Neorhizobium xiangyangense sp. nov., Isolated From a Highland Barley Cultivation Soil in Qamdo, Tibet

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Abstract

A novel gram-negative, aerobic, rod-shaped and non-nitrogen fixing bacterium named strain T786^T was isolated from a highland barley cultivation soil in Qamdo, Tibet Autonomous Region, PR China. Strain T786^T grew at 5–30°C and pH 6.0–10.0 (optimum, 20–25°C and pH 7.0–8.0) with 0–4% (w/v) NaCl (optimum, 0%). The 16S rRNA gene sequences of strain T786^T showed the highest similarity to *Neorhizobium vignae* CCBAU 05176^T (98.70%), followed by *Neorhizobium alkalisoli* CCBAU 01393^T (98.47%), *Neorhizobium tomejilense* T17_20^T (98.43%), *Neorhizobium huautlense* S02^T (98.36%), and *Neorhizobium galegae* ATCC 43677^T (98.00%). Phylogenetic analysis based on 16S rRNA genes indicated that strain T786^T was a new member of the genus *Neorhizobium*. The digital DNA-DNA hybridization and average nucleotide identity values between the genome sequence of strain T786^T and related strains were 20.20–20.60% and 76.64–80.01%, respectively. The genomic DNA G + C content of strain T786^T was 60.2%. The major cellular fatty acids were Summed feature 8 (C_{18:1} ω7c or C_{18:1} ω6c, 51.53%), C_{16:0} (21.74%) and Summed feature 3 (C_{16:1} ω7c or C_{16:1} ω6c, 10.21%). The polar lipids of strain T786^T were consisted of diphosphatidyl glycerol, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl methyl ethanolamine, unidentified phospholipid and unidentified lipids (1–4). The isoprenoid quinones of strain T786^T was consisted of Q-10. The DAP and sugar components of cell wall of strain T786^T were *meso*-DAP and ribose, glucose, respectively. Based on phenotypic, phylogenetic, and genotypic datas, strain T786^T is considered to represent a novel species of the genus *Neorhizobium*, for which the name *Neorhizobium xiangyangense* sp. nov. is proposed. The type strain is T786^T (= JCM 35100^T = CICC 25102^T).

Introduction

Rhizobia are a diverse group of α- and β-Proteobacteria that can enter nitrogen-fixing symbioses with legumes, both symbiotic and non-symbiotic bacteria were included in these genera (Rahi et al. 2020). Until now rhizobial genera include *Allorhizobium*, *Agrobacterium*, *Azorhizobium*, *Burkholderia*, *Bradyrhizobium*, *Cupriavidus*, *Devosia*, *Mesorhizobium*, *Methylobacterium*, *Microvirga*, *Neorhizobium*, *Ochrobactrum*, *Pararhizobium*, *Phyllobacterium*, *Rhizobium*, *Shinella* and *Ensifer* (Jiao et al. 2015). The genus *Neorhizobium* is a recently recategorized genus formed by former *Rhizobium* species, which were highly related to *Rhizobium*-*Agrobacterium*-*Agrobacterium* clade (Mousavi et al. 2014). At the time of writing, the genus *Neorhizobium* consisted of six species, include five validly published species (*N.alkalisoli*, *N.galegae*, *N.huautlense*, *N.tomejilense* and *N.vignae*) and one not validly published species ("*N.lilium*") (Mousavi et al. 2014, Soenens et al.2019, Hördt et al. 2020, Liu et al. 2020). Most of these strains were isolated from root nodules and could nodulate with their hosts. The soil nitrogen (N) deficiency is the major factor contributing to low primary productivity on the Qinghai-Tibet Plateau, so the study on Rhizobia in this region will advantage to improve the research level of nitrogen-cycling in soil (Wang et al. 2019). During the study of culturable bacterial diversity of highland barley cultivation soil from Tibet Autonomous Region, strain T786^T was isolated (Pan et al. 2021). The phylogenetic analysis of 16S rRNA indicated that strain T786^T should be classified as a member of genus *Neorhizobium*. Genomic, phylogenetic and phenotypic datas obtained from the strain T786^T support the definition of a new *Neorhizobium* species, for which the name *Neorhizobium xiangyangense* sp. nov. is proposed.

Materials And Methods

Isolation and cultivation of strain T786^T

In April 2018, a study of microbial diversity in a highland barley cultivation soil sample led to the isolation of a novel species of *Neorhizobium*. The sample site was located in the Zhu Village, Banbar County, Qamdo City, Tibet Autonomous Region, China (30°55'48.9"N, 94°58'13.4"E, Altitude: 4011 m). The sample site belongs to the plateau temperate subhumid climate type, the air temperature range is -40-29.0°C, the annual average air temperature is -1°C and the yearly frozen period is from September to April. The soil sample was diluted, using a tenfold dilution series method, spread on R agar medium and incubated at 4°C. The R agar medium contained (1000 mL): peptone 10.0 g, yeast extract 5.0 g, maltose extract 5.0 g, casein amino acid 5.0 g, beef extract 2.0 g, glycerol 2.0 g, tween-80 50.0 mg, MgSO₄·7H₂O 1.0 g, agar 18.0 g, pH value 7.2–7.6. After 60 d of incubation, a white-coloured colony was collected and named as T786. Strain T786 was routinely cultured on Luria-Broth (LB) at 25°C after repeated purifying. The purified strain was preserved at -80°C with 25% (v/v) glycerol. Strain T786^T has been deposited at the CICC (China Center of Industrial Culture Collection) and JCM (Japan Collection of Microorganisms).

Phenotypic characterization

Five closely related type strains, *N.vignae* LMG 25447^T, *N.alkalisoli* LMG 27728^T (formerly LMG 24763^T), *N.tomejilense* LMG 30623^T, *N.huautlense* LMG 18254^T and *N.galegae* LMG 6214^T, were obtained from Laboratory of Microbiology, Ghent University (LMG) for comparison studies of phenotypic characterization and chemotaxonomic analysis. The phenotypic characteristics were tested on yeast mannitol agar (YMA) in parallel with five reference strains under the same conditions. Cell morphology of the strains cultured at 28°C for 3 days were observed and photographed by light microscopy (CX31, Olympus) and scanning electron microscopy (Hitachi FE-SEM SU8000).

The temperature for optimal growth was tested at 5–45°C (5, 10, 15, 20, 25, 30, 37, 40 and 45°C). The pH range for growth was measured from pH 4.0 to pH 12.0, with an interval of 1.0 units. The salt tolerance was determined with various NaCl concentrations (0, 1, 2, 3, 4, 5 and 6%, w/v). Strain T786^T and related reference strains were cultured on YMA broth for 3 days, and determined by measuring the optical density at 600 nm (OD₆₀₀) in triplicate. Other tests to determine the biochemical characteristics were carried out using API 50CH, API 20E, API 20NE, and API ZYM strips according to the manufacturer's instructions (BioMérieux) (Zhang et al. 2018).

Phylogenetic analysis of 16S rRNA, *nodA*, *nodC* and *nifH* genes

Genomic DNA was extracted from Strain T786^T after growth in Luria-Broth for 48 h, using MiniBEST Bacterial Genomic DNA Extraction Kit Version 2.0 (TaKaRa Biotechnology Co., Tokyo, Japan). Amplification of the 16S rRNA, *nodA*, *nodC* and *nifH* genes were performed using the extracted highly purified genomic DNA as a template under the following conditions: 95°C for 10 min, followed by 94°C for 45 s, 55–58°C for 45 s, and 72°C for 90 s for 30 cycles with a final 10 min extension at 72°C, the PCR products were detected by agarose gel electrophoresis and then sent to GENEWIZ, Inc for sequencing. Primers used for amplification and

sequencing of 16S rRNA, *nodA*, *nodC* and *nifH* genes were as described by Mousavi et al (2016), Deng et al (2020) and Heng et al (2017). 16S rRNA and other genes were aligned in EzBioCloud (<https://eztaxon-e.ezbiocloud.net/>) and GenBank (www.ncbi.nlm.nih.gov), respectively. Maximum-likelihood tree was constructed using MEGA7.0 software with bootstrap values of 1000 replicates (Kumar et al. 2016).

Genome sequencing and comparative genomic analysis

The genomic DNA of strain T786^T was sequenced with BGISEQ-500 platform in China center of industrial culture collection. The genomic sequence information of T786^T had been submitted to the National Centre for Biotechnology Information (NCBI) database under the accession number JAJAWH0000000000. Draft genome assemblies were prepared from the ONT reads using Apades v3.11.0, gene prediction using Glimmer 3.02 software. The predicted coding sequences were translated and used as queries to search the COG database.

Based upon the close relationship with the test strain in phylogenetic analyses, the draft genome sequence of *N. vignae* CCBAU 05176^T, *N. alkalisoli* CCBAU 01393^T, *N. tomejilense* T17_20^T, *N. huautlense* S02^T, *N. galegae* ATCC 43677^T were obtained from NCBI database. The digital DNA-DNA hybridization (dDDH) values and confidence intervals were calculated using the recommended settings of Genome-to-Genome Distance Calculator (GGDC) (Meier-Kolthof et al. 2013). The average nucleotide identity (ANI) was determined between strains T786^T and closely related strains of the genus *Neorhizobium* by OrthANlu (Yoon et al. 2017). The whole-genome orthologous clusters were compared and analyzed by OrthoVenn2 (Xu et al, 2019).

Determination of fatty acid, polar lipids, isoprenoid quinones and chemical components of cell wall

After incubation in YMA broth at 28°C and 180 rpm for 3 days, cells were collected for a series of tests. Fatty acids were saponified, methylated and extracted according to the standard protocol of the Sherlock Microbial Identification System (version 6.2B, MIDI) and identified using gas-chromatograph method (Kroppenstedt RM.1985). Polar lipids and chemical components of cell wall were detected using thin layer chromatography method. Isoprenoid quinones were identified using high-performance liquid chromatography method (Komagata and Suzuki.1987).

Results And Discussion

Morphological, cultural, physiological and biochemical characteristics

Colonies of strain T786^T was white, round, moist, opaque on YMA solid medium (Fig. 1A). Strain T786^T was gram-negative, aerobic, rod-shaped, single or paired, 0.4–0.6 µm×0.8–2.1 µm (Fig. 1B, 1C and 1D). Strain T786^T showed many similar phenotypic characteristics with reference strains of *Neorhizobium*, but there were a few of differences (Table 1). Compared with other type strains in this study, the optimum range of growth temperature, pH and salinity of T786^T were narrow. The activities of β-galactosidase and β-glucosidase of strain T786^T were negative. Strain T786^T can assimilate L-xylose, D-cellobiose, D-melibiose and D-gentiobiose, while it can not utilize β-methyl-D-xylopyranoside and potassium gluconate.

Table 1
Differential phenotypic characteristics of strain T786^T and its closely related species of the genus
Neorhizobium

Characteristic	1	2	3	4	5	6
Cell size (width×length; µm)	0.4– 0.6×0.8– 2.1	0.5– 0.7×0.8– 2.2	0.5– 0.9×1.0– 3.0	0.5– 0.9×0.9– 2.5	0.6– 0.9×1.0– 2.3	0.6– 0.9×0.9– 2.3
Color	white	light orange	white	white	white	light orange
Temperature range (optimum) (°C)	5–30(20– 25)	10– 37(20–30)	10–37 (20–30)	10– 37(20– 30)	5–40(20– 30)	5–30(20– 30)
pH range (optimum)	6.0– 10.0(7.0– 8.0)	5.0– 10.0(6.0– 8.0)	5.0– 10.0(7.0– 8.0)	5.0– 9.0(7.0– 8.0)	5.0– 10.0(6.0– 8.0)	5.0– 9.0(6.0– 8.0)
NaCl tolerance (optimum) (% w/v)	0.0– 4.0(0.0– 1.0)	0.0– 3.0(0.0)	0.0	0.0	0.0– 3.0(0.0– 1.0)	0.0
Motility	-	-	+	-	-	+
β-galactosidase	-	+	+	+	+	+
Alkaline phosphatase	-	+	-	-	-	-
Lipidase (C8)	-	-	-	+ ^w	-	+ ^w
β-glucosidase	-	+	+ ^w	+	+	+
Voges-Proskauer test	-	-	+	+	+	+
Nitrate reduction	+	+	+	-	+	-
D-arabinose	+	+ ^w	+ ^w	+ ^w	+	+
D-ribose	+	+ ^w	+ ^w	-	+	+ ^w
D-xylose	+	+ ^w	+ ^w	+ ^w	+	+
L-xylose	+	-	-	-	-	-
adonite	+	-	-	-	+	-
β-methyl-D- xylopyranoside	-	+	+ ^w	+	+	+
D-glucose	+ ^w	+ ^w	+ ^w	-	+	+ ^w
L-rhamnose	+	+ ^w	+	+ ^w	+ ^w	+

Strains: 1, strain T786^T; 2, *N.vignae* LMG 25447^T; 3, *N.alkalisoli* LMG 27728^T; 4, *N.tomejilense* LMG 30623^T; 5, *N.huautlense* LMG 18254^T; 6, *N.galegae* LMG 6214^T. + positive; -, negative; +^w, weakly positive.

Characteristic	1	2	3	4	5	6
D-cellobiose	+	-	-	-	-	-
D-melibiose	+	-	-	-	-	-
Xylitol	+	-	+ ^w	+ ^w	-	-
D-gentiobiose	+	-	-	-	-	-
D-lyxose	+	+	-	-	+ ^w	+ ^w
D-tagatose	-	+ ^w	-	-	-	-
D-maltose	-	+ ^w	+ ^w	-	+	+ ^w
Potassium gluconate	-	+	+ ^w	+ ^w	+ ^w	+ ^w
Adipic acid	-	+ ^w	-	+ ^w	-	-
Malic acid	+	+	-	+	+	+
Strains: 1, strain T786 ^T ; 2, <i>N.vignae</i> LMG 25447 ^T ; 3, <i>N.alkalisoli</i> LMG 27728 ^T ; 4, <i>N.tomejilense</i> LMG 30623 ^T ; 5, <i>N.huautlense</i> LMG 18254 ^T ; 6, <i>N.galegae</i> LMG 6214 ^T . + positive; -, negative; + ^w , weakly positive.						

Phylogenetic analysis based on 16S rRNA, *nodA*, *nodC* and *nifH* genes

In database of EzBioCloud, the 16S rRNA gene sequence of strain T786^T shared highest similarity with *N.vignae* CCBAU 05176^T (98.70%), followed by *N.alkalisoli* CCBAU 01393^T (98.47%), *N.tomejilense* T17_20^T (98.43%), *N.huautlense* S02^T (98.36%), and *N.galegae* ATCC 43677^T (98.00%). Phylogenetic analysis of 16S rRNA of T786^T confirmed its placement within the *Neorhizobium* genus, but to formed a separate branch of evolution with a high bootstrap support. A ML tree derived from full 16S rRNA alignments is shown in Fig. 2. In this study, PCR products for the *nodA*, *nodC* and *nifH* genes of strain T786^T could not be obtained. Furthermore, genome annotation and local blast results showed that sequences of *nodA*, *nodC* and *nifH* genes could not be found in strain T786^T, only a little number of nitrogen-fixing-related genes (*nodB*, *nodT*, *nifB* and *nifU*) could be annotated.

Genomic characteristics and comparative genomics analysis

The draft genome of strain T786^T contained 126 contigs with an N₅₀ value of 169022 bp and an N₉₀ value of 32097 bp. The genome size of strain T786^T is 5.01 Mb. The genomic DNA G + C content is 60.2 mol%, which is within the range reported for the G + C content of the genus *Neorhizobium* (60.0–61.6 mol%). Genes for nodulation (*nodA* and *nodC*) and nitrogen fixation (*nifH*) were not detected in the draft genome of strain T786^T, the ability to form nodules and fix atmospheric nitrogen of strain T786^T needs to be further studied

(Giraud et al. 2007; Cavalcanti et al. 2020) (Table 2). The distribution of genes into COG functional categories revealed that the 4 major functional genes were assigned to metabolism (46.56%), cellular processes and signaling (21.78%), information storage and processing (15.95%) and poorly characterized (15.71%) (Fig not shown).

Table 2
Genomic properties of *Neorhizobium* species

	<i>N.xiangyangense</i> T786 ^T	<i>N.vignae</i> LMG 25447 ^T	<i>N.alkalisoli</i> LMG 27728 ^T	<i>N.tomejilense</i> LMG 30623 ^T	<i>N.huautlense</i> LMG 18254 ^T	<i>N.galegae</i> LMG 6214 ^T
Assembly quality	Draft	Draft	Draft	Draft	Draft	Complete(2) Draft(8)
Total Size (Mb)	5.01	6.34	5.87	6.62	5.73	4.65
GC (%)	60.2	61.6	60.3	61.5	60.0	61.5
rRNA operons	7	5	4	3	4	9
tRNAs	47	43	48	48	48	48
Gene	4941	5971	5492	6279	5248	6033
<i>nod/nif</i> symbiotic genes	-	-	+	-	+	+

The dDDH and ANI values between strain T786^T and *N.vignae* CCBAU 05176^T, *N.alkalisoli* CCBAU 01393^T, *N.tomejilense* T17_20^T, *N.huautlense* S02^T, *N.galegae* ATCC 43677^T were 20.20–20.60% and 76.64–80.01%, respectively, which were lower than the threshold values of 70% and 95–96% for species discrimination (Chun et al. 2018; Goris et al. 2007) (Table 3). The genomic differences and orthologous clusters between strains T786^T and related *Neorhizobium* species were shown in Fig. 3. Strain T786^T, *N.vignae* CCBAU 05176^T, *N.alkalisoli* CCBAU 01393^T, *N.tomejilense* T17_20^T, *N.huautlense* S02^T and *N.galegae* ATCC 43677^T were had 4941, 5971, 5492, 6279, 5248 and 6033 proteins, respectively, of which 1229, 618, 643, 777, 546 and 652 were singletons, 2796 orthologous clusters were identified among all the six strains. Strain T786^T shared the lowest number of proteins (4941), but the highest number of singletons (1229). This suggests that the unique functions of T786^T was a certain adaptation for its growth in Tibet and strain T786^T was identified as a unique cluster of genus *Neorhizobium*.

Table 3

The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values between strain T786^T and related strains

Reference Strain	Accession number	ANI values with T786 ^T (%)	dDDH values with T786 ^T (%)
<i>N.vignae</i> LMG 25447 ^T	GCA_000732195.1	80.01	20.50
<i>N.alkalisoli</i> LMG 27728 ^T	GCA_002968635.1	79.65	20.20
<i>N.tomejilense</i> LMG 30623 ^T	GCA_002968845.1	76.64	20.60
<i>N.huautlense</i> LMG 18254 ^T	GCA_002968575.1	79.20	20.20
<i>N.galegae</i> LMG 6214 ^T	GCA_000731315.1	79.96	20.40

Fatty acid, polar lipids, isoprenoid quinones and chemical components of cell wall

The predominant fatty acids of strain T786^T (≈10.0% of the total amounts) comprised of Summed feature 8 (C_{18:1} ω7c or C_{18:1} ω6c, 51.53%), C_{16:0} (21.74%) and Summed feature 3 (C_{16:1} ω7c or C_{16:1} ω6c, 10.21%). The major differences between strain T786^T and its closely relatives were higher content of Summed feature 3, C_{18:0} and lower content of Summed feature 8, which can distinguish strain T786^T from its related type strains (Table 4).

Table 4

Cellular fatty acids of strain T786^T and its closely related species of the genus *Neorhizobium*

Fatty acid	1	2	3	4	5	6
C _{10:0}	1.54	–	–	–	–	–
C _{12:0}	1.34	–	–	–	–	–
C _{14:0}	1.05	–	–	–	–	–
C _{16:0}	21.74	15.36	13.19	11.59	13.52	20.49
C _{16:0} 3-OH	–	1.71	–	2.54	2.18	3.89
C _{18:0}	5.39	3.01	–	2.38	3.20	2.21
C _{18:0} 3-OH	–	1.14	–	1.11	1.54	
C _{19:0} cyclo ω8c	–	11.65	–	7.98	7.67	–
Summed feature 2 (C _{14:0} 3-OH and/or iso-C _{16:1} I)	3.45	4.14	4.61	4.12	4.59	5.45
Summed feature 3 (C _{16:1} ω7c and/or C _{16:1} ω6c)	10.21	2.24	1.59	1.30	1.05	1.49
Summed feature 6 (C _{19:1} ω9c and/or C _{19:1} ω11c)	–	–	–	6.12	–	–
Summed feature 7 (C _{19:1} ω6c and/or C _{19:1} ω7c)	–	–	–	2.09	–	–
Summed feature 8 (C _{18:1} ω7c and/or C _{18:1} ω6c)	51.53	59.05	77.79	61.15	62.88	66.14
Strains: 1, strain T786 ^T ; 2, <i>N.vignae</i> LMG 25447 ^T ; 3, <i>N.alkalisoli</i> LMG 27728 ^T ; 4, <i>N.tomejilense</i> LMG 30623 ^T ; 5, <i>N.huautlense</i> LMG 18254 ^T ; 6, <i>N.galegae</i> LMG 6214 ^T . Values are percentages of the total fatty acids. Fatty acids that make up < 1.0% of the total are not shown. – Not detected or <1.0%.						

The polar lipids, isoprenoid quinones and chemical components of cell wall of strain T786^T and its related type strains had no significant difference. The polar lipids of these strains were consisted of diphosphatidyl glycerol, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl methyl ethanolamine, unidentified phospholipid and unidentified lipids (1–4). The isoprenoid quinones of these strains were consisted of Q-10 (100%). The DAP and sugar components of cell wall of these strains were *meso*-DAP and ribose, glucose, respectively.

Description of *Neorhizobium xiangyangense* sp. nov.

Neorhizobium xiangyangense (xiang.yang.ense. N.L. gen. n. *xiangyang* in honour of Dr Xiang-yang Lu from Hunan Agricultural University)

Cells are gram-negative, aerobic, rod-shaped, single or paired, 0.5–0.7 µm in width, and 0.8–2.1 µm in length. Colonies grown on YMA for 3 days are white, round, moist, opaque and 2–3 mm in diameter after incubation for 120 h on YMA at 28°C. Growth occurs in the presence of 0–4% (w/v) NaCl (optimum, 0–1% NaCl), pH 6.0–10.0 (optimum, 7.0–8.0), and 5–30°C (optimum, 20–25°C). Negative for hydrolysis of starch, tweens 80, gelatin and urease. In addition, catalase activity, oxidase activity and urease activity are positive, while citric acid utilization, Voges-Prokauer test, hydrogen sulfide and indole production, β-galactosidase activity, arginine dihydrolase activity, lysine decarboxylase activity, ornithine decarboxylase activity and tryptophan deaminase activity are negative. In API 20NE tests, a positive reaction for nitrate reduction, esculin hydrolysis and negative reactions for glucose fermentation. In the API ZYM kit, C4 esterase, leucine aromatase, trypsin and α-glucosidase are present; however, alkaline phosphatase, C8 esterase lipase, C14 esterase lipase, valine arylaminase, cystine arylaminase, chymotrypsin, α-galactosidase, β-uronidase, β-glucosidase, α-mannosidase, β-fucosidase and N-acetylglucosaminidase are absent. In utilization of sole carbon source tests, positive for D-glucose, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, adonite, D-galactose, D-dextrose, D-fructose, D-mannose, L-rhamnose, mannitol, D-cellobiose, D-melibiose, xylitol, D-gentiobiose, D-lysol, D-fucose, L-fucose and D-arabinol, and negative for glycerine, erythritol, β-methyl-D-xylopyranoside, L-sorbose, euonymol, inositol, sorbitol, salicin, D-maltose, D-lactose, D-sucrose, D-trehalose, D-triose, D-raffinose, amyllum, glycogen, D-toulon, D-tagatose, L-arabinol and potassium gluconate.

The major cellular fatty acids in strain T786^T are summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c), C_{16:0} and summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c). The isoprenoid quinone is Q-10. The polar lipids are diphosphatidyl glycerol, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl methyl ethanolamine, unidentified phospholipid and unidentified lipids (1–4). The DAP and sugar components of cell wall are *meso*-DAP and ribose, glucose, respectively. The type strain is T786^T (= JCM 35100^T = CICC 25102^T), which was isolated from soil in Qamdo, Tibet Autonomous Region, PR China. The genome size of strain T786^T is 5.01 Mbp with a high genomic DNA G + C content of 60.2 mol%. The GenBank accession numbers of 16S rRNA and whole genome sequence of strain T786^T are MT611284 and JAJAWH000000000, respectively.

Declarations

Author contributions

YT and YY conceived the project. HP, ZZ, GH, JZ, KJ, HL, and CW performed the experiments. HP, HY, and XL analyzed the data, and HP and YT drafted and revised the manuscript. All authors have read and approved the final version of the manuscript.

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Data availability

All data have been made fully available to the public.

Conflict of interest

All the authors have declared no conflict of interest.

Consent to participate

All authors gave their consent to participate in this study.

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Figures

Figure 1

Images of strain T786^T. (A) Colonies on YMA solid medium. (B) Gram staining. (C, D) Scanning electron microscope.

Figure 2

Maximum-likelihood tree based on 16S rRNA gene sequences (1471 bp) revealing the relationship between strain T786^T and other species of the genus *Neorhizobium*. Bootstrap values $\geq 50\%$ are shown.

Figure 3

Homologous genes analysis of the sequence. (A) Counts of proteins, clusters, and singletons among strain T786^T, *N.vignae* LMG 25447^T, *N.alkalisoli* LMG 27728^T, *N.tomejilense* LMG 30623^T, *N.huautlense* LMG 18254^T and *N.galegae* LMG 6214^T. (B) Venn diagram displays the distribution of shared orthologous clusters in strain T786^T and related *Neorhizobium* species.