production. Furthermore, five strains have ability to hydrolyse aesculin
209 and Tween20, not for Tween80, gelatin, casein, and DNA. Those five strains can
210 not utilize mannitol, inositol, sucrose and amygdalin to produce acid (API 20E),
211 nor assimilated arabinose, hydroxy- decanoate, and phenylacetic acid (API 20NE).
212 The five reference strains were negative to use as carbon source from most
213 substrates (API 50CH). Five strains were sensitive to the following antimicrobial
214 agents (content per disc): penicillin (1 μg), piperacillin (100 μg), cefradine (30
215 μg), ceftriaxone (30 μg), cefoperazone (75 μg), gentamicin (10 μg), kanamycin
216 (30 μg), norfloxacin (10 μg), ofloxacin (5 μg), ciprofloxacin (5 μg) and bactrim
217 (25 μg), not for minocycline (30 μg). Otherwise, Several different characteristics
218 among five strains and the type species of the genus, *Halomonas elongata* ATCC

3317^T, are shown in Table 1.

Chemotaxonomic characteristics

The predominate identified respiratory quinone of strain TRM 85114^T was Q-9 (34.8%), others were Q-8 (15.2%), Q-6 (4.7%) and an unidentified component (45.2%), similar with the ubiquinone systems of members of the genus *Halomonas.* As shown in Table 2, the primary cellular fatty acids of strain TRM 85114^T were identified as $C_{16:0}$ (18.0%) and $C_{19:0}$ cyclo $\omega 8c$ (10.3%). Several fatty acid profile of strain TRM 85114^T was similar to the closely related species: the fatty acids $C_{10:0}$ (0.8%), $C_{11:0}$ 3-OH (0.3%) and $C_{20:2}$ $\omega 6,9c$ (0.3%) were predominant. Moreover, strain TRM 85114^T differed from four reference strains by the types of fatty acids. In addition, the polar lipids of strain TRM 85114^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidyl-ethanolamine, lyso-phospatidylglycerol, phospholipids of unknown structure containing glucosarmine, and five unidentified lipids (Supplementary Fig. S1). Two component of phosphatidylglycerol, phosphatidylglycerol and phosphatidyl-ethanolamine were detected from most species of the genus *Halomonas*, but lyso-phospatidylglycerol and phospholipids of unknown structure containing glucosarmine were evidently different from the polar lipid profiles of other strains (Lu et al. 2018; Zhang et al. 2016; Ramezani et al. 2020).

Phylogenetic analysis of the 16S rRNA gene sequences

Based on the EzBioCloud analysis, the 16S rRNA gene sequence of strain TRM 85114^T (GenBank accession no. MW584241) had high similarity with members

of the genus *Halomonas*. It shared high 16S rRNA gene sequence similarity with the type strain of *H. korlensis* XK1^T (Li et al. 2008) (97.3%), followed by three other *Halomonas* species, including *H. tibetensis* pyc13^T (Lu et al. 2018) (96.4%), *H. urumqiensis* BZ-SZ-XJ27^T (Zhang et al. 2016) (96.1%), and *H. daqiaonensis* CGMCC 1.9150^T (Qu et al. 2011) (96.0%), and with other recognized *Halomonas* species and known species in another genus in the family *Halomonadaceae* were less than 96.0%. Based on the 16S rRNA gene (1504bp), the maximum-likelihood phylogenetic tree and phylogenomic tree for whole genome-based taxonomic analysis (Fig. 2, 3), strain TRM 85114^T clustered tightly with *H. korlensis* XK1^T, forming an independent sub-cluster supported by high bootstrap values within the genus of *Halomonas* cluster. The neighbor-joining, maximum-parsimony and neighbor-joining algorithm based on the concatenated gene sequences (16S rRNA 1504bp; *gyrB* 472bp; *rpoD* 1243bp) phylogenetic trees (Supplementary Fig. S2, S3, S4) also shown the position of strain TRM 85114^T with respect to other type strains in the genus *Halomonas*. All of the five kinds of phylogenetic trees shown TRM 85114^T formed a distinct clade with others, indicated it was a novel species belonged to the genus *Halomonas*.

Genomic features and analysis

The obtained draft genome of TRM 85114^T (accession no. JAHCLU000000000) was 4,126,476 bp in length with a high sequence, which comprise of 60 contigs (N50 = 190,960 bp) and 52 scaffolds (N50 = 208,915 bp). The content of DNA G+C was 61.6 mol%. The annotated genome encodes a total of 3902 genes,

including 1666 protein-coding genes. A total of 3 rRNAs and 57 tRNAs were identified in the genome. Sequences of two complete 16S rRNA genes found in the genome were identical to those obtained through the Sanger sequencing.

Based on the KEGG orthology-based annotation, a total of 3544 genes (90.9%) were annotated and assigned to putative functions, of which 1389 genes were annotated into metabolism-associated pathways, and 249 genes were annotated into environmental information processing pathways. There are 44 ORFs of enzymes involved in aromatic hydrocarbon degradation ability (Table S1), contig15_2804 (*frmA*, *ADH5*, *adhC*) was annotated for naphthalene degradation function. The dDDH and ANI value among strain TRM 85114^T and four reference strains were conducted. The dDDH value between strain TRM 85114^T with *H. korlensis* XK1^T, *H. tibetensis* pyc13^T, *H. urumqiensis* BZ-SZ-XJ27^T, and *H. daqiaonensis* CGMCC 1.9150^T were 39.2%, 25.0%, 25.7%, and 28.1%. The ANI value between them were 88.9%, 77.8%, 78.6%, and 80.5%. These data were all significantly lower than the threshold values for dDDH (70%) and ANI (95–96%) used to discriminate bacterial species, respectively. The above data show that TRM 85114^T was a novel strain of the genus *Halomonas* with naphthalene degradation ability.

1-Naphthylamine degradation rate

The biodegradation efficiency of the isolate TRM 85114^T was further investigated. The result showed that the retention time of 1-naphthylamine was at 26 minute, and the absorption peak area of 1-naphthylamine decreased significantly with the

285 increase of days (Fig. S5). According to the standard curve of 1-naphthylamine
286 (Fig. S6), the degradation amount of 1-naphthylamine could reach up to 21.6
287 mg/L at the 4th day and 32.0 mg/L at the 14th day (Fig. S7) by strain TRM
288 85114^T. Result indicated TRM 85114^T has a strong ability to degrade
289 1-naphthylamine, it was a strain with environmental remediation ability by
290 treating wastewater which contain 1-naphthylamine.
291
292 **Description of *Halomonas jincaotanensis* sp. nov.**
293 *Halomonas jincaotanensis* (jin.caotan.en′ sis. NL fem. adj. jincaotanensis were
294 pertaining to Jincaotan, Pamir Plateau, Xinjiang, China, from where the type
295 strain was isolated).
296 Strain was Gram-stain-negative, aerobic, short-rod shape, and non-motile. The
297 cell size was 0.9–1.0 × 0.4–0.5 μm. Colonies were circular, cream-white, and
298 convex with smooth surfaces when incubated on 12% MGM agar at 30 ℃ for 4
299 days. The strain can grows at 4–35 ℃ (optimum, 30 ℃), pH of 6.0–9.0 (optimum,
300 pH 6.0), and can tolerate 1−10% (w/v) NaCl (optimum, 3%). Strain have ability
301 to hydrolyze Starch, aesculin, Tween 20, 40 and 60, but not Tween 80, casein,
302 DNA, gelatin, and urea. The strain was positive for catalase, oxidase,
303 phenylalanine deaminase, reduction of nitrate to nitrite, ferment D-glucose to
304 produce acid, respiration on nitrate and nitrite, product H_2S from L-cysteine, and
305 methyl red test, but negative for indole production, Voges-Proskauer production,
306 lysine and ornithine decarboxylases, and *o*-Nitrophenyl-*β*-D-galactopyranosidase

activity. This strain could produce poly-*β*-hydroxyalkanoate, but not for exopolysaccharides. Grow without Mg^{2+}, $SO4^{2-}$ and K^{+}, produce acid from D-glucose, rhamnose, and arabinose, not from mannitol, inositol, sorbitol, sucrose, melibiose, and amygdalin. Besides, strain have ability to assimilation D-mannose and malic acid, not 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 sources. Sensitive to the following antimicrobial: vancomycin, penicillin, piperacillin, cefradine, ceftriaxone, cefoperazone, gentamicin, kanamycin, neomycin, erythromycin, norfloxacin, ofloxacin, ciprofloxacin, polymyxin B and bactrim. Resistant to ampicillin, carbenicillin, oxacillin, cefalexin, tetracycline, minocycline, clindamycin and deoxytetracycline. The predominate respiratory quinone was Q-9. The major fatty acids of the cells were $C_{16:0}$ and $C_{19:0}$ cyclo *ω*8*c*. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, lyso-phospatidylglycerol, phospholipids of unknown structure containing glucosarmine, and five unidentified lipids. The DNA G+C content of the strain was 61.6 mol %. The degradation of 1-naphthylamine could reach up to 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 Pamir Plateau. The GenBank accession numbers for the 16S rRNA gene and the draft genome sequence of

329 TRM 85114^T are MW584241 and JAHCLU000000000, respectively.

330

331 **Acknowledgment**

332 Our research was supported by the National Natural Science Foundation of China
333 (31900007), Bingtuan Science and Technology Program (2018BC008), and
334 Microbial Resources Utilization Innovation Team in Key Field of Xin Jiang
335 Production and Construction Corps (2017CB014), and the principal fund
336 (TDHNLH201604).

337 **Author contributions**

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

343 **Disclosure statement**

344 **Conflict of interest**

345 All authors declare no conflict of interest.

346 **Ethical approval**

347 No specific ethical or institutional permission is required for sampling, and
348 experimental studies do not involve endangered or protected species.

349

350 **The table title and Figure caption**

Table 1 Differential characteristics of strain TRM 85114^T and the type strains of related species of the genus *Halomonas.*

Table 2 Compositions of the cellular fatty acids of strain TRM 85114^T and its four reference strains.

Fig. 1 Scanning electron microscope image of TRM 85114^T cells grown on 12% MGM at 30 ℃ for 3 days. Scale bar, 2 μm.

Fig. 2 Maximum-likelihood tree 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.

Fig. 3 Phylogenomic tree of strains TRM 85114^T and related type strains available on the TYGS database. The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications.

Table S1 KEGG annotation analysis of enzymes involved in aromatic hydrocarbon degradation ability based on TRM 85114^T genome.

Fig. S1 Two-dimensional thin-layer chromatograms showing the polar lipids of strain TRM 85114^T with a different chromogenic agent. a, 10% ethanolic molybdophosphoric acid reagent; b, ninhydrin reagent; c, molybdenum blue reagent; d, anisaldehyde reagent. DPG, diphosphatidylglycerol; PG, phosphatidyl-glycerol; PE, phosphatidylethanolamine; LPG, lyso-phospatidylglycerol; NPG, phospholipids of unknown structure containing glucosarmine; PL, unidentified

lipids.

Fig. S2 Neighbour-joining tree 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.

Fig. S3 Maximum-parsimony tree 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.

Fig. S4 Phylogenetic tree generated with the neighbor-joining algorithm based on the concatenated gene sequences (16S rRNA 1504bp; *gyrB* 472bp; *rpoD* 1243 bp) gene sequences showing the phylogenetic positions of strain TRM 85114^T and related taxa. Bootstrap values with more than 50% are shown on the nodes as percentages of 1,000 replicates. *Pseudomonas frederiksbergensis* DSM 13022^T was used as an outgroup. The scale bar equals 0.01 changes per nucleotide position.

Fig. S5 HPLC analysis of TRM 85114^T grew with 1-naphthylamine in 12% MGM medium under absorption wavelength of 222 nm. The absorb peak of 1-naphthylamine at 26 min. a, TRM85114^T cultured with 1-naphthylamine at 0 day; b, at 2nd day; c, at 4th day; d, 6th day; e, 8th day; f, 10th day; g, 12th day; h, 14th day.

Fig. S6 Standard curve of 1-naphthylamine. 1-Naphthylamine absorb peak area as

the abscissa and content as the longitudinal coordinate. Data obtained by HPLC under absorption wavelength of 222 nm.

Fig. S7 Time course of 1-naphthylamine biodegradation by TRM 85114^T during growth in 12% MGM medium.

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Figures

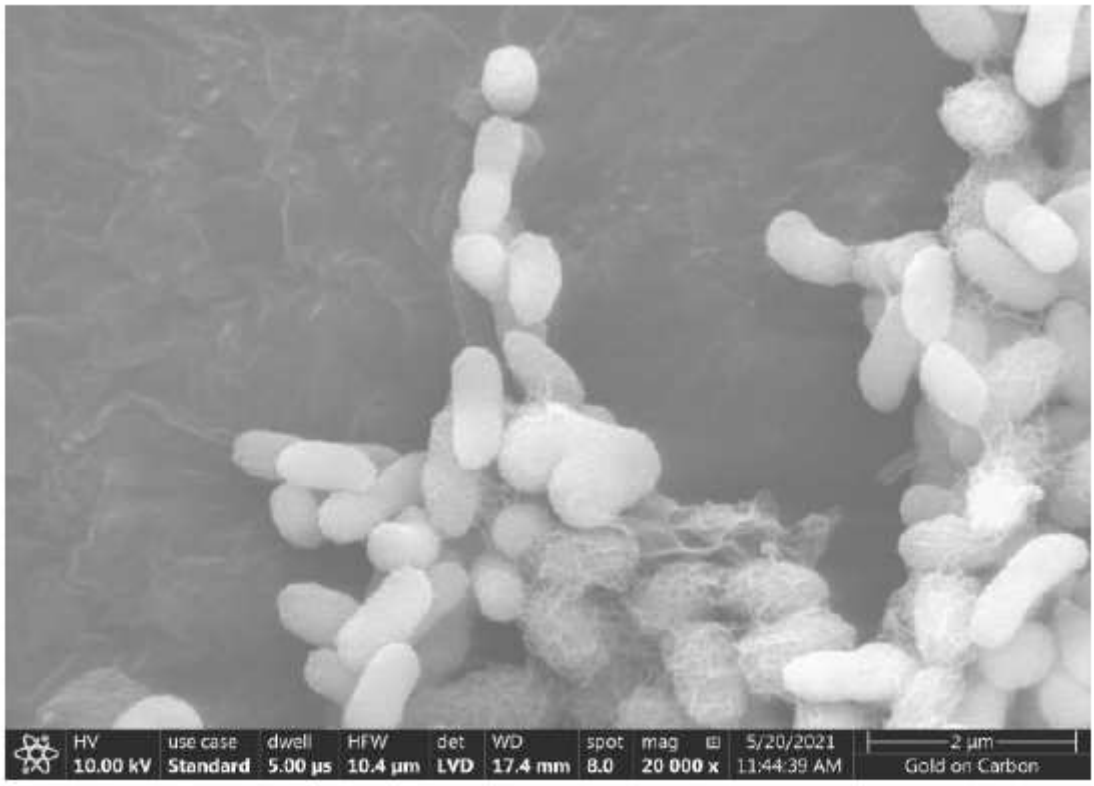

Figure 1

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

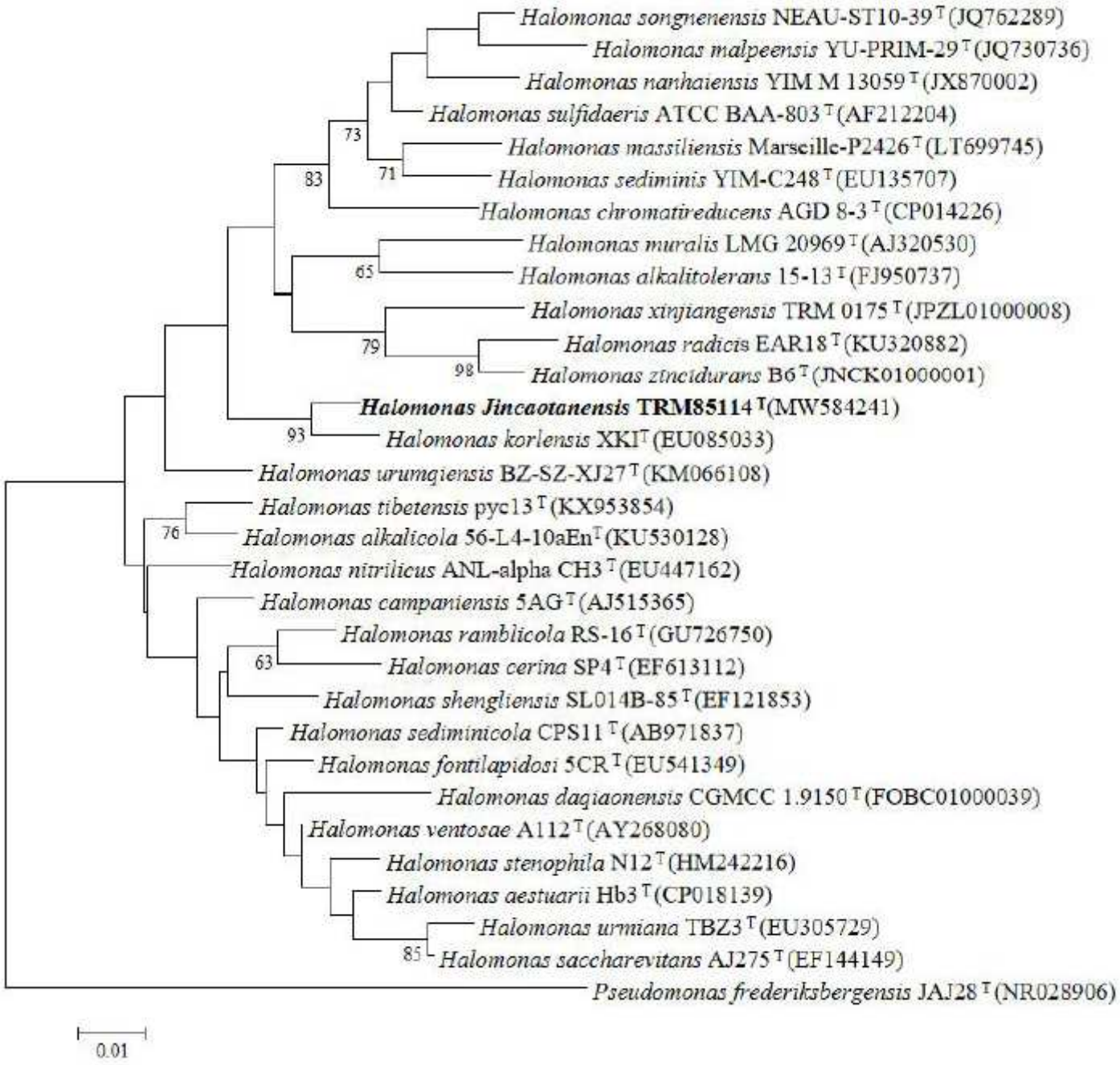

Figure 2

Maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain TRM 85114T and related taxa. Bootstrap values over 50% are shown on the nodes as percentages of 1,000 replicates. Pseudomonas frederiksbergensis DSM 13022T (NR028906) was used as an outgroup. Bars indicate 0.01 changes per nucleotide position.

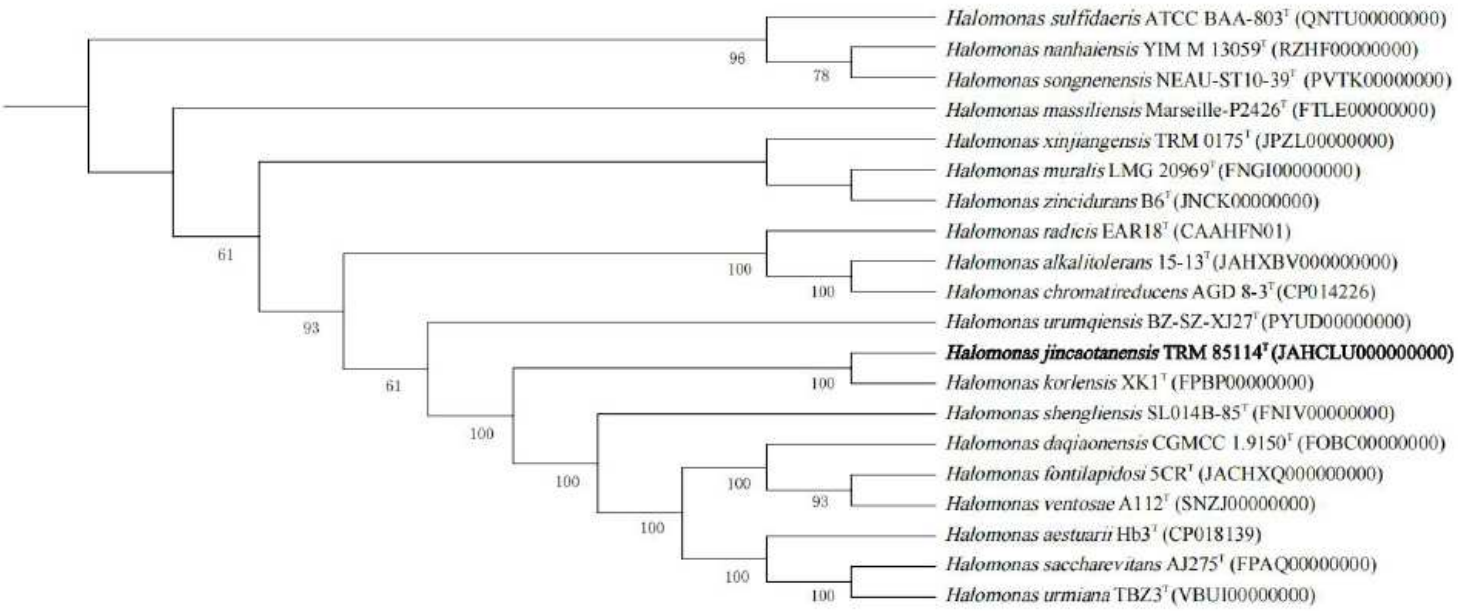

Figure 3

Phylogenomic tree of strains TRM 85114T and related type strains available on the TYGS database. The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications.

Supplementary Files

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