Mesorhizobium camelthorni sp. nov., isolated from *Alhagi sparsifolia*

Wei-Min Chen,¹ Wen-Fei Zhu,¹ Cyril Bontemps,² J. Peter W. Young² and Ge-Hong Wei¹

Correspondence

Ge-Hong Wei
weigehong@yahoo.com.cn

¹College of Life Sciences, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A & F University, Yangling, Shaanxi 712100, PR China

²Department of Biology, University of York, PO Box 373, York YO10 5YW, UK

Nine strains isolated from symbiotic root nodules on *Alhagi sparsifolia* were previously designated as representing genospecies I. Phylogenetic analyses indicated that genospecies I was related closely to *Mesorhizobium alhagi* (genospecies II), and clearly formed a new lineage within the genus *Mesorhizobium*. In this study, we differentiated genospecies I from recognized species of the genus *Mesorhizobium* based on phylogenetic analyses of additional core genes (*recA*, *glnA*), levels of DNA–DNA relatedness (<43.3%), fatty acid profile (58% C_{18:1ω7c}, 19% 11-methyl C_{18:1ω7c}), and biochemical and physiological characteristics. The nine strains are therefore considered to represent a novel species of the genus *Mesorhizobium*, for which the name *Mesorhizobium camelthorni* sp. nov. is proposed. The type strain is CCNWXJ 40-4^T (=HAMBI 3020^T =ACCC 14549^T).

The Xinjiang Autonomous Region of north-west China includes many areas with very poor soils and dry climatic conditions, such as the Taklamakan and Gurbantünggüt deserts. Despite the harsh conditions for plant growth in these areas, the legume *Alhagi sparsifolia* is well adapted to these arid zones. Its success relies in part on its vigorous root system, which can thrive and spread rapidly in many types of soils including sandy, clayey and alkaline and can reach up to 15 m below the soil surface to tap into the water table (Sprent, 2001; Zeng *et al.*, 2002; Siebert *et al.*, 2004). As with many other legumes, another key to the ecological success of *A. sparsifolia* is its ability to form nitrogen-fixing symbioses with soil bacteria (generically termed rhizobia). This commensalism provides the plant with enough nitrogen to enable its growth in nitrogen-poor soils where most other plants cannot grow.

In a previous study, we characterized 33 rhizobia isolated from *A. sparsifolia* which fell into three genospecies based on restriction analysis and sequencing of their 16S rRNA, *dnkA* and *dnaj* genes (Wei *et al.*, 2009). Among them, genospecies II (11 isolates) has been described as *Mesorhizobium alhagi* (Chen *et al.*, 2010). Here, we describe another group of nine isolates from the legume *A. sparsifolia*, previously defined as genospecies I, which

represents a novel species of the genus *Mesorhizobium* based on additional data comprising sequences of two additional core genes (*recA* and *glnA*), one additional symbiosis-related gene (*nifH*), fatty acid profile, levels of DNA–DNA relatedness and numerical taxonomy based on phenotypic characteristics, even though it shares high 16S rRNA gene sequence similarity with *M. alhagi* (genospecies II).

The 16S rRNA gene sequence of one representative strain from genospecies I, CCNWXJ 40-4^T, and of *M. alhagi* CCNWXJ12-2^T clustered in a new branch within the genus *Mesorhizobium*, and were identified as a new *Mesorhizobium* lineage (Wei *et al.*, 2009; Fig. 1a). Strain CCNWXJ 40-4^T shared 99.4% sequence similarity with *M. alhagi* CCNWXJ12-2^T and less than 97.8% with the type strains of other recognized species of the genus *Mesorhizobium*.

For three representative strains of genospecies I, the *recA*, *glnA* and *nifH* genes were amplified and sequenced (Turner & Young, 2000; Gaunt *et al.*, 2001; Laguerre *et al.*, 2001), and were aligned by using CLUSTAL X v.1.81 (Thompson *et al.*, 1997). Phylogenies were reconstructed by the neighbour-joining method with Kimura's two-parameter distance correction and 100 bootstrap replicates by using PHYLIP (Galtier *et al.*, 1996), and by the maximum-likelihood method with the GTR model and 100 bootstrap replicates by using PhyML (Guindon *et al.*, 2005).

The phylogenetic tree based on 440 bp of the *recA* gene differentiated the three novel strains clearly from

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the nine strains reported in this paper are GU220798–GU220806.

A supplementary table is available with the online version of this paper.

Fig. 1. Phylogenetic trees based on 16S rRNA (a), *recA* (b), *glnA* (c) and *nifH* (d) gene sequences, showing the relationships among the novel isolates and recognized species of the genus *Mesorhizobium*. Bootstrap percentages above 70 % are indicated for phylogenies reconstructed by the neighbour-joining (left) and maximum-likelihood (right) methods. Bars, 0.005 (a), 0.01 (b), 0.01 (c) and 0.02 (d) nucleotide substitutions per site.

recognized species of the genus *Mesorhizobium* (Fig. 1b), in which they formed a single clade. Strain CCNWXJ 40-4^T shared 100 % *recA* gene sequence similarity with the other two isolates (CCNWXJ 16-1 and CCNWXJ 32-3). Levels of *recA* gene sequence similarity with the type strains of recognized species of the genus *Mesorhizobium*, however, were less than 93.3 % (except 97.1 % with *M. alhagi* CCNWXJ12-2^T). The tree based on 539 bp of the *glnA* gene (Fig. 1c) was very similar: strain CCNWXJ 40-4^T shared 100 % *glnA* gene sequence similarity with strain CCNWXJ 32-3, 99.8 % with strain CCNWXJ 16-1 and 96.2 % with *M. alhagi* CCNWXJ12-2^T, but only 90.7–93.3 % with the type strains of other species of the genus *Mesorhizobium*.

Sequence determination of the *nodA* and *nodC* genes was described previously (Wei *et al.*, 2009); most *A. sparsifolia* symbionts harboured closely related *nodA* and *nodC* genes that formed a new lineage with *M. alhagi* CCNWXJ12-2^T. The *nifH* gene encodes part of the enzyme nitrogenase, a key enzyme necessary for nitrogen fixation in rhizobia that allows them to form symbioses with legumes. Based on *nifH* gene sequence analysis, the three novel genospecies I strains were intermingled with those of *M. alhagi* in a well-supported clade (100 % bootstrap support) that did not include any other of the database sequences investigated (Fig. 1d). This result differed from those observed with housekeeping gene phylogenies in which genospecies I and *M. alhagi* were always found in distinct clades. This incongruence between housekeeping genes and a symbiosis-related gene suggests the potential for *nifH* lateral gene transfer between genospecies I and *M. alhagi*, as previously suggested for the nodulation genes (Wei *et al.*, 2009).

Total DNA was extracted from the nine test strains and reference strains by using the method of Marmur (1961). The DNA G+C content was measured by using the thermal denaturation method of Marmur & Doty (1962) and *Escherichia coli* K-12 as standard. Levels of DNA–DNA relatedness were determined by using the spectrophotometric method of De Ley *et al.* (1970). DNA–DNA hybridization was carried out at 100 °C for denaturation and 78 °C for renaturation. The DNA G+C content of strain CCNWXJ 40-4^T was 63.7 mol% (Table 1), within the range reported for recognized species of the genus *Mesorhizobium* (59–64 mol%) (Jarvis *et al.*, 1997), and the *T_m* value was 79.6 °C. Levels of DNA–DNA relatedness between strain CCNWXJ 40-4^T and other genospecies I strains ranged from 91.4 to 99.6 %, but were less than 43.3 % with the type strains of recognized species of the genus *Mesorhizobium* (37.9 % with *M. alhagi* CCNWXJ12-2^T). Therefore, the strains in genospecies I can be regarded as representing a single novel species even though they were closely related to *M. alhagi* in the phylogenetic tree based on 16S rRNA gene sequences.

Fatty acid methyl esters were extracted and measured for strain CCNWXJ 40-4^T, *M. alhagi* CCNWXJ12-2^T, *M.*

ciceri USDA 3383^T and *M. loti* NZP 2213^T by using a standard protocol (see Supplementary Table S1 in IJSEM Online). The results indicated that strain CCNWXJ 40-4^T should be assigned to the genus *Mesorhizobium* as it lacked C_{20:3ω6}, 9, 12c and summed feature 3 (one or more of C_{12:0} aldehyde, unknown ECL 10.928, iso-C_{16:1} I and/or C_{14:0} 3-OH) and possessed iso-C_{17:0} fatty acids (Tighe *et al.*, 2000). Strain CCNWXJ 40-4^T differed from recognized species of the genus *Mesorhizobium* in containing higher concentrations of C_{18:1ω7c}.

In total, 125 phenotypic characteristics covering utilization of sole carbon and nitrogen sources, resistance to antibiotics, tolerance to dyes and other chemicals, tolerance to different concentrations of NaCl, temperature and pH ranges for growth, and other characteristics useful for diagnostic purposes with rhizobia were tested for the novel strains. Phenotypic characteristics were analysed by the unweighted pair group method with arithmetic averages (UPGMA) (Sneath & Sokal, 1973). Based on the results of cluster analysis (Fig. 2), the nine isolates from *A. sparsifolia* formed one group while the reference strains were grouped according to species at a similarity level of 76.5 %. The mean similarity for the isolates ranged from 82.0 % for strain CCNWXJ 32-3 to 98.0 % for strain CCNWXJ 25-1. Differential characteristics between strain CCNWXJ 40-4^T and its closest relatives are shown in Table 2 and further features are presented in the species description below.

In nodulation tests, all nine test strains formed pink nodules on the roots of *A. sparsifolia*, which indicated that the nodules were effective in fixing nitrogen, and no nodules formed on the uninoculated control plants in any experiment. In cross-infection tests with legumes that can be found in the sampling areas, strain CCNWXJ 40-4^T was able to nodulate *Sophora alopecuroides*, *Glycyrrhiza inflate* and *Medicago sativa*, but not *Indigofera endecaphylla*, *Vicia cracca* or *Sophora flavescens*. This indicates that strain CCNWXJ 40-4^T has a narrow host range compared with *M. alhagi*, even though the two taxa have similar *nodA* and *nodC* genes.

Description of *Mesorhizobium camelthorni* sp. nov.

Mesorhizobium camelthorni (ca.mel.thor'ni. N.L. n. *camelthornum* camelthorn, a common name for leguminous plants of the genus *Alhagi* in China; N.L. gen. n. *camelthorni* of camelthorn, from which the type strain was isolated).

Gram-negative, aerobic, motile, non-spore-forming rods, 0.3–0.6 µm wide by 1.0–1.2 µm long. Colonies on yeast extract-malt extract agar (YMA) are white, opaque, circular and convex with diameter of 1–2 mm within 5–7 days at 28 °C; generation time ranges from 4.8 to 6.6 h. The optimum temperature and pH for growth are 28 °C and pH 7. Unable to grow on YMA at pH 5.0 or pH 12.0 or at

Table 1. Levels of DNA–DNA relatedness among strains representing the candidate species and the type strains of some recognized species in the genus *Mesorhizobium*

Data from this study. ND, Not determined.

Strain	Host plant	Geographical origin	T _m (°C)	DNA G + C content (mol%)	DNA–DNA relatedness with strain CCNWXJ40-4 ^T (%)
<i>M. camelthorni</i> sp. nov.					
CCNWXJ 40-4 ^T	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	79.6	63.7	100
CCNWXJ 16-1	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	79.0	62.4	97.4
CCNWXJ 32-3	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	79.2	62.9	95.5
CCNWXJ 31-1	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	79.6	63.7	99.4
CCNWXJ 26-3	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	78.6	61.6	91.4
CCNWXJ 34-1	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	79.5	63.6	99.6
CCNWXJ 38-1	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	77.4	59.5	98.7
CCNWAX 41-1	<i>Alhagi sparsifolia</i>	Anxi, Gansu, China	77.8	59.9	93.5
CCNWAX 25-1	<i>Alhagi sparsifolia</i>	Anxi, Gansu, China	78.4	61.2	92.4
<i>M. alhagi</i> CCNWXJ12-2 ^T	<i>Alhagi sparsifolia</i>	Alaer, Xinjiang, China	78.0	60.4	37.9
<i>M. ciceri</i> USDA 3383 ^T	<i>Cicer arietinum</i>	Spain	ND	ND	27.9
<i>M. loti</i> NZP 2213 ^T	<i>Lotus corniculatus</i>	New Zealand	ND	ND	30.3
<i>M. albiziae</i> CCBAU 61158 ^T	<i>Albizia kalkora</i>	Sichuan, China	ND	ND	43.3
<i>M. temperatum</i> SDW 018 ^T	<i>Astragalus adsurgens</i>	Liaoning, China	ND	ND	41.1
<i>M. plurifarium</i> LMG 11892 ^T	<i>Acacia senegal</i>	Senegal	ND	ND	25.8
<i>M. amorphae</i> ACCC 19665 ^T	<i>Amorpha fruticosa</i>	Beijing, China	ND	ND	3.8
<i>M. huakuii</i> CCBAU 2609 ^T	<i>Astragalus sinicus</i>	Nanjing, China	ND	ND	23.6
<i>M. chacoense</i> LMG 19008 ^T	<i>Prosopis alba</i>	Argentina	ND	ND	37.0
<i>M. mediterraneum</i> USDA 3392 ^T	<i>Cicer arietinum</i>	Spain	ND	ND	16.3
<i>M. tianshanense</i> CCBAU 3306 ^T	<i>Glycyrrhiza uralensis</i>	Xinjiang, China	ND	ND	25.7

Fig. 2. Dendrogram based on the results of numerical taxonomy showing the phenotypic similarities among the novel isolates and reference strains representing species of the genus *Mesorhizobium*. Clustering analysis was performed by using the UPGMA method.

Table 2. Differential characteristics between strain CCNWXJ 40-4^T and closely related species of the genus *Mesorhizobium*

Strains: 1, CCNWXJ 40-4^T; 2, *M. alhagi* CCNWXJ12-2^T; 3, *M. ciceri* USDA 3383^T; 4, *M. loti* NZP 2213^T. Data from this study.

Characteristic	1	2	3	4
Utilization as sole carbon source				
Sodium citrate	+	+	–	–
L-Rhamnose	+	+	+	+
D-Xylose	+	+	+	–
D-Fructose	+	+	+	–
D-Sorbitol	+	+	+	+
D-Arabinose	+	+	+	–
Inositol	+	+	+	+
Maltose	+	+	+	+
Sodium succinate	+	+	+	+
Sodium hippurate	–	–	–	–
Melibiose	–	–	–	–
Sodium tartrate	–	–	–	–
Utilization as sole nitrogen source				
D-Arginine	–	+	+	+
D-Aspartate	–	–	+	+
L-Cystine	+	+	+	+
L-Lysine	+	+	+	+
L-Phenylalanine	+	+	+	+
DL-Histidine	–	–	–	–
Glycine	+	+	–	–
Growth at/with:				
pH 5	–	–	+	–
pH 10	–	+	–	–
2 % NaCl	+	+	+	–
Resistance to:				
Ampicillin (50 µg ml ^{–1})	–	–	–	–
Erythromycin (5 µg ml ^{–1})	+	+	+	+
Erythromycin (50 µg ml ^{–1})	–	–	+	+
Streptomycin (5 µg ml ^{–1})	–	–	+	+
Gentamicin (5 µg ml ^{–1})	–	–	+	+
Chloramphenicol (5 µg ml ^{–1})	+	+	+	–
Neutral red 0.2 %	–	–	–	+
Bromothymol blue 0.2 %	–	–	+	–

10 °C. Utilizes sodium citrate, L-rhamnose, D-xylose, D-fructose, D-sorbitol, D-arabinose, inositol and sodium succinate, but not sodium hippurate, DL-histidine, melibiose, sodium tartrate or D-arginine. Unable to grow on YMA supplemented with methylene blue, malachite green, methyl green or methyl red. Sensitive to streptomycin, gentamicin sulfate and amikacin sulfate, but tolerant to erythromycin and gentamicin (all at 5 µg ml^{–1}). Able to grow on YMA in the presence of 2 % (w/v) NaCl. The DNA G+C content is 59.5–63.7 mol%. Can be differentiated clearly from other species of the genus *Mesorhizobium* by both genetic and phenotypic methods, including sequences of core genes (16S rRNA, *recA* and *glnA*), fatty acid composition and levels of DNA–DNA relatedness.

The type strain, CCNWXJ 40-4^T (=HAMBI 3020^T =ACCC 14549^T), was isolated from a root nodule of *A.*

sparsifolia in Alaer, Xinjiang Province, China. The DNA G+C content of strain CCNWXJ 40-4^T is 63.7 mol%.

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