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ORIGINAL PAPER

Mesorhizobium xinjiangense sp. nov., isolated from rhizosphere soil of *Alhagi sparsifolia*

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Abstract

A beige-pigmented, Gram-strain-negative, aerobic, rod-shaped, non-flagellated and non-gliding bacterium, designated strain lm94^T, was isolated from rhizosphere soil of *Alhagi sparsifolia* obtained from Alar city, located in Xinjiang province, China. Growth occurred at 20–45 °C (optimum, 37 °C), in the presence of 0–6% (w/v) NaCl (optimum, 0–1%) and at pH 6.0–9.5 (optimum, pH 7.0–7.5). Phylogenetic analysis based on 16S rRNA gene sequence showed that strain lm94^T belonged to the genus *Mesorhizobium*, with highest sequence similarity to *Mesorhizobium wenxiniae* WYCCWR 10195^T (96.6%). Genome sequencing revealed a genome size of 5 256 375 bp and a G + C content of 63.6 mol%. The average nucleotide identity value and the digital DNA–DNA hybridization value between strain lm94^T and *M. wenxiniae* LMG 30254^T were 75.0% and 20.0%, respectively. The major respiratory quinone was Q-10. The major fatty acids were $C_{19:0}$ cyclo $\omega 8c$ and Summed Feature 8 ($C_{18:1}$ $\omega 6c$ and/or $C_{18:1}$ $\omega 7c$) and its polar lipids consisted of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified phospholipid (PL), phosphatidylcholine (PC), diphosphatidylglycerol (DPG), unidentified aminolipid (AL), unknown glycolipid (GL), unidentified aminophospholipid (APL2) and unidentified polar lipid (L1 and L2). On the basis of these data, strain lm94^T is considered to represent a novel species of the genus *Mesorhizobium*, for which the name *Mesorhizobium xinjiangense* sp. nov. is proposed. The type strain is lm94^T (=KCTC 72863^T=CCTCC AB2019377^T).

Keywords *Mesorhizobium* · Phylogenetic analysis · 16S rRNA gene · Draft genome sequencing · Secondary metabolites

Abbreviations

AAI	Average amino acid identity
ANI	Average nucleotide identity
dDDH	Digital DNA–DNA hybridization
HPLC	High-performance liquid chromatography
MIDI	Microbial identification system
POCP	Percentage of conserved proteins
KCTC	Korean collection for type cultures
CCTCC	China Center for Type Culture Collection
TLC	Thin-layer chromatography

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New taxa–*Proteobacteria*.

The GenBank accession number for the 16S rRNA gene sequence of Mesorhizobium xinjiangense lm94^T is MN519466. The whole genome shotgun project of strain lm94^T has been deposited at DDBJ/ENA/GenBank under the accession number WOAC00000000. The version described in this paper is version WOAC00000000.

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Introduction

The genus *Mesorhizobium*, with the type species *Mesorhizobium loti*, in the family *Phyllobacteriaceae*, phylum *Proteobacteria*, was first described by Jarvis et al. (1997). At the time of writing, the genus *Mesorhizobium* consists of 59 species with validly published names (https://lpsn.dsmz.de/genus/mesorhizobium). Members of the genus *Mesorhizobium* have been isolated from various habitats, such as different leguminous plants, deep-sea sediment (Yuan et al. 2016), soils and seawater (Wang et al. 1999; Fu et al. 2017). Their isolation source has been for the most part the root nodules of various leguminous plants. Members of the genus *Mesorhizobium* are Gram-stain-negative and aerobic. The typical characteristics of the cells include rod-shaped, non-motile

and non-sporulating (Zhang et al. 2012). In addition, the genus *Mesorhizobium* was established for the growth rate which was slower than that of the genus *Rhizobium*. In this study, we describe a novel bacterial strain, designated lm94^T, isolated from rhizosphere soil of *Alhagi sparsifolia*. The aim of the present investigation was to determine the taxonomic position of strain lm94^T based on analysis of phenotypic, phylogenetic, genomic and chemotaxonomic characteristics.

Materials and methods

Isolation, maintenance and culture conditions

Strain lm94^T was isolated from rhizosphere soil of *Alhagi sparsifolia* obtained from Alar city, located in Xinjiang province, China (80° 40′ 63″ E, 40° 25′ 23″ N). The sample was serially diluted in sterile water and plated on LB (yeast extract 5 g, tryptone 10 g, NaCl 10 g, water 1 L) agar and incubated at 30 °C for 10 days. One beige colony, designated lm94^T, was picked and purified by repeated plate streaking. The strain was preserved at − 80 °C in sterile 1% (w/v) saline supplemented with 15% (v/v) glycerol. *M. wenxiniae* LMG 30254 ^{T} was obtained from previous study (Zhang et al. 2018). *M. loti* JCM 21464^T (the type species of this genus) was purchased from the Japan Collection of Microorganisms (JCM). Both strains were used as reference strains.

Phylogenetic and genome sequence analysis

Genomic DNA extraction from strain lm94^T was carried out using a bacterial genomic DNA kit (OMEGA) according to the manufacturer's recommendations. The 16S rRNA gene was amplified by PCR with the primers 27F and 1492R (Mu et al. 2018). The amplicon was cloned into pMD18-T vector (Takara) and recombinant plasmids were reproduced in *Escherichia coli* DH5α cells. Sequencing was performed by TSINGKE Biotechnology (Qingdao, PR China). Then an almost full-length 16S rRNA gene was submitted to GenBank database (accession number is MN519466). Comparison of 16S rRNA with related strains was conducted by EzTaxon server (https://www.ezbiocloud.net/identify) (Yoon et al. 2017). Multiple alignments were performed via the MEGA version 7.0 and phylogenetic trees were reconstructed using the neighbour-joining, maximum-parsimony and minimum-likelihood methods in the computer program MEGA version 7.0 (Kumar et al. 2016). Bootstrap analysis was performed with 1000 replications. The draft genome sequence of strain lm94^T was sequenced at Chinese National Human Genome Center (Shanghai, PR China) using Solexa paired-end sequencing technology. Genome data of *M. wenxiniae* LMG 30254^T (NPKH00000000) and *M. loti* JCM 21464^T (QGGH00000000) were obtained from the NCBI genome database. The predicted coding sequences of each genome were translated and annotated using the KEGG and COG. The average nucleotide identity (ANI) based on the whole genome sequence was calculated using the ANI calculator (www.ezbiocloud.net/tools/ani). The percentage of conserved protein (POCP) was calculated according to previous study (Qin et al. 2014). Digital DNA–DNA hybridization (dDDH) analysis was performed on the DSMZ Genome-to-Genome Distance Calculator platform (Richter et al. 2015). Furthermore, genome mining for the presence of secondary metabolites gene clusters was performed using antiSMASH program (version 5.0) (Blin et al. 2019).

Morphological, physiological and biochemical analyses

For phenotypic characteristics, strain lm94^T was cultivated on LB at 30 °C for 3 days. Microscopic observations were performed using scanning electron microscopy (Hitachi SU-8100). Gram-staining was carried out as described by Cerny (1978). Gliding motility was examined according to the method described by Bowman (2000). The effects of different temperatures on growth were tested at 5, 10, 15, 20, 25, 30, 37, 40, 45, 50, 55, 60 °C for approximately 7 days in liquid medium. Then salt tolerance tests were tested at LB medium with a pH of 7.0, the concentrations of NaCl were ranging from 0 to 10% (w/v, at intervals of 1%). Then the initial pH range for growth was determined at pH 3.0–10.0 intervals of 0.5 units and at 30 °C in LB agar medium. The pH was adjusted by addition of citric acid–sodium citrate buffer (pH 3.0 and 3.5), NaH_2PO_4 buffer (4.0, 4.5 and 5.0), MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0, 9.5 and 10.0). The OD_{600} values of the cultures were measured after incubation for the growth tests mentioned above. Growth under anaerobic (10% H_2, 10% CO_2 and 80% N_2) and microaerobic (5% O_2, 10% CO_2 and 85% N_2) conditions were determined on LB medium with or without 0.1% (w/v) KNO_3 after incubation for 14 days in an anaerobic jar. Oxidase activity was tested using the oxidase reagent kit (BioMérieux) according to manufacturer's instructions. Catalase activity was detected by bubble production in 3% (v/v) H_2O_2. Susceptibility to antibiotics was examined on LB medium using the disc diffusion method as described previously, and according to procedures outlined by the Clinical and Laboratory Standards Institute (Institute 2007). Tests for other physiological and biochemical feature were determined using the API 20E and API 50CHB kits (BioMérieux). Enzyme activities were examined using the API ZYM test system (BioMérieux). The substrate-oxidation profiles were gained using Biolog GEN III MicroPlates. All the API and Biolog tests were performed in duplicate

according to the manufacturer's instructions, except that the suspension media were adjusted to 1% (w/v) NaCl.

Chemotaxonomic characterization

Strain lm94^T and the reference strains were cultured at optimum growth conditions. Cells were harvested in the exponential growth phases and then lyophilized immediately for chemotaxonomic analyses. The fatty acids were analyzed according to the Sherlock Microbial Identification System (MIDI) Sherlock version 6.3. The fatty acid extracts obtained using the standard MIDI protocol were subjected to further analysis by GC–MS as previously described. Peaks were automatically integrated and fatty acid names and percentages were determined using MIS standard software with the TSBA40 database. Polar lipids were separated by two-dimensional silica gel TLC according to the methods of Xu et al. (2007). Polar lipids were then analyzed as described by Minnikin et al. (1984). For analyses of respiratory quinones, respiratory quinones were extracted from freeze-dried cells, and determined by HPLC (Kroppenstedt 1982).

Results and discussion

Phylogenetic and genome analysis

Pairwise comparison of the 16S rRNA gene sequence of strain lm94^T (GenBank accession number MN519466) with the corresponding 16S rRNA gene sequences in the EzBioCloud database indicated that the strain shared 16S rRNA gene sequence similarities of 96.6% and 94.9% with *Mesorhizobium wenxiniae* LMG 30254^T and *Mesorhizobium loti* JCM 21464^T (the type species of this genus), respectively. The topological structure of the phylogenetic neighbor-joining tree (Fig. 1) clearly indicated that strain lm94^T clustered with species of the genus *Mesorhizobium* and was distinctly separated from members of other genera.

Draft genome sequencing of strain lm94^T yielded a genome of 5 256 375 bp in length after assembly which produced 53 contigs, and the N50 value was 177 626 bp. The genome coverage was 561x. The genomic DNA G+C content of strain lm94^T calculated from the draft genome sequence was 63.6 mol%, which was different from the related type strain *Mesorhizobium wenxiniae* LMG 30254^T and *Mesorhizobium loti* JCM 21464^T (Table 1). The whole genome data were used to calculate the average nucleotide identity (ANI; www.ezbiocloud.net/tools/ani) with *M. wenxiniae* LMG 30254^T and *M. loti* JCM 21464^T. The OrthoANI value of strain lm94^T with *M. wenxiniae* LMG 30254^T and *M. loti* JCM 21464^T were 75.0 and 75.2%, respectively, which were lower than the species threshold of 95–96% (Liu et al. 2019). The POCP values between the genomes of strain lm94^T and reference strains were 53.6 and 53.5%, respectively. The dDDH values (the recommended results from formula 2) between strain lm94^T and *M. wenxiniae* LMG 30254^T and *M. loti* JCM 21464^T were 20.0 and 20.1%, respectively. Both were significantly lower than their threshold values (dDDH, 70%) (Meng et al. 2020). Secondary metabolic genes were predicted by antiSMASH (https://antismash.secondarymetabolites.org/). The analysis of secondary metabolic gene clusters revealed that strain lm94^T harboured seven gene clusters, which was different from reference strains (Table 1).

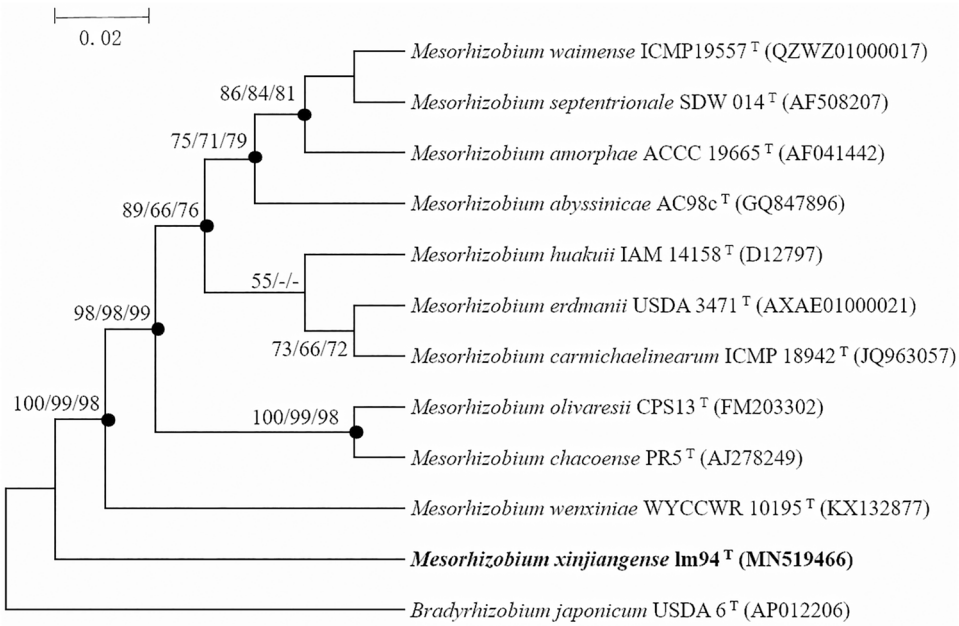

Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain lm94^T among species of the genus *Mesorhizobium*. Bootstrap values (expressed as percentages of 1000 replications) > 50% are shown at branching points. *Bradyrhizobium japonicum* USDA 6 ^{T} (GenBank accession No. AP012206) was used as an outgroup. Filled circles indicate nodes also obtained in both maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position

Table 1 Comparison between the genomes and gene clusters involved in biosynthesis of secondary metabolites of strain lm94^T, *M. wenxiniae* LMG 30254^T and *M. loti* JCM 21464^T

Attribute	lm94^T	*M. wenxiniae*	*M. loti*
Draft genome characteristics			
GenBank ID	WOAC00000000	NPKH00000000	QGGH01000000
Genome size (bp)	5,256,3758	6,681,850	7,451,806
Contigs	53	43	52
N_{50} length (bp)	177,626	386,068	607,128
Coverage	561x	156x	139x
tRNA Numbers	47	62	54
Genes assigned to COGs	4078	5859	6573
Genes assigned to KEGG	2431	2076	2145
G+C content (%)	63.6%	61.9%	62.3%
Secondary metabolite clusters			
Terpene	1	1	1
Betalactone	1	0	0
Hserlactone	2	3	4
NAGGN	1	0	0
Type III PKS	0	1	1
Redox-cofactor	0	1	0
Thioamitides	1	1	0
RiPP-like	1	1	0
Total number of clusters	7	8	6

Morphological, physiological and biochemical characteristics

The cells of strain lm94^T grown on LB agar were circular, smooth, approximately 0.3–0.5 μm in width and 0.7–1.0 μm in length (Fig. S1) and cells without appendages were observed. Cells were Gram-stain-negative without gliding motility. The morphological characteristics between strain lm94^T and reference strains were similar, which was consistent with the result of the phylogenetic analysis. However, the most important phenotypic feature, which can be significantly separated from reference strains, was the colour of cell mass. Anaerobic growth did not occur on LB agar. The catalase and oxidase test of strain lm94^T was positive. Cells of the type strain lm94^T were sensitive to kanamycin, chloramphenicol, streptomycin, gentamicin, neomycin, tetracycline, cefoperazone, spectinomycin, paromomycin, penicillin and erythromycin, while resistant to ampicillin, vancomycin, troleandomycin, rifamycin, minocycline and lincomycin. The detailed morphological, physiological, and biochemical characteristics of strain lm94^T are summarized in the species description and in Table 2.

The major cellular fatty acids (> 10.0%) of strain lm94^T were $C_{19:0}$ cyclo $\omega 8c$ and summed feature 8 ($C_{18:1}$ $\omega 6c$ and/or $C_{18:1}$ $\omega 7c$), which were also the dominant fatty acids in the two reference strains. While the fatty acid composition was similar to that of two related genera, but there were differences in the proportions of some fatty acids. For example, $C_{18:1}$ $\omega 9c$ was found in reference strains, but was absent in strain lm94^T. Moreover, iso-$C_{15:0}$ 3-OH was present in strain lm94^T, but was absent in two related genera. Further comparative information between the proposed new species and two related genera are given in Table S1. The major polar lipids identified in strain lm94^T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified phospholipid (PL), phosphatidylcholine (PC), diphosphatidylglycerol (DPG), unidentified aminolipid (AL), unknown glycolipid (GL), unidentified aminophospholipid (APL2) and unidentified polar lipid (L1 and L2). All three strains contained PE, PG, PC and DPG. However, strain lm94^T contained GL that was absent in two reference strains. The polar lipid profiles of all the strains are represented in Fig. S2. Furthermore, the sole respiratory quinone of strain lm94^T was Q-10, which was also the case with *M. wenxiniae* LMG 30254^T and *M. loti* JCM 21464^T.

Based on the results of the chemotaxonomic examination, phenotypic analysis and phylogenetic analysis from this study, strain lm94^T represents a novel species of the genus *Mesorhizobium*, for which the name *Mesorhizobium xinjiangense* sp. nov. is proposed.

Description of *Mesorhizobium xinjiangense* sp. nov.

Mesorhizobium xinjiangense (xin.jiang.en'se. N.L. neut. adj. xinjiangense referring to Xinjiang province in China, the area where the type strain was isolated).

Cells are Gram-stain-negative, aerobic, non-flagellated, non-gliding, rod-shaped, 0.3–0.5 μm in width,

Table 2 Characteristics that differentiate strain lm94^T from *M. wenxiniae* LMG 30254^T and *M. loti* JCM 21464^T

Characteristic	1	2	3
Pigmentation (Colony colour)	Beige	White	White
Growth range of:			
NaCl (w/v, %)	0–6	0–3	0–2
pH range	6.0–9.5	6.5–8.5	6.6–9.0
Temperature range for growth (°C)	20–45	25–37	25–40
Hydrolysis of			
Lysine decarboxylase	+	–	–
Utilization of citrate	–	–	+
Tryptophan deaminase	+	–	–
Arabinose	+	–	+
Acid production from (API 50 CHB)			
D-arabinose	+	–	–
D-xylose	+	–	+
L-arabinose	+	–	+
D-adonitol	+	–	+
Sorbitol	+	–	–
D-tagatose	–	+	–
Enzymic activities (API ZYM):]			
Alkaline phosphatase	+	–	–
Esterase (C4)	–	+	+
Esterase lipase (C8)	–	+	–
Valine arylamidase	w	–	–
α-glucosidase	–	+	+
Trypsin	+	–	+
Oxidation of (Biolog GEN III)			
D-Maltose	–	+	+
D-Trehalose	–	+	+
D-Cellobiose	–	+	+
D-Fucose	–	+	+
Sucrose	–	+	+
D-Turanose	–	+	+
N-Acetyl-β-D-Mannosamine	–	+	+
D-Serine	+	–	–
α-Hydroxy-Butyric Acid	+	–	–

Strains: 1, lm94^T; 2, *M. wenxiniae* LMG 30254^T; 3, *M. loti* JCM 21464^T. All data are from this study unless otherwise indicated

+ positive; – negative; *w* weakly positive

0.4–1.0 μm in length. Colonies on LB are circular, convex, smooth, opaque, beige-pigmented and approximately 1.0–1.5 mm in diameter after 3 days at 37 °C. Growth occurs at 20–45 °C (optimum 37 °C), pH 6.0–9.5 (optimum pH 7.0–7.5), and in the presence of 0–6% (w/v) NaCl (optimum 0–1%). Cells are catalase-positive and oxidase-positive. They are negative for H_2S production, citrate utilization, indole production, gelatinase production and starch hydrolysing but positive for arabinose, tryptophan deaminase and lysine decarboxylase according to API 20E tests. Acid is produced from D-arabinose, L-arabinose, D-ribose, D-xylose, D-adonitol, D-glucose, D-fructose, L-rhamnose, mannose, ESC, sorbitol, D-lyxose, D-fucose, L-fucose, D-arbaitol and 5-keto-potassium gluconate (API 50 CHB). In API ZYM tests, positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities. In carbon source oxidation tests, cells are positive for D-sorbitol, D-mannitol, D-arabitol, glycerol, D-fructose-6-PO_4, N-acetyl-D-glucosamine, L-alanine, L-arginine, L-glutamic acid, L-histidine, α-D-glucose, D-mannose, D-fructose, L-fucose, D-fucose, L-rhamnose, D-serine, D-glucuronic acid, glucuronamide, L-lactic acid, D-malic acid and L-malic acid. Q-10 is the sole respiratory quinone. The major polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified phospholipid (PL), phosphatidylcholine (PC), diphosphatidylglycerol (DPG), unidentified aminolipid (AL), unknown glycolipid (GL), unidentified aminophospholipid (APL2) and unidentified polar lipid (L1 and L2). The major cellular fatty acids (> 10.0%) are $C_{19:0}$ cyclo $\omega 8c$ and Summed Feature 8 ($C_{18:1}$ $\omega 6c$ and/or $C_{18:1}$ $\omega 7c$). The genomic DNA G+C content of the type strain is 63.6 mol%.

The type strain is lm94^T (=KCTC 72863^T=CCTCC AB2019377^T) and was isolated from rhizosphere soil of *Alhagi sparsifolia*, collected from Xinjiang province, China (80º 40′ 63″ E, 40º 25′ 23″ N).

The GenBank accession number for the 16S rRNA gene sequence of strain lm94^T is MN519466. The whole genome shotgun project of strain lm94^T has been deposited at DDBJ/ENA/GenBank under the accession WOAC00000000.

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Author contributions Author contributions MD, LWM and LQ designed research and project outline. JY, MH, LYL, FXY and HZS performed isolation, deposition, and identification. MD, GPF, DZJ, LWM and LQ drafted the manuscript. All authors read and approved the final manuscript.

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Declarations

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

Human and animal rights This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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