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Methylobacterium cistanchae sp. nov., a novel plant-associated bacterium isolated from diseased Cistanche soil

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Research Article

Keywords: Methylobacterium, Polyphasic taxonomy, Cistanche deserticola, Plant growth-promoting, Antagonistic activity

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10579921/v1

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Additional Declarations: No competing interests reported.

Abstract

A pink-pigmented, Gram-stain-negative, aerobic, rod-shaped bacterium, designated strain JK268^T, was isolated from the rhizosphere soil of *Cistanche deserticola* growing in a *Haloxylon ammodendron* desert forest in Yutian County, Hotan Prefecture, Xinjiang Uygur Autonomous Region, China. The strain was selected for further taxonomic characterization due to its antagonistic activity against *Fusarium acuminatum* and its plant growth-promoting potential toward *C. deserticola*. Growth occurred at 20–40°C (optimum, 28°C), at pH 5.0–10.0 (optimum, pH 8.0), and in the presence of 0–3.0% (w/v) NaCl (optimum, 0.6%). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain JK268^T belonged to the genus *Methylobacterium* and is most closely related to *Methylobacterium isbiliense* AR24^T (97.54% similarity). The DNA G + C content of draft genome was 71.5 mol%. Phylogenomic analyses confirmed its affiliation with the genus *Methylobacterium*, with strain JK268^T clustered most closely with *Methylobacterium isbiliense* DSM 17168^T. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between strain JK268^T and its closest phylogenetic relatives were 80.78–83.57% and 24.50–27.50%, respectively, well below the thresholds for species delineation. Genome analysis revealed genes potentially involved in tryptophan-dependent indole-3-acetic acid (IAA) biosynthesis and phosphate acquisition, supporting the plant growth-promoting potential of strain JK268^T. Moreover, antiSMASH analysis revealed several secondary metabolite biosynthetic gene clusters, which may contribute to the antifungal activity of strain JK268^T against *F. acuminatum*. The major cellular fatty acids were summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c; 73.64%) and $C_{16:0}$ (8.61%). The sole respiratory quinone was ubiquinone Q-10. The major polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. Based on phenotypic, phylogenetic and genomic evidence, strain JK268^T represents a novel species of the genus *Methylobacterium*, for which the name *Methylobacterium cistanchae* sp. nov. is proposed. The type strain is JK268^T (= CCTCC AB 2025129^T = LMG 34231^T). The GenBank accession numbers for the 16S rRNA gene sequence and the whole-genome sequence are PX780827, JBSVZN000000000, respectively.

Introduction

The genus *Methylobacterium*, first described by Patt et al. 1976, belongs to the family Methylobacteriaceae within the class Alphaproteobacteria and currently comprises 76 validly published species (LPSN: https://lpsn.dsmz.de/genus/Methylobacterium). (Parte. 2020). Members of the genus are widely distributed in diverse environments, including soil (Jia et al. 2020), aquatic habitats (Kato et al. 2008; Gallego et al. 2005; Gallego et al. 2005) and plant tissues (Madhaiyan *et al*. 2014). Chemotaxonomically, the major cellular fatty acid of *Methylobacterium* species is $C_{18:1}$ ω7c, and the predominant respiratory quinone is ubiquinone-10 (Q-10). The DNA G + C content ranges from 63.6 to 73.2 mol% (Kelly et al. 2014; Green *et al*. 2014). Several *Methylobacterium* strains have been recognized as plant growth-promoting bacteria (PGPB) due to their ability to induce root nodule formation and nitrogen fixation, synthesize high levels of phytohormones, and enhance plant productivity (Jourand et al. 2004; Sy et al. 2001; Palberg et al. 2022; Krug et al. 2020). In this study, a novel plant growth-promoting strain, designated *Methylobacterium cistanchae* JK268^T, was isolated from the rhizosphere soil of *Cistanche deserticola*, a medicinal and edible plant, growing in a Haloxylon ammodendron desert forest in Yutian County, Hotan Prefecture, Xinjiang Uygur Autonomous Region, China. The distinct phenotypic, chemotaxonomic, genomic and phylogenetic characteristics of strain JK268^T support its classification as a novel species within the genus *Methylobacterium*, for which the name *Methylobacterium cistanchae* sp. nov. is proposed.

Materials and methods

Isolation culturing, and preservation

Methylobacterium cistanchae JK268^T was isolated in October 2024 from rhizosphere soil of *Cistanche deserticola* groing in a Haloxylon ammodendron desert forest in Yutian County, Hotan Prefecture, Xinjiang Uygur Autonomous Region, China (36°51'08.9" N, 81°40'56.6" E). Ten grams of each Soil samples from four sites were collected, and 10 g of each sample was suspended in sterile physiological saline, serially diluted, and spread onto R2A agar plates. After incubation at 28°C for 1–3 weeks, strain JK268^T was obtained. During inatial screening, this isolate was selected for further taxonomic study because it displayed plant growth-promoting traits, including indole-3-acetic acid (IAA) production. The purified culture was routinely maintained on R2A agar slants at 4°C for short-term storage and preserved in 25% (v/v) glycerol suspensions at − 80°C for long-term storage. Strain JK268^T has been deposited in the China Center for Type Culture Collection (CCTCC) and the Belgian Coordinated Collections of Microorganisms (BCCM/LMG) under accession numbers CCTCC AB 2025129^T and LMG 34231^T, respectively.

Phylogeny and genomic characterization

The nearly complete 16S rRNA gene sequence of strain JK268^T was amplified using the universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-AAGGAGGTGATCCAGCC-3') (Weisburg et al. 1991). The resulting PCR product was sequenced and assembled using SeqMan software (DNASTAR 18.1.1). For phylogenetic analyses, a near-complete 16S rRNA gene sequence was extracted from the whole-genome sequence of strain JK268 using the ContEst16S tool in EzBioCloud(https://www.ezbiocloud.net/tools/contest16s) (Lee et al. 2017). Sequence similarities against closely related type strains were calculated using the EzBioCloud database (Kim et al. 2012)(www.ezbiocloud.net) and the NCBI BLAST tool. Phylogenetic trees were reconstructed using MEGA 12 software (Kumar *et al*. 2024) with the maximum-likelihood (ML), neighbour-joining (NJ) (Saitou *et al*. 1987) and maximum-parsimony (MP) methods. The ML tree was inferred using the Tamura-Nei substitution model as implemented in MEGA (Kumar *et al*. 2018). The robustness of the tree topologies was evaluated by bootstrap analysis based on 1000 replications (Felsenstein *et al*. 1985).

Genomic DNA of strain JK268^T was extracted using an enzyme-based minimal extraction protocol (Sun et al. 2016). After quality assessment of the genomic DNA for purity, concentration and integrity, and subsequent validation of the library, sequencing was performed on a PacBio Sequel II platform (Frasergen

Bioinformatics Co., Ltd,Wuhan, PR China). Raw reads were processed using SMRT Link (version 11.0.0) (Walker et al. 2014) and genome assembly was conducted using the *pb_assembly_hifi_microbial* (from SMRT Link 11.0.0). If available, the initial assembly was subsequently polished with Pilon (v1.24) using paired-end reads to obtain the final assembly. Non-coding RNAs, primarily tRNA and rRNA genes, were predicted using tRNAscan-SE (v2.0.9) and RNAmmer (v1.2) (Lowe et al. 1997; Lagesen et al. 2007), respectively. Functional annotation was conducted via the RAST server (https://rast.nmpdr.org/rast.cgi) (Aziz et al. 2008). Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values between strain JK268^T and its closest phylogenetic relatives were determined using the EzBioCloud ANI calculator (www.ezbiocloud.net/tools/ani) (Yoon et al. 2017) and the Genome-to-Genome Distance Calculator (GGDC 2.1; https://ggdc.dsmz.de/ggdc.php) (Meier et al. 2013), respectively. Biosynthetic gene clusters (BGCs) were predicted using antiSMASH 7.0 (https://antismash.secondarymetabolites.org/) with the detection strictness set to “relaxed” (Blin et al. 2023). A genome-based phylogenomic tree was reconstructed using EasyCGTree (v4.2) based on a concatenating GTDB bac120 single-copy marker proteins into a supermatrix, followed by inference with FastTree under the strict trimming option (https://github.com/zdf1987/EasyCGTree4) (Zhang et al. 2023).

Physiological and chemotaxonomic characterization

Cell morphology of strain JK268^T during the exponential growth phase was examined transmission electron microscopy after incubation on R2A agar at 28 ˚C for 24 h. Physiological characteristics were assessed in R2A medium under varied conditions. Growth was monitored within 48 h under routine incubation conditions; for low-temperature conditions (4, 10 and 15 ˚C), the incubation period was extended to up to 7 days to capture slow growth and determine the minimum growth temperature. The growth temperature range was evaluated at various temperatures (4, 10, 15, 28, 30, 37 and 40 ˚C) and the pH range was tested from pH 5.0 to 10.0 (increments of 0.5 units) in R2A medium adjusted with appropriate buffers. NaCl tolerance was detemined in R2A medium supplemented with 0–3.0% (w/v) NaCl (at 0.1% increments), with optimal concentration assessed by measuring OD_{600}. Motility was assessed on semi-solid R2A medium (0.3% agar) by stab inoculation and incubation at 28˚C for 30 h. Flagella were examined by transmission electron microscopy (Fig.S6). Catalase activity was assessed by observing bubble formation in 3% (v/v) H_2O_2 solution. Additional physiological and biochemical characteristics, including Gram staining, nitrate reduction, indole production, H_2S production, hydrolysis of starch/urea/gelatin/cellulose, and oxidase activity, were determined according to the methods of Smibert and Krieg (Gerhardt *et al*. 1994). Enzymatic activities and substrate utilization profiles were further assessed using API 20NE and API ZYM kits according to the manufacturer’s instructions.

For fatty acid analysis, strain JK268^T was incubated on R2A agar at 28 ˚C until the late-exponential phase. Fatty acid methyl esters (FAMEs) were prepared and analysis according to Sakamoto *et al*. (Sakamoto et al. 2002) and analysed using the Sherlock Microbial Identification System (MIDI; v6.3) coupled with an Agilent 6890 N GC, following the manufacturer's protocols. Isoprenoid quinones were extracted from freeze-dried biomass and analysed by LC–MS (Minnikin et al. 1984). Polar lipids were extracted according to Liu et al. (2020) and separated and visualized by two-dimensional thin-layer chromatography (2D-TLC) on silica gel 60 F254 aluminium plates (Kates et al. 1986). Indole-3-acetic acid (IAA) production, were assessed according to the standard protocols described by Glickmann & Dessaux.(1995) and Collavino et al. (2010).

Results and discussion

16S rRNA gene sequence and phylogenetic analysis

The nearly complete 16S rRNA gene sequence of strain *M. cistanchae* JK268^T (1484 bp) was obtained. Comparative analysis based on the NCBI BLAST and EzBioCloud databases revealed that strain JK268^T showed the highest 16S rRNA gene sequence similarity to *Methylobacterium isbiliense* AR24^T (97.54%), followed by *Methylobacterium currus* PR1016A^T (97.30%), *Methylobacterium variabile* DSM 16961^T (96.87%), *Methylobacterium nodulans* ORS 2060^T (96.73%), *Methylobacterium oryzihabitans* TER-1^T (96.09%), and *Methylobacterium crusticola* MIMD6^T (94.54%).

Phylogenetic analyses based on 16S rRNA gene sequences demonstrated that strain JK268^T belongs to the genus *Methylobacterium*. In the maximum-likelihood (ML) phylogenetic tree, strain JK268^T clustered with *M. isbiliense* AR24^T and *M. currus* PR1016A^T, forming a well-supported lineage within the genus (Fig. 1). The phylogenetic trees reconstructed using the maximum-parsimony (MP; Fig. S1) and neighbour-joining (NJ; Fig. S2) methods exhibited topologies consistent with that of the ML tree, further supporting the affiliation of strain JK268^T with the genus *Methylobacterium*. The close but distinct phylogenetic relationship between strain JK268^T and its nearest recognized neighbours, *M. isbiliense*^{T} and *M. currus*^{T}, suggested that strain JK268^T represents a potentially novel species.

Genome analysis

The whole-genome sequence of strain JK268^T has been deposited in GenBank under accession number JBSVZN000000000.1. The draft genome is 7,341,470 bp in length, comprising 66 contigs with an N50 of 198 kb. Genome completeness and contamination were assessed using CheckM v1.0.18 (Parks et al., 2015). Annotation identified 6,865 genes, including 6,719 protein-coding sequences, 28 CRISPR arrays, and 60 RNA genes (54 tRNA genes and 6 rRNA operons). Fifteen putative prophage regions were predicted (total length 310,008 bp; average length 20,667 bp), and the genomic DNA G + C content was 71.5%. Overall genome relatedness comparisons with phylogenetically closest type strains showed ANI values of 80.78–83.57% and dDDH values of 24.50–27.50% (Table 1), both well below the thresholds commonly used for species delineation (ANI ≈ 95–96%, dDDH ≈ 70%), supporting the recognition of strain JK268^T as a distinct species within the genus *Methylobacterium*. The phylogenomic tree indicated that strain JK268^T formed a coherent cluster with *M. isbiliense* DSM 17168^T, *M. currus* PR1016A^T, *M. variabile* DSM 16961^T, *M. nodulans* ORS 2060^T, *M. oryzihabitans* TER-1^T, and *M. crusticola* MIMD6^T, all of which belonged to the genus *Methylobacterium* (Fig.S3). Phylogenomic analysis further confirmed its placement within the genus and provided critical evidence for its designation as a novel species.

Table 1

Genomic features of strain M. cistanchae JK268^T and closely related species of the genus Methylobacterium. Strain: 1, *M. cistanchae* JK268^T; 2, *M. isbilien* 17168^T; 3, *M. currus* PR1016A^T; 4, *M. variabile* DSM 16961^T; 5, *M. nodulans* ORS 2060^T; 6, *M. oryzihabitans* TER-1^T; 7, *M. crusticola* MIMD6^T; /, .not available. I strains 2–7 were obtained from previous studies (Gallego et al. 2005; Park et al. 2018; Gallego et al. 2005; Jourand et al. 2004; Chen et al. 2019; Jia et al. 2

Features	1	2	3	4	5	6	7
Accession number	JBSVZN000000000.1	BPQQ00000000.1	GCA_003058325.1	LABY00000000.1	CP001349.1	SACP00000000.1	QOWC000
Size (Mbp)	7.3	7.0	7.7	7.4	8.8	6.3	7.5
DNA G + C content (%)	71.5	71.0	70.2	70.5	68.5	72.0	73.0
Completeness (%)	99.71	99.21	99.21	92.89	99.24	98.02	96.79
Contamination (%)	4.51	1.88	1.35	1.1	0.25	1.1	2.03
Total number of genes	6865	6767	7277	7261	8727	5865	7177
Protein-coding genes	6719	6531	6940	6907	82.4	5690	6842
rRNA (5S, 16S, 23S)	4, 1, 1	/	36	5	21	1, 3, 2	/
tRNA	54	/	91	58	73	49	/
16S rRNA gene sequence similarity (%)	100	97.54	97.3	96.87	96.73	96.09	94.54
ANI (%) with JK268^T	100	82.81	80.78	81.34	83.57	81.26	81.42
dDDH (%) with JK268^T	100	26.10	24.50	24.80	27.50	24.70	25.00

Genome annotation and antiSMASH predictions revealed a complete tryptophan biosynthesis module (*trpEGDCFBA*) and key genes associated with tryptophan-dependent indole-3-acetic acid (IAA) synthesis, including aromatic amino acid transaminase (*tyrB*), monoamine oxidase (*MAO*), and aldehyde dehydrogenase (ALDH), consistent with the observed IAA-producing phenotype (Table S1; Fig. S4). The co-occurrence of *MAO* and *ALDH* suggests a potential TAM-based IAA pathway, whereas classical IPA or IAM pathways appear incomplete. These features indicate that strain JK268^T may support its functional phenotype via synergistic tryptophan-dependent IAA synthesis and secondary metabolism-mediated ecological interactions. Additional genome features relevant to plant growth promotion include a high-affinity phosphate uptake system (*pstSCAB*) and stress adaptation systems for trehalose (*treS*, *otsAB*) and proline (*proABC*) biosynthesis, whereas complete nitrogen-fixation gene clusters (*nifHDKENB*) and classical glucose dehydrogenase-mediated phosphate solubilization pathways were absent. Collectively, these genomic traits suggest that strain JK268^T promotes plant growth primarily through phytohormone production, phosphate acquisition, and stress tolerance rather than via biological nitrogen fixation. Genome mining using antiSMASH revealed several secondary metabolite biosynthetic gene clusters potentially associated with the antifungal activity of strain JK268^T against *F. acuminatum*. Among these clusters, an NRPS gene cluster showing similarity to the vicibactin biosynthetic pathway and a type I polyketide synthase (T1PKS) cluster were identified, suggesting potential capacities for siderophore-associated metabolite production and bioactive polyketide biosynthesis. In addition, an NRPS-like cluster with limited similarity to known biosynthetic pathways indicated the presence of unexplored secondary metabolite biosynthetic potential. These genomic features may contribute to the antagonistic activity of strain JK268^T against *F. acuminatum*. Consistent with the genome-based predictions, strain JK268^T exhibited antagonistic activity against *F. acuminatum* in vitro, with a 20% (v/v) cell-free fermentation broth showing an inhibition rate of 47.76% (Table S2; Fig. S5).

Morphological, physiological and chemotaxonomic characterization

Cells were Gram-stain-negative, aerobic, motile, non-spore-forming rods with flagella. Colonies on R2A agar at 28 ˚C were pink, opaque, circular, smooth, and moist, with entire margins, measuring approximately 1–2 mm in diameter. Transmission electron microscopy revealed cells 1.0–3.0 μm in length (Fig.S6). Strain JK268^T grew at 20–40 ˚C (optimum, 28 ˚C), at pH 5.0–10.0 (optimum, pH 8.0), and in the presence of 0–3.0% (w/v) NaCl, with optimal growth at 0.6% NaCl.

The major respiratory quinone of strain *M. cistanchae* JK268^T was ubiquinone-10 (Q-10), consistent with the genus *Methylobacterium* (Chen WM et al. 2019;Gallego et al. 2005Jourand et al. 2004). The polar lipid profile consisted predominantly of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine (Fig. S7), consistent with chemotaxonomic characteristics of the genus *Methylobacterium*. Differences in minor polar lipid composition distinguished strain JK268^T from its closest phylogenetic relatives.

The cellular fatty acid profile was dominated by summed feature 8 ($C_{18:1}$ω7c and/or $C_{18:1}$ω6c; 73.64%) and $C_{16:0}$ (8.61%). Other fatty acids included summed feature 5 ($C_{18:2}$ω6,9c and/or anteiso-$C_{18:0}$; 4.93%), $C_{18:1}$ω9c (4.03%), $C_{18:0}$ (3.23%) and $C_{18:0}$ 3-OH (2.22%). The predominance of summed feature 8 is a characteristic feature of members of the genus *Methylobacterium*, futher supporting the affiliation of strain JK268^T with this genus.

Differential physiological and biochemical characteristics between strain JK268^T and its six closest phylogenetic neighbours are summarized (Table 2). Strain JK268^T was positive for catalase and oxidase activities, but negative for nitrate reduction, indole production and glucose fermentation. It did not assimilate D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, citrate or phenylacetic acid.

Table 2

Differential physiological and biochemical characteristics of strain JK268^T and closely related species of the genus Methylobacterium. Strain: 1, *M. cistanchae* JK268^T; 2, *M. isbiliense* DSM 17168^T; 3, *M. currus* PR1016A^T; 4, *M. variabile* DSM 16961^T; 5, *M. nodulans* ORS 2060^T; 6, *M. oryzihabitans* TER-1^T; 7, *M. crusticola* MIMD6^T. All strains with available data are negative for indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, assimilation of maltose and caprate, and the activities of lipase (C14), α-chymotrypsin, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. All strains with available data are positive for alkaline phosphatase, esterase (C4), leucine arylamidase and acid phosphatase activities. Results for strains 2–7 are from previous studies (Gallego et al. 2005; Park et al. 2018; Gallego et al. 2005; Jourand et al. 2004; Chen et al. 2019; Jia et al. 2020). Symbols: +, positive; –, negative; /, no available.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Isolation source	Rhizosphere soil	Drinking water	Car air-conditioning	Drinking wate	Bel-Air area	Water	Lichen biological soil crusts
Colony color	Pink	Pink	Reddish pink	Pink	Not pigmented	Pink	Pink
Temperature range for growth (˚C)	20–40	20–37	18–42	20–30	30–37	15–35	20–37
The pH growth range	5–10	4–10	4.0–9.0	5.0–8.0	6.8–7.5	5–8	6–8
Growth in NaCl (%)	< 3.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Nitrate reduction	-	-	w+	+	+	+	+
Urease	-	+	+	-	+	-	-
Gelatin hydrolysis	-	-	-	-	-	-	+
β-Galactosidase / PNPG	-	-	-	-	-	-	+
Oxidase	+	w+	+	-	w+	-	-
Assimilation							
glucose	-	-	w+	+	-	w+	+
L-arabinose	-	-	+	-	+	+	+
D-mannose	-	-	-	-	-	w+	+
N-acetylglucosamine	-	-	-	-	-	-	+
gluconate	-	-	+	-	+	+	-
adipate	-	/	+	+	/	w+	-
malate	-	+	+	+	+	w+	-
citrate	-	+	+	+	+	+	-
phenylacetate	-	/	-	-	+	-	-
Enzymatic activity				-			
Esterase lipase (C8)	+	/	+	/	/	-	-
Valine arylamidase	-	/	-	+	/	-	-
Cystine arylamidase	-	/	+	/	/	-	-
Trypsin	-	/	+	/	/	-	-
Naphthol-AS-BI-phosphohydrolase	+	/	+	+	/	+	-
β-Galactosidase	-	-	-	-	-	-	+
β-Glucuronidase	-	-	-	-	-	-	+
α-Glucosidase	-	/	-	-	/	-	-
N-acetyl-β-glucosaminidase	-	+	-	-	-	-	-
Chemotaxonomic features							
Polar lipids	DPG, PG, PE, PC	/	DPG, PG, PE, PC	/	/	PE, PG, PC, L1, L2	PMME, DPG, PE, PG, PC, AL, GL

Characteristic	1	2	3	4	5	6	7
Predominant menaquinone	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10
Fatty acids (> 5%)	Summed feature 8 ($C_{18:1}$ω7c / $C_{18:1}$ω6c), 73.64%; $C_{16:0}$, 8.61%	$C_{18:1}$ω7c (88.1%); summed feature 2 (5.2%)	Summed feature 8 ($C_{18:1}$ω7c and/or $C_{18:1}$ω6c), $C_{16:0}$, $C_{18:0}$	Summed feature 8 ($C_{18:1}$ω7c and/or $C_{18:1}$ω6c), 77.5%; $C_{19:0}$ cyclo ω8c, 10.4%	$C_{18:1}$ω7c (85.8%); $C_{18:0}$ (8.8%)	$C_{18:1}$ω7c (80.1%); $C_{16:0}$ (5.5%)	$C_{18:1}$ω7c (87.3%)

In API ZYM tests, strain JK268^T exhibited activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Activities of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, *N-acetyl-β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase were not detected. The physiological and biochemical characteristics indicated that strain JK268^T can be differentiated from the type species of the genus *Methylobacterium* with validly published names. Based on the phylogenetic, genomic, and phenotypic data presented, strain JK268^T reprents a novel species of the genus *Methylobacterium*, for which name *Methylobacterium cistanchae* sp. nov. is proposed.

Description of Methylobacterium cistanchae sp. nov.

Methylobacterium cistanchae (cis.tan'che.i. N.L. gen. n. *cistanchae*, of *Cistanche*, referring to the rhizosphere soil of a *Cistanche* plant from which the type strain was isolated).

Cells are Gram-stain-negative, aerobic, motile, non-spore-forming rods with flagella. Colonies on R2A agar after incubation at 28 ˚C for 48 h are pink, opaque, circular, smooth and moist, with entire margins and diameters of approximately 1–2 mm. Transmission electron microscopy revealed rod-shaped cells 1.0–3.0 μm in length. Growth occurs at 20–40 ˚C (optimum, 28 ˚C), at pH 5.0–10.0 (optimum, pH 8.0), and in the presence of 0–3.0% (w/v) NaCl, with optimal growth at 0.6% NaCl. Catalase and oxidase activities are positive. Nitrate reduction, indole production and glucose fermentation are negative. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, citrate and phenylacetic acid are not assimilated. Strain JK268^T exhibited activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Activities of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-β-glucosaminidase, *α*-mannosidase and *α*-fucosidase were absent. The predominant respiratory quinone is ubiquinone-10 (Q-10). The major cellular fatty acids are summed feature 8 ($C_{18:1}$ω7c and/or $C_{18:1}$ω6c; 73.64%) and $C_{16:0}$ (8.61%). Minor fatty acids include summed feature 5 ($C_{18:2}$ω6,9c and/or anteiso-$C_{18:0}$; 4.93%), $C_{18:1}$ω9c (4.03%), $C_{18:0}$ (3.23%) and $C_{18:0}$ 3-OH (2.22%). The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine.

Genome analysis revealed the presence of plant growth-promotion–related genes, including those involved in tryptophan-dependent indole-3-acetic acid biosynthesis (*trpEGDCFBA*), trehalose biosynthesis (*treS* and *otsAB*), proline biosynthesis (*proABC*) and phosphate metabolism (*pstSCAB*).

The type strain is JK268^T (= CCTCC AB 2025129^T = LMG 34231^T), isolated from rhizosphere soil of *Cistanche deserticola* collected in Yutian County, Hotan Prefecture, Xinjiang Uyghur Autonomous Region, PR China. The genomic DNA G + C content is 71.5%. The GenBank accession numbers are PX780827 for the 16S rRNA gene sequence and JBSVZN000000000 for the whole-genome sequence.

Abbreviations

ANI
average nucleotide identity
dDDH
digital DNA-DNA hybridization
ML
maximum-likelihood
MP
maximum-parsimony
NJ
neighbor-joining
DPG
diphosphatidylglycerol
PG
phosphatidylglycerol
PE
phosphatidylethanolamine
PL
unidentified phospholipid
UL
unidentified lipid

GL
unidentified glycolipid
PGL
unidentified phosphoglycolipid
IAA
indole-3-acetic acid.

Declarations

Author Notes:

The 16S rRNA gene sequence of strain JK268^T determined by Sanger sequencing is available in GenBank under the accession number PV130114. Additionally, a full-length 16S rRNA gene sequence extracted from the complete genome-deposited under accession number JBSVZN000000000 was assigned accession number PX780827 and used for phylogenetic analyses. Supplementary material comprising seven figures and two tables is available online.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

Funding

This work was suppored by Natural Science Foundation of Xinjiang Uygur Autonomous Region (grant no. 2025D01B97) and the National Natural Science Foundation of China (grant no. 32160002).

Author Contribution

All authors contributed to the study's conception and design. S.Y.W. designed and performed the experiments and statistical analyses, produced most of the figures and tables, and wrote the manuscript. X.R.W. and H.X. conducted some of the experiments and analyzed the data. W.Z. and D.L.K. revised the manuscript. W.Z. and S.Y.W contributed to the creation of the concept and the funding acquisition. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors are grateful to Professor Aharon Oren (The Hebrew University of Jerusalem, Israel) for helping with the etymology.

Data availability

The draft genome sequence of strain JK268^T has been deposited in GenBank under the accession number JBSVZN000000000. The raw read sequences are available in the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA1357107. The 16S rRNA gene sequence of strain JK268^T has been deposited in GenBank under the accession number PX780827. Strain JK268^T has been deposited in the China Center for Type Culture Collection (CCTCC) and the Belgian Coordinated Collections of Microorganisms (LMG) under the accession numbers CCTCC AB 2025129^T and LMG 34231^T, respectively.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

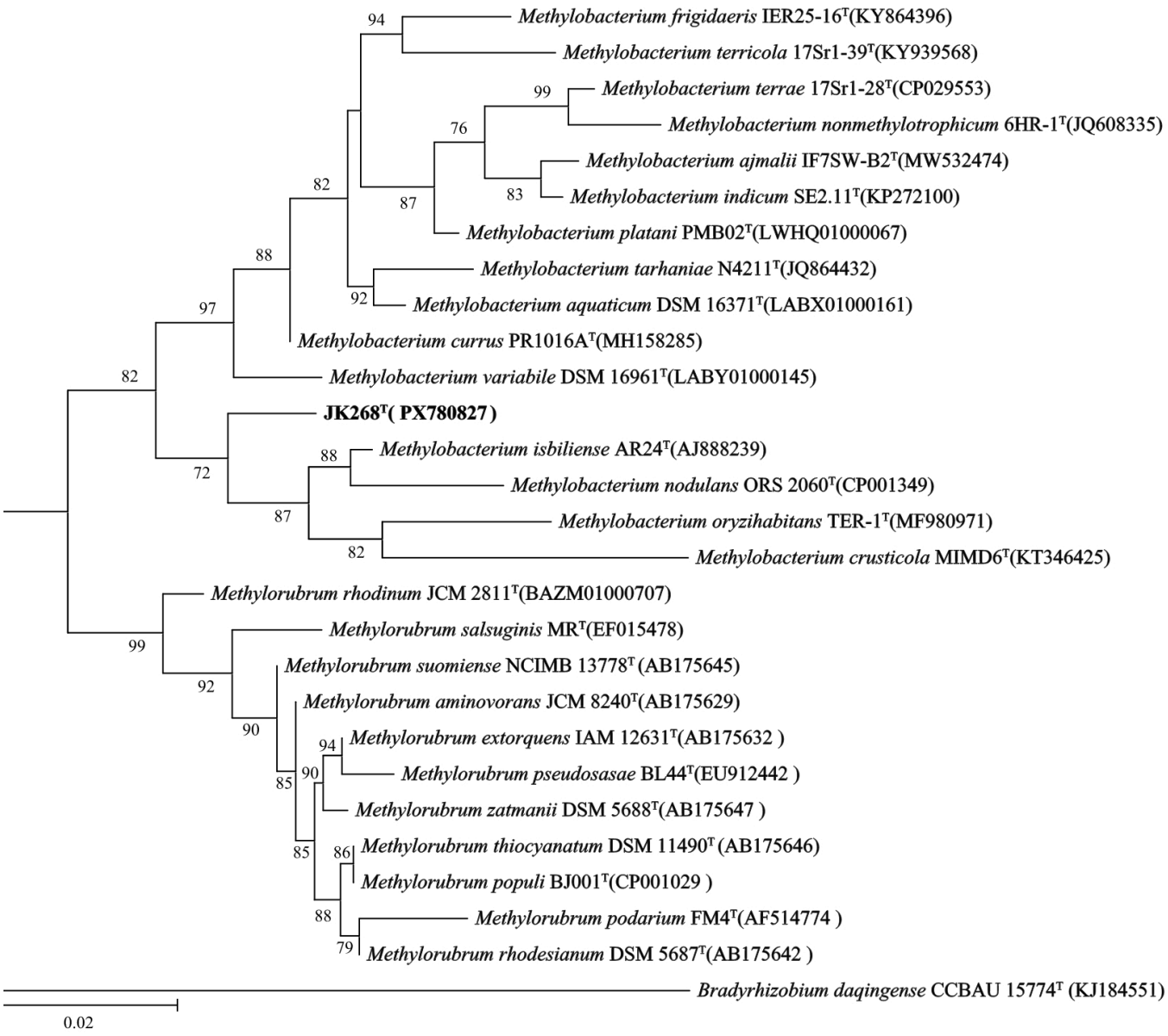


Figure 1

Maximum-likelihood phylogenetic tree based on a comparison of the 16S rRNA gene sequences of strain JK268^T and its closest relatives. GenBank accession numbers are given in parentheses. The tree was constructed using the Tamura-Nei model with the nearest-neighbour-interchange heuristic method. Bar, 0.02 nucleotide substitutions per site. Bootstrap values (>70%) based on 1000 replications are shown at branch nodes.

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