Mesorhizobium albiziae sp. nov., a novel bacterium that nodulates *Albizia kalkora* in a subtropical region of China

Feng Qin Wang,^{1,2} En Tao Wang,³ Jie Liu,^{1,4} Qiang Chen,^{1,5} Xin Hua Sui,¹ Wen Feng Chen¹ and Wen Xin Chen¹

Correspondence

Wen Xin Chen

wenxin_chen@263.net

¹Key Laboratory of Agro-Microbial Resource and Application, Ministry of Agriculture/College of Biological Sciences, China Agricultural University, Beijing 100094, China

²College of Life Science, Henan Agricultural University, Zhengzhou 450002, China

³Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, 11340 México D. F., Mexico

⁴Department of Bioengineering and Biotechnology, College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

⁵College of Resources and Environmental Science, Sichuan Agricultural University, Yaan 625000, China

A novel *Mesorhizobium* group associated with *Albizia kalkora* [Wang *et al.* (2006), *Syst Appl Microbiol* **29**, 502–517] was further characterized. The seven strains in this group showed similar protein patterns and were different from defined *Mesorhizobium* species in SDS-PAGE of whole-cell proteins. The representative strain CCBAU 61158^T formed a novel *Mesorhizobium* lineage in phylogenetic analyses of 16S rRNA, *atpD*, *glnII* and *nifH* genes. However, its *nodC* gene sequence was more similar to that of *Rhizobium gallicum* R602sp^T than to those of *Mesorhizobium* species. DNA–DNA relatedness between CCBAU 61158^T and reference strains of defined *Mesorhizobium* species was lower than 34.1 %. These results indicated that this *Mesorhizobium* group was a unique genomic species. The subtropical distribution, host origin, PCR-RFLP patterns of 16S rRNA genes, fatty acid profile and a series of phenotypic characteristics could be used as distinctive features of this group. This group is therefore proposed as a novel species, *Mesorhizobium albiziae* sp. nov., with CCBAU 61158^T (=LMG 23507^T=USDA 4964^T) as the type strain. Strain CCBAU 61158^T could form effective nodules on *Albizia julibrissin*, *Glycine max*, *Leucaena leucocephala* and *Phaseolus vulgaris*.

Rhizobia are well known for their ability to carry out symbiotic nitrogen fixation by forming nodules on the roots or occasionally on the stems of legume plants. A total of 1485 leguminous species in 172 genera have been recorded in China, and they are distributed in the tropical, subtropical, temperate and frigid climate zones, from the south to the north. 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; Wei *et al.*, 2002, 2003; Xu *et al.*, 1995; Yao *et al.*, 2002). In addition, novel rhizobial lineages are becoming apparent when further studies are carried out to characterize new isolates with enlarged host ranges and geographical origins.

Trees of the genus *Albizia*, belonging to the Mimosoideae, are native to Asia and Africa and have many uses. In addition to use as foliage, in gardening/landscaping, as green manure and as a source of timber for furniture production, the bark of *Albizia* trees is used in herbal medicine and the seeds are a source of oil. There are about 150 species in the genus and 17 of them can be found in the southern regions of China (Wu *et al.*, 1988). Although trees of the genus *Albizia* are of great importance, little is known about the diversity of their microsymbionts. In our previous study, 31 rhizobial strains isolated from *Albizia* species were characterized by a polyphasic taxonomic approach and great

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CCBAU 61158^T, CCBAU 61161 and CCBAU 61156 are DQ100066, DQ311088 and DQ311089, and those for the partial *glnII*, *atpD*, *nodC* and *nifH* gene sequences of strain CCBAU 61158^T are respectively DQ311090–DQ311093.

A dendrogram based on whole-cell protein patterns, an alignment of the insertion within the 16S rRNA genes of the novel strains and results of DNA–DNA hybridization are available as supplementary material with the online version of this paper.

diversity was found (Wang *et al.*, 2006). Seven strains obtained from nodules of *Albizia kalkora* grown in Sichuan Province were defined as a novel group (rDNA type 6) belonging to *Mesorhizobium* by ARDRA (amplified 16S rDNA restriction analysis), SDS-PAGE of whole-cell proteins, numerical taxonomy, rep-PCR and sequencing of 16S rRNA genes (Wang *et al.*, 2006). Interestingly, an insertion sequence of about 80 bp, partially similar to the insertion sequence in the 16S rRNA gene of *Rhizobium tropici* CFN 299, was found in the 16S rRNA gene of CCBAU 61158^T, the representative strain of the novel group. With the intention of verifying the taxonomic position of this novel group, we characterized the seven strains further in this study in comparison with reference strains of defined *Mesorhizobium* species (Table 1) by SDS-PAGE of whole-cell proteins, cellular fatty acid analysis, DNA–DNA hybridization, sequencing of housekeeping genes (16S rRNA, *atpD* and *glnII*) and sequencing of symbiotic genes (*nifH* and *nodC*). All strains were maintained on YMA (Vincent, 1970) at 4 °C for temporary storage and in 20 % glycerol at –20 °C for long-term storage.

In the analysis of SDS-PAGE of whole-cell proteins, strains were incubated in YM broth (Vincent, 1970) at 28 °C; protein extraction and electrophoretic analysis were performed according to the methods described previously (Tan *et al.*, 1997). Normalized densitometric traces of the electrophoretic protein patterns were grouped by using the Gelcompar II software package. The similarity between each pair of samples (strains) was expressed by the Pearson coefficient and a UPGMA dendrogram was constructed (Vauterin & Vauterin, 1992). Very similar electrophoretic protein patterns were distinguished among the seven strains and they formed a cluster at a similarity level of 95 % (Supplementary Fig. S1 in IJSEM Online). The protein patterns of the reference strains were different from each other and from those of the seven strains.

Tighe *et al.* (2000) found that similarities derived from fatty acid analysis are in broad agreement with 16S rRNA gene sequence analysis results and appear to distinguish accurately between most species in *Agrobacterium*, *Rhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Bradyrhizobium*. In this study, the cellular fatty acid compositions of *Mesorhizobium amorphae* ACCC 19665^T, *Mesorhizobium septentrionale* SDW014^T, *Mesorhizobium temperatum* SDW018^T, *Mesorhizobium chacoense* LMG 19008^T and strains CCBAU 61158^T, CCBAU 61162 and CCBAU 61166 from the novel group were analysed. All strains were cultured using methods described previously (Graham *et al.*, 1995; Jarvis & Tighe, 1994) and fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (Microbial ID; MIDI). Extracts were analysed by using a Hewlett Packard model HP6890 gas chromatograph equipped with a flame-ionization detector, an automatic sampler, an integrator and a computer, as described previously (Kämpfer & Kroppenstedt, 1996; Kämpfer *et al.*, 1997). The results obtained were summed and compared with previously reported data (Ghosh & Roy, 2006; Tighe *et al.*, 2000) (Table 2). The novel group could be assigned to *Mesorhizobium* because they lacked 20:3 ω 6,9,12c and summed feature 3 (12:0, unknown ECL 10.928, 16:1 iso 1/14:0 3-OH) and possessed 17:0 iso fatty acids (Tighe *et al.*, 2000). The strains of the novel group were separated from *Mesorhizobium plurifarium* because the former contain 11-methyl 18:1 ω 7c and 15:0 iso and lower concentrations of 19:0 cyclo ω 8c and 16:0 fatty acids. The strains of the novel group could be distinguished from *Mesorhizobium tianshanense* group II (Tighe *et al.*, 2000) and *Mesorhizobium thiogangeticum* based on the lower concentration of summed feature 7 and higher concentrations of 17:0 iso and 15:0 iso 3-OH fatty acids. The novel group differed from other *Mesorhizobium* species by the higher concentration of summed feature 7 and 15:0 iso and lower concentrations of 19:0 cyclo ω 8c and 15:0 iso fatty acids (Table 2).

Table 1. Strains and isolates used in this study

Strain	Host plant	Geographical origin	Reference
rDNA type 6 (<i>Mesorhizobium albiziae</i> sp. nov.)			
CCBAU 61158 ^T , CCBAU 61165, CCBAU 61161, CCBAU 61166, CCBAU 61156, CCBAU 61162, CCBAU 61164	<i>Albizia kalkora</i>	Sichuan, China	Wang <i>et al.</i> (2006)
Reference strains			
<i>Mesorhizobium loti</i> NZP 2213 ^T	<i>Lotus corniculatus</i>	New Zealand	Jarvis <i>et al.</i> (1997)
<i>Mesorhizobium loti</i> NZP 2234	<i>Lotus corniculatus</i>	New Zealand	Jarvis <i>et al.</i> (1997)
<i>Mesorhizobium plurifarium</i> LMG 11892 ^T	<i>Acacia senegal</i>	Senegal	de Lajudie <i>et al.</i> (1998)
<i>Mesorhizobium amorphae</i> ACCC 19665 ^T	<i>Amorpha fruticosa</i>	Beijing, China	Wang <i>et al.</i> (1999)
<i>Mesorhizobium chacoense</i> LMG 19008 ^T	<i>Prosopis alba</i>	Argentina	Velázquez <i>et al.</i> (2001)
<i>Mesorhizobium ciceri</i> USDA 3378 ^T	<i>Cicer arietinum</i>	Spain	Nour <i>et al.</i> (1994)
<i>Mesorhizobium huakuii</i> CCBAU 2609 ^T	<i>Astragalus sinicus</i>	Nanjing, China	Chen <i>et al.</i> (1991)
<i>Mesorhizobium tianshanense</i> CCBAU 3306 ^T	<i>Glycyrrhiza uralensis</i>	Xingjiang, China	Chen <i>et al.</i> (1995)
<i>Mesorhizobium septentrionale</i> SDW014 ^T	<i>Astragalus adsurgens</i>	Liaoning, China	Gao <i>et al.</i> (2004)
<i>Mesorhizobium temperatum</i> SDW018 ^T	<i>Astragalus adsurgens</i>	Liaoning, China	Gao <i>et al.</i> (2004)

Table 2. Major fatty acids of the novel strains (*M. albisiae* sp. nov.) and defined *Mesorhizobium* species

Strains: 1, *M. ciceri* (n=9); 2, *M. huakuii* (n=16); 3, *M. mediterraneum* (n=12); 4, *M. loti* (n=17); 5, *M. tianshanense* group I (n=8); 6, *M. tianshanense* group II (n=4); 7, *M. plurifarium* (n=17); 8, *M. thiogangeticum* SJT^T; 9, *M. amorphae* ACCC 19665^T; 10, *M. septentrionale* SDW014^T; 11, *M. temperatum* SDW018^T; 12, *M. chacoense* LMG 19008^T; 13, *M. albisiae* CCBAU 61158^T; 14, *M. albisiae* CCBAU 61162; 15, *M. albisiae* CCBAU 61166. Values are percentages of total fatty acids. Data in columns 1–7 are mean values for the numbers of strains shown taken from Tighe *et al.* (2000) and data in column 8 were taken from Ghosh & Roy (2006).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
12:0 3-OH	0.27	0.23	0.11	0.34	0.09		0.2			0.34		0.41			
13:0 iso 3-OH	0.24	1.0	0.35	1.23	0.27		0.84		0.5	0.74		0.58			
14:0								0.88		0.46	0.47			0.2	0.36
Unknown (ECL 14.780)	0.16		0.04				0.01								
15:0	0.02		0.04												
15:0 iso 3-OH								0.41			1.22		1.29	1.34	1.03
15:0 anteiso				0.12											
15:0 iso	0.18		0.13	0.13	0.16			8.23	0.38	0.65	2.08	0.29	6.82	6.55	6.43
16:0	13.41	15.83	10.29	14.51	12.25	11.26	11.6	2.46	14.33	12.88	5.97	11.68	4.31	4.57	5.38
16:0 iso										0.26					
16:1 ω 5c						2.65									
17:0	1.29	0.42	1.71	0.5	0.83	0.13	0.69	0.81	1.9	2.02	2.43	3.76	2.69	2.37	1.78
17:0 iso	4.16	5.88	4.19	8.17	3.34		6.26	0.85	3.59	3.16	6.89	3.4	4.42	4.1	4.09
17:0 iso 3-OH											0.33				
17:0 anteiso											0.38		0.32	0.31	0.31
17:0 cyclo	0.84	0.7	0.85	0.69	0.16		0.12		0.46			0.43			
17:1 ω 6c					0.11	0.26	0.09						0.52	0.48	0.43
17:1 ω 8c	0.15	0.25	0.51	0.12	0.52	0.14	0.71	0.51	0.48	0.6	0.92	1.04	1.48	1.29	1.07
Unknown (ECL 17.875)	0.17		0.5												
18:0	3.25	4.1	4.21	4.5	4.99	0.92	2.99	1.28	6.92	5.52	5.49	6.17	2.5	2.65	2.42
18:0 3-OH								0.47							
11-Methyl 18:1 ω 7c	11.92	4.82	5.57	11.14	9.99	0.28		4.23	15.91	22.76	19.81	8.5	6.42	5.88	5.83
18:1 2-OH		0.11		0.47											
18:1 ω 5c			0.06												
18:1 ω 9c	0.12			0.34					0.7	0.75	0.68		0.58	0.52	0.5
Unknown (ECL 18.794)	0.21		0.32	0.08	0.11		0.25								
19:0										0.39					
19:0 cyclo ω 8c	37.27	22.96	33.35	21.93	12.37	0.57	11.33	0.64	18.41	1.72	5.27	19.29	0.7	0.73	0.78
10-Methyl 19:0	0.18	1.16	0.08	1.23	0.16		1.28		0.5	0.6					
20:0	0.05	0.04		0.07			0.04		0.38	0.52					
20:1 ω 7c									0.44	0.62			0.25		0.22
20:1 ω 9t	0.14		0.46	0.15			0.1								
20:2 ω 6,9c	0.95	0.04	0.77	0.05								0.51			
Summed feature 4*	0.34	0.41	0.61	0.51	1.42	0.29	0.44	1.82	0.87	3.73	3.21	0.77	1.25	1.33	2.89
Summed feature 7*	24.69	42.04	35.81	33.73	53.22	83.52	63.02	77.41	34.22	42.27	44.85	43.18	66.45	67.66	66.47

*Summed feature 4 contains 15:0 iso 2-OH and/or 16:1 ω 7c. Summed feature 7 contains 18:1 ω 7c/ ω 9t/ ω 12t and/or 18:1 ω 7c/ ω 9c/ ω 12t.

To verify the phylogenetic relationships of the novel group, two additional strains (CCBAU 61156 and CCBAU 61161) were used in 16S rRNA gene sequencing. Fragments of the 16S rRNA gene amplified by primers P1 and P6 (Tan *et al.*, 1997) were purified and cloned into the pGEM-T Easy vector (Promega) as recommended by the manufacturer. Almost full-length sequences of the cloned 16S rRNA gene

were obtained by the method of Tan *et al.* (1997). The acquired sequences were aligned using the programs in the CLUSTAL X package (Thompson *et al.*, 1994, 1997). A neighbour-joining tree was reconstructed and bootstrapped with 1000 replications of each sequence using the TREECON programs (Van de Peer & De Wachter, 1994). Similar to strain CCBAU 61158^T (Wang *et al.*, 2006), an insertion of

about 80 bp at the 5' end was detected in these two strains between positions 75 and 76 of the *Escherichia coli* 16S rRNA gene sequence (Supplementary Fig. S2). The three strains of the novel group had almost identical 16S rRNA gene sequences and formed a divergent lineage in the genus *Mesorhizobium* (Fig. 1a). The three strains had similarities of 94.8–96.4% with *M. thioglyticum* SJT^T and 98.1–99.2% with other *Mesorhizobium* species when the insertion was not considered.

The 16S rRNA gene is a useful molecular marker and has revealed the general evolutionary history of bacteria; its phylogeny is one of the basic criteria used to delineate bacterial species (Graham *et al.*, 1991; Stackebrandt *et al.*, 2002). However, phylogenetic analysis of the 16S rRNA gene has some shortcomings in describing species. For example, the rRNA genes are too highly conserved to allow distinction among closely related species. Mosaicism, recombination and the presence of multiple copies with distinct sequences

have been found in the 16S rRNA genes of rhizobia (Hashimoto *et al.*, 2003; Haukka *et al.*, 1996; Martínez-Romero *et al.*, 1991; Ueda *et al.*, 1999; van Berkum *et al.*, 2003). The insertion discovered in the novel group may have been obtained by recombination from other sources. To corroborate the reliability of 16S rRNA gene sequence-based definition of bacterial species, sequence analysis of protein-coding genes has been recommended (Stackebrandt *et al.*, 2002). Core metabolic genes such as *atpD*, *dnaK*, *glnI*, *glnII* and *recA* have been used in taxonomic and phylogenetic studies of rhizobia (Gao *et al.*, 2004; Gaunt *et al.*, 2001; Ghosh & Roy, 2006; Parker, 2004; Stepkowski *et al.*, 2003; Turner & Young, 2000; Vinuesa *et al.*, 2005; Wernegreen & Riley, 1999).

In this study, PCR amplification and sequencing of partial *atpD* and *glnII* genes of strain CCBAU 61158^T was undertaken according to Gaunt *et al.* (2001) and Turner & Young (2000), respectively, and neighbour-joining trees

Fig. 1. Simplified phylogenetic trees of the 16S rRNA gene (a), *glnII* (b), *atpD* (c), *nodC* (d) and *nifH* (e), showing the relationships among the novel isolates and defined *Mesorhizobium* species. Insertion fragments in the 16S rRNA genes of the novel isolates were not included in the similarity calculation. The trees were constructed by the neighbour-joining method from Jukes-Cantor distance matrices of the sequences. Bootstrap confidence levels greater than or equal to 50% are indicated at internodes. GenBank accession numbers are shown in parentheses. A., *Agrobacterium*; R., *Rhizobium*; S., *Sinorhizobium*. Bars, 2% (a), 5% (b, c, e) and 10% (d) nucleotide substitutions.

were reconstructed based on the sequences (Fig. 1b, c). The phylogenies of partial *atpD* and *glnII* sequences showed that CCBAU 61158^T was a member of *Mesorhizobium*, supporting the conclusion from 16S rRNA gene analysis (Fig. 1a). However, slight differences were observed among the phylogenies of these three genes. CCBAU 61158^T formed a lineage distantly related to *Mesorhizobium* species in the 16S rRNA gene phylogeny (Fig. 1a), whereas it formed a subcluster together with *M. chacoense* in phylogenetic trees of *glnII* (Fig. 1b) and *atpD* (Fig. 1c) sequences.

The symbiotic genes (*nod* and *nif*) are adaptive genes and in many cases have an evolutionary history independent of the rest of the genome. Comparison of their phylogenies with those derived from housekeeping genes may reveal lateral gene transfer events among rhizobia (Haukka *et al.*, 1998). In this study, partial *nodC* and *nifH* sequences of CCBAU 61158^T were amplified by using the primers and PCR conditions specified by Laguerre *et al.* (2001). The purified PCR products were sequenced directly as described previously (Laguerre *et al.*, 2001). In the phylogenetic analysis of *nifH* (Fig. 1e), CCBAU 61158^T formed a subcluster together with an *M. plurifarium* strain above 93.6 % similarity. The *nodC* gene of CCBAU 61158^T was most similar to that of *Rhizobium gallicum* bv. *gallicum* R602sp^T (Fig. 1d), with 84.3 % similarity, and it had sequence similarity of less than 71.5 % with *Mesorhizobium* species. These results demonstrated that the *nodC* and *nifH* genes of strain CCBAU 61158^T had different origins.

Total DNAs extracted from the strains by the method of Marmur (1961) were used for determination of DNA base composition and for DNA–DNA hybridization. The G + C content of DNA was measured using the thermal denaturation method of Marmur & Doty (1962) and *E. coli* K-12 as a standard. The G + C content of the representative strain CCBAU 61158^T was 59 mol%, which is within the range reported for *Mesorhizobium* (59–64 mol%) (Jarvis *et al.*, 1997). DNA–DNA hybridization was determined by the spectrophotometric method of De Ley (1970) and it showed that the DNA relatedness among CCBAU 61158^T and the other six strains in the novel group varied between 88.8 and 100 %, with a mean of 95 % (Supplementary Table S1). With hybridization values of 12.6–34.1 %, none of the defined *Mesorhizobium* species showed a significant degree of DNA relatedness to the novel group.

The data obtained in this study showed that the seven strains in the novel group were very similar, but were not clones, since their protein patterns and fatty acid compositions were slightly different. Considering the variations observed in the present study and in the previous phenotypic characterization and ARDRA (Wang *et al.*, 2006), we conclude that the seven strains in the novel group cover diverse populations.

No strain of *M. thioganicum* was included in the DNA–DNA hybridization in this work. However, we can estimate that *M. thioganicum* might share low DNA–DNA relatedness based upon the assessment of Vandamme *et al.* (1996),

who concluded that organisms with less than 97 % 16S rRNA gene sequence similarity would not give a DNA relatedness of more than 60 % and suggested that DNA–DNA hybridization might be not necessary when the 16S rRNA gene sequence similarity was below 97 %. In our study, the 16S rRNA gene sequence similarity between the novel group and *M. thioganicum* SJT^T was 94.8–96.4 % when the insertion sequence was not considered.

Host range is an important feature for root- and/or stem-nodule bacteria, and cross-nodulation tests with selected hosts are required for the description of novel rhizobial species (Graham *et al.*, 1991). Strain CCBAU 61158^T, representing the novel *Mesorhizobium* group, was used for cross-nodulation tests with 14 legume species. Seed treatment and inoculation were performed using the standard method of Vincent (1970). All the plants were maintained in glass tubes that were half-filled with semi-solid agar and sealed with cotton. Seedlings were grown in a greenhouse under natural daylight and seedlings of the blank control were not nodulated after 1 month. The results showed that strain CCBAU 61158^T could nodulate *Albizia julibrissin*, *Glycine max*, *Leucaena leucocephala* and *Phaseolus vulgaris*, but not *Pisum sativum*, *Astragalus adsurgens*, *Melilotus suaveolens*, *Lotus corniculatus*, *Trifolium repens*, *Glycyrrhiza glabra*, *Vigna radiata*, *Vigna unguiculata*, *Sesbania* sp. or *Robinia pseudoacacia*.

Based on the results obtained in this study and in our previous work (Wang *et al.*, 2006), we believe that the seven strains represent a novel species in the genus *Mesorhizobium*. This species could be differentiated by SDS-PAGE of proteins, cellular fatty acid analysis, numerical taxonomy, DNA–DNA hybridization and sequencing of the 16S rRNA, *atpD*, *glnII*, *nodC* and *nifH* genes. According to the current criteria for rhizobial species description, we propose the name *Mesorhizobium albiziae* sp. nov. for the seven strains; distinctive features of this species are summarized in Table 3.

Description of *Mesorhizobium albiziae* sp. nov.

Mesorhizobium albiziae (al.bi'zi.ae. N.L. gen. n. *albiziae* of *Albizia*, a genus of leguminous plants, referring to the isolation of the first strains from *Albizia kalkora*).

Gram-negative, aerobic, motile, non-spore-forming rods, 0.3–0.5 µm wide by 1–3 µm long. Colonies on YMA are circular, convex, white, opaque and usually 1–2 mm in diameter within 5–7 days at 28 °C. The generation time is 7–8 h in PY broth at 28 °C. Strains of this species can use fructose, lactose, L-malate, maltose, melibiose, D-raffinose, D-sorbose, sucrose, L-xylose and *meso*-erythritol as sole carbon sources and DL-α-alanine, L-arginine, L-glutamic acid, L-isoleucine and L-phenylalanine as sole nitrogen sources. None of the strains can use L-arabinose, rhamnose, salicin, dulcitol, citrate or L-malonate as sole carbon sources or L-cystine as a sole nitrogen source. Resistant to 300 µg ampicillin (sodium salt) ml⁻¹, 50 µg kanamycin ml⁻¹,

Table 3. Distinctive features of the novel strains and defined *Mesorhizobium* species

Strains: 1, *M. loti* NZP 2213^T; 2, *M. mediterraneum* USDA 3392^T; 3, *M. ciceri* USDA 3378^T; 4, *M. amorphae* ACCC 19665^T; 5, *M. tianshanense* CCBAU 3306^T; 6, *M. huakuii* USDA 2609^T; 7, *M. chacoense* LMG 19008^T; 8, *M. temperatum* SDW018^T; 9, *M. plurifarium* LMG 11892^T; 10 *M. septentrionale* SDW014^T; 11, *M. thio-gangeticum* SJT^T (data from Ghosh & Roy, 2006); 12, *M. albiziae* sp. nov. (seven strains). Unless indicated, data were obtained in this study. ND, No data available/not determined.

Character	1	2	3	4	5	6	7	8	9	10	11	12
Use as sole carbon source												
Lactose	+	+	+	+	+	+	+	+	+	+	+	+
D-Raffinose	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+	+
meso-Erythritol	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+	+
L-Malonate	+	+	+	+	+	+	+	+	+	+	+	+
D-Sorbose	+	+	+	+	+	+	+	+	+	+	+	+
L-Xylose	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+
L-Malate	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+
Resistance to ($\mu\text{g ml}^{-1}$):												
Kanamycin (50)	+	+	+	+	+	+	+	+	+	+	+	+
Kanamycin (100)	+	+	+	+	+	+	+	+	+	+	+	+
Ampicillin sodium (5)	+	+	+	+	+	+	+	+	+	+	+	+
Ampicillin sodium (50)	+	+	+	+	+	+	+	+	+	+	+	+
Ampicillin sodium (300)	+	+	+	+	+	+	+	+	+	+	+	+
Dihydrostreptomycin (5)	+	+	+	+	+	+	+	+	+	+	+	+
Dihydrostreptomycin (50)	+	+	+	+	+	+	+	+	+	+	+	+
Dihydrostreptomycin (100)	+	+	+	+	+	+	+	+	+	+	+	+
Dihydrostreptomycin (300)	+	+	+	+	+	+	+	+	+	+	+	+
Chloromycetin (5)	+	+	+	+	+	+	+	+	+	+	+	+
Chloromycetin (50)	+	+	+	+	+	+	+	+	+	+	+	+
Chloromycetin (100)	+	+	+	+	+	+	+	+	+	+	+	+

100 μg dihydrostreptomycin ml^{-1} and 100 μg chloromycetin ml^{-1} . Strains can grow on YMA plates with 0.1% erythrosin B or 1% NaCl, but can not grow in LB broth. Alkali is produced when strains are grown in litmus milk. The species can be differentiated at the molecular level from other *Mesorhizobium* species by PCR-based RFLP of the 16S

rRNA gene, phenotypic characterization and numerical taxonomy (Wang *et al.*, 2006), fatty acid composition, SDS-PAGE of whole-cell proteins, total DNA hybridization and 16S rRNA gene sequencing.

The type strain is strain CCBAU 61158^T (= LMG 23507^T = USDA 4964^T). Its G + C content is 59.0 mol% (T_m).

Acknowledgements

This work was supported by the Foundation of State Key Basic Research and Development Plan of China (project no. 2001CB108905), the National Natural Science Foundation of China (project no. 30400001) and National Project for Basic S & T Platform Construction (project no. 2005DKA30560-1).

References

- Chen, W. X., Yan, G. H. & Li, J. L. (1988). Numerical taxonomy study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. *Int J Syst Bacteriol* **38**, 392–397.
- Chen, W. X., Li, G. S., Qi, Y. L., Wang, E. T., Yuan, H. L. & Li, J. L. (1991). *Rhizobium huakuii* sp. nov., isolated from the root nodules of *Astragalus sinicus*. *Int J Syst Bacteriol* **41**, 275–280.
- Chen, W., Wang, E., Wang, S., Li, Y., Chen, X. & Li, Y. (1995). Characteristics of *Rhizobium tianshanense* sp. nov., a moderately and slowly growing root nodule bacterium isolated from an arid saline environment in Xinjiang, People's Republic of China. *Int J Syst Bacteriol* **45**, 153–159.
- Chen, W. X., Tan, Z. Y., Gao, J. L., Li, Y. & Wang, E. T. (1997). *Rhizobium hainanense* sp. nov., isolated from tropical legumes. *Int J Syst Bacteriol* **47**, 870–873.
- de Lajudie, P., Willems, A., Nick, G., Moreira, F., Molouba, F., Hoste, B., Torck, U., Neyra, M., Collins, M. D. & other authors (1998). Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarium* sp. nov. *Int J Syst Bacteriol* **48**, 369–382.
- De Ley, J. (1970). Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.
- Gao, J. L., Turner, S. L., Kan, F. L., Wang, E. T., Tan, Z. Y., Qiu, Y. H., Gu, J., Terefework, Z., Young, J. P. W. & other authors (2004). *Mesorhizobium septentrionale* sp. nov. and *Mesorhizobium temperatum* sp. nov. isolated from *Astragalus adsurgens* growing in the northern regions of China. *Int J Syst Evol Microbiol* **54**, 2003–2012.
- Gaunt, M. W., Turner, S. L., Rigottier-Gois, L., Lloyd-Macgilp, S. A. & Young, J. P. W. (2001). Phylogenies of *atpD* and *recA* support the small subunit rRNA-based classification of rhizobia. *Int J Syst Evol Microbiol* **51**, 2037–2048.
- Ghosh, W. & Roy, P. (2006). *Mesorhizobium thio-gangeticum* sp. nov., a novel sulfur-oxidizing chemolithoautotroph from rhizosphere soil of an Indian tropical leguminous plant. *Int J Syst Evol Microbiol* **56**, 91–97.
- Graham, P. H., Sadowsky, M. J., Keyser, H. H., Barnett, Y. M., Bradley, R. S., Cooper, J. E., De Ley, J., Jarvis, B. D. W., Roslycky, E. B. & other authors (1991). Proposed minimal standards for the description of new genera and species of root- and stem-nodulation bacteria. *Int J Syst Bacteriol* **41**, 582–587.
- Graham, P. H., Sadowsky, M. J., Tighe, S. W., Thompson, J. A., Howieson, J. G. & Thomas, R. (1995). Differences among strains of *Bradyrhizobium* in fatty acid-methyl ester analysis. *Can J Microbiol* **41**, 1038–1042.

- Hashimoto, J. G., Stevenson, B. S. & Schmidt, T. M. (2003). Rates and consequences of recombination between rRNA operons. *J Bacteriol* **185**, 966–972.
- Haukka, K., Lindström, K. & Young, J. P. W. (1996). Diversity of partial 16S rRNA sequences among and within strains of African rhizobia isolated from *Acacia* and *Prosopis*. *Syst Appl Microbiol* **19**, 352–359.
- Haukka, K., Lindström, K. & Young, J. P. W. (1998). Three phylogenetic groups of *nodA* and *nifH* genes in *Sinorhizobium* and *Mesorhizobium* isolates from leguminous trees growing in Africa and Latin America. *Appl Environ Microbiol* **64**, 419–426.
- Jarvis, B. D. W. & Tighe, S. W. (1994). Rapid identification of *Rhizobium* species based on cellular fatty acid analysis. *Plant Soil* **161**, 31–41.
- Jarvis, B. D. W., van Berkum, P., Chen, W. X., Nour, S. M., Fernandez, M. P., Cleyet-Marel, J. C. & Gillis, M. (1997). Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. *Int J Syst Bacteriol* **47**, 895–898.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Denner, E. B. M., Meyer, S., Moore, E. R. B. & Busse, H.-J. (1997). Classification of “*Pseudomonas azotocolligans*” Anderson 1955, 132 in the genus *Sphingomonas* as *Sphingomonas trueperi* sp. nov. *Int J Syst Bacteriol* **47**, 577–583.
- Laguerre, G., Nour, S. M., Macheret, V., Sanjuan, J., Drouin, P. & Amarger, N. (2001). Classification of rhizobia based on *nodC* and *nifH* gene analysis reveals a close phylogenetic relationship among *Phaseolus vulgaris* symbionts. *Microbiology* **147**, 981–993.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Martínez-Romero, E., Segovia, L., Mercante, F. M., Franco, A. A., Graham, P. & Pardo, M. A. (1991). *Rhizobium tropici*: a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. *Int J Syst Bacteriol* **41**, 417–426.
- Nour, S. M., Fernandez, M. P., Normand, P. & Cleyet-Marel, J. C. (1994). *Rhizobium ciceri* sp. nov., consisting of strains that nodulate chickpeas (*Cicer arietinum* L.). *Int J Syst Bacteriol* **44**, 511–522.
- Parker, M. A. (2004). rRNA and *dnaK* relationships of *Bradyrhizobium* sp. nodule bacteria from four papilionoid legume trees in Costa Rica. *Syst Appl Microbiol* **27**, 334–342.
- Scholla, M. H. & Elkan, G. H. (1984). *Rhizobium fredii* sp. nov., a fast-growing species that effectively nodulates soybeans. *Int J Syst Bacteriol* **34**, 484–486.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in Bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Stepkowski, T., Czaplinska, M., Miedzinska, K. & Moulin, L. (2003). The variable part of the *dnaK* gene as an alternative marker for phylogenetic studies of rhizobia and related alpha *Proteobacteria*. *Syst Appl Microbiol* **26**, 483–494.
- Tan, Z. Y., Wang, E. T., Gao, J. L., Martínez-Romero, E. & Chen, W. X. (1997). Phylogenetic and genetic relationships of *Mesorhizobium tianshanense* and related rhizobia. *Int J Syst Bacteriol* **47**, 874–879.
- Tan, Z. Y., Kan, F. L., Peng, G. X., Wang, E. T., Reinhold-Hurek, B. & Chen, W. X. (2001). *Rhizobium yanglingense* sp. nov., isolated from arid and semi-arid regions in China. *Int J Syst Evol Microbiol* **51**, 909–914.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tighe, S. W., de Lajudie, P., Dipietro, K., Lindström, K., Nick, G. & Jarvis, B. D. W. (2000). Analysis of cellular fatty acids and phenotypic relationships of *Agrobacterium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* species using the Sherlock Microbial Identification System. *Int J Syst Evol Microbiol* **50**, 787–801.
- Turner, S. L. & Young, J. P. W. (2000). The glutamine synthetases of rhizobia: phylogenetics and evolutionary implications. *Mol Biol Evol* **17**, 309–319.
- Ueda, K., Seki, T., Kudo, T., Yoshida, T. & Kataoka, M. (1999). Two distinct mechanisms cause heterogeneity of 16S rRNA. *J Bacteriol* **181**, 78–82.
- van Berkum, P., Beyene, D., Bao, G., Campbell, T. A. & Eardly, B. D. (1998). *Rhizobium mongolense* sp. nov. is one of three rhizobial genotypes identified which nodulate and form nitrogen-fixing symbioses with *Medicago ruthenica* [(L.) Ledebour]. *Int J Syst Bacteriol* **48**, 13–22.
- van Berkum, P., Terefework, Z., Paulin, L., Suomalainen, S., Lindström, K. & Eardly, B. D. (2003). Discordant phylogenies within the *rrn* loci of rhizobia. *J Bacteriol* **185**, 2988–2998.
- Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K. & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* **60**, 407–438.
- Van de Peer, Y. & De Wachter, Y. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.
- Vauterin, L. & Vauterin, P. (1992). Computer-aided objective comparison of electrophoresis patterns for grouping and identification of microorganisms. *Eur Microbiol* **1**, 37–41.
- Velázquez, E., Igual, J. M., Willems, A., Fernández, M. P., Muñoz, E., Mateos, P. F., Abril, A., Toro, N., Normand, P. & other authors (2001). *Mesorhizobium chacoense* sp. nov., a novel species that nodulates *Prosopis alba* in the Chaco Arido region (Argentina). *Int J Syst Evol Microbiol* **51**, 1011–1021.
- Vincent, J. M. (1970). *Manual for the Practical Study of the Root-Nodule Bacteria*, IBP Handbook 15. Oxford: Blackwell.
- Vinuesa, P., León-Barrios, M., Silva, C., Willems, A., Jarabo-Lorenzo, A., Pérez-Galdona, R., Werner, D. & Martínez-Romero, E. (2005). *Bradyrhizobium canariense* sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (Papilionoideae: Genisteeae) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium* genospecies α and *Bradyrhizobium* genospecies β . *Int J Syst Evol Microbiol* **55**, 569–575.
- Wang, E. T., van Berkum, P., Sui, X. H., Beyene, D., Chen, W. X. & Martínez-Romero, E. (1999). Diversity of rhizobia associated with *Amorpha fruticosa* isolated from Chinese soil and description of *Mesorhizobium amorphae* sp. nov. *Int J Syst Bacteriol* **49**, 51–65.
- Wang, F. Q., Wang, E. T., Zhang, Y. F. & Chen, W. X. (2006). Characterization of rhizobia isolated from *Albizia* spp. in comparison with microsymbionts of *Acacia* spp. and *Leucaena leucocephala* grown in China. *Syst Appl Microbiol* **29**, 502–517.
- Wei, G. H., Wang, E. T., Tan, Z. Y., Zhu, M. E. & Chen, W. X. (2002). *Rhizobium indigoferae* sp. nov. and *Sinorhizobium kummerowiae*

sp. nov., respectively isolated from *Indigofera* spp. and *Kummerowia stipulacea*. *Int J Syst Evol Microbiol* **52**, 2231–2239.

Wei, G. H., Tan, Z. Y., Zhu, M. E., Wang, E. T., Han, S. Z. & Chen, W. X. (2003). Characterization of rhizobia isolated from legume species within the genera *Astragalus* and *Lespedeza* grown in the Loess Plateau of China and description of *Rhizobium loessense* sp. nov. *Int J Syst Evol Microbiol* **53**, 1575–1583.

Wernegreen, J. J. & Riley, M. A. (1999). Comparison of the evolutionary dynamics of symbiotic and housekeeping loci: a case for the genetic coherence of rhizobial lineages. *Mol Biol Evol* **16**, 98–113.

Wu, T. L., Chen, P. Y., Wei, C. F., Chen, T. C., Hu, C. C., Cheng, H. C. & Li, L. C. (1988). *Flora Republicae Popularis Sinicae*. Tomus 39, Angiospermae, Dicotyledoneae, Laguminosae (1). Beijing: Science Press.

Xu, L. M., Ge, C., Cui, Z., Li, J. & Fan, H. (1995). *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. *Int J Syst Bacteriol* **45**, 705–711.

Yao, Z. Y., Kan, F. L., Wang, E. T., Wei, G. H. & Chen, W. X. (2002). Characterization of rhizobia that nodulate legume species of the genus *Lespedeza* and description of *Bradyrhizobium yuanmingense* sp. nov. *Int J Syst Evol Microbiol* **52**, 2219–2230.