Mesorhizobium gobiense sp. nov. and *Mesorhizobium tarimense* sp. nov., isolated from wild legumes growing in desert soils of Xinjiang, China

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Twenty-four *Mesorhizobium* strains were isolated from desert soils in the Xinjiang region of China and were characterized by a polyphasic approach. These strains grouped into three clusters in IGS-RFLP, SDS-PAGE analysis of whole-cell proteins and BOX-PCR analysis, corresponding to genomic species V, VI and VII as found in a previous study. The results were supported by sequencing analyses of *rrs*, IGS, *atpD* and *recA* genes. Genospecies VII was most related to *Mesorhizobium septentrionale*, while genospecies V and VI were both most closely related to *Mesorhizobium tianshanense*, but were distinct from each other and from *M. tianshanense*. The DNA–DNA hybridization value between the representative strain CCBAU 83284 (genospecies VII) and the type strain of *M. septentrionale* was 90.1 %. Genospecies VII was thus defined as *M. septentrionale*. The DNA–DNA relatedness value for representative strains of genospecies V or VI with the related reference strains of recognized species were always lower than 60 %. Low values of DNA–DNA hybridization (32.79 %) between representative strains of genospecies V (CCBAU 83330^T) and of VI (CCBAU 83306^T) were also observed. Based upon these results, two novel species are proposed: *Mesorhizobium gobiense* sp. nov. represented by genospecies V (type strain, CCBAU 83330^T=LMG 23949^T=HAMBI 2974^T) and *Mesorhizobium tarimense* sp. nov. represented by genospecies VI (type strain, CCBAU 83306^T=LMG 24338^T=HAMBI 2973^T). Strain CCBAU 83278 grouped as the most peripheral member with genospecies VI in SDS-PAGE of whole-cell proteins and BOX-PCR analysis and in the phylogenetic tree of 16S–23S rRNA intergenic spacer (IGS) sequences. The results of analyses of *rrs*, *atpD* and *recA* gene sequences, as well as those of DNA–DNA hybridization studies, strongly supported the suggestion that this strain belonged to a species quite different from genospecies V and VI and from any other recognized species of the genus *Mesorhizobium*. As only one strain has been

Abbreviations: ARDRA, amplified rDNA restriction analysis; BOX-PCR, BOX-repeat-based PCR; IGS, 16S–23S rRNA intergenic spacer.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences for *M. gobiense* sp. nov. strains CCBAU 83330^T, CCBAU 83511 and CCBAU 83346, *M. tarimense* sp. nov. strains CCBAU 83306^T and CCBAU 83321, strain CCBAU 83278 and *M. septentrionale* strains CCBAU 83281 and 83284 are EF035064, EF549381, EF549388, EF035058, EF549387, EU368831, EF549379 and EF035063, respectively. Those for partial IGS are EF050768, EF549537, EF050769, EF050771, EF050772, EF050770, EF549535 and EF050773, respectively. Accession numbers for the partial *atpD* gene sequences are EF549409, EF549411, EF549413, EF549410, EF549412, EU371996, EF549407 and EF549415, respectively. For partial *recA* gene sequences, the accession numbers are EF549481, EF549480, EF549478, EF549482, EF549479, EU371997, EF549484 and EF549485, respectively. The partial *nodC* gene sequences are EF050784, EF549525, EF549524, EF050786, EF549514, EF549527, EF549511 and EF050783, respectively. The partial *nifH* gene sequences are EU252606, EU252604, EU252605, EU252607, EU252608, EU252603, EU252601 and EU252602, respectively.

Dendrograms constructed from IGS-RFLP, BOX-PCR and SDS-PAGE analyses and additional phylogenetic trees based on IGS, *atpD*, *recA*, *nodC* and *nifH* gene sequences are available as supplementary figures with the online version of this paper. A supplementary table giving details of cellular fatty acid contents is also available.

isolated to date, strain CCBAU 83278 was not proposed as a novel species in this study. *Mesorhizobium gobiense* sp. nov. and *Mesorhizobium tarimense* sp. nov. could be differentiated from each other as well as from recognized species of the genus *Mesorhizobium* on the basis of phenotypic characteristics. The symbiotic loci (*nodC* and *nifH*) of the two novel species formed two phylogenetic branches related to *Mesorhizobium loti* and *M. tianshanense*. The type strains of the two novel species were able to nodulate *Glycyrrhiza uralensis*, *Lotus corniculatus*, *Oxytropis glabra* and *Robinia pseudoacacia* but not *Astragalus membranaceus*, *Leucaena leucocephala*, *Phaseolus vulgaris*, *Pisum sativum* or *Medicago sativa*.

The family Leguminosae is one of the largest families of green plants, containing more than 17 900 species distributed throughout the world (Allen & Allen, 1981). Most legume species have a unique and important characteristic that enables them to form nodules on roots and/or stems and establish symbiosis with soil bacteria, collectively called rhizobia. Within nodules, rhizobia reduce N₂ to ammonia which can then be used by the legume plants. Due to the ecological and economic importance of rhizobia, their diversity has been extensively investigated and rhizobial taxonomy has been greatly improved in the last two decades. So far, more than 50 species of rhizobia have been reported (Wang *et al.*, 2006). In China, a total of 1485 leguminous species in 172 genera have been recorded and they are distributed in the tropical, subtropical, temperate and frigid climate zones, from the south to the north (Wang *et al.*, 2007). Diverse rhizobia isolated from Chinese legumes have been characterized and several novel species have been proposed (Chen *et al.*, 1988, 1991, 1997; Gao *et al.*, 2004; Scholla & Elkan, 1984; Tan *et al.*, 2001; van Berkum *et al.*, 1998; Wang *et al.*, 1999, 2007; Wei *et al.*, 2002, 2003; Xu *et al.*, 1995; Yao *et al.*, 2002).

Xinjiang is the largest province of China and has an area of 1.66 million km², roughly 1/6 of the total Chinese territory. Most of Xinjiang is covered by desert. It has a typical continental climate, with low rainfall but very high evaporation potential. The soils in this area are poor in organic matter with high salinity and alkalinity (Chen *et al.*, 1995). In a previous study (Han *et al.*, 2008), we characterized 111 rhizobial strains isolated from multiple wild legume species growing natively in Xinjiang by amplified rDNA restriction analysis (ARDRA) and sequence analysis of 16S rRNA genes (data not shown). Twenty-four of the strains that were isolated from the nodules of *Astragalus filicaulis*, *Lotus frondosus*, *Lotus tenuis*, *Oxytropis glabra* and *Robinia pseudoacacia* (Table 1) were classified within the genus *Mesorhizobium* based on ARDRA. These 24 strains formed three genomic species and genospecies V and VI were phylogenetically closely related to *Mesorhizobium tianshanense*, while genospecies VII was closely related to both *Mesorhizobium amorphae* and *Mesorhizobium septentrionale*. In this study, these strains were further analysed to clarify their taxonomic status by 16S–23S rRNA intergenic spacer (IGS) RFLP (IGS-RFLP), SDS-PAGE analysis of whole-cell proteins, BOX-repeat-based PCR (BOX-PCR) fingerprinting, analysis of phenotypic characteristics, DNA–DNA hybridization and

sequence analyses of *rrs*, IGS, *atpD*, *recA*, *nodC* and *nifH* genes.

IGS-RFLP, considered to be a useful tool for investigating the relatedness of closely allied rhizobial strains (Jensen *et al.*, 1993; Vinuesa *et al.*, 1998; Laguerre *et al.*, 1996), was performed with the primers FGPS1490 (Navarro *et al.*, 1992)/FGPL132' (Ponsonnet & Nesme, 1994) and *MboI*, *MspI* and *AluI* restriction endonucleases. The results showed that the test strains formed three clusters corresponding to the three genomic species defined in the previous study at a similarity of 76 % at which all the reference strains were distinguished according to their species (see Supplementary Fig. S1 in IJSEM Online). Genomic species VII contains four strains isolated from *Robinia pseudoacacia* and was found to cluster with *M. amorphae* and *M. septentrionale*, indicating that these species are closely related to genomic species VII. In contrast, genomic species V and VI formed a cluster that was separate and distinct from the recognized species of the genus *Mesorhizobium* analysed here, suggesting that they may represent two novel species.

SDS-PAGE of whole-cell proteins was conducted according to a previously described method (Tan *et al.*, 1997). The normalized densitometric traces of the electrophoretic protein patterns were grouped using the Gelcompar II software package (Applied Maths). The similarity between each pair of samples (strains) was expressed by DICE and an UPGMA dendrogram was constructed (Vauterin & Vauterin, 1992). All the test strains in the SDS-PAGE analysis of whole-cell proteins formed three clusters corresponding to genomic species V, VI, and VII, respectively, at a similarity level of 87 %, where all the reference strains of *Mesorhizobium* included in the study could be well separated (see Supplementary Fig. S2 in IJSEM Online). Genospecies VII clustered with *M. septentrionale* at a similarity level of about 87.5 %, implying that this genospecies belongs to *M. septentrionale*. Genospecies V and VI produced different protein patterns from those of the strains for recognized *Mesorhizobium* species at the similarity level of 87 %.

To reveal the genetic diversity of the novel isolates, BOX-PCR was performed using the BOXAIR primer (Versalovic *et al.*, 1994). PCRs were carried out as described by Nick & Lindström (1994). The fingerprints were analysed by the Gelcompar II program (Applied Maths) and an UPGMA

Table 1. Isolates and reference strains used in this study

Strains	Host plant	Geographical origin
<i>M. septentrionale</i> (genomic species VII) CCBAU 83281, CCBAU 83282, CCBAU 83284, CCBAU 83285	<i>Robinia pseudoacacia</i>	Xinjiang, China
<i>M. gobiense</i> sp. nov. (genomic species VI) CCBAU 83508, CCBAU 83509, CCBAU 83511	<i>Astragalus filicaulis</i>	Xinjiang, China
CCBAU 83307, CCBAU 83310, CCBAU 83341, CCBAU 83342	<i>Lotus frondosus</i>	Xinjiang, China
CCBAU 83346	<i>Lotus tenuis</i>	Xinjiang, China
CCBAU 83329, CCBAU 83330 ^T , CCBAU 83331	<i>Oxytropis glabra</i>	Xinjiang, China
<i>M. tarimense</i> sp. nov. (genomic species V) CCBAU 83306 ^T , CCBAU 83308, CCBAU 83311, CCBAU 83312, CCBAU 83339, CCBAU 83338, CCBAU 83343	<i>Lotus frondosus</i>	Xinjiang, China
CCBAU 83321	<i>Lotus tenuis</i>	Xinjiang, China
<i>Mesorhizobium</i> sp. (genomic species V) CBAU 83278	<i>Robinia pseudoacacia</i>	Xinjiang, China
Reference strains		
<i>M. loti</i> NZP 2213 ^T	<i>Lotus corniculatus</i>	New Zealand
<i>M. loti</i> NZP 2234	<i>Lotus corniculatus</i>	New Zealand
<i>M. plurifarium</i> LMG 11892 ^T	<i>Acacia senegal</i>	Senegal
<i>M. amorphae</i> ACCC 19665 ^T	<i>Amorpha fruticosa</i>	Beijing, China
<i>M. chacoense</i> LMG 19008 ^T	<i>Prosopis alba</i>	Argentina
<i>M. ciceri</i> USDA 3378 ^T	<i>Cicer arietinum</i>	Spain
<i>M. huakuii</i> CCBAU 2609 ^T	<i>Astragalus sinicus</i>	Nanjing, China
<i>M. tianshanense</i> CCBAU 3306 ^T	<i>Glycyrrhiza uralensis</i>	Xinjiang, China
<i>M. septentrionale</i> SDW014 ^T	<i>Astragalus adsurgens</i>	Liaoning, China
<i>M. temperatum</i> SDW018 ^T	<i>Astragalus adsurgens</i>	Liaoning, China
<i>M. mediterraneum</i> USDA 3392 ^T	<i>Cicer arietinum</i>	Spain

dendrogram was constructed as described above. BOX-PCR is a high resolution molecular fingerprinting method that is able to distinguish closely related isolates up to the intraspecies level (Cho & Tiedje, 2000; Healy *et al.*, 2005; Rademaker *et al.*, 2000). In this analysis, 16 BOX patterns were produced among 24 test strains, revealing a high genetic diversity (see Supplementary Fig. S3 in IJSEM Online). Three corresponding clusters were observed. Genospecies V and VI were clearly separated from all the recognized species of the genus *Mesorhizobium* used in the BOX-PCR analysis. These results were in an agreement with those of the IGS-RFLP and SDS-PAGE analyses described above.

The 16S rRNA gene and IGS sequence of representative strains for each genomic species were amplified with the primers P1/P6 (Chen *et al.*, 1995) for *rrs* and FGPS1490 (Navarro *et al.*, 1992)/FGPL132' (Ponsonnet & Nesme, 1994) for IGS. The *atpD* and *recA* genes of the representative strains were also amplified and sequenced by using the method of Vinuesa *et al.* (2005a, b), with primer sets atpD255F/atpD782R and recA41F/recA640R, respectively. The sequencing of partial *nodC* and *nifH* genes of representative strains was performed with the primers nodCfor540/nodCrev1160 of Sarita *et al.* (2005) and nifH1F/nifH1R of Rivas *et al.* (2002), respectively. The PCR protocol for the *nifH* gene was as described by Rivas *et al.* (2002), while that for the *nodC* gene was as following: an initial denaturation at 95 °C for 5 min, 30 cycles of

denaturation at 94 °C for 45 s, annealing at 56–52 °C with $-0.1\text{ }^{\circ}\text{C s}^{-1}$ for 40 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 6 min. All the amplified fragments of each gene were directly sequenced as described previously (Hurek *et al.*, 1997). These sequences were compared with those held in the GenBank database. All the sequences obtained from this work and from the GenBank database were aligned using CLUSTAL W software (Thompson *et al.*, 1994). The distances were calculated according to the Jukes & Cantor (1969), the Kimura two-parameter (Kimura, 1983), Tajima & Nei (1984) and Tamura & Nei (1993) models. Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1993) and maximum-parsimony (Swofford, 1993) methods. Bootstrap analysis was based on 1000 replications. All of the trees were analysed by the MEGA3 package (Kumar *et al.*, 2004). As no significant topological differences were found among the phylogenetic trees obtained by the different methods assayed, only those trees constructed by using the neighbour-joining method after distance analysis of aligned sequences according to the Jukes & Cantor model are shown.

In the present study, strains CCBAU 83330^T, CCBAU 83346 and CCBAU 83511 representing genospecies V, strains CCBAU 83306^T, CCBAU 83321 and CCBAU 83278 representing genospecies VI, and strains CCBAU 83281 and CCBAU 83284 representing genospecies VII were selected for sequence analyses of 16S rRNA, IGS, *atpD*,

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships among *M. gobiense* sp. nov., *M. tarimense* sp. nov. and the reference strains for recognized species of the genus *Mesorhizobium*. The neighbour-joining tree was derived from a 16S rRNA gene sequence distance matrix (Jukes–Cantor). Bootstrap confidence levels $\geq 50\%$ are indicated at the nodes. Bar, 0.2 % nucleotide substitutions.

recA, *nodC* and *nifH* genes. The phylogenetic trees for each locus are shown in Fig. 1 and Supplementary Figs S4–S8 (available in IJSEM Online). The strains of each genomic species had almost identical 16S rRNA gene sequences, except for strain CCBAU 83278 which showed a similarity level of only 99.01 % with the other two representative strains of genospecies VI as calculated from a 1327 bp sequence. Strain CCBAU 83284 had an identical 16S rRNA gene sequence to both *M. amorphae* and *M. septentrionale* for the sequenced IGS region. Further analyses of the IGS, *atpD* and *recA* gene sequences clearly supported the observation that genospecies VII was most related to *M. septentrionale*. Strain CCBAU 83330^T shared 99.85 % 16S rRNA gene sequence similarity with strain CCBAU 83306^T. *M. tianshanense* was the closest species to both strains CCBAU 83330^T and CCBAU 83306^T, with 16S rRNA gene sequence similarity values of 99.77 % and 99.92 %, respectively. These data indicate that genospecies V, VI and *M. tianshanense* are the most closely related species; this was also confirmed by the phylogenetic analyses based on IGS, *atpD* and *recA* gene sequences. It should be noted that strain CCBAU 83278 formed quite separate lineages in the phylogenetic trees based on *atpD* and *recA* gene sequences. This could be explained by the fact that strain CCBAU 83278 is the most peripheral member of genospecies VI in BOX-PCR and SDS-PAGE analyses and in the phylogenetic tree based on IGS sequences. These results indicated that strain CCBAU 83278 belongs to a different species from strain CCBAU 83306^T and from any of the recognized species of the genus *Mesorhizobium*. Strain CCBAU 83278 was analysed further by DNA–DNA hybridization experiments.

The symbiotic genes (*nod* and *nif*) of rhizobia are adaptive genes that in many cases have an evolutionary history independent of the housekeeping genes (Wang *et al.*, 2007). A comparison of their phylogenies with those derived from housekeeping genes may reveal lateral gene transfer events

among rhizobia (Haukka *et al.*, 1998). In the present study, *nodC* and *nifH* gene fragments were amplified by PCR from the strains with different host plants and were sequenced directly as described above. In the phylogenetic tree based on the *nodC* gene (see Supplementary Fig. S7 in IJSEM Online), strains CCBAU 83306^T, CCBAU 83321, CCBAU 83511 and CCBAU 83346 clustered with *M. tianshanense* and strain CCBAU 83330^T clustered with *Mesorhizobium loti*. Strains CCBAU 83281 and CCBAU 83284, as well as strain CCBAU 83278, which were isolated from *Robinia pseudoacacia*, formed a separate lineage that was most closely related to the *M. amorphae*/*Mesorhizobium huakuii* lineage. Generally, the *nifH* gene-based tree (see Supplementary Fig. S8 in IJSEM Online) showed similar phylogenetic relationships to that based on the *nodC* gene sequences. The exception was that strain CCBAU 83511, isolated from *Astragalus filicaulis*, was closest to *M. tianshanense* in the *nifH* gene-based phylogenetic tree but was closest to *M. loti* in the *nodC* gene-based phylogenetic tree.

Since DNA–DNA hybridization is considered as a standard method for species definition (Wayne *et al.*, 1987; Graham *et al.*, 1991), representative strains CCBAU 83306^T, CCBAU 83321, CCBAU 83278, CCBAU 83330^T, CCBAU 83521, CCBAU 83346, CCBAU 83284 and related reference strains were chosen for hybridization assays. Total DNA was extracted from each strain by the method of Marmur (1961). DNA–DNA hybridization was performed using the spectrophotometric method (De Ley, 1970). The G+C content of the DNA was measured using the thermal denaturation method of Marmur & Doty (1962) using *Escherichia coli* K-12 as the standard. The hybridization values among the representative strains and the reference strains for the related recognized species of the genus *Mesorhizobium* are summarized in Table 2. Except for strain CCBAU 83278, the DNA–DNA relatedness values

Table 2. Values for DNA–DNA hybridization (%) among the representative strains for the novel groups and the reference strains for recognized species of the genus *Mesorhizobium*

Strains	Hybridization (%) with DNA from:						
	1*	2	3*	4*	5	6	CCBAU 83284
1. <i>M. tarimense</i> sp. nov. CCBAU 83306 ^T			17.04	32.79			
2. <i>M. tarimense</i> sp. nov. CCBAU 83321	85.91						
3. <i>Mesorhizobium</i> sp. CCBAU 83278	31.03	37.82					
4. <i>M. gobiense</i> sp. nov. CCBAU 83330 ^T	32.79		41.44				
5. <i>M. gobiense</i> sp. nov. CCBAU 83511				100.0			
6. <i>M. gobiense</i> sp. nov. CCBAU 83346				78.17			
<i>M. tianshanense</i> CCBAU 3306 ^T	43.45	61.52	34.52	57.45	49.26	56.85	
<i>M. mediterraneum</i> USDA 3392 ^T	27.23		3.16	51.66			
<i>M. temperatum</i> SDW018 ^T	12.07		17.68	30.57			
<i>M. amorphae</i> ACCC 19665 ^T	24.42		8.45	40.94			60.4
<i>M. septentrionale</i> SDW014 ^T	56.02		34.0	43.42			90.1
<i>M. loti</i> NZP 2213 ^T	17.64			25.3			
<i>M. loti</i> NZP 2227	24.24			17.3			

*Data are mean values of at least five repetitions while the others are for 2–3 repetitions.

among the representative strains of each genomic species were always higher than 70 %, ranging from 78.2 % to 100 %. However, genospecies VI strain CCBAU 83278 shared low DNA–DNA relatedness with strains CCBAU 83306^T (31.03 %) and CCBAU 83321 (37.82 %), which was in agreement with the results of sequence analyses and strongly suggested that they are distinct species. Strains CCBAU 83306^T, CCBAU 83278, CCBAU 83330^T and CCBAU 83284 were further hybridized with *M. tianshanense* CCBAU 3306^T, *Mesorhizobium temperatum* SDW018^T, *Mesorhizobium mediterraneum* USDA 3392^T, *M. septentrionale* SDW014^T, *M. amorphae* ACCC 19665^T, *M. loti* NZP 2213^T and *M. loti* NZP 2234. The DNA–DNA relatedness values of strain CCBAU 83284 with *M. amorphae* ACCC 19665^T and *M. septentrionale* SDW014^T were 60.4 % and 90.1 %, respectively, clearly indicating that genospecies VII belongs to *M. septentrionale*. In contrast, levels of DNA–DNA relatedness between strains CCBAU 83330^T, CCBAU 83306^T, CCBAU 83278 and the seven reference strains of recognized *Mesorhizobium* species were very low (Table 2). These results, as well as those of the sequence analyses, led to the conclusion that the strains of genomic species V and VI belong to three distinct novel species based on the recommendation of a threshold value of 70 % DNA–DNA relatedness for species delineation (Wayne *et al.*, 1987; Graham *et al.*, 1991). The DNA G+C content values ranged from 57.23 % to 57.86 % with a mean value of 57.65 % for genospecies V, and 57.44 % to 57.85 % with a mean value of 57.51 % for genospecies VI.

Strains representing the three novel species including strains CCBAU 83306^T, CCBAU 83521, CCBAU 83330^T, CCBAU 83345 and CCBAU 83278 and six type strains of related recognized species were chosen for phenotypic characterization. A total of 107 phenotypic features were

examined using methods described previously (Gao *et al.*, 1994). The characteristics examined included utilization of sole carbon and nitrogen sources; resistance to antibiotics (5, 50, 100 and 300 µg ml⁻¹); tolerance to NaCl (1–5.0 %; w/v); production of catalase, urease, L-phenylalaninase and oxidase; the methyl blue reduction reaction, Nile blue reduction and nitrate reduction; litmus milk reaction and the pH and temperature ranges for growth. Analysis of cellular fatty acids was performed for strains CCBAU 83278, CCBAU 83330^T and CCBAU 83306^T according to methods described by Sasser (1990) and Tighe *et al.* (2000). The two novel species could be distinguished from each other and from the closely related species of the genus *Mesorhizobium* by several phenotypic characteristics (Table 3) and by their fatty acid contents (see Supplementary Table S1 in IJSEM Online).

Based upon all the results obtained in this study, we believe that genomic species V and VI represent three separate novel species in the genus *Mesorhizobium*. Since only one strain, CCBAU 83278, was identified as representing one of the novel species, a name is not yet proposed for this taxon. According to the current criteria for descriptions of rhizobial species, we propose two novel species, *Mesorhizobium gobiense* sp. nov., represented by genomic species V (type strain CCBAU 83330^T), and *Mesorhizobium tarimense* sp. nov. represented by genomic species VI (type strain CCBAU 83306^T).

Host range is an important feature for root- and/or stem-nodule bacteria and cross-nodulation tests were performed with the representative strains CCBAU 83306^T and CCBAU 83330^T with the hosts of origin and the related hosts *Astragalus membranaceus*, *Glycyrrhiza uralensis*, *Lotus corniculatus*, *Oxytropis glabra*, *Robinia pseudoacacia* and

Table 3. Distinctive features of *M. gobiense* sp. nov., *M. tarimense* sp. nov. and the closest related recognized species of the genus *Mesorhizobium*

Taxa: 1, CCBAU 83278; 2, *M. gobiense* sp. nov. CCBAU 83330^T; 3, *M. gobiense* sp. nov. CCBAU 83346; 4, *M. tarimense* sp. nov. CCBAU 83306^T; 5, *M. tarimense* sp. nov. CCBAU 83321; 6, *M. tianshanense* CCBAU 3306^T; 7, *M. temperatum* SDW018^T; 8, *M. mediterraneum* USDA 3392^T; 9, *M. septentrionale* SDW014^T; 10, *M. amorphae* ACCC 19665^T; 11, *M. loti* NZP 2213^T. +, Positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Substrates used as sole carbon source											
D-Amygdalin	+	–	–	–	–	–	–	–	–	–	–
Calcium gluconate	+	+	+	–	–	–	–	–	–	–	–
Dextrin	+	+	+	+	+	–	–	+	–	–	–
meso-Erythritol	+	+	+	–	–	–	–	+	–	+	–
Melezitose	+	–	–	–	–	–	–	–	–	–	–
Raffinose	+	–	–	–	–	–	–	–	+	+	+
Salicin	+	–	–	–	–	–	–	–	–	–	–
Sodium acetate	+	–	–	–	–	+	+	+	+	–	–
Sodium citrate	+	+	+	–	–	+	–	–	–	+	–
Sodium formate	–	+	+	–	–	+	–	+	+	–	–
Sodium D-gluconate	+	+	+	–	–	–	–	–	–	–	–
Sorbose	+	–	+	–	–	–	–	–	–	–	–
DL-Proline	+	+	+	+	+	–	–	+	–	–	+
Substrates used as sole nitrogen source											
DL-Alanine	–	+	+	–	–	–	–	–	–	–	–
L-Arginine	+	+	+	+	+	+	+	+	+	+	–
L-Cystine	+	+	+	–	+	+	–	+	+	–	+
D-Glutamic acid	+	+	+	–	–	+	–	+	+	–	–
L-Isoleucine	+	+	+	–	–	+	–	+	+	+	+
L-Lysine	+	+	+	+	–	+	–	+	+	+	–
L-Phenylalanine	+	+	+	–	–	+	–	+	+	–	+
D-Threonine	–	+	–	–	–	+	+	+	+	–	–
L-Valine	+	+	+	–	–	+	–	+	+	–	+
Glycine	–	+	+	–	–	+	+	+	+	–	–
Resistance to:											
Ampicillin (5 µg ml ^{–1})	+	+	+	–	–	–	+	+	+	+	+
Chloramphenicol (5 µg ml ^{–1})	+	+	+	–	–	–	+	+	–	–	+
Erythromycin (5 µg ml ^{–1})	+	+	+	–	+	+	+	+	–	+	–
Kanamycin (5 µg ml ^{–1})	+	+	+	–	–	+	+	–	–	–	+
Streptomycin (50 µg ml ^{–1})	–	–	–	–	–	–	–	–	–	–	+
Streptomycin (5 µg ml ^{–1})	+	+	+	–	–	–	–	+	–	–	+
Growth at/in:											
NaCl (1 %)	+	+	+	–	–	–	–	–	–	–	–
pH 9.0	+	+	+	+	–	–	+	+	–	–	+
37 °C	+	+	+	–	–	–	–	–	–	–	–
Other reactions											
Oxidase reaction	+	+	+	–	–	+	+	+	+	+	+
Methyl blue reduction	+	+	+	–	–	–	–	–	–	–	–
Acid reaction in litmus milk	+	+	+	+	–	–	–	+	–	+	–
Alkaline reaction in litmus milk	–	–	–	–	+	+	+	–	+	–	+
Peptonization in litmus milk	–	–	–	–	–	–	–	–	+	+	–
Curd in litmus milk	–	–	–	–	–	–	–	–	–	+	–
Reduction reaction in litmus milk	+	+	+	+	+	–	+	+	+	+	+

the recommended standard plant species *Leucaena leucocephala*, *Phaseolus vulgaris*, *Pisum sativum* and *Medicago sativa* (Graham *et al.*, 1991). The seeds of the host plants were surface-sterilized, germinated and inoculated according to a standard procedure (Vincent, 1970). The inoculated seedlings were grown in Gibson tubes (Gibson,

1980) supplemented with a nitrogen-free plant nutrient solution (Brunel *et al.*, 1996), while non-inoculated plants were used as controls. Three replicates were prepared for each treatment. Plants were incubated in a greenhouse under natural sunlight. After 45–60 days, the plants were harvested and the nodules were observed. The results

showed that both strain CCBAU 83306^T and CCBAU 83330^T could nodulate *Glycyrrhiza uralensis*, *Lotus corniculatus*, *Oxytropis glabra* and *Robinia pseudoacacia* but could not nodulate *Astragalus membranaceus*, *Leucaena leucocephala*, *Phaseolus vulgaris*, *Pisum sativum* or *Medicago sativa*.

Description of *Mesorhizobium gobiense* sp. nov.

Mesorhizobium gobiense (go.bi.en'se. N.L. neut. adj. *gobiense* pertaining to the Gobi desert, where the strains reported in this study were isolated)

Gram-negative, aerobic, non-spore-forming rods that are 0.3–0.6 µm wide by 1–3 µm long. Colonies on YMA medium are circular, convex, white, opaque and usually have a diameter of 1–2 mm after 5–7 days incubation at 28 °C. The optimum growth temperature is 25–30 °C and growth can take place at 37 °C. Growth is inhibited at 10 °C or 60 °C. The optimum pH for growth is 6–8 and growth can take place at pH 5–9. All strains grow on YMA in the presence of 1 % NaCl. Able to utilize D-arabinose, calcium gluconate, dextrin, *meso*-erythritol, D-fructose, (+)-D-galactose, D-glucose, inositol, lactose, sodium malate, maltose, D-mannose, turanose, sodium pyruvate, L-rhamnose, sodium citrate, sodium D-gluconate, sodium succinate, sucrose, trehalose, D-xylose, DL-arginine, DL-aspartic acid and DL-proline as sole carbon sources. Utilizes L-arginine, (+)-L-aspartic acid, L-cystine, D-glutamic acid, (+)-L-glutamic acid, hypoxanthine, L-isoleucine, L-lysine, L-phenylalanine, L-valine, L-threonine and L-hydroxyproline as sole nitrogen sources. Does not utilize adipic acid, inulin, sodium hippurate, soluble starch, syringic acid, sodium tartrate, vanillic acid, glycine and L-methionine as sole carbon sources. Resistant to 5 µg ml⁻¹ ampicillin, chloramphenicol, erythromycin, kanamycin and streptomycin. Catalase and oxidase activities are present, but L-phenylalaninase and urease activities are not. No reduction of nitrate or Nile blue, but reduction of methyl blue is observed. Acid and reduction reactions are produced in litmus milk.

The type strain is strain CCBAU 83330^T (=LMG 23949^T=HAMBI 2974^T). The DNA G + C content of the type strain is 57.86 mol%.

Description of *Mesorhizobium tarimense* sp. nov.

Mesorhizobium tarimense (ta.ri.men'se. N.L. neut. adj. *tarimense* referring to the Tarim basin, from where the strains reported in this study were isolated)

Gram-negative, aerobic, non-spore-forming rods that are 0.3–0.6 µm wide by 1–3 µm long. Colonies on YMA medium are circular, convex, white, opaque and usually have a diameter of 1–2 mm after 5–9 days growth at 28 °C. The optimum growth temperature is 25–30 °C and no growth occurs at 10 °C or 37 °C. The optimum pH for growth is 6–8 and growth can take place on YMA plates at pH 5. Does not grow on YMA in the presence of 1 % NaCl.

Is able to utilize D-arabinose, dextrin, D-fructose, (+)-D-galactose, D-glucose, inositol, lactose, sodium malate, maltose, D-mannose, turanose, sodium pyruvate, L-rhamnose, sodium succinate, sucrose, trehalose, D-xylose, DL-arginine, DL-aspartic acid and DL-proline as sole carbon sources. Can use L-arginine, (+)-L-aspartic acid, (+)-L-glutamic acid, hypoxanthine, L-threonine and L-hydroxyproline as sole nitrogen sources. Does not utilize adipic acid, D-amygdalin, calcium gluconate, *meso*-erythritol, inulin, melezitose, raffinose, salicin, sodium acetate, sodium citrate, sodium formate, sodium D-gluconate, sodium hippurate, sorbose, soluble starch, syringic acid, sodium tartrate, vanillic acid, glycine or L-methionine as sole carbon sources. Does not utilize DL-alanine, D-glutamic acid, L-isoleucine, L-phenylalanine, D-threonine, L-valine or glycine as sole nitrogen sources. Resistant to 5 µg ml⁻¹ erythromycin. Catalase production is detected but no production of oxidase, L-phenylalaninase or urease. No reduction of nitrate, methyl blue or Nile blue. Acid and reduction reactions are produced in litmus milk.

The type strain is CCBAU 83306^T (=LMG 24338^T=HAMBI 2973^T). The DNA G + C content of the type strain is 57.85 mol%.

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