Arch Microbiol (2002) 178 : 301–305
DOI 10.1007/s00203-002-0448-9

SHORT COMMUNICATION

En Tao Wang · M. Antonio Rogel · Xin Hua Sui
Wen Xin Chen · Esperanza Martínez-Romero
Peter van Berkum

Mesorhizobium amorphae, a rhizobial species that nodulates *Amorpha fruticosa*, is native to American soils

Received: 5 March 2002 / Revised: 6 May 2002 / Accepted: 17 May 2002 / Published online: 29 June 2002

Abstract *Amorpha fruticosa* was inoculated with rhizosphere soil from Iowa, USA, and 140 rhizobia isolated from root nodules were compared with *Mesorhizobium amorphae* originating from Chinese soils. PCR-RFLP patterns of the 16S rRNA gene from the isolates and from *M. amorphae* were the same. All isolates had a symbiotic plasmid of the same size with a single *nifH* gene. DNA:DNA hybridization values, DNA G+C content, and induced Nod factor patterns also were similar. We concluded that the four genotypes distinguished among 53 representative American isolates were *M. amorphae*. Since *A. fruticosa* is native to the Americas and is highly specific in its nodulation requirement, *M. amorphae* probably was transmitted to China.

Keywords *Amorpha fruticosa* · *Mesorhizobium amorphae* · Rhizobia · Symbiosis · Nitrogen fixation · Plant-microbe interaction

E.T. Wang
Departamento de Microbiología,
Escuela Nacional de Ciencias Biológicas, IPN,
México D. F., México

E.T. Wang · M.A. Rogel · E. Martínez-Romero
Programa de Ecología Microbiana Molecular,
Centro de Investigación sobre Fijación de Nitrógeno, UNAM,
Cuernavaca, Morelos, México

X.H. Sui · W.X. Chen
Department of Microbiology, College of Biology,
China Agricultural University,
Beijing 100094, People's Republic of China

P. van Berkum (✉)
Soybean Genomics and Improvement Laboratory,
Agriculture Research Service,
U. S. Department of Agriculture, ARS,
HH-4, Bldg010, BARC-West,
10300 Baltimore Blvd., Beltsville, MD 20705, USA
e-mail: pberkum@ba.ars.usda.gov,
Tel.: +1-301-504-7280, Fax: +1-301-504-5728

Introduction

Amorpha fruticosa is a leguminous shrub indigenous to North America (Allen and Allen 1981) and was introduced to China as a windbreak and as soil cover for erosion control. The majority of rhizobia forming a symbiosis with *A. fruticosa* growing in different regions of China were characterized as a species of *Mesorhizobium*. These *Mesorhizobium* isolates formed effective nitrogen-fixing symbioses with *A. fruticosa* (Wang et al. 1999). Wang et al. (1999) also identified that a few of the Chinese isolates were related to *Rhizobium* and *Bradyrhizobium*, but these cultures were ineffective for nitrogen fixation with *A. fruticosa*.

An extensive analysis of the isolates originating from China led to the description of the new species, *Mesorhizobium amorphae* (Wang et al. 1999). This species harbors a 930-kb symbiotic plasmid, which is unusual since, with the exception of *Mesorhizobium huakuii* (Guo et al. 1999; Xu and Murooka 1995; Zou et al. 1997), most of the *Mesorhizobium* spp. carry the symbiotic genes in their chromosomes. Nodulation appears to be specifically limited to *A. fruticosa* since other legume species formed no nodules in a cross-nodulation test with *M. amorphae* (Wang et al. 1999).

The characterization of rhizobia nodulating *A. fruticosa* included only isolates originating from China (Wang et al. 1999). Considering that North America is the center of origin of *A. fruticosa* (Allen and Allen 1981) and that formation of an effectively nitrogen-fixing symbiosis is specific for *M. amorphae*, we theorized that *M. amorphae* possibly also originated from North America and had found its way to China together with the host plant. Therefore, we isolated rhizobia using *A. fruticosa* as a trap host with soils obtained from around the roots of this legume growing in Iowa with the aim of comparing them with strains originating from China.

Material and methods

Isolation of bacteria

Rhizosphere soil samples were collected from *A. fruticosa* growing in an experimental field in Ames, Iowa and were used as a source of rhizobia for plants grown in Leonard jars (Leonard 1943) as described before (Wang et al. 1999). Isolations from root nodules were made using modified arabinose-gluconate (MAG) bacterial growth medium (van Berkum 1990) and the procedure described by Vincent (1970). The isolates and the reference strains used in our study (Table 1) were stored in 50% glycerol at –70 °C and are kept in the collection center of USDA.

DNA analyses

DNA was extracted from 5-ml peptone yeast extract (PY) cultures (Noel et al. 1984) in exponential growth phase using a DNA/RNA isolation kit (Chirgwin et al. 1979). The G+C content was measured according to the method of De Ley (1970). DNA:DNA hybridization was determined by using the filter method described by van Berkum et al. (1996). The procedure to amplify the16S rRNA genes by PCR using primers fD1 and rD1 (Weisburg et al. 1991) was as described previously (van Berkum et al. 1998). These PCR products were digested with *Msp*I, *Hin*fI, *Hha*I, or *Sau*3AI and the restriction fragments were separated according to molecular size by electrophoresis in 3% agarose gels (Wang et al. 1998). A subsample of each DNA preparation was digested with *Eco*RI or *Bam*HI for horizontal agarose gel electrophoresis, Southern transfer, and Southern hybridization using a 600-bp *nifH* fragment of *Rhizobium etli* CFN42 (Morett et al. 1988) as probe as described by Wang et al. (1998). The Eckhardt (1978) method, as modified by Hynes and McGregor (1990), was used to determine cellular plasmid content. These gels were prepared for Southern hybridization to identify pSym as described (Wang et al. 1998) using a 600-bp PCR-amplified *nifH* internal fragment from *Sinorhizobium meliloti* strain USDA 1002 (Eardly et al. 1992) as probe. The molecular size of each plasmid was estimated by comparing their migration distances with those of plasmids from *R. etli* strain CFN42 as reference (Wang et al. 1999).

Multilocus gel electrophoresis analysis

Protein extracts were prepared from 40 ml of PY broth cultures as described by Caballero-Mellado and Martínez-Romero (1994). Proteins were separated by electrophoresis in starch gels; eight enzymes, esterase (EST), glucose-6-phosphate dehydrogenase (G6P), NADP-dependent glutamate dehydrogenase (GD2), isocitrate dehydrogenase (IDH), indophenol oxidase (IPO), NADP-malate dehydrogenase (ME), phosphoglucose isomerase (PGI) and phosphoglucomutase (PGM), were analyzed by selective staining as described by Selander et al. (1986). The method of Nei and Li (1979) was used for cluster analysis of the data.

Nod factors

Nod factors were analyzed according to the method of Laeremans et al. (1996). A mixture of apigenin, naringenin and chrysin, each at a final concentration of 0.4 µM, was used to induce *nod* gene expression. Products, labeled with 0.1 µCi of D-[^{14}C-(U)]-glucosamine, were separated by thin-layer chromatography and visualized by radioautography. *Rhizobium tropici* strain CFN299 was used as a reference.

Results and discussion

All 140 isolates (Table 1) obtained from individual nodules were slow-growing, acid-producing rhizobia that on MAG solid media after 7 days incubation at 28 °C formed single colonies less than 1 mm in diameter. Each of the

Table 1 Bacteria used in this work and their relevant characteristics. The molecular sizes of the plasmids were estimated according to their mobility in 0.7% Eckhardt gels (Eckhardt 1978) using the computer program of Rhoads and Roufa (1989) and plasmids of *Rhizobium etli* CFN42 as molecular size reference markers. *ET* Electrophoretic type based on Multilocus gel electrophoresis analysis of eight enzyme loci

Isolate or strain	ET	Plasmids (kb)	Source or reference
Isolates from the USA soils			
1–1, 3–5, 5–2, 6–7, 7–1, 7–3, 7–5, 9–1, 9–3, 10–2, 10–4, 11–2, 11–4, 11–15	1	(930, 550, 250)	This study
6–4, 7–6, 8–6, 11–1	1	(930, 550)	This study
9–5, 10–1	1	(930, 250)	This study
1–2, 1–3, 2–1, 2–32, 2–33, 2–36, 2–38, 2–39, 3–1, 3–2, 3–3, 3–4, 4–1, 4–2, 4–3, 4–4, 5–1, 5–8, 6–1, 6–3, 6–5, 7–4, 8–1, 8–2, 8–3, 8–4, 8–5, 9–2, 9–4, 10–5, 11–5	2	(930)	This study
1–6	3	(730)	This study
5–4	4	(730, 360)	This study
Reference strains from China			
M. amorphae, B289	9	(930)	Wang et al. (1999)
M. amorphae, ACCC19660	8	(930, 150)	Wang et al. (1999)
M. amorphae, B109	10	(930)	Wang et al. (1999)
M. amorphae, ACCC19665	5	(930)	Wang et al. (1999)
M. amorphae, ACCC19663	6	(930)	Wang et al. (1999)
M. amorphae, SH190012	7	(930)	Wang et al. (1999)
Isolate B276 from *A. fruticosa*	11	(930)	Wang et al. (1999)
Isolate SH1503 from *A. fruticosa*	12	(930)	Wang et al. (1999)
Cloning plasmid			
PEM15 (pSup205 derivative)		*nif* genes of *R. etli* CFN42	Morett et al. (1988)

Table 2 Allele variation of eight metabolic enzymes among rhizobia isolated from nodules of *Amorpha fruticosa* and originating from Iowa. The number of alleles implies bands with different mobility for each enzyme. $h=[1-\Sigma(Xi)^2]\times[n/(n-1)]$. *H* is the average of the *h* values, as described by Selander et al. (1986)

Enzyme locus[a]	Four ETs of the USA isolates		Six ETs of Chinese strains		All ten *M. amorphae* ETs	
	Number of alleles[b]	Genetic diversity (h)[c]	Number of alleles	Genetic diversity (h)	Number of alleles	Genetic diversity (h)
IDH	3	0.833	2	0.600	5	0.844
IPO	2	0.500	1	0.000	2	0.525
ME	2	0.500	1	0.000	2	0.200
GD2	1	0.000	1	0.000	1	0.000
PGM	2	0.500	1	0.000	2	0.200
G6P	1	0.000	1	0.000	1	0.000
PGI	2	0.500	3	0.600	4	0.644
EST	3	0.833	3	0.733	5	0.800
Mean	2.0	H=0.458	1.6	H=0.214	2.8	H=0.44

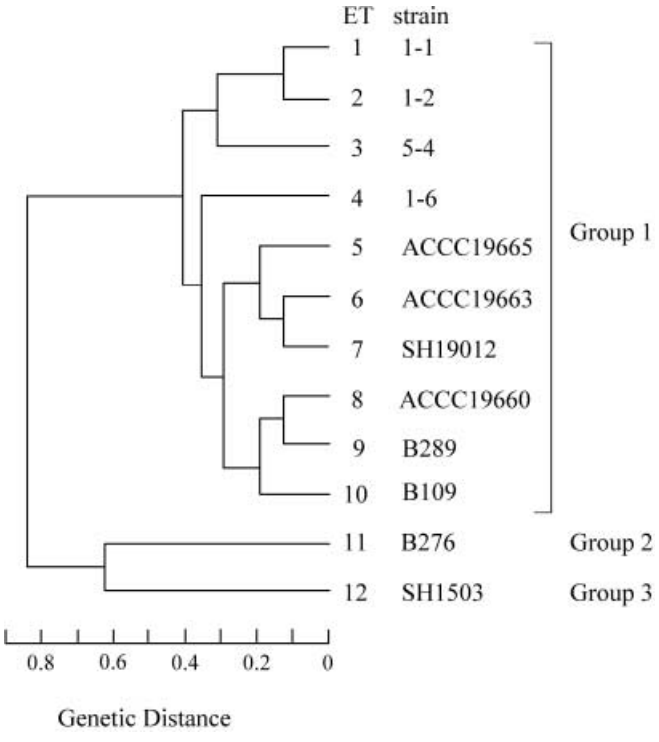

Fig. 1 Data from multilocus gel electrophoresis analysis using eight enzyme loci to construct a dendrogram showing the genetic relationships among the *Amorpha fruticosa*-nodulating rhizobia originating from the American and Chinese soils. The strains ACCC 19665, B276 and SH15003 represent groups 1, 2 and 3 originally defined according to Wang et al. (1999). For the number of strains in each electrophoretic type, see to Table 1

140 isolates had 16S rRNA gene restriction patterns identical with that of the *M. amorphae* type strain (data not shown). The DNA G+C content of randomly chosen isolates 1–1 and 1–2 were 63 mol% and 62 mol%, respectively. This value is within the range for species of *Mesorhizobium* and is close to 64 mol%, the value reported for *M. amorphae* (Wang et al. 1999). DNA:DNA hybridization between isolate 1–1 and the *M. amorphae* type strain ACCC19665 was 70±8%, which indicated close genetic homogeneity between the two strains representing the isolates from Iowa and from China, respectively.

Genetic diversity among 53 randomly chosen isolates was limited (Table 2) and only four electrophoretic types (ETs) were identified (Table 1). The majority of the isolates had enzyme profiles characteristic of either ET1 or ET2. Only the single isolates 1–6 and 5–4 had unique enzyme profiles and were assigned to ET3 and ET4, respectively. Although distinct from the reference strains originating from China, the American isolates were placed with the group of ETs formed by *M. amorphae* (Fig. 1). The mean genetic diversity among the isolates of American origin was H=0.42, which was more than among *M. amorphae* originating from China (H=0.21).

Among the 140 isolates, six distinctive plasmid patterns were observed (Table 1). The number of plasmids among these isolates ranged from one to three and most contained a 930-kb plasmid that in strains 1–1, 1–2, 1–3, 3–5, 7–3 and 9–5 was identified by Southern hybridization as the symbiotic plasmid. Isolates 1–6 and 5–4 were the exception because they harbored a symbiotic plasmid of 730 kb instead of 930 kb. The presence of a 930-kb symbiotic plasmid in most of the American isolates concurs with a symbiotic plasmid of equal molecular size in strains originating from China (Wang et al. 1999). In the case of isolates 1–1, 1–6, and 5–4, one hybridization fragment carrying *nifH* was observed in both restriction digests. This result is similar to that obtained with the type strain ACCC19665 of *M. amorphae* (Fig. 2). However, the symbiotic plasmids among these four cultures probably do differ in base sequences because the molecular sizes of the hybridizing signals varied among them in one or both digests (Fig. 2). The *nod* genes of the cultures originating from China and the Americas probably produce similar or identical products since Nod factor TLC patterns among the *M. amorphae* type strain and isolates 1–2 and 3–5 were identical (data not shown).

North America is the center of origin of *A. fruticosa*; therefore, the presence of rhizobia that form nitrogen-fixing symbioses with this legume in soils of this continent can be expected. However, it was unknown whether the isolates from China and the Americas would be similar or different. Unknown also was whether or not *M. amorphae* was an opportunistic rhizobial species of another legume native to China, with an inherent ability to nodulate *A. fruticosa*. The rhizosphere soils collected from roots of

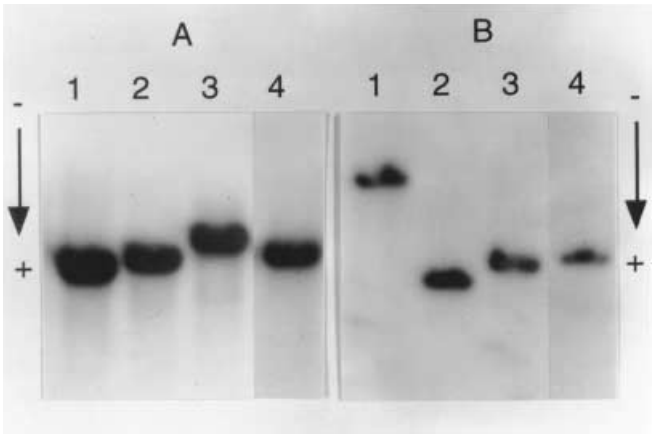

Fig. 2 Southern hybridization analysis of the *nifH* gene among the *A. fruticosa*-nodulating isolates from the American soils compared with that of *M. amorphae* type strain ACCC19665. Total DNA was digested with (A) *Eco*RI or (B) *Bam*HI and hybridized to a 600-bp PCR-amplified *nifH* fragment from *Sinorhizobium meliloti* USDA1002 using the procedure of Eardly et al. (1992). *Lane 1* Isolate 1–1, *lane 2* isolate 1–6, *lane 3* isolate 5–4, *lane 4* type strain for *M. amorphae*, ACCC19665

A. fruticosa growing in Iowa, USA, harbored bacteria with characteristics common to *M. amorphae*. Therefore, it must be concluded that both soils of China and the Americas contain *M. amorphae* and that this bacterial species is a natural microsymbiont of *A. fruticosa*. Considering our data, we would disagree with a conclusion that *M. amorphae* evolved in Chinese soils and nodulated *A. fruticosa* by being opportunistic or by acquisition of the genetic information through genetic transfer and recombination.

The difficulty is to explain the presence of *M. amorphae* in Chinese soils considering that this legume is not inoculated when grown in China. Two theories have been proposed based upon the study of common bean (*Phaseolus vulgaris*) and the rhizobia that form symbioses with this leguminous crop species. The first theory, to explain the presence of presumptive *R. etli* and *R. tropici* in the soils of Europe and Western Africa (Diouf et al. 2000; Herrera-Cervera et al. 1999; Sessitsch et al. 1997), is that the rhizobia of bean were transmitted from the Americas along with the grain when it was exported (Perez-Ramirez et al. 1998). This suggestion would seem plausible, but the usefulness of bean as a model for the study of the distribution of rhizobia from the Americas to other geographic locations is hindered by the promiscuity of this legume as a host (Michiels et al. 1998). For example, *Rhizobium mongolense* nodulates and forms effectively nitrogen-fixing symbioses with bean even though *Medicago ruthenica* is its natural host and both plant and microbe originated from the steppes of Inner Mongolia, where bean has not been introduced (van Berkum et al. 1998). A second theory, to explain nodulation of bean by *Rhizobium leguminosarum*, *Rhizobium gallicum* and *Rhizobium giardinii*, includes the exchange of the genes for symbiosis with bean from unintentionally introduced *R. etli* to native bacterial populations present in the fields where bean is cultivated outside of the Americas (Herrera-Cervera et al. 1999). This suggestion also would seem possible, except for the distribution of particularly *R. leguminosarum* and *R. gallicum*. For example, Illinois is the origin of the type strain of *R. leguminosarum* (Keyser and Griffin 1987); the Mexican bean isolate FL27 is a strain of *R. gallicum* that nodulates at least three other hosts besides bean (Sessitch et al. 1997) and bean-nodulating *R. leguminosarum* are present in soils of Colombia (Eardly et al. 1995) and the prairies of North America (Graham et al. 1999). Also, the presence of just a single copy of *nifH* in *R. gallicum* (Amarger et al. 1997) is inconsistent with three copies in *R. etli* (Martinez et al. 1985). Possibly the theory is more convincing in the case of *R. giardinii*; however, distribution of this species appears very limited (Amarger et al. 1997; Herrera-Cervera et al. 1999) and on bean the symbiosis is either ineffective or very poor (Amarger et al. 1997). Although some arguments may be made for genetic differences among the two biovars of both *R. gallicum* and *R. giardinii*, the promiscuous nature of bean is as likely an explanation for the nodulation of this legume host by both species as is the acquisition of the genetic determinants from *R. etli*. Our data are significant by providing more substantial support for a possibility that rhizobia may be transmitted between continents. The basis of this support is that *A. fruticosa* and *M. amorphae* are specific for their nodulation requirements, and the bacteria from soils of China and Iowa are genetically more homogeneous.

Acknowledgements This work was partly supported by USDA-FAS-OICD-RSED, USA and by DGAPA IN203097 from UNAM, Mexico. We thank K. Lee Nash for technical support.

References

Allen ON, Allen EK (1981) The leguminosae: a source book of characteristics, uses, and nodulation. University of Wisconsin Press, Madison, Wisconsin

Amarger N, Macheret V, Laguerre G (1997) *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris* nodules. Int J Syst Bacteriol 47:996–1006

Caballero-Mellado J, Martínez-Romero E (1994) Limited genetic diversity in the endophytic sugarcane bacterium *Acetobacter diazotrophicus*. Appl Environ Microbiol 60: 1532–1537

Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biological active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299

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

Diouf A, de Lajude P, Neyra M, Kersters K, Monique G, Martinez E, Gueye M (2000) Polyphasic characterization of rhizobia that nodulate *Phaseolus vulgaris* in West Africa (Senegal and Gambia). Int J Syst Evol Microbiol 50:159–170

Eardly BD, Young JPW, Selander RK (1992) Phylogenetic position of *Rhizobium* sp. strain Or 191, a symbiont of both *Medicago sativa* and *Phaseolus vulgaris*, based on partial sequences of the 16S rRNA and *nifH* genes. Appl Environ Microbiol 58: 1809–1815

Eardly BD, Wang FS, Whittam TS, Selander RK (1995) Species limits in *Rhizobium* populations that nodulate the common bean (*Phaseolus vulgaris)*. Appl Environ Microbiol 61:507–512

Eckhardt T (1978) A rapid method for the identification of plasmid deoxyribonucleic acid in bacteria. Plasmid 1:584–588

Graham PH, Ballen KG, Montealegre C, Jones RK, Fischer B, Luque E (1999) Characterization of rhizobia associated with Dalea spp. in natural prairies and revegetation areas in Minnesota. In: Martinez E, Hernandez G (eds) Highlights of nitrogen fixation research. Kluwer Academic/Plenum, New York, pp 69–75

Guo XW, Zhang XX, Zhang ZM, Li FD (1999) Characterization of *Astragalus sinicus* rhizobia by restriction fragment length polymorphism analysis of chromosomal and nodulation genes regions. Curr Microbiol 39:358–0364

Herrera-Cervera JA, Caballero-Mellado J, Laguerre G, Tichy HV, Requena N, Amarger N, Martínez-Romero E, Olivares J, Sanjuán J (1999) At least five rhizobial species nodulate *Phaseolus vulgaris* in a Spanish soil. FEMS Microbiol Ecol 30:87–97

Hynes MF, McGregor NF (1990) Two plasmids other than the nodulation plasmid are necessary for formation of nitrogen-fixing nodules by *Rhizobium leguminosarum*. Mol Microbiol 4: 567–574

Keyser HH, Griffin RF (1987) Beltsville Rhizobium Culture Collection Catalogue. US Department of Agriculture, Agricultural Research Service, ARS-60, p.79

Laeremans T, Caluwaerts I, Verreth C, Rogel MA, Vanderleyden J, Martínez-Romero E (1996) Isolation and characterization of the *Rhizobium tropici* Nod factor sulfation genes. Mol Plant-Microbe Interact 9:492–500

Leonard LT (1943) A simple assembly for use in the testing for cultures of rhizobia. J Bacteriol 45:523–527

Martinez E, Pardo MA, Palacios R, Cevallos MA (1985) Reiteration of nitrogen fixation gene sequences and specificity of *Rhizobium* in nodulation and nitrogen fixation in *Phaseolus vulgaris*. J Gen Microbiol 131:1779–1786

Michiels J, Dombrecht B, Vermeiren N, Xi C, Luyten E, Vanderleyden J (1998) *Phaseolus vulgaris* is a non-selective host for nodulation. FEMS Microbiol Ecol 26:193–205

Morett E, Moreno S, Espin G (1988) Transcription analysis of the three *nifH* genes of *Rhizobium phaseoli* with gene fusion. Mol Gen Genet 213:499–504

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Noel KD, Sanchez F, Fernandez F, Leemans J, Cevallos A (1984) *Rhizobium phaseoli* symbiotic mutants with transposon *Tn*5 insertions. J Bacteriol 158:148–155

Pérez-Ramirez NO, Rogel-Hernández MA, Wang ET, Martínez-Romero E (1998) Seeds of *Phaseolus vulgaris* bean carry *Rhizobium etli*. FEMS Microbiol Ecol 26:289–296

Rhoads DD, Roufa DJ (1989) SEQAID II tm, version 3.5. Molecular Genetics Laboratory, Kansas State University, Kansas

Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS (1986) Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 51:873–884

Sessitsch A, Ramirez-Saad H, Hardarson G, Akkermans ADL, de Vos WM (1997) Classification of Austrian rhizobia and the Mexican isolate FL27 obtained from *Phaseolus vulgaris* L. as *Rhizobium gallicum*. Int J Syst Bacteriol 47:1097–1101

Van Berkum P (1990) Evidence for a third uptake hydrogenase phenotype among the soybean bradyrhizobia. Appl Environ Microbiol 56:3835–3841

Van Berkum P, Beyene D, Eardly BD (1996) Phylogenetic relationships among rhizobium species nodulating the common bean (*Pheaseolus vulgaris* L.). Int J Syst Bacteriol 46:240–244

Van Berkum P, Beyene D, Bao G, Campbell TA, Eardly BD (1998) *Rhizobium mongolense* sp. nov. is one of three rhizobial genotypes identified which nodulate and form nitrogen-fixing symbiosis with *Medicago ruthenica* [(L.) Ledebour]. Int J Syst Bacteriol 48:687–699

Vincent JM (1970) A manual for the practical study of the root nodule bacteria. In: International Biological Programme IBP Handbook 15. Blackwell Scientific, Oxford

Wang ET, van Berkum P, Beyene D, Sui XH, Dorado O, Chen WX, Martínez-Romero E (1998) *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* that has a close phylogenetic relationship with *Rhizobium galegae*. Int J Syst Bacteriol 48:687–699

Wang ET, van Berkum P, Sui XH, Beyene, D, Chen WX, 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

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 43:305–313

Xu Y, Murooka Y (1995) A large plasmid isolated from *Rhizobium huakuii* bv Renge that includes genes for both nodulation of *Astragalus sinicus* cv. Japan and nitrogen-fixation. J Ferment Bioeng 80:276–279

Zou XH, Li FD, Chen HK (1997) Characteristics of plasmids in *Rhizobium huakuii*. Curr Microbiol 35:215–220