Mesorhizobium caraganae sp. nov., a novel rhizobial species nodulated with *Caragana* spp. in China

Su Hua Guan,[1] Wen Feng Chen,[1] En Tao Wang,[2] Yang Li Lu,[1] Xue Rui Yan,[3] Xiao Xia Zhang[4] and Wen Xin Chen[1]

Correspondence
Wen Feng Chen
chenwf@cau.edu.cn

[1]Key Laboratory of Agro-Microbial Resource and Application, Ministry of Agriculture/College of Biological Sciences, China Agricultural University, Beijing 100193, PR China

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

[3]College of Plant Protection, Shenyang Agricultural University, Shenyang 110161, PR China

[4]Agricultural Cultural Collection of China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China

Five rhizobial strains representing the largest group in the genus *Mesorhizobium* associated with *Caragana* spp. in China were characterized taxonomically. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these microsymbionts belonged to the genus *Mesorhizobium*, with *Mesorhizobium tianshanense* USDA 3592^T, *Mesorhizobium temperatum* SDW018^T and *Mesorhizobium mediterraneum* UPM-Ca36^T as the closest neighbours ($\geqslant$99.5 % 16S rRNA gene sequence similarity). Genotypic fingerprinting by whole-cell protein electrophoresis, DNA–DNA hybridization, comparative housekeeping sequence analysis of the *atpD*, *glnII* and *recA* genes, fatty acid profiles and a series of phenotypic and physiological tests allowed the novel group to be differentiated from all previously recognized species of the genus *Mesorhizobium*. This group therefore represents a novel species, for which the name *Mesorhizobium caraganae* sp. nov. is proposed with the type strain CCBAU 11299^T (=LMG 24397^T=HAMBI 2990^T). Cross-inoculation tests showed that strain CCBAU 11299^T could form effective nodules on *Caragana microphylla*, *Caragana intermedia*, *Glycyrrhiza uralensis*, *Astragalus adsurgens* and *Phaseolus vulgaris*.

Caragana, a genus of the subfamily Papilionoideae in the Leguminosae, is a perennial leguminous shrub that is highly tolerant to drought, salt and extreme cold and grows in relatively poor or sandy, well-drained soil. In China, over 60 species have been recorded within this genus. They are often used as windbreaks to protect soils from desertification in the northern regions of China (Su *et al.*, 2005). They are also used for livestock forage and as high-energy firewood. Their flowers are a good source of food for bees and their seeds are used as herbal medicines (Xiang *et al.*, 2005).

Rhizobia are soil bacteria that fix nitrogen (diazotrophy) after becoming established inside the root nodules of legumes. Most rhizobia symbiosed with *Caragana* spp. growing in Liaoning Province of China have been placed within the genus *Mesorhizobium* (Yan *et al.*, 2007). This genus was established by Jarvis *et al.* (1997) to describe rhizobia whose growth rate was slower than the fast-growing members of the genus *Rhizobium* and faster than the slow-growing members of the genus *Bradyrhizobium*. At the time of writing, 12 species are included in the genus *Mesorhizobium* (for details see http://www.bacterio.cict.fr/m/mesorhizobium.html). In our previous studies, a group of mesorhizobia associated with *Caragana* spp. (designated *Mesorhizobium* sp. III) in the Liaoning Province of China showed unique protein and BOX-PCR patterns different from those of the recognized species of this genus (Yan *et al.*, 2007). In this study, two strains (CCBAU 11299^T and

Abbreviation: NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the partial 16S rRNA, *atpD*, *recA*, *glnII*, *nodC* and *nifH* gene sequences of *Mesorhizobium caraganae* sp. nov. CCBAU 11299^T are EF149003, EU249379, EU249394, EU249384, EU130405 and EU130422, respectively.

Tables giving details of fatty acid contents and DNA–DNA relatedness of the novel strains compared with recognized species of the genus *Mesorhizobium* and figures showing phylogenetic trees based on the *nodC* and *nifH* genes and a phenogram derived from UPGMA analysis and SDS-PAGE analysis of whole-cell proteins of strain CCBAU 11299^T are available as supplementary materials in IJSEM Online.

CCBAU 11300) representing *Mesorhizobium* sp. III and three additional strains (CCBAU 01502, CCBAU 01519, CCBAU 01528), isolated from *Caragana intermedia* in Inner Mongolia, China, a region adjacent to Liaoning Province, were studied in more detail by comparing the SDS-PAGE of whole soluble proteins, 16S rRNA gene sequences, housekeeping genes (*atpD*, *recA* and *glnII*), symbiotic genes (*nodC* and *nifH*), DNA–DNA hybridization and biochemical characteristics with the recognized species of the genus *Mesorhizobium*. Comparisons of fatty acid profiles were also made between the novel group and the recognized species of the genus. Based on the results of this polyphasic taxonomic approach, *Mesorhizobium* sp. III, together with the three isolates from Inner Mongolia, formed a coherent group and thus a novel species is proposed. The strains were maintained on YMA (Vincent, 1970) at 4 °C for temporary storage and in 20 % glycerol at −70 °C for long-term storage.

The five novel strains and the reference strains of the genus *Mesorhizobium* (Table 1) were subjected to SDS-PAGE analysis of whole-cell soluble proteins. Cellular protein extracts were prepared and electrophoretic analysis was performed as described previously (Tan *et al.*, 1997). Digitization, normalization and numerical analyses of the protein profiles were performed with the GelCompar II version 4.5 software (Applied Maths). The similarity between pairs of protein patterns was expressed by the Pearson's coefficient and a UPGMA dendrogram was constructed (Vauterin & Vauterin, 1992). The strikingly similar protein patterns observed among the five novel strains indicated considerable homogeneity (see Supplementary Fig. S1a in IJSEM Online; the profile of strain CCBAU 11300 was the same as CCBAU 11299 and is therefore not shown in this figure). However, the isolates were not duplicates as they had been isolated from different regions and different species of *Caragana* (Table 1). A comparison of the protein patterns with those of other recognized species of the genus *Mesorhizobium* revealed that none of the protein profiles of the recognized strains were highly similar to those of the novel group (only 67 % similarity, see Supplementary Fig. S1b in IJSEM Online).

Fatty acid profiling is a popular method for characterizing the microbial communities of natural systems (Schutter & Dick, 2000) and is a useful tool for the identification of root-nodule bacteria (Tighe *et al.*, 2000). In this study, all the mesorhizobia were grown on YMA medium for 72 h at 28 °C and then approximately 40 mg of the well-grown cells was harvested. Fatty acid methyl esters were prepared and separated using a previously described method (Sasser, 1990) and identified with the MIDI Sherlock Microbial Identification System. The cellular fatty acid profiles of the novel group and related species of the genus *Mesorhizobium* are shown in Supplementary Table S1 (available in IJSEM Online). The novel group could be assigned to the genus *Mesorhizobium* because they lacked 20 : 3ω6,9,12*c* and summed feature 2 (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; Wang *et al.*, 2007). The

Table 1. Strains used in this study and relevant information

Strain	Host plant	Geographical origin	Reference
***Mesorhizobium caraganae* sp. nov.**			
CCBAU 11299^T	*Caragana microphylla*	Liaoning, China	Yan *et al.* (2007)
CCBAU 11300	*Caragana microphylla*	Liaoning, China	Yan *et al.* (2007)
CCBAU 01502	*Caragana intermedia*	Inner Mongolia, China	This study
CCBAU 01519	*Caragana intermedia*	Inner Mongolia, China	This study
CCBAU 01528	*Caragana intermedia*	Inner Mongolia, China	This study
Reference strains			
M. albiziae CCBAU 61158^T	*Albizia kalkora*	Sichuan, China	Wang *et al.* (2007)
M. loti NZP 2213^T (=LMG 6125^T=USDA 3471^T)	*Lotus corniculatus*	New Zealand	Jarvis *et al.* (1997)
M. plurifarium LMG 11892^T (=ICMP 13640^T)	*Acacia senegal*	Senegal	de Lajudie *et al.* (1998)
M. amorphae ACCC 19665^T (=ICMP 15022^T =LMG 18977^T)	*Amorpha fruticosa*	Beijing, China	Wang *et al.* (1999)
M. chacoense LMG 19008^T (=Pr-5^T=ICMP 14587^T)	*Prosopis alba*	Argentina	Velázquez *et al.* (2001)
M. ciceri USDA 3383^T (=UPM-Ca7^T)	*Cicer arietinum*	Spain	Nour *et al.* (1994)
M. huakuii CCBAU 2609^T (=IFO 15243^T=USDA 4779^T)	*Astragalus sinicus*	Nanjing, China	Chen *et al.* (1991)
M. mediterraneum USDA 3392^T (=UPM-Ca-36^T)	*Cicer arietinum*	Spain	Nour *et al.* (1995)
M. septentrionale SDW014^T	*Astragalus adsurgens*	Liaoning, China	Gao *et al.* (2004)
M. temperatum SDW018^T	*Astragalus adsurgens*	Liaoning, China	Gao *et al.* (2004)
M. tianshanense CCBAU 3306^T (=USDA 3592^T =A-1BS)	*Glycyrrhiza pallidiflora*	Xinjiang, China	Chen *et al.* (1995)

strains of the novel group differed from *Mesorhizobium mediterraneum* by their much higher concentrations of 16 : 0, summed feature 4 (17 : 1 iso I/anteiso B and/or 17 : 1 anteiso B/iso I), summed feature 8 (18 : 1ω7*c* and/or 18 : 1ω6*c*) and lower concentrations of 11-methyl 18 : 1ω7*c*, 19 : 0 cyclo ω8*c*. The novel group differed from *Mesorhizobium temperatum* by the presence of 15 : 0 iso, 17 : 0, 17 : 1ω8*c*, 10-methyl 19 : 0, 20 : 0, 20 : 1ω7*c* and a higher concentration of summed feature 8 (18 : 1ω7*c* and/or 18 : 1ω6*c*). When compared with *Mesorhizobium tianshanense*, the members of the novel group possessed 11-methyl 18 : 1ω7*c*, 19 : 0 cyclo ω8*c* and summed feature 8 but lacked of summed feature 7 (18 : 1 *c*11/*t*9/*t*6 and/or 18 : 1 *trans* 9/*t*6/*c*11). The novel strains differed from *Mesorhizobium septentrionale* in that they lacked 16 : 1*c*9, 18 : 1*c*9, unknown fatty acid (ECL19.368), summed feature 5 (17 : 1 iso I/anteiso B and/or 17 : 1 anteiso B/I I) and summed feature 7 but did contain 11-methyl 18 : 1ω7*c*, 19 : 0 cyclo ω8*c*, summed feature 3 (16 : 1ω7*c*/16 : 1ω6*c* and/or 16 : 1ω6*c*/16 : 1ω7*c*) and summed feature 8. The novel strains differed from other recognized *Mesorhizobium* species by containing higher amounts of summed feature 8, lower amounts of 11-methyl 18 : 1ω7*c* and lacking fatty acids of summed features 5 and 7.

The 16S rRNA gene was amplified and sequenced for the five novel strains isolated from *Caragana* spp. using primers P1 and P6 according to the technique of Tan *et al.* (1997). The sequences were aligned with those of related species of the genus *Mesorhizobium* using the CLUSTAL W program from the MEGA 4.0 software package (Tamura *et al.*, 2007). Aligned sequences were analysed using MEGA to produce a Jukes–Cantor distance (Jukes & Cantor, 1969) and to construct an optimal unrooted tree using the neighbour-joining method (Saitou & Nei, 1987). The robustness of the tree topology was calculated from bootstrap analysis using 1000 replications of the sequences (Felsenstein, 1985). All five novel strains had identical 16S rRNA gene sequences and were closely related to *M. temperatum* SDW018^T, *M. mediterraneum* UPM-Ca36^T and *M. tianshanense* USDA 3592^T (Fig. 1a), with more than 99.5 % similarity between strain CCBAU 11299^T and these three species.

Housekeeping genes (*atpD*, *glnI*, *glnII*, *recA*, *dnaK* etc.) 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; Wang *et al.*, 2007). Most recently, multilocus sequence analysis (MLSA) of ten housekeeping genes was performed and the results were compared with data from DNA–DNA hybridization experiments for 34 representatives of the genus *Ensifer* (Martens *et al.*, 2008). The results obtained by Martens *et al.*, 2008) indicated that sequencing of housekeeping genes was superior to DNA–DNA hybridization for the assessment of genetic relatedness between species of the genus *Ensifer*. To investigate the genomic diversity of the root nodule isolates from *Caragana* spp., we sequenced the variable regions of three housekeeping genes, *atpD* (gene encoding the ATP synthase β-subunit), *recA* (homologous recombination protein A) and *glnII* (glutamine synthetase gene) and compared them with the same genes from recognized species of the genus *Mesorhizobium*. In this study, PCR amplification and sequencing of partial *atpD* and *recA* genes were undertaken according to Gaunt *et al.* (2001) and a 600 bp intragenic fragment of *glnII* was amplified according to Turner & Young (2000). The five novel *Mesorhizobium* strains had identical partial *atpD* sequences and shared 94.5 % sequence similarity with *M. septentrionale* SDW014^T. Strain CCBAU 11299^T had 95.6 % gene sequence similarity to *M. tianshanense* and 94 % similarity to *M. septentrionale* SDW014^T for the *recA* sequence. In the analysis of the partial *glnII* gene, the five novel strains differed by only 2 or 4 bp and showed similarity levels of $\leqslant$92.7 % with the type strains of recognized species of the genus *Mesorhizobium*. Neighbour-joining (NJ) trees constructed with the same methods as used for the 16S rRNA gene analysis are shown in Fig. 1b, c and d based on the respective sequences. The groupings and precise branching patterns for the three different genes were not always congruent with those found for the 16S rRNA gene sequence tree, and the bootstrap values were not always high. This may be due to the occurrence of lateral gene transfer events and the fact that fewer taxa were included for the construction of the phylogenetic trees. When further highly similar strains were added to the analysis, as studied in this research, the five novel strains clustered very closely together in each tree and the branches were supported by high bootstrap values ranging from 96 to 100 % (Fig. 1a, b, c and d).

The symbiotic genes (*nod* and *nif*) are required for the successful establishment of highly specific symbiosis between rhizobia and legumes and are important determinants of rhizobial host specificity. Therefore, comparisons of these symbiotic genes may reveal the host ranges of rhizobia. In this study, partial sequences of the symbiotic genes *nodC* and *nifH* for the five novel strains were amplified by using the previously described primers (nodCF and nodCI, nifHF and nifHI) and PCR conditions as described by Laguerre *et al.* (2001). The *nodC* gene sequences for the five strains isolated from *Caragana* were closely related, with sequence similarities ranging from 99 to 100 %. In the NJ tree (see Supplementary Fig. S2a in IJSEM Online), the five strains were closely placed to the type strains of *M. tianshanense*, *M. temperatum* and *M. septentrionale* and had >96.7 % *nodC* gene sequence similarity with these strains. The *nifH* gene sequence of the novel strains isolated from *Caragana* spp. showed sequence similarities that ranged from 98 to 100 % and showed >91.2 % sequence similarity to those of *M. tianshanense*, *M. temperatum* and *M. septentrionale* (see Supplementary Fig. S2b in IJSEM Online).

The high similarities between the symbiotic genes from the mesorhizobia isolated from *Caragana* spp. and the type

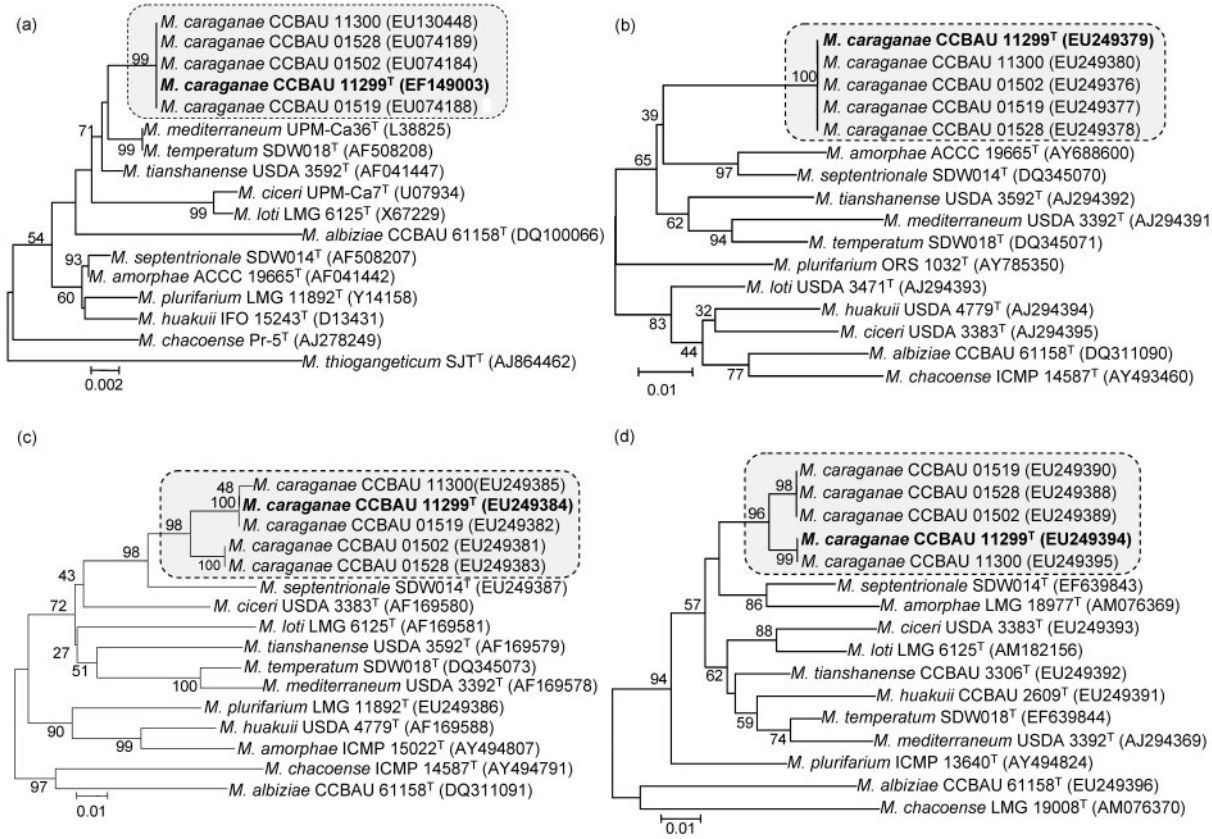

Fig. 1. Comparison of 16S rRNA (a), *atpD* (b), *glnII* (c) and *recA* (d) gene phylogenies, showing the relationships among the novel group (*Mesorhizobium caraganae* sp. nov.) (hatched and framed) and recognized species of the genus *Mesorhizobium*. Trees were constructed by the neighbour-joining method with a Jukes–Cantor distance matrix. Bootstrap values (%) are based on 1000 replications and are shown at each node. Bars, expected number of changes per site.

strains of *M. temperatum*, *M. septentrionale* and *M. tianshanense* demonstrated that these strains may have common host ranges, as suggested by Laguerre *et al.* (2001). Strains of *M. septentrionale* and *M. temperatum* were originally isolated from nodules of *Astragalus adsurgens* in Liaoning (Gao *et al.*, 2001), from where the *Caragana* microsymbionts were isolated in this study (Yan *et al.*, 2007). *M. tianshanense* A-1BS^T was isolated from *Glycyrrhiza pallidiflora* (Chen *et al.*, 1995) in the Xinjiang province of China, a region about 5000 km away from Liaoning. However, two strains (032B and 91X11) within the species *M. tianshanense* were isolated from nodules of *Caragana polourensis*, while strain A-1BS^T could form effective nodules with *Caragana polourensis* (Chen *et al.*, 1995). Therefore, the high sequence similarities of the symbiotic genes, especially for the *nod* genes, might indicate that these bacteria could share their hosts, as suggested by Laguerre *et al.* (2001). To test whether the *Caragana* mesorhizobia shared the same hosts with these three species, cross-inoculation between these strains and the recognized *Mesorhizobium* species and their original hosts was performed. Seed treatment and inoculation of *Caragana microphylla* and *Astragalus adsurgens* were conducted using the standard method of Vincent (1970). Since seeds of the original host plant, *Glycyrrhiza pallidiflora*, were not available, we used *Glycyrrhiza uralensis* as the test plant. Seeds of *Glycyrrhiza uralensis* were first immersed in concentrated sulfuric acid for 4 h before surface-sterilization. Seedlings inoculated with different strains were grown in a greenhouse under natural daylight for 6 weeks. The non-inoculated seedlings were used as controls and were cultured under the same conditions. The results confirmed that each of the five novel strains from *Caragana* spp. and the three type strains (*M. tianshanense*, *M. temperatum* and *M. septentrionale*) could form effective nodules on *Glycyrrhiza uralensis*, *Caragana microphylla*, *Caragana intermedia* and *Astragalus adsurgens*. The representative strain, CCBAU 11299^T, of the novel *Mesorhizobium* group was also used for cross-nodulation tests with another 11 legume species. The results showed that only *Phaseolus vulgaris*, a promiscuous host to many kinds of rhizobia, could be nodulated with CCBAU 11299^T, while no nodules were found on seedlings of *Glycine max*, *Trifolium pratense*, *Medicago sativa*, *Pisum*

sativum, *Melilotus albus*, *Amorpha fruticosa*, *Lespedeza cuneata*, *Dunbaria rotundifolia*, *Vigna radiata* and *Vicia septum*. The nodulation spectra of strain CCBAU 11299^T were different from those of strains *M. tianshanense* USDA 3592^T, *M. septentrionale* SDW014^T and *M. temperatum* SDW018^T, indicating that the novel group of isolates could have unique host-specific genes.

For the determination of the DNA base composition and DNA–DNA relatedness, total DNA was extracted from each strain using the method of Marmur (1961). Using the thermal denaturation method (Marmur & Doty, 1962) and *Escherichia coli* DH5α as a standard, the DNA G+C content of strain CCBAU 11299^T was found to be 59.7 mol% (T_m). This value was within the range reported for members of the genus *Mesorhizobium* (59–64 mol%; Jarvis *et al.*, 1997). DNA relatedness values between the strains of the novel group and the most closely related *Mesorhizobium* species were determined using the spectrophotometric method of De Ley (1970). The DNA–DNA relatedness values between CCBAU 11299^T and recognized *Mesorhizobium* species ranged from 7 to 35% (see Supplementary Table S2 in IJSEM Online) and suggested that the novel group represented a novel genomic species in the genus *Mesorhizobium*.

The phenotypic features of the novel group of isolates were determined and compared with those of related species of the genus *Mesorhizobium* according to the method described by Gao *et al.* (1994). The tested features included the utilization of sole carbon and nitrogen sources, resistance to antibiotics and tolerance of NaCl, and the pH and temperature ranges for growth (Gao *et al.*, 1994). The distinctive features of the novel group are shown in Table 2 and further features are presented in the description of the novel species.

Based on the results obtained in this study, we believe that the five new strains represent a novel species in the genus *Mesorhizobium*. This species could be differentiated by SDS-PAGE of cellular proteins, fatty acid profiles, phenotypic characteristics, DNA–DNA relatedness and by the sequencing of the *atpD*, *glnII*, *recA*, *nodC* and *nifH* genes. The name *Mesorhizobium caraganae* sp. nov. is proposed for the most abundant rhizobial group, group III, represented by the five novel strains associated with *Caragana* spp. growing in the Liaoning Province of China.

Description of *Mesorhizobium caraganae* sp. nov.

Mesorhizobium caraganae (ca.ra.ga′na.e. N.L. gen. n. *caraganae* of *Caragana*, a genus of leguminous plants, referring to the rhizobium isolated from root nodules of *Caragana* spp.).

Gram-negative, aerobic, motile, non-spore-forming rods, 0.5 μm wide by 2–3 μm long. Colonies on yeast mannitol

Table 2. Distinctive features of strains of *Mesorhizobium caraganae* sp. nov. and recognized species of the genus *Mesorhizobium*

Strains: 1, *M. ciceri* USDA 3383^T; 2, *M. chacoense* LMG 19008^T; 3, *M. huakuii* CCBAU 2609^T; 4, *M. albiziae* CCBAU 61158^T; 5, *M. amorphae* ACCC 19665^T; 6, *M. loti* NZP 2213^T; 7, *M. mediterraneum* USDA 3392^T; 8, *M. tianshanense* CCBAU 3306^T; 9, *M. septentrionale* SDW014^T;10, *M. temperatum* SDW018^T; 11, *M. plurifarium* LMG 11892^T; 12, *M. caraganae* sp. nov. CCBAU 11299^T; 13, *M. caraganae* CCBAU 11300; 14, *M. caraganae* CCBAU 01502; 15, *M. caraganae* CCBAU 01519; 16, *M. caraganae* CCBAU 01528. +, Growth or resistant; −, no growth or sensitive; ND, not detected or cannot be distinguished.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Utilization as sole carbon source:																
D-Arabinose	+	ND	ND	−	−	−	−	−	−	+	+	+	+	+	+	+
Inulin	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+
Dextrin	−	−	+	−	−	−	−	−	+	−	−	+	+	+	+	+
Dulcitol	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
meso-Erythritol	+	−	−	−	+	−	−	−	+	+	−	+	+	+	+	+
D-Amygdalin	+	−	−	−	−	−	−	−	+	−	−	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	−	+	−	+	+	+	+	−	−	+	+	+	+	+	+
Sodium DL-malate	+	−	+	+	+	+	−	−	−	−	+	+	+	−	+	+
Maltose	+	−	+	−	+	+	+	−	+	−	+	+	+	+	+	+
Melibiose	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−
Sodium pyruvate	−	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−
Raffinose pentahydrate	−	+	+	+	−	−	−	−	+	+	+	−	−	+	+	+
L-Rhamnose	+	−	+	−	−	+	+	−	+	+	+	+	+	+	+	+
Salicin	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
D-Ribose	+	−	+	−	−	−	−	−	+	+	+	+	+	+	+	+
Sodium acetate	+	−	+	−	−	−	−	−	+	+	+	+	+	+	+	+
Sodium citrate	−	−	−	−	+	−	−	−	−	−	+	−	−	+	+	+

Table 2. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sodium formate	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sodium hippurate	−	−	+	−	−	−	−	−	+	−	+	+	+	+	+	−
Sodium succinate	+	−	−	+	+	+	−	+	+	−	+	+	+	+	+	+
D-Sorbitol	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+
Sorbose	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−
Sucrose	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Sodium tartrate	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Trehalose	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+
D-Xylose	+	−	+	−	−	−	−	−	+	+	+	+	+	+	+	+
L-Arginine	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Glycine	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
L-Methionine	−	−	+	−	−	−	+	−	−	−	−	+	+	−	−	−
L-Threonine	−	−	−	−	−	−	−	−	+	−	+	+	+	−	−	−
Utilization as sole nitrogen source:																
DL-α-Aminopropionic acid	+	−	+	−	+	+	−	+	−	−	+	+	+	+	+	+
D-Arginine	+	−	+	+	+	+	−	+	−	−	+	+	+	−	+	+
D-Aspartic acid	+	−	+	+	+	+	−	−	+	−	+	+	+	+	+	+
L-Cystine	+	−	+	+	+	+	−	+	−	−	+	+	+	+	+	+
D-Glutamic acid	+	−	+	−	+	+	−	−	+	−	+	+	−	+	+	+
(+)-L-Glutamic acid	+	−	+	−	+	+	+	+	−	−	+	+	+	−	−	−
Hypoxanthine	+	−	+	−	+	+	+	+	−	−	+	+	+	+	+	+
L-Isoleucine	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+
L-Phenylalanine	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Threonine	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−
L-Valine	+	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+
L-Methionine	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
L-Threonine	+	−	+	+	+	+	−	+	+	+	+	+	+	−	+	+
Growth at:																
pH 5	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
1 % NaCl	+	−	+	−	−	−	−	−	+	−	+	−	−	−	−	−
Resistance to antibiotics (μg ml^{-1}):																
Ampicillin (50)	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
Ampicillin (100)	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
Ampicillin (300)	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
Kanamycin sulfate (5)	+	−	+	+	+	+	−	+	−	−	+	+	+	+	+	−
Kanamycin sulfate (50)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Neomycin sulfate (5)	+	−	+	+	+	+	−	−	−	−	+	+	+	+	+	−
Erythromycin (5)	+	−	+	+	+	+	+	+	+	−	+	+	+	−	+	+
Erythromycin (50)	+	−	+	+	+	+	−	+	+	−	+	+	+	−	+	+
Erythromycin (100)	+	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−
Erythromycin (300)	+	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−
Streptomycin sulfate (5)	+	−	+	+	−	+	−	−	+	−	+	+	+	−	−	−
Streptomycin sulfate (50)	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Streptomycin sulfate (100)	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Streptomycin sulfate (300)	−	−	ND	ND	−	+	−	−	−	−	−	−	−	−	−	−
Spectinomycin (5)	−	−	+	+	−	+	−	−	+	−	−	+	−	−	−	−
Gentamicin (5)	+	−	+	+	+	+	−	−	−	−	+	−	+	−	−	−
Bacitracin (5)	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+
Bacitracin (50)	+	−	+	+	+	+	−	+	−	−	+	+	+	+	−	+
Bacitracin (100)	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−
Chloramphenicol (5)	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−

agar (YMA) plates are circular, convex, white, opaque and usually 1–2 mm in diameter within 5–7 days incubation at 28 °C. The generation time is about 8 h in PY broth at 28 °C. The optimum temperature and pH for growth are 28 °C and pH 7, respectively. The maximum temperature for growth is 40 °C. Able to survive being heated at 60 °C for 10 min. Catalase-positive, but oxidase-negative. The type strain can utilize D-galactose, glucose, inositol, D-mannose and the sugars listed in Table 2 as sole carbon sources. Able to utilize almost all the tested sole nitrogen sources except for D-threonine. Does not utilize adipic acid, calcium gluconate, calcium malonate, melezitose, sodium D-gluconate, soluble starch, syringic acid, vanillic acid, DL-asparagine or L-proline as a sole carbon source. Resistance and sensitivity to antibiotics is shown in Table 2 and is also sensitive to (μg ml^{-1}) kanamycin sulfate (100), neomycin sulfate (50), spectinomycin (50), gentamicin (50), bacitracin (300), tetracycline hydrochloride (5), chloramphenicol (50). Synthesizes the following fatty acids (full details are given in Supplementary Table S1 in IJSEM Online) : 13 : 0 2-OH, 14 : 0, 15 : 1 isoG, 16 : 0, 17 : 0, 17 : 0 iso, 18 : 0, 11-methyl 18 : 1ω7*c*, 18 : 1ω9*c*, 19 : 0 cyclo ω8*c*, 20 : 0, 20 : 1ω7*c*, summed feature 1 (15 : 1 iso H/13 : 0 3-OH and/or 13 : 0 3-OH/15 : 1 i H), summed feature 3 (16 : 1ω7*c*/16 : 1ω6*c* and/or 16 : 1 ω6*c*/16 : 1ω7*c*), summed feature 4 (17 : 1 iso I/anteiso B and/or 17 : 1 anteiso B/iso I) and summed feature 8 (18 : 1ω7*c* and/or 18 : 1ω6*c*).

The type strain, CCBAU 11299^T (=LMG 24397^T=HAMBI 2990^T), was isolated from root nodules of *Caragana microphylla* growing in Beipiao city, Liaoning Province, China. The DNA G+C content of the type strain is 59.7 mol% (T_m).

Acknowledgements

This work was financed by the National Natural Science Foundation of China (project no. 30400001 and 30670001), National Program for Basic S & T Platform Construction (No. 2005DKA21201-10) and National Basic Research Program of China (2006CB100206, 2006AA10A213). We thank Professor J. P. Euzéby for his suggestion for the spelling of the specific epithet. We also thank Professor Janet Sprent and Professor Terry Godfrey for their embellishment of the manuscript.

References

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. X., Wang, E. T., Wang, S. Y., Li, Y. B., Chen, X. Q. & 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.

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.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gao, J. L., Sun, J. G., Li, Y., Wang, E. T. & Chen, W. X. (1994). Numerical taxonomy and DNA relatedness of tropical rhizobia isolated from Hainan Province, China. *Int J Syst Bacteriol* **44**, 151–158.

Gao, J. L., Terefework, Z., Chen, W. X. & Lindström, K. (2001). Genetic diversity of rhizobia isolated from *Astragalus adsurgens* growing in different geographical regions of China. *J Biotechnol* **91**, 155–168.

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. & 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., Lioyd-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 thiogangeticum* sp. nov., a novel sulfur-oxidizing chemolithoautotroph from rhizosphere soil of an Indian tropical leguminous plant. *Int J Syst Evol Microbiol* **56**, 91–97.

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.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

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.

Martens, M., Dawyndt, P., Coopman, R., Gillis, M., De Vos, P. & Willems, A. (2008). Advantages of multilocus sequence analysis for taxonomic studies: a case study using 10 housekeeping genes in the genus *Ensifer* (including former *Sinorhizobium*). *Int J Syst Evol Microbiol* **58**, 200–214.

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.

Nour, S. M., Cleyet-Marel, J.-C., Normand, P. & Fernandez, M. P. (1995). Genomic heterogeneity of strains nodulating chickpeas (*Cicer arietinum* L.) and description of *Rhizobium mediterraneum* sp. nov. *Int J Syst Bacteriol* **45**, 640–648.

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.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Schutter, M. E. & Dick, R. P. (2000). Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. *Soil Sci Soc Am J* **64**, 1659–1668.

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.

Su, Y. Z., Zhang, T. H., Li, Y. L. & Wang, F. (2005). Changes in soil properties after establishment of *Artemisia halodendron* and *Caragana microphylla* on shifting sand dunes in semiarid Horqin Sandy Land, northern China. *Environ Manage* **36**, 272–281.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

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.

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.

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). The cultivation, isolation and maintenance of rhizobia. In *A Manual for the Practical Study of the Root-Nodule Bacteria*, pp. 1–13. Edited by J. M. Vincent. Oxford: Blackwell Scientific.

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: Genisteae) 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 soils and description of *Mesorhizobium amorphae* sp. nov. *Int J Syst Bacteriol* **49**, 51–65.

Wang, F. Q., Wang, E. T., Liu, J., Chen, Q., Sui, X. H., Chen, W. F. & Chen, W. X. (2007). *Mesorhizobium albiziae* sp. nov., a novel bacterium that nodulates *Albizia kalkora* in a subtropical region of China. *Int J Syst Evol Microbiol* **57**, 1192–1199.

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.

Xiang, T., Uno, T., Ogino, F., Ai, C., Duo, J. & Sankawa, U. (2005). Antioxidant constituents of *Caragana tibetica*. *Chem Pharm Bull (Tokyo)* **53**, 1204–1206.

Yan, X. R., Chen, W. F., Fu, J. F., Lu, Y. L., Xue, C. Y., Sui, X. H., Li, Y., Wang, E. T. & Chen, W. X. (2007). *Mesorhizobium* spp. are the main microsymbionts of *Caragana* spp. grown in Liaoning Province of China. *FEMS Microbiol Lett* **271**, 265–273.