Mesorhizobium silamurunense sp. nov., isolated from root nodules of *Astragalus* species

Chun Tian Zhao,^{1,2} En Tao Wang,³ Yan Ming Zhang,¹ Wen Feng Chen,¹ Xin Hua Sui,¹ Wen Xin Chen,¹ Hong Can Liu⁴ and Xiao Xia Zhang⁵

Correspondence

Wen Xin Chen
wenxin@cau.edu.cn

¹State Key Laboratories for AgroBiotechnology/Department of Microbiology and Immunology, College of Biological Sciences, China Agricultural University, Beijing 100193, PR China

²College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310014, PR China

³Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México DF 11340, México

⁴Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

⁵Agricultural Cultural Collection of China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China

Four rhizobial strains representing a previously defined novel group in the genus *Mesorhizobium* and isolated from *Astragalus* species in China were further characterized using a polyphasic approach. Phylogenetic analysis of 16S rRNA gene sequences showed that these Gram-negative bacteria belonged to the genus *Mesorhizobium*, with *Mesorhizobium plurifarum* LMG 11892^T as the closest neighbour sharing a sequence similarity of 99.8 %. Comparative sequence analysis of the *atpD*, *recA*, *glnII*, *rpoB*, *nodC* and *nifH* genes, SDS-PAGE of whole-cell soluble proteins, DNA–DNA hybridization, fatty acid profiles and a series of phenotypic and physiological tests differentiated the novel group from all recognized species of the genus *Mesorhizobium*. Based on the data obtained in the present and previous studies, this group represents a novel species within the genus *Mesorhizobium*, for which the name *Mesorhizobium silamurunense* sp. nov. is proposed. The type strain is CCBAU 01550^T (=HAMBI 3029^T=LMG 24822^T), and could form effective nodules on *Astragalus membranaceus*, *Astragalus adsurgens* and *Caragana intermedia*, and ineffective nodules on *Phaseolus vulgaris* in cross-nodulation tests.

The genus *Astragalus*, comprising up to 2000 species of annual or perennial herbs, subshrubs and shrubs, is the largest genus in the plant family Fabaceae (Allen & Allen, 1981), which is widely distributed in temperate regions of the world, principally in Asia, Europe and North America. In China, 401 species (221 endemic) have been recorded within this genus (Wu *et al.*, 2010), which are used as herbal medicine, forage, green manure, resources for honey

or gum production, ornamental plants, windbreaks and indicators of selenium and uranium contamination (Laguerre *et al.*, 1997).

Approximately 90 species of the genus *Astragalus* have been recorded to form symbioses with root nodule bacteria (Allen & Allen, 1981), which are mainly classified as four species: *Mesorhizobium huakuii* (Chen *et al.*, 1991; Jarvis *et al.*, 1997), *Mesorhizobium septentrionale*, *Mesorhizobium temperatum* (Gao *et al.*, 2004), and *Rhizobium loessense* (Wei *et al.*, 2003). The genus *Mesorhizobium* (Jarvis *et al.*, 1997) belongs to the *Alphaproteobacteria* and at the time of writing consisted of 22 species with validly published names, including the recently described species: *Mesorhizobium alhagi* (Chen *et al.*, 2010), *Mesorhizobium robiniae* (Zhou *et al.*, 2010) and *Mesorhizobium camelthorni* (Chen *et al.*, 2011).

In our previous study, diverse genomic species of the rhizobia associated with *Astragalus* species grown in the temperate regions of China were identified, and included a novel group, *Mesorhizobium* sp. I, of 29 strains isolated

The GenBank/EMBL/DDBJ accession numbers for the partial *rpoB* gene sequences of strain CCBAU 01550^T, CCBAU 45265, CCBAU 25300 and CCBAU 45272, and are FJ393290–FJ393293, respectively. The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA and *nifH* gene sequences of strain CCBAU 25300 are FJ824658 and FJ824661, respectively. The GenBank/EMBL/DDBJ accession number for the partial 16S rRNA gene sequence of strain CCBAU 45272 is FJ824659. The GenBank/EMBL/DDBJ accession numbers for the partial *rpoB* gene sequences of other species of the genus *Mesorhizobium* are listed in the phylogenetic tree (Fig. 2d).

Three supplementary figures and a supplementary table are available with the online version of this paper.

from the root nodules of *Astragalus membranaceus* and *Astragalus adsurgens* (Zhao *et al.*, 2008). To determine their taxonomic status, four representative strains were further analysed in the present study and compared with reference strains of defined species by phenotypic, genotypic, phylogenetic and symbiotic characterizations.

The four representative strains (Table 1) of *Mesorhizobium* sp. I, CCBAU 01550^T, CCBAU 45265, CCBAU 45272 and CCBAU 25300, were isolated from root nodules of *Astragalus membranaceus* and *Astragalus adsurgens* growing in Inner Mongolia, Henan and Shandong provinces, which are located along the Yellow River in China (Zhao *et al.*, 2008). All strains were maintained on yeast mannitol agar (YMA) slants at 4 °C for temporary storage or in 20 % (w/v) glycerol at –80 °C for long-term storage. Unless otherwise indicated, strains were incubated at 28 °C for experiments in this study.

The almost complete full-length 16S rRNA gene sequences (about 1350 bp) were amplified by PCR and sequenced directly by using universal primers P1 and P6 according to Tan *et al.* (1997). The corresponding sequences of type strains of the recognised species of the genus *Mesorhizobium* were retrieved from the GenBank database. Multiple nucleotide sequence alignments were generated by using the CLUSTAL W program in the MEGA 4.0.2 software package (Tamura *et al.*, 2007). Phylogenetic trees were constructed with MEGA version 4.0.2 software using the neighbour-joining (NJ) method with the Jukes–Cantor model (Jukes & Cantor, 1969), and the maximum-parsimony (MP) method. Bootstrap analysis was based on 1000 replicates to validate the reliability of the branching pattern of the trees. The four representative strains had identical 16S rRNA gene sequences and were closely related to *Mesorhizobium plurifarum* LMG

11892^T (99.8 % gene sequence similarity) with 92 % bootstrap support, and shared 98.1 % 16S gene sequence similarity with *Mesorhizobium loti* LMG 6125^T, the type species of the genus *Mesorhizobium* (Fig. 1). The neighbour-joining tree and maximum-parsimony tree (not shown) had similar topologies.

The phylogenetic relationships of the four representative strains to recognized species of the genus *Mesorhizobium* were confirmed by sequencing analysis of the housekeeping genes *atpD* (ATP synthase, beta subunit), *recA* (recombinase A), *glnII* (glutamine synthetase type II) and *rpoB* (RNA polymerase, beta subunit), which are frequently used as alternative phylogenetic markers within rhizobia (Gaunt *et al.*, 2001; Martens *et al.*, 2007; Martens *et al.*, 2008; Turner & Young, 2000). Partial *atpD*, *recA* and *glnII* gene sequences were amplified and sequenced in our previous study (Zhao *et al.*, 2008) with primer pairs *atpD*255F/*atpD*782R, *recA*41F/*recA*640R and *glnII*12F/*glnII*689R, respectively (the primer coordinates correspond to *Bradyrhizobium japonicum* USDA110 gene positions), using previously reported protocols (Vinuesa *et al.*, 2005). PCR amplification and sequencing of partial *rpoB* gene sequences were performed with primers *ropB*82F (5'-AAC CTC ATC GAG GTT CAG AAG GC-3') and *ropB*1580R (5'-TGG TCC ATG AAC TGC GAG AGC TG-3'), corresponding to the *rpoB* gene sequence of *Sinorhizobium meliloti* 1021 (GenBank/EMBL/DDBJ accession number AL591688) (Capela *et al.*, 2001). Phylogenetic analyses were performed with the MEGA 4.0.2 software package (Tamura *et al.*, 2007) using the neighbour-joining method with Kimura's two-parameter model and 1000 bootstrap replications. The four representative strains had identical *recA* gene sequences and were most similar to *M. plurifarum* USDA 4413^T with 93.3 % gene sequence similarity. *rpoB* gene sequences for the four

Table 1. Strains used in this study

Strain	Host plant	Geographical origin
<i>Mesorhizobium silamurunense</i> sp. nov.*		
CCBAU 01550 ^T	<i>Astragalus membranaceus</i>	Inner Mongolia, China
CCBAU 45265	<i>Astragalus membranaceus</i>	Henan, China
CCBAU 45272	<i>Astragalus membranaceus</i>	Henan, China
CCBAU 25300	<i>Astragalus adsurgens</i>	Shandong, China
<i>Mesorhizobium loti</i>		
NZP 2213 ^T (=LMG 6125 ^T =USDA 3471 ^T)	<i>Lotus corniculatus</i>	New Zealand
NZP 2234	<i>Lotus corniculatus</i>	New Zealand
<i>Mesorhizobium amorphae</i> ACCC 19665 ^T (=ICMP 15022 ^T =LMG 18977 ^T)	<i>Amorpha fruticosa</i>	Beijing, China
<i>Mesorhizobium huakuii</i> CCBAU 2609 ^T (=NBRC 15243 ^T =USDA 4779 ^T)	<i>Astragalus sinicus</i>	Nanjing, China
<i>Mesorhizobium mediterraneum</i> USDA 3392 ^T (=UPM-Ca36 ^T)	<i>Cicer arietinum</i>	Spain
<i>Mesorhizobium plurifarum</i> LMG 11892 ^T (=ICMP 13640 ^T)	<i>Acacia senegal</i>	Senegal
<i>Mesorhizobium septentrionale</i> SDW014 ^T (=HAMB1 2582 ^T)	<i>Astragalus adsurgens</i>	Liaoning, China
<i>Mesorhizobium temperatum</i> SDW018 ^T (=HAMB1 2583 ^T)	<i>Astragalus adsurgens</i>	Liaoning, China
<i>Mesorhizobium tianshanense</i> CCBAU 3306 ^T (=USDA 3592 ^T =A-1BS ^T)	<i>Glycyrrhiza pallidiflora</i>	Xinjiang, China
<i>Mesorhizobium opportunistum</i> LMG 24607 ^T	<i>Biserrula pelecinus</i>	Australia

*These four strains represent a novel group containing 29 strains.

Fig. 1. Neighbour-joining tree based on sequence distance matrix (Jukes-Cantor) of 16S rRNA gene sequences showing the relationships among *Mesorhizobium silamurunense* sp. nov. and recognized species of the genus *Mesorhizobium*. Bootstrap confidence levels are indicated at the nodes. Bar, 0.01 expected changes per site.

representative strains were also identical and most similar to *M. plurifarum* LMG 11892^T with 95.7% gene sequence similarity. The four strains had almost identical sequences for a 487 bp intragenic fragment of the *atpD* gene and a 590 bp internal region of the *glnII* gene. They were also most similar to *M. plurifarum* strains ICMP 13640^T and LMG 11892^T respectively, with 93.8% and 93.6% sequence similarity for *atpD* and *glnII* genes, respectively (Fig. 2). The phylogenetic relationships based on the four house-keeping gene sequences were consistent with that obtained for 16S rRNA gene sequences.

Three representative strains of the novel group (CCBAU 01550^T, CCBAU 45272 and CCBAU 45265) and the type strains of eight closely related species of the genus *Mesorhizobium* were subjected to SDS-PAGE analysis of whole-cell soluble proteins as described previously (Tan *et al.*, 1997).

Digitization, normalization and numerical analyses of the protein profiles were performed with GelCompar II version 4.5 software (Applied Maths). The similarity of protein patterns between strain pairs was expressed by Pearson's coefficient and a UPGMA dendrogram was constructed (Vauterin & Vauterin, 1992). Strains CCBAU 01550^T, CCBAU 45272 and CCBAU 45265 formed a cluster with strikingly similar protein patterns, indicating their considerable homogeneity, but they were significantly different from the protein profiles of related species of the genus *Mesorhizobium* (Fig. S1 available in IJSEM Online). These results also demonstrated that strains CCBAU 01550^T, CCBAU 45272 and CCBAU 45265 were not clones.

Analysis of cellular fatty acids is a useful method to characterize and identify microbes, especially in the study

Fig. 2. Comparison of *atpD* (a), *recA* (b), *glnII* (c) and *rpoB* (d) gene phylogenies, showing the relationships among *Mesorhizobium silamurunense* sp. nov. and recognized species of the genus *Mesorhizobium*. Trees were constructed by the neighbour-joining method with a Kimura two-parameter distance matrix. Bootstrap values (%) are based on 1000 replicates and are shown at each node. Bars, 0.01 (a, c, d) or 0.02 (b) expected changes per site.

of species of the genus *Mesorhizobium* (Schutter & Dick, 2000; Tighe *et al.*, 2000). For analysis of cellular fatty acids, strains were grown on YMA medium (pH 7.0) (Vincent, 1970) at 28 °C. Cells of the tested strains in the stationary growth phase were harvested and approximately 40 mg were used for the preparation and extraction of fatty acid methyl esters according to methods described by Sasser (1990). Identification of these fatty acid methyl esters was completed with the MIDI Sherlock Microbial Identification System version 6.0 using the TSBA6 database for identification of the peaks. The comparison of fatty acid profiles between strain CCBAU 01550^T and six related species of the genus *Mesorhizobium* (*M. loti* N2P 2234, *M. plurifarium* LMG 11892^T, *M. huakuii* CCBAU 2609^T, *M. septentrionale* SDW014^T, *M. amorphae* ACCC 19665^T and *M. opportunistum* LMG 24607^T) is shown in Table S1. Strain CCBAU 01550^T could be assigned to the genus *Mesorhizobium* because it lacked 20:3 ω 6,9,12c and summed feature 2 (12:0, unknown ECL 10.928, 16:1 iso I and/or 14:0 3-OH), but contained 17:0 iso fatty acids (Tighe *et al.*, 2000). Furthermore, strain CCBAU 01550^T had a distinctly different

fatty acid profile compared to the other recognized species of the genus *Mesorhizobium* (Table S1).

DNA–DNA hybridization was performed using the spectrophotometric method (De Ley, 1970). The DNA G+C content was measured using the thermal denaturation (T_m) method of Marmur & Doty (1962) using *Escherichia coli* K-12 as the standard. The DNA G+C content of strain CCBAU 01550^T was 62.4 mol%, which was within the range reported for the genus *Mesorhizobium* (59–64 mol%) (Jarvis *et al.*, 1997). DNA–DNA relatedness was greater than 80 % among the representative strains of the novel group and ranged from 12.4 to 38.5 % between CCBAU 01550^T and the type strains of related species of the genus *Mesorhizobium* (Table 2). Taking the recommended species threshold of 70 % DNA–DNA relatedness (Graham *et al.*, 1991; Wayne *et al.*, 1987), these results indicated that the novel group should be considered as a novel species in the genus of *Mesorhizobium*.

Phenotypic characteristics of CCBAU 01550^T, CCBAU 45265, CCBAU 45272 and CCBAU 25300 and the type strains of closely related species of the genus were determined and

Table 2. DNA–DNA relatedness (%) between strain CCBAU 01550^T and the type strains of related species of the genus *Mesorhizobium*

Strain	DNA–DNA relatedness (%) with CCBAU 01550 ^T
CCBAU 45265	84.1
CCBAU 45272	85.4
<i>M. plurifarium</i> LMG 11892 ^T	38.5
<i>M. loti</i> NZP 2213 ^T	19.3
<i>M. septentrionale</i> SDW014 ^T	38.2
<i>M. amorphae</i> ACCC 19665 ^T	32.3
<i>M. temperatum</i> SDW018 ^T	38.0
<i>M. huakuii</i> CCBAU 2609 ^T	28.5
<i>M. mediterraneum</i> USDA 3392 ^T	23.8
<i>M. tianshanense</i> CCBAU 3306 ^T	12.4

compared 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 dyes; and the ranges of NaCl concentration, pH and temperature for growth (Gao *et al.*, 1994). Biochemical tests including catalase and oxidase production; methyl red and Voges–Proskauer reactions; and hydrolysis of casein, gelatin, starch, DNA and Tween 80 were performed according to Smibert & Krieg (1994). Production of hydrogen sulfide from cysteine, and production of indole from tryptophan were examined as described by Barrow & Feltham (2003). The results demonstrated that the novel group could be distinguished from related species of the genus *Mesorhizobium* by a combination of phenotypic characteristics, such as growth at pH 10.0, but not in 1 % (w/v) NaCl; utilization of glucose, D-mannose, D-fructose, melibiose, D-glutamic acid, L-phenylalanine and L-valine, but not D-arabinose raffinose, DL-asparagine, and L-arginine; resistance to ($\mu\text{g ml}^{-1}$) ampicillin (5), erythromycin (100) and Congo red (0.1), but sensitivity to kanamycin (50), streptomycin (5), bromothymol blue (0.1) and erythrosine bluish (0.1) (Table 3). Additional features are presented in the species description. In the cluster analysis based upon phenotypic features, strain CCBAU 01550^T was clearly separated from the type strains of related species of the genus *Mesorhizobium* (Fig. S3).

The symbiotic genes (*nod* and *nif*) are required for the successful establishment of highly specific symbiosis between rhizobia and legumes. Partial *nodC* (931 bp) and *nifH* (784 bp) gene fragments were amplified with the primer pairs *nodCF/nodCI* and *nifHF/nifHI*, respectively, using protocols described by Laguerre *et al.* (2001). Neighbour-joining trees were constructed with the same methods used for the housekeeping gene analyses. In the phylogenetic tree based on *nodC* gene sequences, strains CCBAU 01550^T, CCBAU 45272, CCBAU 45265 and CCBAU 25300 formed a cluster sharing sequence similarities from 99.1 % to 100 %. They were most similar to *M. septentrionale* SDW014^T (99.3 % sequence similarity) (Fig. S2a). In the phylogenetic tree based

Table 3. Differential phenotypic characteristics of strains of *Mesorhizobium silamurunense* sp. nov. and closely related species of the genus *Mesorhizobium*

Strains: 1, CCBAU 01550^T; 2, CCBAU 25300; 3, CCBAU 45265; 4, CCBAU 45272; 5, *M. plurifarium* LMG 11892^T; 6, *M. septentrionale* SDW014^T; 7, *M. amorphae* ACCC 19665^T; 8, *M. huakuii* CCBAU 2609^T; 9, *M. tianshanense* CCBAU 3306^T. +, Growth or resistant; –, no growth or sensitive.

Characteristic	1	2	3	4	5	6	7	8	9
Growth at:									
NaCl (1 %)	–	–	–	–	+	–	–	+	–
pH 10.0	+	+	+	+	+	–	–	+	+
Sole carbon source									
Glucose	+	+	+	+	+	–	+	+	+
D-Arabinose	–	–	–	–	+	–	–	+	+
D-Mannose	+	+	+	+	+	+	–	+	–
D-Fructose	+	+	+	+	+	–	+	+	+
Melibiose	+	+	+	+	+	–	+	+	+
Raffinose	–	–	–	–	+	+	+	+	–
D-Xylose	+	+	+	+	+	–	+	+	+
Sorbose	–	–	–	–	+	–	–	–	–
Inulin	–	+	+	–	+	+	–	–	–
Sodium acetate	+	+	+	–	+	–	–	+	–
Sodium citrate	–	–	–	–	+	–	+	–	+
Sodium pyruvate	–	–	–	–	+	–	–	+	+
Sodium sorbate	+	+	+	+	+	+	–	+	–
DL-Sodium malate	+	+	+	+	+	–	+	+	+
Galactitol	+	+	+	–	–	–	–	–	–
meso-Erythritol	+	+	+	–	–	–	+	–	+
Lactose	–	–	–	–	+	–	–	+	+
Sole nitrogen source									
L-Aspartic acid	+	+	+	–	+	–	–	+	–
D-Glutamic acid	+	+	+	+	+	–	–	+	–
L-Phenylalanine	+	+	+	+	+	–	–	+	–
L-Valine	+	+	+	+	+	+	+	–	–
Resistance to ($\mu\text{g ml}^{-1}$):									
Ampicillin (5)	+	+	+	+	+	–	–	–	+
Kanamycin sulfate (50)	–	–	–	–	+	–	–	+	–
Streptomycin sulfate (5)	–	–	–	–	+	–	–	+	–
Erythromycin (100)	+	+	+	+	+	–	–	+	+
Chloramphenicol (5)	–	+	–	+	–	–	+	+	+
Tolerance of (%):									
Bromothymol blue (0.1)	–	–	–	–	+	–	–	–	–
Congo red (0.1)	+	+	+	+	+	+	+	+	–
Erythrosine bluish (0.1)	–	–	–	–	+	–	–	–	–
Sodium deoxycholate (0.1)	+	+	–	+	+	–	–	+	–

on *nifH* gene sequences, the four novel strains were divided into three lineages (Fig. S2b): 1, strain CCBAU 45265 and CCBAU 45272 sharing 100 % sequence similarity; 2, strain CCBAU 01550^T and *M. septentrionale* SDW014^T sharing 97.2 % *nifH* gene sequence similarity; and 3, strain CCBAU 25300 sharing 90.9 % and 93.3 % *nifH* gene sequence similarity with the former two lineages, respectively. These results demonstrated that *nod* genes in the strains of the novel species may have the same origin, but *nif* genes may have

divergent origins, possibly via lateral gene transfer as reported by Sullivan & Ronson (1998).

Host range is an important feature of root-nodule bacteria. Cross-nodulation tests were performed for strain CCBAU 01550^T with the original plant (*Astragalus membranaceus*) and the other selected leguminous plants including *Astragalus adsurgens*, *Glycine max*, *Pisum sativum*, *Phaseolus vulgaris*, *Vigna radiate*, *Medicago sativa*, *Lotus corniculatus*, *Trifolium repens*, *Caragana intermedia*, *Lepedeza cuncta* and *Leucaena leucocephala*. The seeds of the leguminous plants were surface-sterilized, germinated and inoculated according to a standard procedure (Vincent, 1970). The inoculated seedlings were grown in Gibson tubes supplied 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 nodules were examined. Strain CCBAU 01550^T formed pink root nodules on *Astragalus membranaceus*, *Astragalus adsurgens* and *Caragana intermedia*, indicating that the nodules were effective for nitrogen fixation. White (ineffective) nodules were found on *Phaseolus vulgaris* inoculated with CCBAU 01550^T. No nodules were found on *Glycine max*, *Pisum sativum*, *Vigna radiate*, *Medicago sativa*, *Lotus corniculatus*, *Trifolium repens*, *Lepedeza cuncta* or *Leucaena leucocephala* inoculated with CCBAU 01550^T, or on non-inoculated control plants.

The differences in the phenotypic and genotypic characteristics of strains of the novel group indicated that they represented a novel species within the genus *Mesorhizobium*, for which the name *Mesorhizobium silamurunense* sp. nov. is proposed.

Description of *Mesorhizobium silamurunense* sp. nov.

Mesorhizobium silamurunense [si.la.mu.ru.nen'se. N.L. neut. adj. *silamurunense* of or belonging to silamurun (Yellow River in Mongolian), referring to the original isolation of this bacteria from the Yellow River valley].

Cells are Gram-negative, aerobic, motile, non-spore-forming rods, 1.2–1.8 µm wide by 2.1–4.2 µm long. Colonies on YMA plates are circular, convex, white and opaque, usually 1–2 mm in diameter within 5–7 days of incubation 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. Positive result in tests for catalase, indole production, hydrolysis of DNA, and litmus milk acid production, acid coagulation and peptonization. The ability to hydrolyse DNA varies among the novel isolates; strain CCBAU 45272 shows the highest DNA hydrolysis activity. Negative result in tests for oxidase, urease, nitrate reductase, L-phenylalanine deaminase, 3-ketolactose, H₂S production, and hydrolysis of starch, gelatin and Tween 80. Reduces litmus milk, methylene blue and Nile blue, but methyl red and Voges–Proskauer reactions are negative. Utilizes D-galactose, D-glucose, sucrose,

D-fructose, inositol, inulin (except strains CCBAU 01550^T and CCBAU 45272), DL-sodium malate, galactitol (except CCBAU 45272), meso-erythritol (except CCBAU 45272), maltose, D-mannose, melibiose, L-rhamnose, sodium acetate (except CCBAU 45272), sodium succinate, D-sorbitol, trehalose and D-xylose as sole carbon sources for growth, but not adipic acid, D-amygdalin, D-arabinose, calcium gluconate, calcium malonate, dextrin, melezitose, sodium pyruvate, raffinose, lactose, salicin, sodium citrate, sodium formate, sodium D-gluconate, hippuric acid, sorbose, soluble starch, syringic acid, potassium sodium tartrate, L-arginine, DL-asparagine, glycine, L-methionine and L-proline. Utilizes L-threonine, L-α-aminopropionic acid, L-arginine, L-aspartic acid (except strain CCBAU 45272), L-cystine, D-glutamic acid, L-glutamic acid, hypoxanthine, L-isoleucine, L-lysine, L-phenylalanine and L-valine as sole nitrogen sources. Resistant to (µg ml⁻¹) erythromycin (100), ampicillin (5), kanamycin sulfate (5), neomycin sulfate (5) and gentamicin sulfate (5). Sensitive to (µg ml⁻¹) streptomycin sulfate (5), tetracycline hydrochloride (5), ampicillin (50), chloramphenicol (50), kanamycin sulfate (50), neomycin sulfate (50), gentamicin sulfate (50) and erythromycin (300). Strains CCBAU 01550^T and CCBAU 45265 are sensitive to chloramphenicol (5 µg ml⁻¹), but strains CCBAU 25300 and CCBAU 45272 are resistant to it. Strains grow on YMA plates containing 0.1 % (w/v) Congo red or sodium deoxycholate (except strain CCBAU 45265), but do not grow in 1 % (w/v) NaCl in YMA. Synthesizes the following fatty acids (full details in Table S1): 12:0 3-OH, 14:0, 13:0 iso 3-OH, summed feature 3 (16:1 ω7c/16:1 ω6c and/or 16:1 ω6c/16:1 ω7c), 16:0, 17:0 iso, 17:1 ω8c, 17:0, summed feature 8 (18:1 ω7c and/or 18:1 ω6c), 18:0, 19:0 cyclo ω8c and 20:0.

The type strain, CCBAU 01550^T (=HAMBI 3029^T=LMG 24822^T), was isolated from the root nodules of *Astragalus membranaceus* collected from Inner Mongolia, China. The DNA G+C content of the type strain is 62.4 mol% (*T_m*).

Acknowledgements

This work was financed by the National Natural Science Foundation of China (project no. 30870004 and 30970004), Chinese Universities Scientific Fund (No. 2009JD19) and National Basic Research Program of China (2010CB126500). We thank Professor Martha E. Trujillo (Universidad de Salamanca, Spain) for providing thoughtful and constructive comments on the manuscript.

References

- Allen, O. N. & Allen, E. K. (1981). *The Leguminosae: A Source Book of Characteristics, Uses, and Nodulation*. Madison, WI: University of Wisconsin Press.
- Barrow, G. I. & Feltham, R. K. A. (2003). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.
- Brunel, B., Rome, S., Ziani, R. & Cleyet-Marel, J. C. (1996). Comparison of nucleotide diversity and symbiotic properties of *Rhizobium meliloti* populations from annual *Medicago* species. *FEMS Microbiol Ecol* 19, 71–82.

- Capela, D., Barloy-Hubler, F., Gouzy, J., Bothe, G., Ampe, F., Batut, J., Boistard, P., Becker, A., Boutry, M. & other authors (2001). Analysis of the chromosome sequence of the legume symbiont *Sinorhizobium meliloti* strain 1021. *Proc Natl Acad Sci U S A* **98**, 9877–9882.
- 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. M., Zhu, W. F., Bontemps, C., Young, J. P. & Wei, G. H. (2010). *Mesorhizobium alhagi* sp. nov., isolated from wild *Alhagi sparsifolia* in north-western China. *Int J Syst Evol Microbiol* **60**, 958–962.
- Chen, W. M., Zhu, W. F., Bontemps, C., Young, J. P. & Wei, G. H. (2011). *Mesorhizobium cameltorni* sp. nov., isolated from *Alhagi sparsifolia*. *Int J Syst Evol Microbiol* **61**, 574–579.
- 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., 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., 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., Lloyd-Macgilp, S. A. & Young, J. P. (2001). Phylogenies of *atpD* and *recA* support the small subunit rRNA-based classification of rhizobia. *Int J Syst Evol Microbiol* **51**, 2037–2048.
- Graham, P. H., Sadowsky, M. J., Keyser, H. H., Barnet, Y. M., Bradley, R. S., Cooper, J. E., DeLey, D. J. D., 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-nodulating bacteria. *Int J Syst Bacteriol* **41**, 582–587.
- 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., van Berkum, P., Amarger, N. & Prévost, D. (1997). Genetic diversity of rhizobial symbionts isolated from legume species within the genera *Astragalus*, *Oxytropis*, and *Onobrychis*. *Appl Environ Microbiol* **63**, 4748–4758.
- 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. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Martens, M., Delaere, M., Coopman, R., De Vos, P., Gillis, M. & Willems, A. (2007). Multilocus sequence analysis of *Ensifer* and related taxa. *Int J Syst Evol Microbiol* **57**, 489–503.
- 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.
- 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.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Sullivan, J. T. & Ronson, C. W. (1998). Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. *Proc Natl Acad Sci U S A* **95**, 5145–5149.
- 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., Xu, X. D., Wang, E. T., Gao, J. L., Martinez-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. (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. (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.
- Vincent, J. M. (1970). *A Manual for the Practical Study of Root Nodule Bacteria*. Oxford: Blackwell Scientific.
- Vinuesa, P., Silva, C., Werner, D. & Martínez-Romero, E. (2005). Population genetics and phylogenetic inference in bacterial molecular systematics: the roles of migration and recombination in *Bradyrhizobium* species cohesion and delineation. *Mol Phylogenet Evol* **34**, 29–54.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- 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.
- Wu, Z. Y., Raven, P. H. & Hong, D. Y. (editors) (2010). *Flora of China*. vol. 10, (*Fabaceae*). Beijing and St. Louis: Science Press and Missouri Botanical Garden Press.
- Zhao, C. T., Wang, E. T., Chen, W. F. & Chen, W. X. (2008). Diverse genomic species and evidences of symbiotic gene lateral transfer detected among the rhizobia associated with *Astragalus* species grown in the temperate regions of China. *FEMS Microbiol Lett* **286**, 263–273.
- Zhou, P. F., Chen, W. M. & Wei, G. H. (2010). *Mesorhizobium robiniae* sp. nov., isolated from root nodules of *Robinia pseudoacacia*. *Int J Syst Evol Microbiol* **60**, 2552–2556.