



# *Pseudomonas lophurensis* sp. nov., an endophytic bacterium isolated from *Populus euphratica* at the ancient Ugan river

Tursunay Mamtimin · Nusratgul Anwar · Mehruzem Abdurahman · Marygul Kurban · Manziram Rozahon · Hormathan Mamtimin · Buayshem Hamood · Erkin Rahman · Min Wu

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**Abstract** A Gram-stain negative, aerobic, rod-shaped, motile by a single polar flagellum, non-spore-forming bacterium, designated strain AL-54<sup>T</sup>, was isolated from the storage liquid in the stems of *Populus euphratica* tree at the ancient Ugan River in Xinjiang, PR China. Isolated AL-54<sup>T</sup> grew optimally at pH 7.0 and temperature 35 °C in the presence of 3% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequence demonstrated that the isolate belonged

to the genus *Pseudomonas* and was closely related to *Pseudomonas songnenensis* NEAU-ST5-5<sup>T</sup> (97.6%), *Pseudomonas zhaodongensis* NEAU-ST5-21<sup>T</sup> (97.5%), *Pseudomonas alcaliphila* AL15-21<sup>T</sup> (97.3%), *Pseudomonas toyotomiensis* HT-3<sup>T</sup> (97.3%), *Pseudomonas oleovorans* subsp. *lubricantis* RS1<sup>T</sup> (97.3%), *Pseudomonas stutzeri* ATCC 17588<sup>T</sup> (97.3%), *Pseudomonas chengduensis* CGMCC 2318<sup>T</sup> (97.2%), and *Pseudomonas xanthomarina* KMM 1447<sup>T</sup> (97.1%). Multilocus Sequences Analysis (MLSA) of strain AL-54<sup>T</sup> based on the three house-keeping genes, *rpoB*, *rpoD* and *gyrB* further confirmed the phylogenetic assignment of the isolates. The G+C content was 64.7 mol%. The DNA-DNA hybridization with *P. songnenensis* NEAU-ST5-5<sup>T</sup>, *P. zhaodongensis* NEAU-ST5-21<sup>T</sup>, *P. alcaliphila* AL15-21<sup>T</sup>, *P. toyotomiensis* HT-3<sup>T</sup>, *P. oleovorans* subsp. *lubricantis* RS1<sup>T</sup>, *P. stutzeri* ATCC 17588<sup>T</sup>, *P. chengduensis* CGMCC 2318<sup>T</sup> and *P. xanthomarina* KMM 1447<sup>T</sup>

Tursunay Mamtimin and Nusratgul Anwar have contributed equally to this study and are joint first authors.

The Gen Bank/EMBL/DDBJ accession numbers for the partial 16S rRNA, *rpoB*, *rpoD* and *gyrB* gene sequences of the strain AL-54<sup>T</sup> are MW138096, KJ577580, KJ577581 and KY008251 respectively. GenBank Accession Numbers for the whole genome sequences of strain AL-54<sup>T</sup> is JADDIX000000000.

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T. Mamtimin  
School of Life Sciences, Lanzhou University,  
Lanzhou 730000, People's Republic of China

T. Mamtimin · M. Abdurahman · M. Kurban ·  
M. Rozahon · H. Mamtimin · B. Hamood ·  
E. Rahman (✉)  
College of Life Science and Technology, Xinjiang

University, Urumqi, Xinjiang 830046, People's Republic  
of China  
e-mail: erkin1106@163.com

N. Anwar · M. Wu (✉)  
College of Life Science, Zhejiang University,  
Hangzhou 310058, People's Republic of China  
e-mail: wumin@zju.edu.cn

revealed 44.0%, 44.7%, 60.1%, 48.7%, 49.1%, 60.1%, 58.9% and 60.2% relatedness respectively. The predominant quinone system is ubiquinone-9 (Q-9). The major components of the cellular fatty acids (>10%) were summed feature 8 (comprising C<sub>18:1</sub> ω7c /C<sub>18:1</sub> ω6c), summed feature 3 (comprising C<sub>16:1</sub> ω7c /C<sub>16:1</sub> ω6c) and C<sub>16:0</sub>. The detected major polar lipids were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and phosphatidylcholine (PC). On the basis of phenotypic data, chemotaxonomic and phylogenetic properties, strain AL-54<sup>T</sup> can consider as a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas lopnurensis* sp. nov. is proposed. The type strain is AL-54<sup>T</sup> (= JCM 19136<sup>T</sup> = CCTCC AB 2013066<sup>T</sup> = NRRL B-59987<sup>T</sup>).

**Keywords** *Pseudomonas lopnurensis* sp. nov. · *Populus euphratica* trees · Phylogenetic analysis

## Introduction

*Pseudomonas* are widely distributed in nature and belongs to the family *pseudomonadaceae* within the class *Gammaproteobacteria*. Which have been reported as a metabolically and genetically diverse bacterial group (Anzai et al. 2000; Kersters et al. 1996; Moore et al. 1996; Mulet et al. 2010). The genus was first described by Migula (1894) and currently comprises a large number of species, and new species are described continuously till now (Mulet et al. 2018). Most species from this genus were isolated from a variety of distinctive habitats, such as water, soil, and eukaryotic hosts (Silby et al. 2011). Although some species or strains were shown to be pathogenic for humans, animals or plants, most of the *Pseudomonas* species are beneficial to their eukaryotic hosts or harmless as commensal members of the microbiota (e.g., plant growth-promoting rhizobacteria) (Haas et al. 2005; Ramette et al. 2011; Almario et al. 2014). Members of the genus *Pseudomonas* are characterized as gram-stain negative, aerobic, non-spore-forming, motile by one or several polar flagella and rod-shaped bacteria. *Pseudomonas* species of group I based on rRNA–DNA relatedness in the original classification of Palleroni (1984) form the genus *Pseudomonas*. Members of other species of *Pseudomonas* have been

transferred to other existing genera or to new genera (Kersters et al. 1996). In this study, we firstly described the *Pseudomonas* sp. AL-54<sup>T</sup> belongs to the family *Pseudomonadaceae* and the phylum *Proteobacteria*, which was isolated from ancient Ugan river (which river has been dried-up for 123 years), and a high density of natural *Populus euphratica* forest reside along the river. There the physiological-biochemical, chemotaxonomic, and genomic characterization were analyzed for the new strain AL-54<sup>T</sup>, and there is still need of further investigation to optimize more functions of the isolated gene.

## Materials and methods

### Isolation and culture conditions

During the course of a study on culturable endophytic bacteria diversity and community structure of *Populus euphratica* at Ugan River in Xinjiang Uyghur Autonomous Region, PR China. One endophytic bacterial strain, designated AL-54<sup>T</sup>, was isolated from the storage liquid in the stem of *Populus euphratica* stands at the ancient Ugan River (N 41°03'246" E 85° 02'361") in April, 2011. samples were collected according to the method as described by Rozahon et al. (2014). For the isolation, serial dilutions of the samples with sterilized 0.85% saline solution were spread on the LB agar medium and incubated at 35–37 °C for up to 10 days. Colonies were picked and repeatedly re-streaked on the same medium, the pure cultures were obtained from isolated colonies on the plates and preserved at – 80 °C as glycerol suspension for further characterization. To determine the exact taxonomic position of strain AL-54<sup>T</sup> by using a polyphasic approach, The seven species of genus *Pseudomonas* were used as reference strains for phenotypic characterization and fatty acid analysis: *P. songnenensis* NEAU-ST5-5<sup>T</sup>, *P. zhaodongensis* NEAU-ST5-21<sup>T</sup> and *P. oleovorans subsp. lubricantis* RS1<sup>T</sup> were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ); *P. alcaliphila* AL15-21<sup>T</sup>, *P. toyotomiensis* HT-3<sup>T</sup> and *P. xanthomarina* KMM 1447<sup>T</sup> were obtained from the Japan Collection of Microorganisms (JCM); *P. stutzeri* ATCC 17588<sup>T</sup> was obtained from American Type Culture Collection (ATCC).

## Morphological, physiological, and biochemical analyses

Phenotypic characterizations were performed as described methods previously by Macián et al. (2005) and Lucena et al. (2010). Cell morphology and presence of flagella was determined by transmission electron microscopy (Philips CM-20) with cells from exponentially growing cultures. For this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The motility of cells was tested by the hanging drop method (Murray et al., 1994). To determine the growth of strains 50 ml Erlenmeyer flask was used and bacterial growth was estimated by monitoring the OD<sub>600nm</sub>. Growth at various temperatures (4, 10, 15, 20, 25, 30, 37, 40, 45 and 50 °C) was measured in LB medium. The pH range for growth (6–9, with interval of 0.5) was determined using the appropriate biological buffers: MOPS (Sigma) (pH 6.0–8.0) and boric acid/borax (pH 8.5 and 9.0), each at a final concentration of 50 mM (Scheidle et al. 2011). The salinity range supporting growth was determined at various NaCl concentrations (0–7%, w/v with interval of 0.5%). Accumulation of poly-beta-hydroxy-butyrate (PHB) and formation of fluorescent pigments were tested on King A and King B medium according to King et al. (1954). For the basic identification various biochemical test was performed such as catalase activity was determined by bubble production after the addition of a drop of 3% H<sub>2</sub>O<sub>2</sub> to bacteria. Oxidase activity was determined using 1% (w/v) N,N,N',N'-tetramethyl-1,4-phenylenediamine dihydrochloride. Methyl red and Voges–Proskauer tests were performed as described previously (Smibert and Krieg 1994). Other enzyme activities and physiological properties were determined using the API 20E, API 20 NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions, using distilled water supplemented with 5% (w/v) NaCl to suspend the cells. Carbon and nitrogen source utilization were studied by using the Biolog GN3 system.

## Phylogenetic analyses based on 16S rRNA gene and MLSA

The 16S rRNA gene of the strain AL-54<sup>T</sup> was amplified using PCR by using universal primers 27F (5'-AGAGTTTGATC (A/C) TGGCTCAG-3') and

1492R (5'-ACGG(C/T) TACCTTGTTACGACTT-3'). Sequence traces were edited manually and consensus sequences generated using the program SEQMAN, version 7 (DNASTAR). To ascertain the phylogenetic position of the novel isolate, the 16S rRNA gene sequence of strain AL-54<sup>T</sup> was compared with sequences obtained from NCBI full form (<http://blast.ncbi.nlm.nih.gov/blast.cgi>; Altschul et al. 1997) and the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net/>; Kim et al. 2012). For further determination of phylogenetic position of the strain AL-54<sup>T</sup>, the Multilocus Sequences (MLS) *gyrB*, *rpoB* and *rpoD* genes were analyzed. The amplification and sequencing of *gyrB*, *rpoB* and *rpoD* housekeeping genes was performed as described by Mulet et al (2010), using the primers PsEG30F/PsEG790R for *rpoD* gene, LAPS5F/LAPS27R for *rpoB* gene (Tayeb et al. 2005), and primers UP-1 and UP-2r for *gyrB* gene respectively (Yamamoto and Harayama 1998). To construct the phylogenetic trees, 16S rRNA, *gyrB*, *rpoB*, and *rpoD* sequences were collected of related species with valid names from EzTaxon server and the NCBI (<https://www.ncbi.nlm.nih.gov/>). The phylogenetic analysis was performed with MEGA 6 software (Molecular Evolutionary Genetics Analysis, version 6.0; Tamura et al., 2013). The sequences were aligned by using the CLUSTAL\_X (1.83) program (Thompson et al. 1997). Distances were calculated according to Kimura's two-parameter model (Kimura 1983) and clustering was performed by using the neighbour-joining (Saitou and Nei 1987) and maximum-parsimony (Fitch 1971) methods with bootstrap values based on 1000 replications (Felsenstein 1985).

## Genome sequencing analysis

The whole-genome sequences were determined using the Illumina HiSeq 2000 sequencing platform (Beijing Genomics Institute) with Solexa PE150 sequencing technology to generate sub-reads set (approximate 878-fold genome coverage). The de-novo assembly of the reads was performed using ABySS 1.5.2 (Simpson et al. 2009). The assembly k-value was tested from 32 to 64 to find the optimal k-value using abyss-pe script. The quality of microbial genome was assessed using Check M (Parks et al. 2015). Gene annotation were conducted through the NCBI prokaryotic genome annotation pipeline ([https://www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](https://www.ncbi.nlm.nih.gov/genome/annotation_prok/)) and RAST (Rapid

Annotation by Subsystem Technology) annotation server. The average nucleotide identity (ANI) was calculated using <https://www.ezbiocloud.net/tools/ani> (Yoon et al. 2017). The genome sequences of the reference strains were obtained from the Ezbiocloud database (<https://www.ezbiocloud.net/genome/list?tn>) under the Ezbiocloud project accession numbers: *P. songnenensis* NEAU-ST5-5<sup>T</sup> (GCA\_003696315.1), *P. zhaodongensis* NEAU-ST5-21<sup>T</sup> (GCA\_003696365.1), *P. alcaliphila* AL15-21<sup>T</sup> (GCA\_900101755.1), *P. toyotomiensis* HT-3<sup>T</sup> (GCA\_900115695.1), *P. oleovorans* subsp. *lubricantis* RS1<sup>T</sup> (GCA\_900455615.1), *P. chengduensis* CGMCC 2318<sup>T</sup> (GCA\_900102635.1), *P. stutzeri* ATCC 17588<sup>T</sup> (GCA\_000219605.1), and *P. xanthomarina* KMM 1447<sup>T</sup> (GCA\_900129835.1) to investigate the genomic average nucleotide identity (ANI). The genomic ANI values of strain AL-54<sup>T</sup> and the other reference strains were calculated using the modified OrthoANu algorithm, which uses USEARCH instead of BLAST (Lee et al. 2016). The DNA G+C content of strain AL-54<sup>T</sup> was determined by reversed-phase HPLC using the method of Mesbah et al. (1989) with *Escherichia coli* DH5a (CICC 10399) as a control. DNA–DNA hybridization (DDH) experiments were carried out to evaluate the DDH relatedness between strain AL-54<sup>T</sup> and its reference strains using the optical renaturation rate method (Gillis et al. 1970) and a Perkin Elmer Lambda 35 UV/VIS spectrophotometer by the service of CICC (for *P. chengduensis* CGMCC 2318<sup>T</sup> by the service of CGMCC).

#### Chemotaxonomic characterization

For total cellular fatty acid analysis, cells of isolated strain AL-54<sup>T</sup> and the reference strains were harvested on TSA (Bacto<sup>TM</sup>) plates after incubation for two days at 30 °C under aerobic conditions. Cellular fatty acids were extracted and analyzed according to the standard protocol of the Sherlock Microbial Identification system (MIDI; Sasser 1990) version 6.0 and peaks were identified using the peak naming table TSBA6 6.00. Isoprenoid quinones of strain AL-54<sup>T</sup> were extracted according to the method of Komagata and Suzuki (1987) and analyzed using reversed-phase HPLC and an YMC ODS-A (25064.6 mm) column. Polar lipids were extracted from 100 mg freeze-dried cell material by using two-stage method as described previously (Tindall 1990) and separated by silica gel TLC by two-dimensional chromatography. For

polar lipid analysis, TLC plates were stained with 10% ethanolic molybdophosphoric acid solution (Sigma-Aldrich) followed by heating at 150 °C for 10 min and further characterized by spraying with molybdenum blue (specific for phosphates),  $\alpha$ -naphthol (specific for sugars), ninhydrin (specific for amino groups) and dragendorff reagent (quaternary nitrogen compounds) (Ventosa et al. 1993).

## Results and discussion

### Morphological, physiological and biochemical characteristics

The morphological features of strain AL-54<sup>T</sup> were studied on LB agar plates and formed circular, smooth and creamy colonies 1.5–2 mm in diameter after 48h incubation at 35 °C. Cells of strain AL-54<sup>T</sup> were Gram-stain negative, aerobic, rod-shaped (length 0.5–2.1  $\mu$ m, width 0.5–0.7  $\mu$ m), motile with one polar flagellum (Supplementary Fig. S1), non-spore-forming and not pigmented on King A and King B medium. The isolate grew aerobically between 10–45 °C, but not at 50 °C, optimum growth was observed at 35 °C. The pH range for growth was 6.5–8.0, with an optimum at pH 7.0. Growth in the presence of NaCl (1–5%), optimum growth was at 3% (w/v) NaCl. Detailed morphological, physiological and biochemical characteristics of strain AL-54<sup>T</sup> are given in the species description. The phenotypic characteristics distinguishing this novel strain AL-54<sup>T</sup> and type strains of the most closely related species of the genus *Pseudomonas* are shown in the Table 1. Some characteristics of strain AL-54<sup>T</sup> were in accordance with other types of strains, and some characteristics differences such as NaCl, temperature and pH from reference strains. For instance, the pH range of isolated strain (AL-54<sup>T</sup>) and type strains: *P. alcaliphila* AL15-21<sup>T</sup>, *P. stutzeri* ATCC 17588<sup>T</sup> and *P. oleovorans* subsp. *lubricantis* RS1<sup>T</sup> were 6–9; *P. toyotomiensis* HT-3<sup>T</sup> and *P. xanthomarina* KMM 1447<sup>T</sup> were 5–10, and *P. songnenensis* NEAU-ST5-5<sup>T</sup> and *P. zhaodongensis* NEAU-ST5-21<sup>T</sup> showed growth at 7–12 range, because these two strains were isolated from alkaline soil, hence they have showed growth at alkaline conditions. Besides, in the biochemical test only strain AL-54<sup>T</sup> and reference strain *P. xanthomarina* KMM 1447<sup>T</sup> were positive (+) for potassium nitrate and L-fructose, but other type strains were

**Table 1** Cellular fatty acid profiles of strain AL-54<sup>T</sup> and type strains of the seven most closely related species of the genus *Pseudomonas*

Characteristic	1	2	3	4	5	6	7	8
PHB accumulation	–	–	–	–	–	+	–	+
pH range for growth	6.5–8	7–12	7–11	6.5–9	5.5–10	6–9	6–8	5–10
Growth at/in:								
4 °C	–	–	–	+	+	+	–	+
45 °C	+	+	–	–	–	–	+	–
NaCl range (%)	1–5	0–5	0–5	0–10	0.5–8	1–3	0–8	0–8
Enzyme activity API ZYM:								
Alkaline phosphatase	+	+	+	–	–	+	–	–
Esterase (C4)	+	+	+	–	–	+	–	+
Esterase lipase (C8)	+	+	+	–	–	+	–	+
Lipase (C14)	–	+	+	+	–	+	–	+
Leucine arylamidase	–	+	+	–	–	+	–	–
Acid phosphatase	+	–	–	–	+	+	–	+
a-Galactosidase	–	–	–	–	–	–	+	–
a-Glucosidase	–	–	–	–	–	–	+	–
P-Glucosidase	–	–	–	–	+	+	–	–
API 20 NE:								
Potassium nitrate	+	–	–	–	–	–	–	+
Adipic acid	–	–	–	–	+	–	–	–
Dextrin	–	+	+	w	w	–	–	+
L-fructose	+	–	–	–	–	–	+	–
Acetoacetic acid	+	–	–	–	w	+	–	+
D-serine	+	–	–	w	w	–	+	+
Minocycline	+	–	–	w	w	–	+	–
Lithium chloride	–	+	+	w	w	w	+	–
Potassium tellurite	+	–	+	w	w	–	w	+
G+C content (mol %)	64.7	59.0*	65.0*	62.3–63.2*	65.1*	62.2*	61.0–66.0*	59.1*

Strains: 1, *P. Lopnurensis* AL-54<sup>T</sup>; 2, *P. songnenensis* NEAU-ST5-5<sup>T</sup>; 3, *P. zhaodongensis* NEAU-ST5-21<sup>T</sup>; 4, *P. alcaliphila* AL15-21<sup>T</sup>; 5, *P. toyotomiensis* HT-3<sup>T</sup>; 6, *P. oleovorans* subsp. *lubricantis* RS1<sup>T</sup>; 7, *P. stutzeri* ATCC 17588<sup>T</sup>; 8, *P. xanthomarina* KMM 1447<sup>T</sup>. +, Positive; –, negative; w, weakly positive.

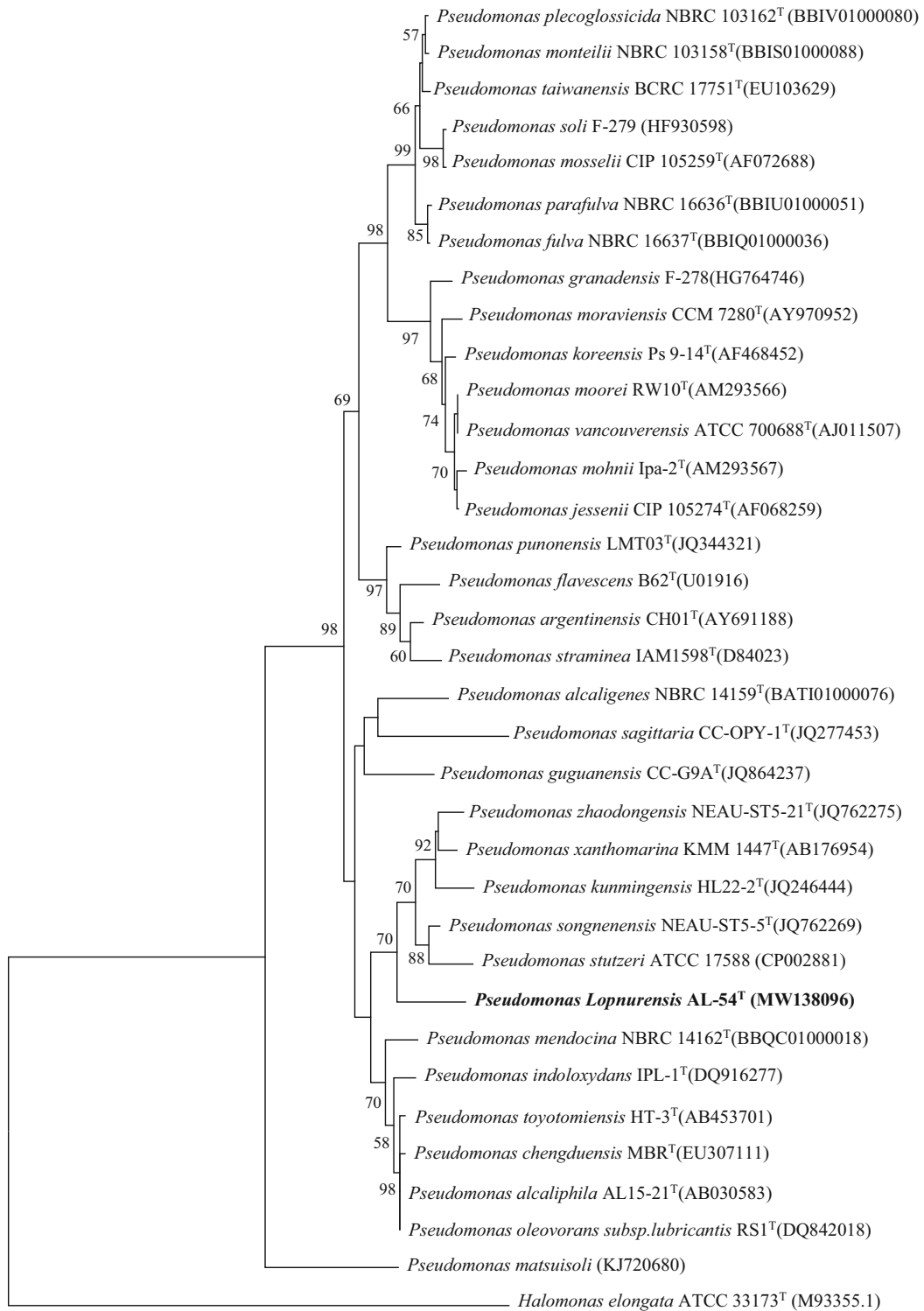
\*Data are from the present study (Lei Zhang et al. (2015); Yumoto I et al. 2001; Hirota et al. (2011); Saha et al. 2010; Romanenko et al. 2005).

negative (–). However, morphological, physiological and biochemical characteristics of isolated strain AL-54<sup>T</sup> and other reference strains were no significant difference (Table 1).

Phylogenetic analyses based on 16S rRNA gene and MLSA

Almost-complete 16S rRNA gene sequences (1489 bp) of strain AL-54<sup>T</sup> was obtained and compared with 16S rRNA gene sequences available in Gene Bank

using BLAST searches. Phylogenetic analyses of the strain AL-54<sup>T</sup> based on 16S rRNA gene sequences showed that the new strain belonged to the genus *Pseudomonas* and exhibited 16S rRNA gene sequence similarity to the type strains *P. songnenensis* NEAU-ST5-5<sup>T</sup>, *P. zhaodongensis* NEAU-ST5-21<sup>T</sup>, *P. alcaliphila* AL15-21<sup>T</sup>, *P. toyotomiensis* HT-3<sup>T</sup>, *P. oleovorans* subsp. *lubricantis* RS1<sup>T</sup>, *P. stutzeri* ATCC 17588<sup>T</sup>, *P. chengduensis* CGMCC 2318<sup>T</sup> and *P. xanthomarina* KMM 1447<sup>T</sup> with 97.6%, 97.5%, 97.3%, 97.3%, 97.3%, 97.3%, 97.2% and 97.1%,



0.01

◀ **Fig. 1** Phylogenetic tree based on 16S rRNA gene sequences showing relationships between strain AL-54<sup>T</sup> and related species of the genus *Pseudomonas*. The 16S rRNA gene sequence of *Halomonas elongata* ATCC 33173<sup>T</sup> was used as an outgroup. Tree was reconstructed by using the neighbour-joining method with the Kimura two-parameter model. The significance of each branch is indicated by a bootstrap value (percentage) calculated for 1000 subsets (only values greater than 55 % are indicated). Bar, 0.005 substitutions per nucleotide position

respectively (Table 2). In the neighbour-joining tree based on 16S rRNA gene sequences, strain AL-54<sup>T</sup> fell within the clade comprising species of the genus *Pseudomonas* and occupied a branch related to a cluster formed by *P. songnenensis* NEAU-ST5-5<sup>T</sup>, *P. zhaodongensis* NEAU-ST5-21<sup>T</sup>, *P. stutzeri* ATCC 17588<sup>T</sup>, *P. xanthomarina* KMM 1447<sup>T</sup>, *P. alcaliphila* AL15-21<sup>T</sup>, *P. oleovorans* subsp. *lubricantis* RS1<sup>T</sup>, *P. toyotomiensis* HT-3<sup>T</sup> and *P. chengduensis* CGMCC 2318<sup>T</sup> (Fig. 1). Sequence analyses of the 16S rRNA gene showed that strain AL-54<sup>T</sup> was phylogenetically related to the members of the genus *Pseudomonas*, but distinct from all the defined species. The three partial housekeeping genes *rpoB* (1086 bp), *gyrB* (705 bp), *rpoD* (585 bp) sequences were obtained and compared with other species of the genus *Pseudomonas* in GeneBank using BLAST searches. The three housekeeping genes sequences of rest species analyzed in this paper were obtained from public databases. Strain AL-54<sup>T</sup> exhibited with the highest *rpoB* gene sequence similarity to *P. stutzeri* ATCC 17588<sup>T</sup> (94%), and followed by *P. toyotomiensis* HT-3<sup>T</sup> (90%) and *P. alcaliphila* CIP 108031<sup>T</sup> (90%), respectively. The *gyrB* gene sequence similarity to *P. stutzeri* ATCC 17588<sup>T</sup> was 90% and other species in the genus *Pseudomonas* were <90%. It shared lower than 91% *rpoD* gene sequence similarity to type strains of species of the genus *Pseudomonas*. Phylogenetic trees were reconstructed based on the concatenated 16S rRNA, *rpoD*, *gyrB* and *rpoB* gene sequences were using the MEGA 6 software as for the 16S rRNA gene analysis. As shown in neighbour-joining phylogenetic trees based on the concatenated 16S rRNA, *rpoD*, *gyrB* and *rpoB* gene sequences (Fig. 2) and separated partial *rpoD*, *gyrB* and *rpoB* gene sequences, respectively (Fig. S2–S4). The strain AL-54<sup>T</sup> also formed a distinct phylogenetic branch within the genus *Pseudomonas*. All these phylogenetic trees supported the

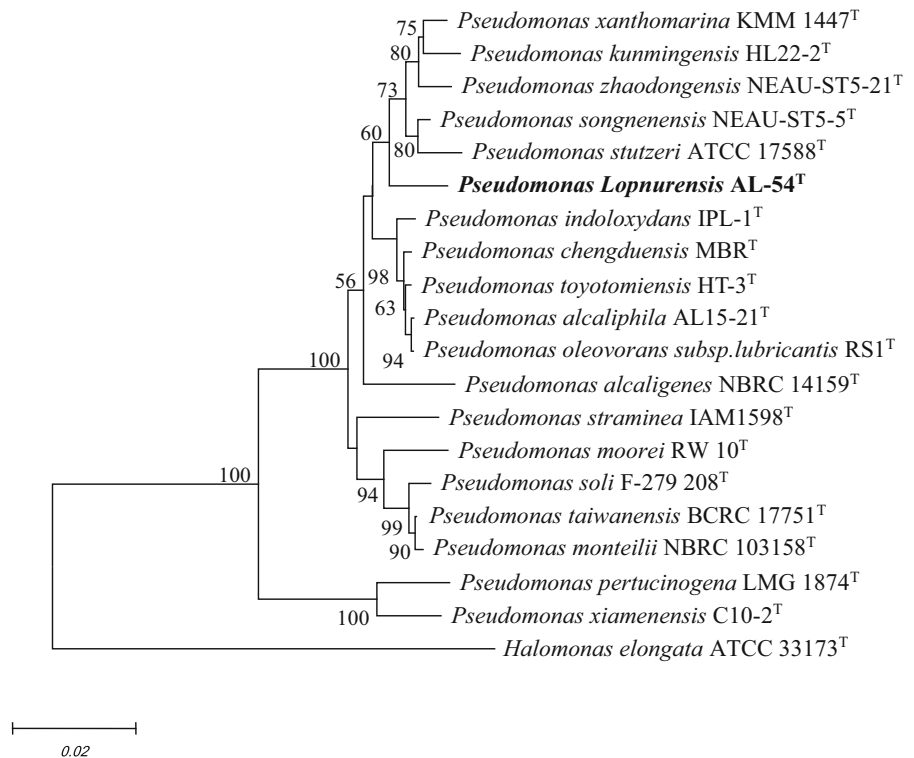
affiliation of strain AL-54<sup>T</sup> as a novel member of the genus *Pseudomonas*.

#### Genome sequencing analysis and DDH relatedness

The draft genome sequence of strain AL-54<sup>T</sup> generated 583 Mb of clean data, and the genome completeness was 99.89% with 0.55% contamination, which considered as good reference genome for deeper analysis ( $\geq 95\%$  completeness,  $\leq 5\%$  contamination) (Parks et al. 2015). The draft genome sequence of strains AL-54<sup>T</sup> has a genome size of 4,914,518 bp and produced 100 contigs after assembly. Genome statistics are presented in Supplementary Table S2 and subsystem distribution of strain AL-54<sup>T</sup> is depicted in Supplementary Fig. S5 based on the RAST (Rapid Annotation by Subsystem Technology) annotation server. Almost 85% total genes were annotated with all sorts of putative functions, including genes coding for flagellar related proteins (flagellar biosynthesis protein FlhA, B and F; flagellar assembly protein FliH; flagellar motor protein MotD), peroxide stress protein YaaA, Oxidase-related enzyme (catalase/peroxidase HPI, glycine oxidase ThiO, glycolate oxidase subunit GlcD, GlcE and GlcF, cytochrome C oxidase Cbb3), heavy metal resistance proteins (arsenical resistance protein ArsH, copper resistance protein B, copper resistance system multicopper oxidase). Furthermore, multiple dioxygenases and related enzymes coding genes involved in the degradation of aromatic compounds were observed, like benzoate 1,2-dioxygenase small subunit, benzoate 1,2-dioxygenase large subunit, 4-hydroxy-3-polyprenylbenzoate decarboxylase, catechol 1,2-dioxygenase, catechol 2,3-dioxygenase.

The genomic average nucleotide identity (ANI) values between AL-54<sup>T</sup> and other type strains were 78.46–85.06% as showed in Table 2. The ANI values were supported the proposed threshold values of less than 95–96% to ANI for the delineation on species level (Chun et al. 2018). The genomic DNA G+C content of strain AL-54<sup>T</sup> was 64.7 mol%, which is consistent with values reported for members of the genus *Pseudomonas* (58 ~ 70 mol%). Three repeats of DDH hybrids showed that the DDH relatedness values between strain AL-54<sup>T</sup> and other type strains revealed 44.0–60.2% (Table 2), clearly below the 70% threshold value generally accepted for species delineation (Stackebrandt et al. 2002). These results strongly

**Fig. 2** Neighbour-joining phylogenetic tree based on concatenated 16S rRNA, rpoD, gyrB and rpoB gene sequences showing the inter-species relationships of strain AL-54<sup>T</sup> and type strains of the closely related species. The concatenated 16S rRNA, rpoD and gyrB gene sequence of *Halomonas elongata* ATCC 33173<sup>T</sup> was used as an outgroup and which all gene sequence given in the file \*all sequence 6423f.txt\*. Bootstrap values > 50 % (based on 1,000 replications) are shown at branch points. Bar, 0.02 substitutions per nucleotide position. The accession numbers of the sequences used in the concatenated phylogenetic tree were shown in Supplementary Table S1.



demonstrated that strain AL-54<sup>T</sup> should be a representative novel species of genus *Pseudomonas*.

#### Chemotaxonomic characterization

The major cellular fatty acids of strain AL-54<sup>T</sup> were summed feature 8 (comprising C<sub>18:1</sub> ω7c / C<sub>18:1</sub> ω6c) (28.0%), summed feature 3 (comprising C<sub>16:1</sub> ω7c / C<sub>16:1</sub> ω6c) (25.5%) and C<sub>16:0</sub> (23.0%), C<sub>12:0</sub> (7.5%), C<sub>19:0</sub> cyclo ω8c (3.3%), C<sub>17:0</sub> cyclo (3.1%). The major cellular hydroxy fatty acids were C<sub>10:0</sub> 3-OH (3.4%) and C<sub>12:0</sub> 3-OH (2.8%). Detailed fatty acid profiles of strain AL-54<sup>T</sup> and the type strains were of seven closely related species of the genus *Pseudomonas* were shown in Table 3. This profile of fatty acids matched those of the reference strains, although there were some differences in the proportions of some components. As given in Table 3, strain AL-54<sup>T</sup> contains a combination of fatty acids found in other *Pseudomonas* species, confirming that strain AL-54<sup>T</sup> is a member of the genus *Pseudomonas* including C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c (28 %), C<sub>16:1</sub> ω7c/C<sub>16:1</sub> ω6c (25.5 %) and C<sub>16:0</sub> (23.7 %). The same fatty acids as

detected in the *P. songnenensis* NEAU-ST5-5<sup>T</sup>, *P. zhaodongensis* NEAU-ST5-21<sup>T</sup>, *P. alcaliphila* AL15-21<sup>T</sup>, *P. toyotomiensis* HT-3<sup>T</sup>, *P. stutzeri* ATCC 17588<sup>T</sup> and *P. xanthomarina* KMM 1447<sup>T</sup>, with the exception of *P. oleovorans subsp. lubricantis* RS1<sup>T</sup>, which have less amount of C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c (Summed Feature8: 15.0 %). Notably, the ratio of the cyclo fatty acids of strain AL-54<sup>T</sup> was different from the above-mentioned reference strains (Table 3). For instance, the isolated strain AL-54<sup>T</sup> contained larger amount of C<sub>17:0</sub> cyclo (3.0%) and C<sub>19:0</sub> cyclo ω8c (3.3%), but other types of strains contained less amount of C<sub>17:0</sub> cyclo < 1.5% and C<sub>19:0</sub> cyclo ω8c < 0.5% (Table 3). Analysis of respiratory lipoproteins indicated that the isolate contained ubiquinone-9 (Q-9), which is compatible with other *Pseudomonas* species (Pascual et al. 2012). Strain AL-54<sup>T</sup> had phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylcholine (PC) as major components, which is in agreement with data published previously for *Pseudomonas* species (Ventosa et al. (1993). Minor amounts of several unidentified polar lipids



**Table 2** Genome comparison of AL-54<sup>T</sup> with available whole genomes of members of the genus *Pseudomonas* on the basis of 16S rRNA sequence similarity, ANI, DDH and G+C mol% differences value (%)

Type strains of genus <i>Pseudomonas</i>	16S rRNA Sequence similarity (%)	ANI (%)	DDH (%)	G+C Difference (%)
<i>P. songnenensis</i> NEAU-ST5-5 <sup>T</sup>	97.6	84.56	44.0	5.7
<i>P. zhaodongensis</i> NEAU-ST5-21 <sup>T</sup>	97.5	78.52	44.7	0.3
<i>P. alcaliphila</i> AL15-21 <sup>T</sup>	97.3	78.46	60.1	1.7
<i>P. toyotomiensis</i> HT-3 <sup>T</sup>	97.3	78.47	48.7	0.4
<i>P. oleovorans</i> subsp. lubricantis RS1 <sup>T</sup>	97.3	78.92	49.1	2.5
<i>P. stutzeri</i> ATCC 17588 <sup>T</sup>	97.3	85.06	60.1	0.7
<i>P. chengduensis</i> CGMCC 2318 <sup>T</sup>	97.2	78.78	58.9	2.4
<i>P. xanthomarina</i> KMM 1447 <sup>T</sup>	97.1	79.24	60.2	4.7

**Table 3** Cellular fatty acid profiles of strain AL-54<sup>T</sup> and type strains of the seven most closely related species of the genus *Pseudomonas*

Fatty acid	1	2	3	4	5	6	7	8
<i>Saturated fatty acids</i>								
C12: 0	7.5	7.4	7.2	6.7	6.6	7.5	7.8	8.1
C16: 0	23.0	19.1	21.4	22.2	25.0	22.0	20.8	23.0
<i>Hydroxy fatty acids</i>								
C10: 03-OH	3.5	3.6	3.3	4.1	4.5	5.2	3.4	4.5
C12: 03-OH	2.8	3.4	3.0	2.3	2.5	3.0	3.7	2.67
<i>Unsaturated fatty acids</i>								
C17 1 ff>8c	1.8	-	0.4	2.2	0.1	0.1	1.8	1.1
C17: 0 cyclo	3.0	0.6	0.6	1.5	0.8	1.2	0.4	0.5
C190 cyclo co8c	3.3	0.5	0.3	0.1	0.1	0.4	0.5	0.1
Summed feature 3*	25.5	28.7	27.8	17.4	16.5	23.0	22.2	20.8
Summed feature 8*	28.0	33.4	33.4	39.1	35.0	15.0	31.0	25.6

\*Summed feature 8 C181 ra7c/C181 ra6c Strains: 1, *P. Lopnurensis* AL-54<sup>T</sup>; 2, *P. songnenensis* NEAU-ST5-5<sup>T</sup>; 3, *P. zhaodongensis*NEAU-ST5-21<sup>T</sup>; 4, *P. alcaliphila* AL15-21<sup>T</sup>; 5, *P. toyotomiensis* HT-3<sup>T</sup> (AB453701); 6, *P. oleovorans subsp. lubricantis* RS1<sup>T</sup>; 7, *P. stutzeri* ATCC 17588<sup>T</sup>; 8, *P. xanthomarina* KMM 1447<sup>T</sup>. All strains were tested under the same growth conditions. (All data in the table were obtained in this study).

were also present, for example, a moderate amount of unknown phospholipids (PL1 ~ PL3) and unidentified lipids L1 (Supplementary Fig. S6).

**Conclusion**

The morphological, chemotaxonomic and phylogenetic analysis strongly supported the affiliation of strain AL-54<sup>T</sup> belongs to the genus *Pseudomonas*. Many different characteristic features such as

physiological and biochemical characteristics, G+C content, fatty acid profile, 16S rRNA gene sequence similarity and DNA-DNA hybridization can be used to distinguish this strain from phylogenetically related taxa. Thus, according to the results of this study, the strain AL-54<sup>T</sup> represents a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas lopnurensis* sp. nov. is proposed. It is concluded that isolated strain is more efficient than previously isolated strains of *pseudomonas* species.

## Description of *Pseudomonas lopnurensis* sp. nov.

*Pseudomonas lopnurensis* sp. nov. (*lop.nur.en'sis*. N.L. fem. adj. *lopnurensis* pertaining to *Lopnur* county in Xinjiang Uyghur autonomous region, PR China, where the type strain was isolated).

Cells are Gram-stain negative, aerobic and rod-shaped (length 0.5–2.1 µm, width 0.5–0.7 µm), motile with one polar flagellum, non-spore-forming. Colonies on LB are creamy, circular, smooth and measure 1.5–2 mm in diameter after incubation for 48 h at 35 °C. The temperature, pH and NaCl concentration ranges for growth are 10–45 °C (Optimum 35 °C), pH 6.5–8.0 (Optimum pH 7) and 1–5% (w/v) NaCl (Optimum 3%, w/v). Tests for catalase and oxidase activities are positive. Hydrolyses Tween 80 extracellularly but not alginate, starch, casein, gelatin or DNA. positive for assimilation of caprate, potassium nitrate, adipate, and negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolyase, urease, hydrolysis of aesculin and gelatin. The major components of the cellular fatty acids (>10%) were summed feature 8 (comprising C<sub>18:1</sub> ω7c /C<sub>18:1</sub> ω6c), summed feature 3 (comprising C<sub>16:1</sub> ω7c /C<sub>16:1</sub> ω6c) and C<sub>16:0</sub>. The major isoprenoid quinone was Q-9. The major polar lipids were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and phosphatidylcholine (PC), minor amounts of several unidentified polar lipids were also present. The DNA G+C content of strain AL-54<sup>T</sup> was 64.7 mol%.

The type strain AL-54<sup>T</sup> (=JCM 19136<sup>T</sup> = CCTCC AB 2013066<sup>T</sup> = NRRL B-59987<sup>T</sup>) was isolated from the storage liquid in the stems of *Populus euphratica* trees at the ancient Ugan River in Xinjiang, PR China.

## Supplementary material

The transmission electron micrograph of the cell, polar lipids, phylogenetic tree (based on the separated partial *rpoD*, *gyrB* and *rpoB* gene sequences), and Genome statistics are available as supplementary materials. Supplementary data associated with this article can be found in the online version.

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**Author contributions** T.M and N.A: responsible for the major experiments, analyze data and prepared the manuscript; M.A, M.R and M.K: isolated strain AL-54<sup>T</sup> and undertake physiological and biochemical experiments; H.M and B.H: responsible for purchasing reference strains; A.R and M.W: designed the experiments and guided the manuscript writing. All authors have read and approved the final manuscript.

## Compliance with ethical standards

**Conflicts of interests** Authors declare that there are no conflicts of interest regarding the publication of this paper.

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