Archives of Microbiology (2022) 204:249
https://doi.org/10.1007/s00203-022-02869-y

ORIGINAL PAPER

Pseudomonas tumuqii sp. nov., isolated from greenhouse soil

Delong Kong[1] · Qingqing Li[1] · Yanzheng Zhou[2] · Yan Wang[1,3] · Xu Jiang[1] · Zhiye Wang[4] · Zhiyong Ruan[1,3,5]

Received: 8 November 2021 / Revised: 22 March 2022 / Accepted: 23 March 2022 / Published online: 9 April 2022

Abstract

A Gram-stain-negative, aerobic, rod-shaped and motile bacterium, named LAMW06^T, was isolated from greenhouse soil in Beijing, China. In the 16S rRNA gene sequence comparison, strain LAMW06^T had the highest similarity with *Pseudomonas cuatrocienegasensis* 1N^T. Phylogenetic analysis based on the 16S rRNA and three housekeeping gene sequences (*gyrB*, *rpoB* and *rpoD*) indicated that strain represented a member of the genus *Pseudomonas*. The genome sequence size of the isolate was 5.5 Mb, with a DNA G + C content of 63.5 mol%. The average nucleotide identity and DNA–DNA hybridization values between strain LAMW06^T and closely related members of *Pseudomonas borbori* R-20821^T, *Pseudomonas taeanensis* MS-3^T and *P. cuatrocienegasensis* 1N^T were 90.9%, 82.4%, 81.5% and 43.0%, 25.9%, 24.6% respectively. The major fatty acids contained summed feature 3 ($C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c), $C_{18:1}$ ω7c and $C_{16:0}$. The primary respiratory quinone was ubiquinone-9. The main polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, six aminophospholipids, six phospholipids, one aminolipid and one glycolipid. According to the genotypic, phylogenetic and chemotaxonomic data, strain LAMW06^T represents a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas tumuqii* sp. nov. is proposed. The type strain is LAMW06^T (= GDMCC 1.2003^T = KCTC 72829^T).

Keywords *Pseudomonas* · 16S rRNA gene · Polyphasic taxonomy

Introduction

The first species of genus *Pseudomonas* was described by Migula in 1894 (1900). At the time of writing, there are 256 validly named species (https://lpsn.dsmz.de/genus/pseudomonas) with validly published names on the List of Prokaryotic names with Standing in Nomenclature (Parte et al. 2020). Members of the genus *Pseudomonas* are described to be Gram-negative, rod-shaped, motile and catalase- and oxidase-positive (Anwar et al. 2017; Wang et al. 2020). The DNA G + C contents calculated from available genomes ranges from 58 to 69 mol% and the major respiratory quinone is ubiquinone-9 (Sun et al. 2018). Members of the genus *Pseudomonas* have been isolated from a wide variety of environments comprising soils (Zou et al. 2019), waters (Romanenko et al. 2008), plants (Timilsina et al. 2018) and animals (Lick et al. 2020). We isolated a novel strain designated as LAMW06^T from soil sample collected from a greenhouse. Based on the genotypic, phylogenetic, phenotypic and chemotaxonomic characterization, the isolate is considered to represent a new species of the genus *Pseudomonas*.

Communicated by Erko Stackebrandt.

Delong Kong and Qingqing Li have contributed equally to this work.

✉ Zhiyong Ruan
ruanzhiyong@caas.cn

1 CAAS-CIAT Joint Laboratory in Advanced Technologies for Sustainable Agriculture, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China

2 Jining Academy of Agricultural Sciences, Jining 272000, People's Republic of China

3 College of Resources and Environment, Tibet Agricultural and Animal Husbandry University, Linzhi 860000, People's Republic of China

4 Key Laboratory of Microbial Resources Exploitation and Application of Gansu Province, Institute of Biology, Gansu Academy of Sciences, Lanzhou 730000, People's Republic of China

5 College of Life Sciences, Yantai University, Yantai 264005, People's Republic of China

Materials and methods

Isolation and culture conditions

Strain LAMW06^T was isolated from soil sample collected from a greenhouse in Beijing (39°96.73' N, 116°33.53' E), using an isolation medium of trypticase soy agar (TSA; Difco). The soil samples were diluted and cultured on the TSA medium after at 30 °C for 3 days, separated colonies were picked and serially streaked onto TSA plates incubating at 30 °C to obtain single colony. Strain LAMW06^T was obtained after several re-streaking and transfer onto TSA plates. The pure culture of strain LAMW06^T was preserved at − 80 °C in tryptic soy broth (TSB; Difco) medium with 25% (v/v) glycerol. The strains *Pseudomonas cuatrocienegasensis* DSM 23418^T (= 1N^T) and *Pseudomonas borbori* DSM 17834^T (= R-20821^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM); *Pseudomonas taeanensis* JCM 16046^T (= MS-3^T) was obtained from the Japan Collection of Microorganisms (JCM). All the strains were chosen as reference strains in this study.

Bacterial strain and culture condition

Cells morphology were examined using light microscope (Nikon 80i, Tokyo, Japan) and transmission electron microscope (Hitachi 7500, Tokyo, Japan) after incubation on TSA agar plates for 3 days at 30 °C (Ruan et al. 2014). The temperature range for growth was determined by incubating strain LAMW06^T at 10, 15, 20, 25, 30, 35, 37, 40 and 45 °C. The pH value range was determined in tryptic soy broth (TSB; Difco) with pH values of 4.0–11.0 at intervals of 0.5 pH units. The optimal concentration of NaCl for growth was investigated using NaCl-free TSB medium (prepared according to the TSB formula without NaCl) with different NaCl concentrations (0, 0.5 and 1–12% at 1.0% increments, w/v). The pH of the media was adjusted using the following buffer systems: MES (pH 4.0–6.0), MOPS (pH 7.0), Tricine (pH 8.0), TAPS (pH 9.0), CAPS (pH 10.0) and Na_2CO_3/ $NaHCO_3$ (pH 11.0) (Wang et al. 2017). Catalase and oxidase activities were detected by observing bubble production in 3% (v/v) H_2O_2 solution and color variance of 1% (w/v) tetramethyl-*p*-phenylenediamine, respectively. Cell motility was examined using the hanging-drop technique. Anaerobic growth was tested on TSA plates at 30 °C for 10 days in GasPak EZ anaerobic container system (BD) added with sodium nitrite (10 mM) or sodium nitrate (20 mM) as potential electron acceptors (Skerman 1967). Gram-stain reaction, H_2S production and methyl red test, hydrolysis of starch, gelatin and Tween 20, 40, 60 and 80 were detected as described by Smibert and Krieg (1994). Additional physiological and biochemical features were determined using API ZYM, 20NE and 50CH strips (bioMérieux) according to the manufacturers' instructions. Antibiotic susceptibility test was performed on TSA plates using antibiotic disks (Hangzhou Microbial Reagent) containing the following concentrations (μg per disk unless otherwise stated): tetracycline (30 μg), gentamicin (10 μg), erythromycin (15 μg), ampicillin (10 μg), sulfaisoxazole (300 μg), vancomycin (30 μg), clindamycin (2 μg) and amikacin (30 μg).

Phylogenetic analysis based on 16S rRNA gene sequences

The 16S rRNA gene of strain LAMW06^T was amplified by PCR using the bacterial universal primers 27F and 1492R (Weisburg et al. 1991) and compared with available sequences using the EzBioCloud identify service (Yoon et al. 2017a). The genomic DNA was prepared using the method described by Sun et al. (2016). The purified PCR product was inserted into pGEM-T vector and sequenced by Shanghai Life Technologies Company. Multiple sequences were aligned using CLUSTAL X program (Thompson et al. 1997). Phylogenetic relationships were analysed with neighbour-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981) and maximum-parsimony (MP) methods (Fitch 1971) using MEGA 7.0 software (Kumar et al. 2016). According to the algorithm of the Kimura's two-parameter model, evolutionary distances were calculated using the NJ, ML and MP trees. The topologies of phylogenetic trees were evaluated by bootstrap analysis based on 1000 replicates (Kimura 1980). Multilocus sequence analysis (MLSA) was performed based on the three housekeeping genes *gyrB*, *rpoB* and *rpoD* that gathered from the genome assembly (Table S1), following the method described by Anurat et al. (2019) based on the same taxonomic sampling.

Genomic analysis

The genomic DNA G + C content was calculated according to the draft genome sequence of strain LAMW06^T, which was done on the Illumina MiSeq platform by Guangzhou Magigene Company. SOAPdenovo assembler software was applied to assemble the reads (Yoon et al. 2017b). Gene annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline and Swiss-Prot database. The genes involved in metabolic pathways were analysed using the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database. Other genome sequences of closest phylogenetic relatives were obtained from the GenBank sequence database. The average nucleotide identity (ANI) and in silico DNA–DNA hybridization (DDH) values were calculated

according to the minimal standards proposed by Chun et al. (2018). The ANI values between strain LAMW06^T and the relative reference species of strains *P. cuatrocienegasensis* 1N^T (accession number FOFP00000000 of the NCBI GenBank database), *P. borbori* R-20821^T (FOWX00000000), *P. neuropathica* P155^T (JACOPX000000000) and *P. taeanensis* MS-3^T (AWSQ00000000), and *P. peli* DSM 17833^T (JAAQXQ000000000) were calculated using the OrthoANIu algorithm (https://www.ezbiocloud.net/tools/ani). The in silico DDH value between two strains was calculated by Genome-to-Genome Distance Calculator 2.1 (http://ggdc.dsmz.de). Antibiotic resistance genes (ARGs) were predicted with Comprehensive Antibiotic Resistance Database (CARD) software based on genome, protein or metagenomics data (Jia et al. 2017).

Chemotaxonomic analyses

For fatty acids analyses, strain LAMW06^T, *P. cuatrocienegasensis* DSM 23418^T, *P. taeanensis* JCM 16046^T and *P. borbori* DSM 17834^T were incubated on TSA medium after 2 days at 30 °C. According to the manufacturers' instructions, cellular fatty acids were analysed by the Sherlock Microbial Identification System with the standard MIS Library Generation Software (VERSION 6.0 and Date 4, Microbial ID) and a 6890 N gas chromatograph (Agilent) (Sakamoto et al. 2002). Respiratory quinones of strain LAMW06^T were extracted from freeze-dried cells and analysed using LC–MS (Minnikin et al. 1984). The polar lipid extracts were isolated by two-dimensional TLC using silica gel 60 F 254 aluminium-backed thin-layer plates (Merck) and identified by the method described by Xu et al. (2011). Molybdophosphoric acid was used for the detection of all lipids, ninhydrin reagent for aminolipids, molybdenum blue reagent for phospholipids and p-anisaldehyde reagent for glycolipids (Kates 1986).

Results

Morphological and physiological characteristics

Cells of strain LAMW06^T were 1.2–2.0 µm length and 0.6–1.0 µm width rod-shaped in Fig. S1. Positive for oxidase and catalase activities, negative for hydrolysis of starch, casein, chitin, Tween 20, 40 and 60 as same as the related strains *P. cuatrocienegasensis* DSM 23418^T, *P. borbori* DSM 17834^T and *P. taeanensis* JCM 16046^T. Negative for gelatin hydrolysis unlike *P. cuatrocienegasensis* DSM 23418^T. In the API ZYM strip test, esterase lipase (C8) and leucine arylamidase were positive for strain LAMW06^T and the related strains *P. cuatrocienegasensis* DSM 23418^T, *P. borbori* DSM 17834^T and *P. taeanensis* JCM 16046^T. The lists of differing physiological and biochemical characteristics of strain LAMW06^T and their closely related species are shown in Table 1. Strain LAMW06^T was resistant to ampicillin, sulfaisoxazole and clindamycin, intermediately susceptible to gentamicin, and susceptible to tetracycline, erythromycin, vancomycin and amikacin. According to the antibiotic susceptibility tests.

Table 1 Differential characteristics of strain LAMW06^T and its relative reference strains in the genus *Pseudomonas*

Characteristic	1	2	3	4	5
Hydrolysis of					
Gelatin	−	+	−	−	−
Tween 80	−	−	+	+	−
Enzyme activities					
Alkaline phosphatase	−	+	−	−	−
Esterase (C4)	+	+	−	+	−
Lipase (C14)	−	+	−	−	−
Valine arylamidase	−	w	−	−	−
Trypsin	−	+	w	−	−
Acid phosphatase	−	+	−	+	−
Naphthol-AS-BI-phosphohydrolase	+	−	+	+	+
Assimilation of					
Glucose	+	−	+	+	−
Mannitol	−	−	−	+	−
Maltose	+	−	+	−	+
Gluconate	+	+	+	+	−
Capric acid	+	+	+	−	−
Citrate	+	+	−	+	−
Acid production from					
D-Glucose	−	−	+	−	−
D-Fructose	−	−	w	−	−
D-Mannose	−	−	−	−	−
L-Sorbose	−	−	−	−	−
L-Rhamnose	−	+	−	−	−
D-Sucrose	−	−	+	−	−
Potassium gluconate	−	+	+	−	−
Nitrate reduction	+	−	+	+	−
DNA G+C content (mol%)	63.5	61.9[a]	60.7[b]	57.6[c]	60.7[b]

Strains: 1, LAMW06^T; 2, *P. cuatrocienegasensis* DSM 23418^T; 3, *P. borbori* DSM 17834^T; 4, *P. taeanensis* KCTC 22612^T; 5, *P. peli* LMG 23201^T, data from Vanparys et al. 2006. All strains were cultivated with the same medium and growth conditions. Data are from this study unless indicated. Symbols: +, positive; −, negative; w, weak reaction

[a]Data from (Escalante et al. 2009)

[b]Data from (Vanparys et al. 2006)

[c]Data from (Lee et al. 2010)

Phylogenetic analysis

Based on 16S rRNA gene sequence similarity, strain LAMW06^T (1418 bp) was closely related to *P. cuatrocienegasensis* 1N^T (97.4%), *P. borbori* R-20821^T (97.3%), *P. neuropathica* P155^T (97.0%), *P. taeanensis* MS-3^T (97.0%) and lower than 97.0% to other species. Phylogenetic analysis based on the NJ, ML and MP method revealed that strain LAMW06^T did not cluster with other type species (Fig. 1, S2 and S3). The phylogenetic trees analysis indicated that strain LAMW06^T belonged to the genus *Pseudomonas*. Alignment and comparison of the *gyrB*, *rpoB* and *rpoD* genes indicated that strain LAMW06^T and the type strain *Pseudomonas borbori* R-20821^T formed a clade by its own (Fig. 2).

Genomic analysis

The genome of strain LAMW06^T was 5.51 Mbp including 65 contigs with N50 as 351,006 coding sequences. The DNA G + C content of strain LAMW06^T was 63.5 mol%, which was consistent with the range of 58–69 mol% reported for the genus *Pseudomonas*. The ANI value between strain LAMW06^T and the relative reference species of genus *Pseudomonas* were less than 91.0%, for example, with *P. cuatrocienegasensis* 1N^T, *P. borbori* R-20821^T, *P. neuropathica* P155^T and *P. taeanensis* MS-3^T, and *P. peli* DSM 17833^T were 81.5%, 90.9%, 76.8% and 82.4%, respectively, which was significantly less than the threshold used for species recognition (94–96%) (Meier-Kolthoff et al. 2013). The in silico DDH values between strain LAMW06^T and the relative reference species of genus *Pseudomonas* were less than 43.0%, for example, with *P. cuatrocienegasensis* 1N^T, *P. borbori* R-20821^T, *P. neuropathica* P155^T and *P. taeanensis* MS-3^T were 24.6%, 43.0%, 21.4% and 25.9% (Table S2), respectively, which was also lower than the threshold value recommended for the assignment of strains to the same genomic species (70%) (Goris et al. 2007). Strain LAMW06^T and the relative reference species of *P. cuatrocienegasensis* 1N^T, *P. borbori* R-20821^T, *P. neuropathica* P155^T and *P. taeanensis* MS-3^T had the similar antibiotic resistance genes *ade*F and *rsm*A from genomes that involved in the resistance-nodulation-cell division (RND) antibiotic efflux pump (Table S3).

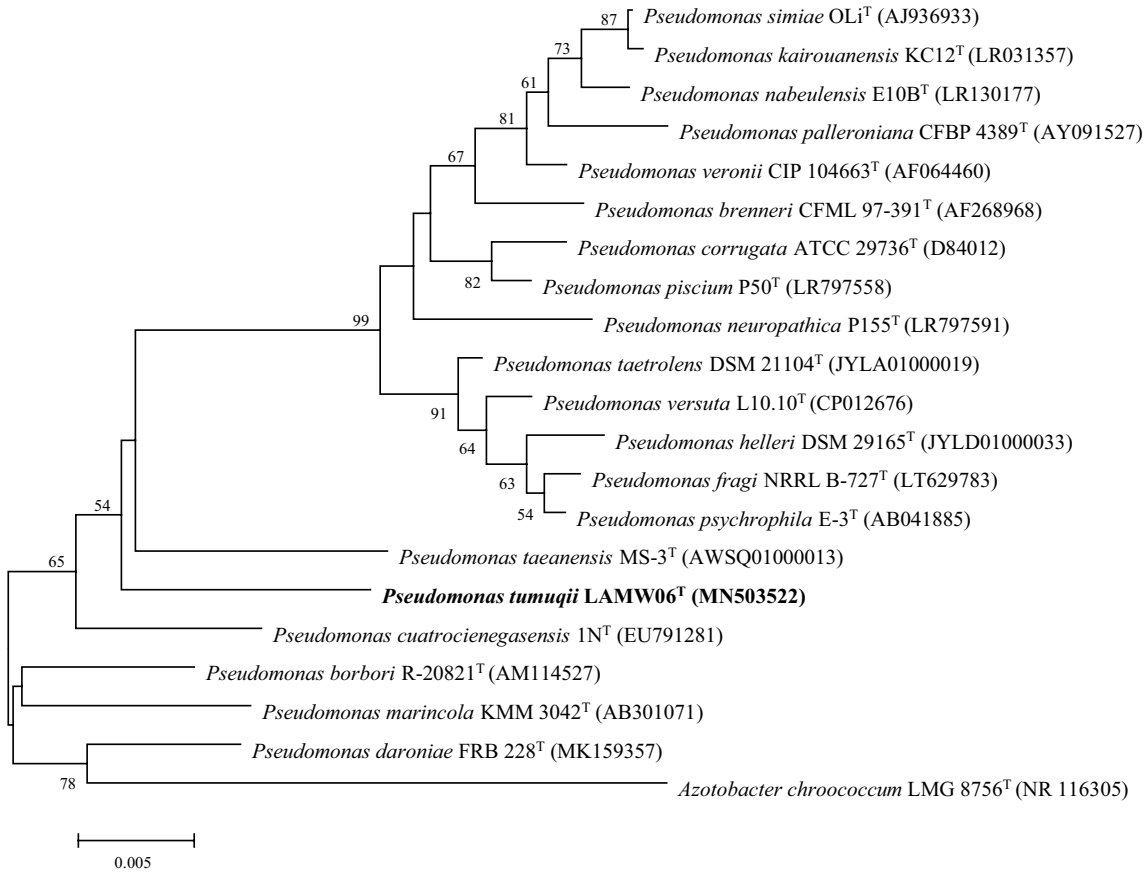

Fig. 1 Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain LAMW06^T and its related taxa. The sequence of *Azotobacter chroococcum* LMG 8756^T was used as outgroup. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood method and maximum-parsimony method. Bootstrap values (those above 50%) are shown as percentages of 1000 replicates. Bar, 0.005 substitutions per nucleotide position

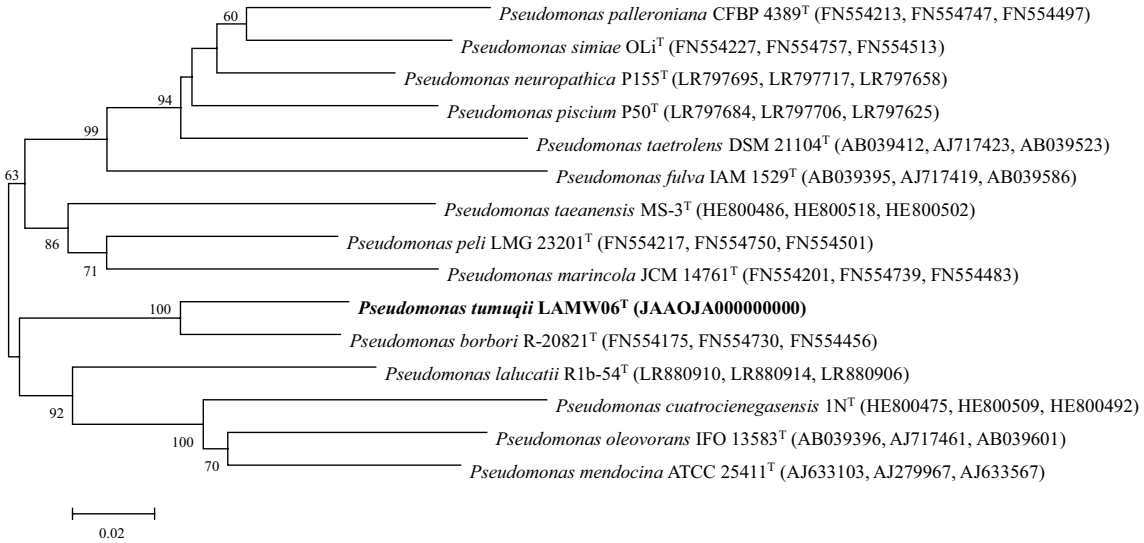

Fig. 2 Phylogenetic tree based on MLSA using the three housekeeping genes *gyrB*, *rpoB* and *rpoD* of type strains of species of the genus *Pseudomonas*, reconstructed using the neighbour-joining method. Bootstrap values were expressed based on 1000 replications; only values 50% or above are shown at the nodes. Bar, 0.02 nucleotide substitutions per 100 nucleotides

Chemotaxonomic characteristics

The predominant fatty acids of strain LAMW06^T (> 10%) were summed feature 3 ($C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c) (38.2%), $C_{18:1}$ ω7c (18.1%) and $C_{16:0}$ (14.9%) consistent with strains *P. cuatrocienegasensis* DSM 23418^T, *P. borbori* DSM 17834^T and *P. taeanensis* JCM 16046^T. Strain LAMW06^T contained iso-$C_{12:0}$ (6.3%) was lower than that of strain *P. cuatrocienegasensis* DSM 23418^T shown in Table S4. The predominant menaquinone of strain LAMW06^T was ubiquinone-9 (Q-9) like other species of the genus *Pseudomonas*. Major polar lipids were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), six aminophospholipids (APL1-6), six phospholipids (PL1-6), one aminolipid (AL) and one glycolipid (GL) (Fig S4).

Therefore, based on the above phenotypic, phylogenetic analysis and genotypic data, it is proposed that strain LAMW06^T should be classified as representative of a novel species of the genus *Pseudomonas* with the name *Pseudomonas tumuqii* sp. nov.

Description of *Pseudomonas tumuqii* sp. nov.

Pseudomonas tumuqii (tu.mu'qi.i. N.L. gen. n. tumuqii, of the Tumuqi biotechnological company, China, where taxonomic studies on this species were performed).

Cells are Gram-stain-negative, rod-shaped (0.6–1.0 × 1.2–2.0 μm), and motile by means of a single flagellum. Colonies are circular, smooth, light brown and with approximately 1.0–2.5 mm in diameter incubation on TSA after 3 days at 30 °C. Growth occurs at 15–37 °C (optimum, 30 °C), pH 5.0–10.0 (optimum, 8.0) and 0–4% (w/v) NaCl (optimum, 1%). Positive for catalase and oxidase, and negative for hydrolysis of starch, gelatin, casein, chitin, Tween 20, 40, 60 and 80. In the API ZYM and 20NE strip tests, positive for esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase, nitrate reduction, and assimilation of glucose, maltose, gluconate, capric acid, malic acid and citrate. In the API 50CH strip test, all results are negative. The major cellular fatty acids (> 10%) are summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c), summed feature 3 ($C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c) and $C_{16:0}$. The major menaquinone and polar lipids are ubiquinone-9 and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, six aminophospholipids, one aminolipid, six phospholipids and one glycolipid, respectively. The genomic DNA G + C content is 63.5 mol%.

The type strain LAMW06^T (= GDMCC 1.2003^T = KCTC 72829^T), was isolated from greenhouse soil in Beijing, China.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-02869-y.

Funding This research was partially supported by the National Natural Science Foundation of China (32070004, 31670006). This study was also supported by the Fundamental Research Funds for Central Nonprofit Scientific Institution (1610132020009), Central Public-interest Scientific Institution Basal Research Fund (Y2021GH18) and Key Laboratory of Microbial Resources Exploitation and Application of Gansu Province (GK2019-01).

Data availability The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LAMW06^T is MN503522. The

GenBank accession number for the draft genome sequence of strain LAMW06^T is JAAOJA000000000.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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