Methylobrevis albus sp. nov., isolated from freshwater lake sediment

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Abstract

An aerobic, Gram-stain-negative, rod-shaped bacterium with flagellum, designated L22^T, was isolated from freshwater of Hulun Lake, Inner Mongolia, China. The organism was found to grow optimally at 30 °C in medium containing 0-0.75%(w/v) NaCl at pH 7.5. The major fatty acid identified was summed feature 8 (C_{16:1} ω7c). The dominant polar lipids were phosphomonoester, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine. The main ubiquinone was Q-10. The G + C content of genomic DNA was 69.8 mol%. The 16S rRNA gene sequences indicated that strain L22^T was affiliated with the genus *Methylobrevis* within the family *Pleomorphomonadaceae*, being most closely related to *M. pamukkalensis* JCM 30229^T with 95.9% sequences similarity. Based on taxonomic results in this study, we proposed that strain L22^T was placed into a novel species in the genus *Methylobrevis* of the family *Pleomorphomonadaceae*, for which the name *Methylobrevis albus* sp. nov. is proposed. The type strain is L22^T (= KCTC 72858^T = MCCC 1H00432^T).

Introduction

The genus *Methylobrevis* was first described by Doronina *et al.* (2015) as a member of the class *Alphaproteobacteria* within the order *Rhizobiales*. The genus *Methylobrevis* is comprised of Gram-stain-negative, strictly aerobic bacteria who do not form endospores or prosthesis. Up to now only one described strain, *M. pamukkalensis*(Doronina *et al.* 2015), has been confirmed to belong to the genus *Methylobrevis* within the family *Pleomorphomonadaceae*, which was recently revised with genus *Chthonobacter*(Kim *et al.* 2017), *Hartmannibacter*(Suarez *et al.* 2014), *Methylobrevis*(Doronina *et al.* 2015), *Mongoliimonas*(Jiao *et al.* 2017), *Oharaeibacter*(Lv *et al.* 2017) and *Pleomorphomonas*(Xie and C.-H. 2005) supposed as the only six type strains base on LPSN (LPSN, List of Prokaryotic Names with Standing in Nomenclature)(Parte)(Hrdt *et al.* 2020). In October 2019, we isolated the strain L22^T from freshwater of Hulun Lake, Inner Mongolia, China. Based on the phenotypic, genotypic and chemotaxonomic characteristics, the paper aimed to propose a novel species of the genus *Methylobrevis* within the newly identified family *Pleomorphomonadaceae*, represented by strain L22^T.

Materials And Methods

Inoculum source, isolation and preservation

Samples of fresh water and sediment were collected from the middle part of Hunlun lack (117°01' 10" E, 48°30' 40" N), Inner Mongolia, the fourth biggest freshwater lack in China in July 2019. The freshwater samples and sediment samples were mixed evenly and then diluted serially to 10⁻³ with sterile water. 0.1 ml aliquots of each dilution were spread onto 1/2-strength R₂A agar. After incubating in an aerobic environment at 28 °C for 15 days, strain L22^T was picked among several tiny white colonies and purified on R₂A agar by routinely repetitious streaking. The strain was stored in sterile 15 % (v/v) glycerol supplemented with 1 % (w/v) NaCl suspensions at -80 °C. Strain L22^T was cultivated on R2A agar at 30

°C unless otherwise mentioned. For comparison and analysis, we used *M. pamukkalensis* JCM 30229^T as a related strain, which was cultured under the same conditions as strain L22^T.

16S rRNA gene sequence analysis

Universal bacterial primers 1492R and 27F as previously described (Q.-Q. et al. 2014) were used for 16S rRNA gene sequence PCR amplification. All PCR production was purified and ligated into the PMD-18 vector (Takara). Cloned *Escherichia coli* DH5α cells (Trans-Gen Biotech) were then transformed with the obtained recombinant plasmids. The positive clones were selected by LB agar contained 0.01% (w/v) ampicillin and sequenced at BGI Co. Ltd (Qingdao, China) using the ABI 3730XL system. The almost complete 16S rRNA gene sequence of strain L22^T was compared with those available from the EzTaxon server (<http://www.ezbiocloud.net/identify>) (Kim et al. 2011) and NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) for further phylogenetic analysis. Sequences of the related type strains and the almost completed 16S rRNA gene sequence of strain L22^T were aligned using MUSCLE (Robert and Edgar 2004). A phylogenetic tree was reconstructed utilizing the maximum-likelihood (ML) algorithm (Felsenstein 1981) implemented in the software package MEGA 7.0 (www.megasoftware.net) (Sudhir et al. 2016). The overall mean distance of aligned 16S rRNA sequences was calculated and showed suitable compatibility, thus a neighbour-joining (NJ) (N et al. 1987) tree was reconstructed to verify the confidence of ML tree. For all tree-making calculations, variance estimates were taken on for 1000 replication by the method of bootstrap analysis to ensure accurate calculation results.

Genome sequence analysis

The genomic DNA of strain L22^T was extracted and purified using a bacterial genomic DNA kit (Takara). The draft genomic sequence of strain L22^T was sent to Shanghai OE Biotech Co., Ltd (Shanghai, China) and sequenced using Illumina technology. An Illumina shotgun library using the Illumina TruSeq Nano DNA Sample Prep Kit was reconstructed and sequenced using the pair-end 350-bp protocol on the Illumina HiSeq Xten platform (Illumina Inc., San Diego, USA). Raw sequencing data was generated by Illumina base-calling software CASAVA v1.8.2 (<http://support.illumina.com/>) according to its corresponding manuscript. The sequenced reads were assembled using SOAPdenovo software (Ruiqiang Lisup/sup and Jun Wangsup/sup 2009). Gene content was annotated using the NCBI Prokaryotic Genome Annotation Pipeline and the genes involved in metabolic pathways were analyzed in detail by using information from the KEGG database (Minoru et al. 2016). Protein-encoding regions were identified with the Rapid Annotations using Subsystem Technology (RAST) server (Aziz et al. 2008). A further phylogenetic and taxonomic multilocus sequence analysis was made to identify the accurate taxonomic status of strain L22^T. Genomes of the order *Hyphomicrobiales* which were available from the NCBI were aligned with MUSCLE v.3.8.31. The phylogenomic relationship was analysed by Genome Taxonomy Database (GTDB; <https://gtdb.ecogenomic.org>), and the phylogenetic trees were constructed by using FastTree (Luke 2002) using GTR+CAT parameters and IQTree (Jana et al. 2016) using GTR+F+I+G4 model and 1000 bootstrap replicates. The genome of *Ochrobactrum lupini* ATCC 49188^T served as the outgroup

to compute the phylogeny for the DNA and protein sequences. The tree was collapsed and formatted using iTOL v4 (Letunic Ivica;Bork Peer 2019).

The average amino acid identity (AAI) between genomes of strain L22^T and strain 30229^T was calculated by CompareM (<https://github.com/dparks1134/CompareM>). Digital DNA-DNA hybridization (dDDH) value was calculated using the Genome-to-Genome Distance Calculator (GGDC 2.1; <http://ggdc.dsmz.de>). The orthologous average nucleotide identity (ANI) was calculated using an online server (<http://enve-omics.ce.gatech.edu/ani/>) (Chun et al. 2015).

Morphological, physiological and biochemical analyses

Morphological features of strain L22^T were tested using the biomass incubated on R₂A agar at 30 °C for 3 days. Light microscopy (E600; Nikon) and transmission electron microscopy (JEM1200, Japan) were used to observe the microstructures of cells of strain L22^T. The motility was tested using the hanging-drop method and gliding motility was confirmed as described by Bowman (Bowman 2000). Gram-staining was performed using a Gram stain kit (bioMérieux) according to the manufacturer's instructions. Different gradients of 0, 4, 10, 15, 20, 25, 28, 30, 33, 37 and 40 °C were set up to find the temperature range for growth on R₂A agar. The tolerances to various NaCl concentrations were examined by incubating strain L22^T on R₂A agar where 0.0-10.0 % (w/v) NaCl at intervals of 0.25 % (w/v) was added. The pH range for growth was determined by a series of adjusted R₂A broth adding a series of 20mM Microbial buffer: 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), CAPSO (pH 9.0, 9.5 and 10.0) and CAPS (pH 10.5 and 11.0). Growth at an anaerobic (10 % H₂, 10 % CO₂ and 80 % N₂) environment was confirmed after 14 days' incubation in an anaerobic bag with or without 0.1 % (w/v) KNO₃. All experiments were performed with three replicates. The reduction of nitrate was tested following a previous statement of Cowan and Steel (Cowan ST and Steel KJ 1974). Oxidase activity was tested using an oxidase reagent kit (bioMérieux) according to the manufacturer's instructions. Catalase activity was determined by the application of 3 % (v/v) hydrogen peroxide solution and observing bubble production. Tests for the hydrolysis of alginate, carboxymethylcellulose, starch, casein, Tweens 20, 40, 60 and 80 were performed as previously mentioned (Cowan et al. 1966). DNase activity was investigated by DNase agar (HopeBio) according to the manufacturer's instruction. Sensibility to antibiotics was investigated on R₂A agar using the disc diffusion method following the procedures outlined by the Clinical and Laboratory Standards Institute (CLSI 2018). Activities of other enzymes were evaluated in virtue of API ZYM (bioMérieux) kits according to the manufacturer's instruction. Oxidation of various carbons as the sole carbon source was assessed in Biolog GEN μ microplates (<http://www.biolog.com/>) according to the manufacturer's instruction. Acid production of different compounds as sole sources of carbon and energy was determined using API 50CHB (bioMérieux) kits according to the manufacturer's instruction. Other biochemical tests were performed with the API 20E (bioMérieux) kits according to the manufacturer's instruction. Each one of those API and Biolog tests was carried out using the biomass of strain L22^T grown on R₂A agar at 30 °C for 3 days and repeated for two times simultaneously with the related type strain.

Chemotaxonomic characterization

Late-exponential-growth-phase biomass of strain L22^T and its relative grew on R₂A agar at 30 °C were collected in centrifuge tubes and impermanently preserved at -20 °C ready for imminent investigations. The cellular fatty acid methyl esters were prepared and extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.1)(Athalye et al. 2010). The subsequent identification was performed by an Agilent 6890N gas chromatography basing on the TSBA40 database of the microbial identification system. Polar lipids were obtained by a series of centrifugation and extraction and separated via TLC (thin layer chromatography) as described previously (Minnikin et al. 1984). The ubiquinone was extracted from 300 mg of freeze-drying biomass and analysed using reverse-phase HPLC (High-Performance Liquid Chromatography), according to the method used previously (Minnikin et al. 1984).

Results And Discussion

16S rRNA gene sequence and phylogenetic analysis

The almost complete 16S rRNA (1,457 bp) gene sequence of strain L22^T was obtained through conventional Sanger sequencing. The result of pairwise comparison of 16S rRNA gene sequences in the GenBank database of NCBI server (<https://www.ncbi.nlm.nih.gov>) showed that the most related strain was *M. pamukkalensis* JCM 30229^T with 95.9 % 16S rRNA gene sequence similarity, followed by *C. albigriseus* JCM 30603^T (95.0 %), *O. diazotrophicus* DSM 102969^T (94.5 %), *N. lucknowense* DSM 24322^T (93.8 %) and *N. indicus* C115^T (93.8 %). The 16S rRNA gene sequence of strain L22^T showed a maximum identity of 95.9 % with validly published species, which is below the 98.7 % species threshold identity but higher than the 94.5% genus threshold identity. Following the 16S rRNA-based taxonomic identity, the species rank thus seems appropriate for this strain. Besides, the phylogenetic maximum-likelihood tree based on 16S rRNA gene sequences (Fig. 1) showed than strain L22^T belonged to the genus *Methylobrevia*. The topology of the phylogenetic tree constructed by neighbour-joining arithmetic (Fig. S1) proved the conclusion that strain L22^T and *M. pamukkalensis* JCM 30229^T formed a stable cluster which had a distinct phylogenetic lineage with other genera within the family *Pleomorphomonadaceae*. These overall data indicated that strain L22^T was regarded as a novel species within the genus *Methylobrevia*.

Genome sequence analysis

The draft genome sequence of strain L22^T consisted of 4,354,788 bp. The combined assembly yielded 74 contigs with the largest being 618,762 bp and the coting N50 value was 249,401 bp. An average 473× coverage depth was accomplished. The DNA G+C content of strain L22^T was 69.8 mol%, which was following reported value for strain *M. pamukkalensis* JCM 30229^T (67.9 mol%) (Doronina et al. 2015). Gene prediction and annotation identified 3,927 genes, 3,866 protein-encoding genes, 51 tRNA genes, 6 rRNA genes. Two 16S rRNA gene sequences (1,167 bp and 1,473 bp) was extracted from the draft

genome and were compared with the almost complete 16S rRNA (1,457 bp) gene sequence of strain L22^T obtained through conventional Sanger sequencing. One showed 99.9 % and the other 99.8 % gene sequence similarity. Moreover, according to annotation from KEGG database, genes assigned to functional categories are involved in the environmental information processing (318), carbohydrate metabolism (250), protein families: genetic information processing (227), protein families: signaling and cellular processing (222), genetic information processing (164) and amino acid metabolism (147). Gene *katE* (encoding catalase) and *katE*-intracellular protease were found which meant strain L22^T got the ability to hydrolyze hydrogen peroxide. A complete phosphatidate cytidyltransferase [EC:2.7.7.41] pathway and gene *pmtA* were proved to exist and thus Phosphatidylethanolamine (PE) can be produced and transformed into Phosphatidylcholine (PC), in accord with the result of polar lipids experiment. Results on KEGG told that strain L22^T can reduce sulfate to APS, PAPS, sulfite and finally sulfide step by step. However, the experiment showed that sodium thiosulfate could not be reduced to H₂S. Phylogenetic analysis of the genomic amino acid sequences performed with the FastTree and IQTree approaches demonstrates that strain L22^T formed a stable lineage within the genus *Methylobrevia* (Fig. 2 & Fig. 3).

The AAI value between strain L22^T and *M. pamukkalensis* JCM 30229^T was 71.8 %, significantly below the proposed cut-off for a species boundary of 85-90 % and exceed the threshold value for a genus boundary 55-60 % (Rodriguez-R and Konstantinidis 2014). The results of ANI and dDDH between strain L22^T and *M. pamukkalensis* JCM 30229^T were 78.6 % and 22.3 %, both below the thresholds (ANI: 95-96 %; dDDH: 70 %) for new species identification (Goris J et al. 2007) (Meier-Kolthoff et al. 2013). These results supported the finding that strain L22^T was a novel member of the genus *Methylobrevia*.

Morphological, physiological and biochemical characteristics

Cells of strain L22^T were Gram-stain-negative, aerobic, 0.3-0.4 µm wide and 0.9-1.1 µm long, gliding, short rods with long flagella (Fig. S2), thus made them possible to move quickly in a liquid environment. Colonies of strain L22^T were milky white, convex and round could grow up to 0.5 mm in diameter after incubation on R₂A agar at 30 °C for 3 days. Strain L22^T grow at 4-37 °C (optimum 30 °C), at pH 6.0-9.5 (optimum 7.5). Interestingly, though strain L22^T was isolated from the fourth biggest freshwater lake in China, its tolerance to NaCl was high up to 4.5 % (w/v) (optimum 0-0.75 %). Strain L22^T was susceptible to chloramphenicol (30 µg), ofloxacin (5 µg), norfloxacin (10 µg), ceftriaxone (30 µg), tetracycline (30 µg), cefotaxime (30 µg), penicillin (10 µg) and ampicillin (10 µg), resistant to clarithromycin (15 µg), rifampicin (5 µg), gentamicin (10 µg), vancomycin (30 µg), erythromycin (15 µg), streptomycin (10 µg), lincomycin (2 µg), neomycin (30 µg) and tobramycin (10 µg). The strain was negative for hydrolyzes of starch, casein, alginate, cellulose, Tweens 20, 60 and 80 but positive for Tween 40. Strain L22^T show many typical characteristics of the genus *Methylobrevia*, such as cells are Gram-stain-negative, aerobic, rods, positive for catalase and oxidase activities, and negative for nitrate reduction (Doronina et al. 2015). However, differences remained that strain *M. pamukkalensis* JCM 30229^T showed positive for starch and negative gelatinase activity while strain L22^T was not. The other morphological, physiological, and

biochemical characteristics that differentiate strain L22^T from its related type strain are provided in Table 1.

Chemotaxonomic characterization

The major fatty acid (>10.0 %) of strain L22^T was summed feature 8 (C_{16:1} ω7c) (69.1 %) same as the major fatty acid extracted from its related strain strain *M. pamukkalensis* JCM 30229^T. The polar lipids of strain L22^T comprised phosphomonoester (PME), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), one unknown glycolipid (GL) and seven unknown lipids (L). What deserved to be mentioned was that strain L22^T and strain *M. pamukkalensis* JCM 30229^T both possessed phosphomonoester (PME), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylcholine (PC) as the major polar lipids (Fig. S3). Strain L22^T contained unknown lipid 1 to 9, while strain 30229^T did not have unknown lipid 8 and unknown lipid 9 but had unknown lipid 10 to 13. Details of cellular fatty acids and polar lipids compositions were listed in Table 2. The major ubiquinone of strain L22^T was Q-10, which is accordant to the description of the family *Pleomorphomonadaceae* (Hrdt et al. 2020).

According to the phylogenetic analyses, differential chemotaxonomic data and other phenotypic properties, strain L22^T was classified as a new member of the genus *Methylobrevis*.

Description of *Methylobrevis albus* sp. nov.

Methylobrevis albus (al'bus L. masc. adj. *albus*, white, describing the white aerial mycelium)

Cells are Gram-stain-negative, aerobic, gliding motility, 0.3-0.4 μm wide and 0.9-1.1 μm long rods with flagella. When grown on R₂A agar, colonies were 0.5 mm in diameter after 3 days of growth at 30 °C at pH 7.5, milky white, convex and circular, glossy surfaces, with entire edges. Growth occurs at 4-37 °C (optimum 30 °C) and pH 6.0-9.5 (optimum 7.5). Able to tolerate up to 4.5 % (w/v) NaCl (grows optimally at 0-0.75 % NaCl). Activities of oxidase and catalase are positive. Unable to reduce nitrate. The strain was negative for hydrolyzes of starch, casein, alginate, cellulose, Tweens 20, 60 and 80 but positive for Tween 40. Positive for urease, gelatinase, alkaline phosphatase, lipoidase (C4), white aramidase, valine aramidase, cystine aramidase, trypsin, naphthol-AS-BI-phosphohydrolase and α-glucosidase, but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan dehydrogenase, acid phosphatase, esterase lipase (C8), lipase (C14), chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-glucosinase, α-mannitase, β-fucosidase. Has the ability to produce acetyl methyl carbinol from 3-hydroxy ethyl ketone but not able to produce H₂S either indole. Oxidation can be found with these carbon sources: sucrose, α-d-glucose, d-mannose, d-fructose, d-galactose, d-fucose, l-fucose, l-rhamnose, Inosine, d-sorbitol, d-mannitol, d-arabitol, myo-inositol, glycerol, d-fructose-6-PO₄, l-alanine, l-serine, pectin, d-galacturonic acid, l-galactonic acid lactone, d-gluconic acid, d-glucuronic acid, glucuronamide, quinic acid, methyl pyruvate, d-lactic acid methyl ester, l-lactic acid, α-Keto-glutaric acid, d-malic acid, l-malic acid, bromo-succinic acid, β-hydroxy-d,l-butyric acid, acetoacetic

acid, acetic acid and formic acid. Acids can be produced from various substrates: mannitol, l-arabinose, d-ribose, d-xylose, d-side calendula β , d-fructose, d-sucrose, xylitol, d-lyxose, d-fucose and l-fucose. The major fatty acid is summed feature 8 (C_{16:1} ω 7c). The polar major lipids consist of phosphomonoester, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine. The sole ubiquinone is Q-10.

The type strain is L22^T (=KCTC 72858^T=MCCC 1H00432^T), which is isolated from fresh water of Hulun Lake (117°01' 10" E, 48°30' 40" N), Inner Mongolia, PR China. The genomic DNA G+C content of the type strain is 69.8 mol %. The 16S rRNA gene sequence and the draft genome sequences are available in the NCBI GenBank database under the accession number MW195049 and JADZLT000000000.

Declarations

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Conflicts of interests and ethical statements

The authors declare that they have no conflict of interest.

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Tables

Table 1. Characteristics that differentiate strain L22^T and related type strain.

Characteristic	1	2
Cell shape and size (µm)	Short rod	Short rod
	0.3-0.4×0.9-1.1	0.7-1.6×1.5-2
Colony color	Milky white	Pale brown
Growth at/in		
NaCl (optimum) (%)	0-4.5 (0-0.75)	0-5.8 (0.5)
pH (optimum)	6.0-9.5 (7.5)	6.0-9.0 (7.5-8.0)
Hydrolysis of:		
Starch	-	+
Acid production from:		
Mannitol	+	-
d-Side calendula ☐	+	-
d-Galactose	-	+
d-Fructose	+	-
Heptachlor iron citrate	-	+
d-Sucrose	+	-
Xylitol	+	-
l-Fucose	+	-
Enzyme activities:		
Valine aramidase	w	+
Cystine arylamidase	w	+
Acid phosphatase	-	+
Naphthol-AS-BI-phosphohydrolase	+	-
Polar lipids	PME, PE, PG, PC, GL, L1, L2, L3, L4, L5, L6, L7, L8, L9	PME, PE, PG, PC, GL, L1, L2, L3, L4, L5, L6, L7, L10, L11, L12, L13
DNA G+C (mol%)	69.8	67.9

Strains: 1, L22^T; 2, *M. pamukkalensis* JCM 30229^T (Doronina et al. 2015).

+, positive; w, weakly positive; –, negative.

All strains are Gram-stain-negative, catalase and oxidase positive. The predominant respiratory quinone of all strains of the genus *Methylobrevia* is Q-10.

Data come from this study and earlier articles (Doronina et al. 2015).

Table 2. Fatty acid compositions of strain L22^T and related type strain.

Fatty acid	1	2
Branched		
anteiso-C _{12:0}	1.4	Tr
Unsaturated		
C _{18:1} ω 7 c 11-methyl	4.8	Tr
Straight-chain		
C _{12:0}	1.4	Tr
C _{18:0}	3.9	2.4
C _{16:0}	4.4	6.3
Hydroxylated		
C _{16:0} 3-OH	1.6	1.1
Unidentified		
Summed Feature 2*	2.7	2.6
Summed Feature 3*	3.9	5.7
Summed Feature 8*	69.1	71.2

Strains: 1, L22^T; 2, *M. pamukkalensis* JCM 30229^T.

Data are from this study. Tr, traces (<1.0 %). Fatty acids amounting (<1.0 %) are not shown, dominant fatty acids (≥10.0 %) are highlighted in bold.

*Summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete equivalent chain-lengths (ECLs) as well as those where the ECLs are not reported separately.

Summed feature 2 contained C_{12:0} and/or aldehyde;

Summed feature 3 contained C_{16:1} ω7c and/or C_{16:1} ω6c;

Summed feature 8 contained C_{18:1} ω7c.

Figures

Figure 1

Maximum-likelihood tree based on 16S rRNA gene sequences, showing the the phylogenetic position of strain L22T among closely related taxa. Percentage bootstrap values (1000 replications) greater than 50 % are shown at nodes. GenBank accession numbers are given in parentheses. Filled circles at nodes indicate branches that were also recovered using the Neighbor-joining algorithms. Numbers on nodes represent bootstrap values (ML/NJ) based on 1000 replications. *Escherichia coli* ATCC 11775T (X80725) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Figure 2

Phylogenetic analysis of the genomic amino acid sequences of performed with the IQ tree showing the phylogenetic position of strain L22T among closelyrelated taxa. *Ochrobactrum lupini* ATCC 49188T was used as an outgroup.

Figure 3

Phylogenetic analysis of the genomic amino acid sequences of performed with the fast tree showing the phylogenetic position of strain L22T among closelyrelated taxa. *Ochrobactrum lupini* ATCC 49188T was used as an outgroup.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterialforL22.docx](#)