Terrihabitans rhizophilus sp. nov., isolated from the rhizosphere soil of plant in temperate semi-arid steppe

Runze Bao
Inner Mongolia Agricultural University
Huiling Guo
Inner Mongolia Agricultural University
Yungang Liang
Inner Mongolia Agricultural University
Kai Tang
Inner Mongolia Agricultural University
Fuying Feng
Inner Mongolia Agricultural University
Jianyu Meng
mengjianyu3@163.com

Inner Mongolia Agricultural University

Research Article

Keywords: Terrihabitans rhizophilus sp. nov., Hyphomicrobiales, Rhizosphere soil

Posted Date: January 15th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3852304/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Antonie van Leeuwenhoek on April 12th, 2024. See the published version at https://doi.org/10.1007/s10482-024-01966-w.

Abstract

The bacterial strain was isolated from the rhizosphere of *Elymus dahuricus* Turcz. in temperate semi-arid steppe, located in the northern of Inner Mongolia Autonomous Region, China. Strain PJ23^T is Gram-stain-negative, aerobic, light-pink, short rod-shaped, and does not form spores. Cell growth could be observed at 4-29 ℃ (optimum, 24 ℃), pH 6.0-8.6 (optimum, 8.0) and in the presence of 0-5 % (w/v) NaCl (optimum, 2.5 %). The major fatty acids of strain PJ23^T were Summed feature 8 ($C_{18:1\ \omega6c}$ and/or $C_{18:1\ \omega7c}$) 39.42 %) and $C_{16:0}$ (9.60 %). The polar lipid profile was composed of phosphatidylcholine, two unidentified glycolipids, one unidentified aminophospholipid, and two other unidentified polar lipids. The sole respiratory quinone was ubiquinone-10. Phylogeny on full length of 16S rRNA gene sequences retrieved from the genomes showed that, the strain was closely related to *Terrihabitans soli* IZ6^T and *Flaviflagellibacter deserti* SYSU D60017^T with the sequence similarities of 96.79% and 96.15%, respectively. The G+C content was 65.23 mol% calculated on draft genome sequencing. Between the strains PJ23^T and *T.* soli IZ6^T, the average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) was 73.39 % and 15.7 %, these values are lower than the proposed and generally accepted species boundaries of dDDH and ANI. Based on phenotypic, chemotaxonomic, and phylogenetic characteristics, strain PJ23^T represents a novel species of *Terrihabitans*, for which the name *Terrihabitans rhizophilus* sp. nov. is proposed. The type strain is PJ23^T (= KCTC 92977^T=CGMCC 1.61577^T).

INTRODUCTION

For the recovery of degraded ecosystems, soil microorganisms, an important driver of plant community development, mainly plant growth-promoting rhizobacteria (PGPR), is a major one of the determinants in successfully restoring terrestrial ecosystems[1] Dorbet steppe, located in the temperate semi-arid northern of Mongolia Plains, China, is subjected to continuous drought, leading to the depletion of constructive plant species (e.g. *Elymus dahuricus* Turcz.), and soil degradation and desertification, where PGPR were expected to promote the recovery of the steppe. In the PGPR investigation of *Elymus dahuricus* Turcz., strain PJ23^T was isolated and its 16S rRNA gene fragment obtained through PCR amplification with 27F and 1492 primer pair was most similar to those of *Terrihabitans soli* IZ6^T and *Flaviflagellibacter deserti* SYSU D60017^T with the similarities of 96.15% and 96.09%, suggesting that strain PJ23^T may represent a novel species. These were novel proposed genera assigned to the order of Rhizobiales recently emended as Hyphomicrobiales[2]. but not to any named family, and at the writing time has IZ6^T (Nakai et al., 2021) and SYSU D60017^T (Dong et al., 2019) as only type species published (https://www.bacterio.net/), respectively. In this study, poly-phasic taxonomic classification was conducted using *Flaviflagellibacter deserti* CGMCC 1.16444^T and *Terrihabitans soli* NBRC 106741^T as reference species and the results showed that strain PJ23^T represent a novel species of Genus *Terrihabitans*.

METHODS AND RESULTS

Isolation

A bacterial strain, designated PJ23^T, was obtained from rhizosphere soil of oat (*Elymus dahuricus* Turcz.) collected in the temperate semi-arid Dorbet steppe (42°24.077', 111°22.781') at the northern Mongolia Plains, PR China. The strain was isolated and purified on 1/2 strength R2A medium[3]. incubated at 25 ℃ using the standard dilution plating technique and preserved in a glycerol suspension (18%, w/v) at -80 ℃.

Phylogeny on 16S rRNA gene sequences

The genomic DNA of strain PJ23^T was extracted and purified by using a genomic DNA isolation kit (Tiangen Biotech). The partial 16S rRNA gene was amplified with universal bacterial primer pair of 24F and 1492R[4],. To identify the nearest taxa, the sequences of closely related type species were retrieved and calculated their similarities in EzBioCloud (www.ezbiocloud.net/) [5].. Multiple alignments were performed with the MUSCLE program[6]. The phylogenetic trees were reconstructed using the neighbor-joining (NJ)[7], maximum-likelihood (ML)[8] and minimum-evolution (ME)[9] methods through MEGA version 11.0 [6]. A bootstrap analysis with 1000 replicate datasets [10] was performed to assess support for grouping. The topology of the trees was estimated by bootstrap analysis based on 1000 replications.

Using PCR, a qualified fragment of 16S rRNA gene 1476 nt long of PJ23^T (, was obtained and deposited in GenBank (accession number of OR801651. Based on the sequence, EzBioCloud analysisdemonstrated that the strain was mostly related to strain *Terrihabitans soli* IZ6^T (96.15%), *F. deserti* SYSU D60017^T (96.09%), *R tumorigenes* 1078^T (93.03%), *Methylopila capsulata* IM1^T (92.87%) and *M. musalis* MUSA^T (92.86%). In the phylogenetic trees of ML, MP, and NJ, PJ23^T was clustered in a minimum branch with strains *T. soli* IZ6^T (Fig. 1; Supplementary Fig. S1, available in the online version of this paper). Based on their high similarity in the 16S rRNA gene phylogeny, *T. soli* IZ6^T and *F. deserti* SYSU D60017^T were selected as the reference type species. *F. deserti* SYSU D60017^T was provided by the Lab of Biogeography and Bioresource in Arid Land at the Xinjiang Institute of Ecology and Geography, and *T. soli* IZ6^T was purchased from the NBRC (Biological Resource Center, NITE) Culture Collection.

Genomic characterization

Genomic DNA was extracted as the above. The draft genome sequence was determined and analyzed as described by Aserse et al. [11]. The Illumina HiSeq platform (Majorbio Biopharm Technology) was employed for sequencing. The genomic sequence was annotated using the IMG/ER annotation method and the data was subsequently deposited in GenBank[12] and the GenBank accession number is No. JAXAFJ000000000. The resulting draft genome of strain PJ23^T was 3 415 191 bp long and comprised 25 scaffolds with an N50 of 364 001 bp. A total of 3271 proteins, three rRNAs and 49 tRNAs were predicted (Table S1). The draft genome of strain PJ23^T indicated that the DNA G + C content was 65.23 mol%, higher than 62.2% of *T. soli* IZ6^{T}and 63.8 mol% of *F. deserti* SYSU D60017^T. Based on the KEGG pathway analysis, genes associated with gibberellin synthesis (K01775), cytokinin synthesis (K00788), phosphatase activity (K01126), and polyphosphate kinase (K00937), as cataloged in the Kyoto

Encyclopedia of Genes and Genomes (KEGG) database, have been annotated within the genome of strain PJ23^T. Taxonomic position on genome analysis via TrueBac ID Beta (https://www.truebacid.com/)[13] also supported that strain PJ23^T belongs into Genus *Terrihabitans*. Average Nucleotide Identity (ANI) and digital DNA–DNA hybridization (dDDH; Genome- to Genome Distance Calculator, GGDC) were calculated using online severs (https://www.Ezbiocloud.net/tools/ani)[14], (ggdc.dsmz.de/ggdc.php)[15], respectively. The results showed that ANI and dDDH values were 73.39%-74.93% and 15.7%-17.4% between strain PJ23^T and *T. soli* IZ6^T and *F. deserti* SYSU D60017^T, lower than the threshold values of ANI 95.0% and dDDH 70.0% for bacteria species definition respectively. The phylogenetic tree was constructed using the Composition Vector (CV) approach based on whole- genome sequences (http://tlife.fudan.edu.cn/cvtree/cvtree/)[16] and the resulting topology for for strain PJ23^T and *T. soli* IZ6^T (Fig. S1) is distinct from that observed in the 16S rRNA gene trees.

Phenotypic and chemotaxonomic characterization

In the characterization, *Terrihabitans soli* IZ6^T and *Flaviflagellibacter deserti* SYSU D60017^T as reference type strains were tested along with strain PJ23^T using identical media under the same culture condition. The Gram reaction of strain PJ23^T was examined using the non-staining method described by Buck[17]. Cell morphology was examined by using a scanning electron microscopy (JSM-IT500A, JEOL). Cell motility was observed by the hanging-drop method [18]. The temperature range for growth of the isolates was assessed using 1/2 R2A plates incubated at 0–40 ℃ in increments of approximately 5 ℃. The pH range for growth at final pH 4.0–10.0 (in 0.2 pH unit increments) by using acetate buffer (pH 4–6), Na_2HPO_4/NaH_2PO_4 (pH 6–7) and Tris/HCl buffer (pH 7–10) was determined in 1/2 R2A broth by assessing OD_{600} changes of the cultures incubated at 24 ℃ on an orbital shaker at 100 r.p.m. for 10 days. Salt tolerance was tested with 0–5% (w/v) NaCl (at intervals of 0.5% NaCl, w/v) in 1/2 strength R2A agar. Catalase and oxidase activities were tested according to standard methods[19]. A microplate reader (Synergy H4 Hybrid Reader, BioTek) was used for the spectra measurements. Enzyme activities, substrates that could be utilized as sole carbon sources and some physiological characteristics were determined by using API 20NE, API 20E and API ZYM strips (BioMe rieux) and Biolog GENIII plates (Biolog) according to the manufacturers' instructions, and the temperature of incubation was 24 ℃. Antibiotic sensitivity was determined by the disc diffusion method[20].

Cells of strain PJ23^T were Gram-staining-negative, aerobic, light-pink, non-spore-forming, nonmotile rods (approximately 0.3–0.5 μm wide and 1.5–2.0 μm long) (Fig. S2). Colonies on 1/2 R2A were globular circular, smooth, light-pink, transparent and slimy, indicating it is a exopolysaccharide producer. The phenotypic traits of strain PJ23^T showed some differences from those of the reference type species and some other related species (Table 1). *F. deserti* SYSU D60017^T showed motility while PJ23^T, and IZ6^T did not. Strain PJ23^T could not pass through a 0.2 μm filter membrane at 4℃, where strain IZ6^{T}could [21]. Cells of strain PJ23^T grew at salinities from 0 to 3% (optimum, 2.5% NaCl, w/v), temperatures from 4–29 ℃ (optimum, 24 ℃) and pH values from 6.0 to 8.6 (optimum, pH 8.0). The carbon source utilization of strain PJ23^T was different from that of the closely related strains. Strain PJ23^T could utilize 22 substrates

but failed to utilize 49 substrates in the Biolog GENIII tests. Strain PJ23^T showed strong positive activities of D-galacturonic acid, L-galactonic acid lactone, L-lactic acid, D-glucuronic acid and glucuronamide and could not utilize pectin, D-gluconic acid, methyl pyruvate, D-lactic acid methyl ester, bromo-succinic acid, tween 40 and α-keto-butyric acid, which was distinguished from strain SYSU D60017^T. In contrast to strain SYSU D60017^T, strain PJ23^T showed strong tolerance to rifamycin SV, minocycline, lincomycin, potassium tellurite and sodium bromate, and strain PJ23^T could not tolerate 1% sodium lactate, nalidixic acid or lithium chloride. Lipase (C14) and Trypsine activities of strain PJ23^T were different from those of strain SYSU D60017^T. Other phenotypic characteristics determined using the API ZYM (bioMérieux), API 20NE (bioMérieux) and GEN III MicroPlate system (Biolog) are provided in supplementary(Table S2).

Table 1

Differential phenotypic characteristics of strain PJ23^T and reference type species of the order Hyphomicrobiales. Strain: 1, PJ23^T; 2, *Terrihabitans soli* IZ6^T; 3, *Flaviflagellibacter deserti* SYSU D60017^T. +, Positive; −, negative; +w, weakly positive;

Characteristics	**1**	**2**	**3**
Isolation source	rhizosphere soil of *Elymus dahuricus Turcz*	Forest soil Nonmotile	Desert soil Motile
pH range for growth	6-8.6	5–11	5–8
Temperature range for growth(℃)	4–29	10–30	4–37
Optimum NaCl for growth (%, w/v)	2.5	< 0.5	1.5
Assimilation of glucose	-	-	+
Enzymatic activities:			
Lipase (C14)	-	-	
Leucine arylamidase	+	-	+
Valine arylamidase	+	-	+
Cystine arylamidase	+	-	+
DNA G + C content (mol%)	65.2	62.2	63.8

Cells of strain PJ23^T and the reference type strains were grown on 1/2 R2A plates at 24 ℃ and harvested at logarithmic growth phase for the analysis of fatty acids, polar lipids and respiratory quinone. According to the method described by Sasser [22], cellular fatty acids were extracted, methylated and analyzed on Sherlock Microbial Identification System version 6.1 (MIDI) according to the supplier's instructions with the Agilent GC 7890. The predominant fatty acids (> 9%) present were Summed feature 8 ($C_{18:1\omega7c}$) 39.42%) and $C_{16:0}$ (9.60%) in PJ23^T. while $C_{18:1\omega7c}$ and $C_{18:1\ \omega6c}$ in *T. soli* IZ6^T.The main fatty

acids of PJ23^T are similar to the fatty acids of Strain SYSU D60017^T. However, there are some differences in some fatty acids with lower contents (Table 2).

Table 2

Cellular fatty acid composition (%) of strain PJ23^T and its close phylogenetic neighbors Strains: 1, Strain PJ23^T; 2, *Terrihabitans soli* IZ6^T; 3, *Flaviflagellibacter deserti* SYSU D60017^T. Culture conditions: strains 1, 2, and 3 were grown on 1/2 R2A agar for 5 days at 24 °C. Fatty acids that represented less than 1% in all the strains are not shown. ND, not detected/not reported.

Fatty acid	**1**	**2**	**3**
Straight-chain saturated			
$C_{12:0}$	ND	ND	3.16
$C_{13:0}$	ND	ND	2.08
$C_{14:0}$	2.30	ND	4.68
$C_{16:0}$	9.60	5.26	13.47
$C_{17:0}$	ND	2.94	1.43
$C_{18:0}$	5.93	2.38	10.39
Branched saturated			
Iso-$C_{13:0}$	1.27	ND	1.11
iso -$C_{16:0}$	1.44	ND	1.24
anteiso -$C_{14:0}$	ND	ND	1.96
anteiso -$C_{15:0}$	2.08	ND	1.72
anteiso -$C_{13:0}$	ND	ND	1.25
anteiso -$C_{16:0}$	1.93	ND	1.60
anteiso -$C_{17:0}$	2.45	ND	2.34
Cyclo saturated			
cyclo -$C_{17:0}$	ND	ND	1.11
cyclo-$C_{19:0\ \omega 8c}$	5.45	5.48	4.03
Hydroxy saturated			
$C_{16:0}$ 3-OH	1.12	1.99	ND

Fatty acid	1	2	3
$C_{18:0}$ 3-OH	2.10	ND	ND
Monounsaturated			
$C_{18:1\ \omega9c}$	ND	ND	2.88
$C_{20:1\ \omega7c}$	6.71	ND	ND
Summed feature*			
1	1.19	ND	ND
2	1.86	ND	2.90
3	5.83	5.9	2.25
7	1.28	ND	ND
8	39.42	71.6	31.24

* Summed Features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 contains $C_{16:1\ \omega7c}$ and $C_{16:1\omega6c}$. Summed feature 4 contains iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

Polar lipids were extracted according to the method of Liu et al.[23] and examined by two-dimensional TLC (10×10 cm; Qingdao Haiyang Chemical; stained by primuline and viewed under ultraviolet light). Three kinds of spray reagents were used to detect the corresponding lipids, including molybdenum blue spray reagent (Sigma) for phosphorus-containing lipids, ninhydrin reagent (ninhydrin/ethanol, 0.5:100, m/v) for lipids containing free aminolipids and a-naphthol reagent (a-naphthol/methanol/distilled water, 0.5:50:50, m/v/v and sulfuric acid/ethanol, 1:1, v/v) for glycolipids. The respiratory quinone was extracted by chloroform/methanol (2 : 1) and separated into their different classes (e.g., menaquinones, ubiquinones) by TLC on silica gel with nhexane/ether (17 : 3, v/v), then further analyzed by HPLC (Agilent 1260 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer)[24]. The polar lipids were phosphatidylcholine, two unidentified glycolipids, one unidentified aminophospholipid, and two other unidentified polar lipids(Fig. S3). This is similar to the polar lipid compositions of Strain SYSU D60017^T, but there were differences in the amounts of unidentified polar lipids and unidentified aminophospholipids. The major respiratory quinone of strain PJ23^T was ubiquinone-10.

Based on its phylogenetic position and phenotypic characteristics, combined with the unique combination of chemotaxonomic and biochemical properties, strain PJ23^T represents a novel species of the genus *Terrihabitans*, and *Terrihabitans elymus* is proposed as the name.

DESCRIPTION OF Terrihabitans rhizophilus sp. nov.

Terrihabitans rhizophilus (rhi.zo'phi.lus. Gr. fem. n. *rhiza*, root; N.L. masc. adj. suff. *-philus* (from Gr. masc. adj. *philos*), loving; N.L. masc. adj. *rhizophilus* root-loving).Cells are Gram-stain-negative, aerobic, non-spore-forming, nonmotile rods (approximately 0.3–0.5 μm wide and 1.5–2.0 μm long). Colonies on R2A are globular, circular, smooth, light-pink, transparent and slimy. Cells could grow at salinity from 0 to 4% (optimum, 2.5%; NaCl, w/v), temperature from 4 to 29°C (optimum, 24°C), and pH value from 6.0 to 8.6 (optimum, pH 8.0). Oxidase and catalase-positive.

Positive for phosphatase alkaline, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, phosphatase acide, and naphthol-AS-BI-phosphohydrolase. It cannot hydrolyze pectin, D-gluconic acid, methyl pyruvate, D-lactic acid methyl ester, bromo-succinic acid, tween 40 and α-keto-butyric acid. Able to utilize D-galacturonic acid, L-galactonic acid lactone, L-lactic acid, D-glucuronic acid and glucuronamide. The major fatty acids are Summed feature 8 ($C_{18:1\omega7\ c}$) and $C_{16:0}$. The main polar lipids were phosphatidylcholine, two unidentified glycolipids, one unidentified aminophospholipid, and two other unidentified polar lipids. The sole respiratory quinone was ubiquinone-10.

The type strain is PJ23^T (= KCTC 92977^T = CGMCC 1.61577^T), isolated from rhizosphere soil of *Elymus dahuricus* Turcz. in the temperate semiarid steppe, at the northern of Mongolia Plains, China. The genomic DNA G + C content of the type strain is 65.23 mol%, The draft genome has been deposited under accession number JAXAFJ000000000..

Abbreviations

ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; R2A, Reasoner's 2A.

Declarations

Conflicts of interest

The authors declare that there are no conflicts of interest.

Funding information

This work was mainly supported by the Applied Technology Research and Development Fund of Inner Mongolia (2021GG0360), Natural Science Foundation of Inner Mongolia Autonomous Region (2023LHMS03030) and, Science and Technology Program of Inner Mongolia Autonomous Region (2023YFHH0028), .

Author Contribution

Runze Bao, Kai Tang and Fuying Feng wrote the main manuscript text, Huiling Guo organized the data, while Yungang Liang prepared Figures 1 and S1-S3. All authors reviewed the manuscript.

References

1. Coban O, De Deyn GB, van der Ploeg M. Soil microbiota as game-changers in restoration of degraded lands. Science 2022;375:abe0725.
2. Volpiano CG, Sant'Anna FH, Ambrosini A, de São José JFB, Beneduzi A, *et al.* Genomic Metrics Applied to Rhizobiales (Hyphomicrobiales): Species Reclassification, Identification of Unauthentic Genomes and False Type Strains. Front Microbiol 2021;12:614957.
3. Zeng Y, Feng F, Medová H, Dean J, Koblížek M. Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. Proc Natl Acad Sci USA 2014;111:7795–7800.
4. Phylogenetic Group-Specific Oligodeoxynucleotide Probes for Identification of Single Microbial Cells. J Bacteriol 1988;170:2418.
5. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International journal of systematic and evolutionary microbiology 2017;67:1613.
6. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Molecular biology and evolution 2021;38:3022–3027.
7. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution 1987;4:406–425.
8. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of molecular evolution 1981;17:368–376.
9. Rzhetsky A, Nei M. A simple method for estimating and testing minimum-evolution trees. Molecular Biology and Evolution 1992;9:945–945.
10. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *evolution* 1985;39:783–791.
11. Aserse AA, Woyke T, Kyrpides NC, Whitman WB, Lindström K. Draft genome sequence of type strain HBR26 T and description of Rhizobium aethiopicum sp. nov. Standards in genomic sciences 2017;12:1–16.
12. Markowitz VM, Chen I-MA, Palaniappan K, Chu K, Szeto E, *et al.* IMG 4 version of the integrated microbial genomes comparative analysis system. Nucl Acids Res 2014;42:D560–D567.
13. Ha S-M, Kim CK, Roh J, Byun J-H, Yang S-J, *et al.* Application of the Whole Genome-Based Bacterial Identification System, TrueBac ID, Using Clinical Isolates That Were Not Identified With Three Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) Systems. Ann Lab Med 2019;39:530–536.

14. Yoon S-H, Ha S, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek 2017;110:1281–1286.
15. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 2013;14:60.
16. Zuo G, Hao B. CVTree3 Web Server for Whole-genome-based and Alignment-free Prokaryotic Phylogeny and Taxonomy. Genomics, Proteomics & Bioinformatics 2015;13:321–331.
17. Buck JD. Nonstaining (KOH) method for determination of gram reactions of marine bacteria. Appl Environ Microbiol 1982;44:992–993.
18. Wolfe AJ, Berg HC. Migration of bacteria in semisolid agar. Proc Natl Acad Sci U S A 1989;86:6973–6977.
19. Identification of Pseudomonas pyocyanea by the Oxidase Reaction | Nature. https://www.nature.com/articles/178703a0 (accessed 15 October 2023).
20. Antibiotic susceptibility testing by a standardized single disk method - PubMed. https://pubmed.ncbi.nlm.nih.gov/5325707/ (accessed 13 June 2023).
21. Nakai R, Naganuma T, Tazato N, Kunihiro T, Morohoshi S, *et al.* Characterization of Terrihabitans soli gen. nov., sp. nov., a Novel 0.2 μm-Filterable Soil Bacterium Belonging to a Widely Distributed Lineage of Hyphomicrobiales (Rhizobiales). Diversity 2021;13:422.
22. Sasser M. *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI technical note 101. Newark, DE: MIDI inc; 1990.
23. Marinobacter fuscus sp. nov., a marine bacterium of Gammaproteobacteria isolated from surface seawater - PubMed. https://pubmed.ncbi.nlm.nih.gov/30095388/ (accessed 19 June 2023).
24. Komagata K, Suzuki K-I. 4 Lipid and Cell-Wall Analysis in Bacterial Systematics. In: Colwell RR, Grigorova R (editors). *Methods in Microbiology*. Academic Press. pp. 161–207.

Figures

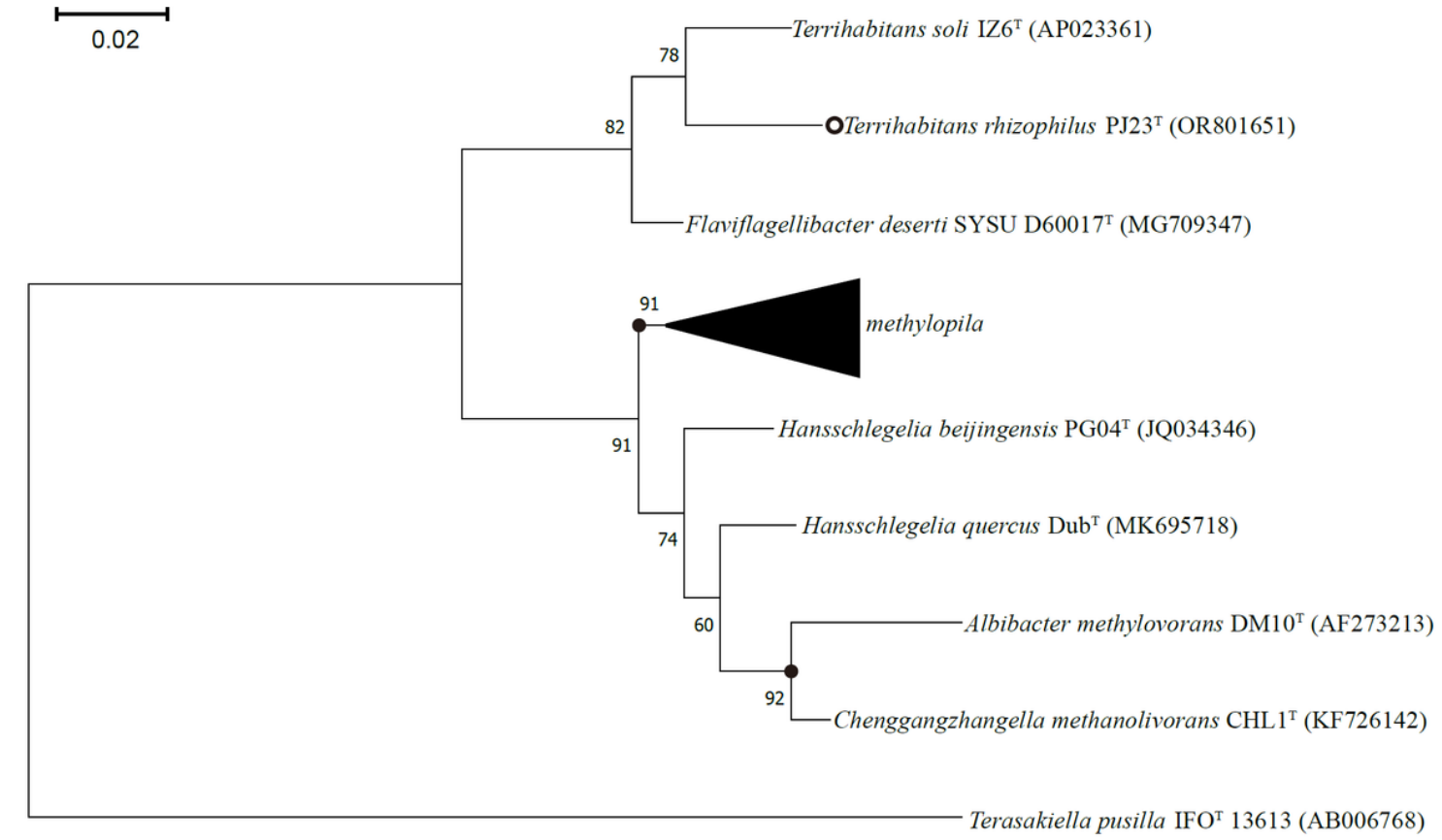

Figure 1

Maximum-likelihood tree showing the phylogenetic positions of strain PJ23^T within the Rhizobiales phylogenetic clade based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) ≥50 % are shown at branch nodes. Filled circles show that a node is common in maximum- likelihood and maximum- parsimony analyses; Filled circles show that a node is common in maximum-parsimony analyze; no marked node is different. Terasaskiella pusilla IFO^T was used as the outgroup. Bar, 0.02 nt substitution rate (Knuc) units.

Supplementary Files

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

- Supplementarymaterials.docx