

## Phylogenetic Relationships and Host Range of *Rhizobium* spp. That Nodulate *Phaseolus vulgaris* L.†

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**We determined the nucleotide sequences of 16S rRNA gene segments from five *Rhizobium* strains that have been isolated from tropical legume species. All share the capacity to nodulate *Phaseolus vulgaris* L., the common bean. Phylogenetic analysis confirmed that these strains are of two different chromosomal lineages. We defined the host ranges of two strains of *Rhizobium etli* and three strains of *R. tropici*, comparing them with those of the two most divergently related new strains. Twenty-two of the 43 tested legume species were nodulated by three or more of these strains. All seven strains have broad host ranges that include woody species such as *Albizia lebbbeck*, *Gliricidia maculata*, and *Leucaena leucocephala*.**

Members of the genus *Rhizobium* form nitrogen-fixing nodules on the roots of leguminous plants and thus are of great ecological and agronomic significance. These bacteria originally were assigned to species on the basis of their host specificity (8), a practice that has been seriously criticized (22, 41, 45). New approaches that consider genetic characteristics as well as symbiotic phenotypes now are available, and they have become useful in defining relationships among rhizobia (11). Analysis of rRNA genes is at present the most useful means to achieve this goal (42), and the phylogeny of the known members of the *Rhizobiaceae* is under revision on the basis of 16S rRNA gene sequences (6, 28, 40, 43, 44).

This approach has also been useful for defining new *Rhizobium* species, including two of particular interest to us: *Rhizobium etli* (29) and *R. tropici* (23). Both of these organisms establish effective symbioses with bean, *Phaseolus vulgaris* L. *R. etli*, which has multiple copies of *nif* genes (21, 26), most likely is the symbiotic species that coevolved with bean. *R. tropici*, which has single copies of *nif* genes, nodulates *Leucaena* spp. in addition to bean (20), and it is tolerant of stress conditions such as high temperature and acidity (10, 13).

*R. leguminosarum* bv. phaseoli and other *Rhizobium* spp. of uncertain taxonomic affinities also are capable of nodulating and fixing nitrogen in association with *P. vulgaris* (6, 21, 25). Nodulation of *P. vulgaris* by a wide range of strains from tropical legumes originally was reported some years ago (18, 41). More recently, bean-nodulating strains have been isolated from *Leucaena* spp., as well as from *Dalea leporina*, *Clitoria ternatea*, and other tropical legumes (2, 12, 21, 33). Strains of *R. fredii* (13, 27) and *R. meliloti* (3) also share this capacity. Collectively, these observations are focusing attention on bean and the variety of different organisms that enter into symbiosis with it.

The purpose of the work described here was twofold. First, we wished to define the genetic relationships of a group of strains that are capable of nodulating bean but were originally isolated from other tropical legumes. Second, we wanted to

systematically investigate the host ranges of a diverse group of strains with the common capacity to nodulate bean. We selected two divergent strains: BR816 (38), originally from Brazil; and CFN234, originally from Mexico. These were compared with *R. tropici* CFN299 (type A), CIAT899 (type B, the type strain for the species), and UMR1173 and *R. etli* CFN42 (the type strain for the species) and F16.

**Analysis of 16S rRNA sequences.** The partial nucleotide sequences of the 16S rRNA genes from *Rhizobium* sp. strains BR816 and CFN234 (from *Leucaena leucocephala*), CFN244 (from *Macroptilium gibbosifolium*), CFN265 (from *L. escul-*

Strains:	1				50
BR816	AGCGGCAGAC	GGGTGAGTAA	CGCGTGGGAA	TCTACCCCTTT	TCTACCGGAAT
CFN244	....CAGAC	GGGTGAGTAA	CGCGTGGGAA	CGTACCCTTT	ACTACCGGAAT
CFN234	..GGCAGAC	GGGTGAGTAA	CGCGTGGGAA	CGTACCCTTT	ACTACCGGAAT
CFN265	AGCGGCAGAC	GGGTGAGTAA	CGCGTGGGAA	CGTACCCTTT	ACTACCGGAAT
Cl180	AGCGGCAGAC	GGGTGAGTAA	CGCGTGGGAA	CGTACCCTTT	ACTACCGGAAT
Consensus	----CAGAC	GGGTGAGTAA	CGCGTGGGAA	--TACCCTTT	-CTACCGGAAT
	51				100
BR816	AACGCAGGGA	AACCTGTGCT	AATACCGTAT	GAGCCCTTCG	GGGAAAGAT
CFN244	AACGCAGGGA	AACCTGTGCT	AATACCGTAT	GTGCCCTTCG	GGGAAAGAT
CFN234	AACGCATGGA	AACGTGTGCT	AATACCGTAT	GTGCCCTTCG	GGGAAAGAT
CFN265	AACGCAGGGA	AACCTGTGCT	AATACCGTAT	GTGCCCTTCG	GGGAAAGAT
Cl180	AACGCATGGA	AACGTGTGCT	AATACCGTAT	GTGCCCTTCG	GGGAAAGAT
Consensus	AACGCA-GGA	AAC-TGTGCT	AATACCGTAT	G-GCCCTT-G	GGGAAAGAT
	101				150
BR816	TTATCGGGAA	AGGATCGGCC	CGCGTTGGAT	TAGCTAGTTG	GTGGGGTAAA
CFN244	TTATCGGTAA	GGGATCGGCC	CGCGTTGGAT	TAGCTAGTTG	GTGGGGTAAA
CFN234	TTATCGGTAA	AGGATCGGCC	CGCGTTGGAT	TAGCTAGTTG	GTGGGGTAAA
CFN265	TTATCGGTAA	AGGATCGGCC	CGCGTTGGAT	TAGCTAGTTG	GTGGGGTAAA
Cl180	TTATCGGTAA	AGGATCGGCC	CGCGTTGGAT	TAGCTAGTTG	GTGGGGTAAA
Consensus	TTATCGG-AA	-GG-T--GCC	CGCGTTGGAT	TAGCTAGTTG	GTGGGGTAAA
	151				200
BR816	GGCCTACCAA	GGCGACGATC	CTAAGCTGCT	CTGAGAGGAT	GATCAGCCAC
CFN244	GGCCTACCAA	GGCGACGATC	CATAGCTGCT	CTGAGAGGAT	GATCAGCCAC
CFN234	GGCCTACCAA	GGCGACGATC	CATAGCTGCT	CTGA.....	.....
CFN265	GGCCTACCAA	GGCGACGATC	CATAGCTGCT	CTGAGAGGAT	GATCAGCCAC
Cl180	GGCCTACCAA	GGCGACGATC	CATAGCTGCT	CTGAGAGGAT	GATCAGCCAC
Consensus	GGCCTACCAA	GGCGACGATC	C--AGCTGCT	CTGA-----	-----
	201				219
BR816	ATTGGGACTG	AGACAC...			
CFN244	ATTGGGACTG	AGACACGGC			
CFN234	.....	.....			
CFN265	ATTG.....	.....			
Cl180	ATTGGGACTG	AGACAC...			
Consensus	ATTG.....	-----			

FIG. 1. Aligned sequences of portions of the rRNA genes of five *Rhizobium* strains capable of nodulating bean.

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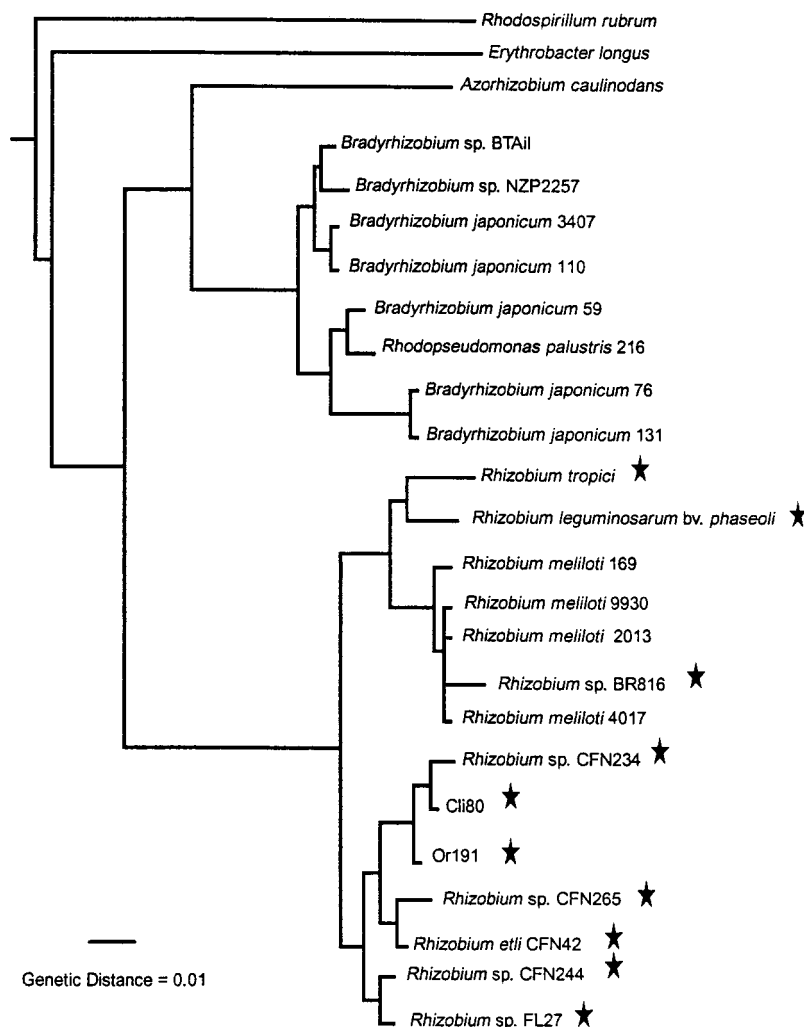


FIG. 2. Phylogenetic tree obtained by neighbor joining groups derived from a Jukes-Cantor distance matrix of the aligned sequences of 16S rRNA fragments from *Rhizobium* spp. and related bacteria. Strains capable of nodulating *P. vulgaris* are indicated by stars.

*lenta*), and Cli80 (from *C. ternatea*) were determined by directly sequencing PCR products. A DNA region corresponding to nucleotides 20 to 338 of the *Escherichia coli* 16S rRNA was amplified from each strain with primers Y1 (5'-TGGCTCAG AACGAACGCTGGCGGC-3') and Y2 (5'-CCCACTGCTG CCTCCCGTAGGAGT-3') as described previously (44). DNA sequencing was performed with a T7 DNA Sequencing Kit from Pharmacia LKB, and sequences have been placed in GenBank under accession numbers L20762 for CFN265, L20763 for CFN244, L20764 for Cli80, L20765 for BR816, and L20766 for CFN234 (Fig. 1). We created a multiple alignment with the PILEUP program of the University of Wisconsin Genetics Computer Group Package (5). The neighbor joining algorithm from the Neighbor Program of Felsenstein's Phylip 3.5 (15) was used to define the phylogenetic relationships (24). We rerooted the tree with the program RETREE (15) and included sequences from two other bean-nodulating strains, FL27 and OR191 (7).

The resulting phylogenetic tree (Fig. 2) shows that the five *Rhizobium* strains represent distinct chromosomal lineages that have evolved the capacity to form nitrogen-fixing nodules with bean. Four of the strains form a cluster with similar 16S rRNA gene sequences, and they all have a diagnostic pattern

of restriction sites that is characteristic of *R. etli* (6). All of these strains have affinity to *R. etli* FL27 and OR191 and to genomic species 1, which was isolated in France by Laguerre and associates (17). These bacteria also share a common isoelectric form of glutamine synthetase II (32), an enzyme proposed for use as a marker of species or groups of strains in the *Rhizobiaceae* (31); thus, they probably derive from a common ancestor. *Rhizobium* sp. strain BR816, which has a different geographical origin, is separated from this group and clustered with *R. meliloti* and allied rhizobia, including *R. fredii*, *Rhizobium* sp. strain NGR234, and the recently proposed *Sinorhizobium saheli* and *S. teranga* (4). The 16S rRNA sequence of BR816 contains four key restriction sites that are diagnostic for division A of *R. meliloti* and in fact identical to that of the *R. meliloti* type strain, ATCC 9930 (6).

**Analysis of host range.** Table 1 summarizes the results of nodulation experiments that were conducted in replicated tests under a controlled environment (16) and that to our knowledge represent the most extensive published database on the host range of bean symbionts. The host ranges of *Rhizobium* sp. strain CFN234, a member of the *R. etli* lineage, and BR816, an organism more closely related to *R. meliloti*, were selected for comparison with two strains of *R. etli* and three strains of *R.*

TABLE 1. Host range of rhizobia capable of nodulating bean<sup>a</sup>

Legume	Characteristic(s) of nodules formed with:						
	<i>R. tropici</i>		<i>R. etli</i>			<i>Rhizobium</i> sp.	
	CIAT899	UMR1173	CFN299	F16	CFN42	CFN234	BR816
<i>Albizia lebbbeck</i> (L.) Benth.	N + F	N + F	N + F	F	N	N	F
<i>Cajanus cajan</i> (L.) Millsp.	N	N	N	N	N	N	N
<i>Canavalia ensiformis</i> (L.) DC	N	N	N	N	N	N	N
<i>Clianthus formosus</i> (G. Don) Ford & Vick.	F	N	F	F	F	F	F
<i>Crotalaria sericia</i> Retz.	N <sup>s</sup> + F	N <sup>s</sup> + F	N <sup>s</sup> + F	F	F	F	N
<i>Cyamopsis tetragonoloba</i> (L.) Taub.	0	0	0	F	N <sup>s</sup>	F	F
<i>Desmanthus illinoensis</i> (Michx.) MacM.	F	0	F	F	F	F	F
<i>Desmodium canadense</i> (L.) DC	F	0	F	N + F	N	N + F	N
<i>Flemingia congesta</i> Roxb.	0	0	0	N	N	N	N
<i>Gliricidia maculata</i> HBK	F	F	F	F	F	F	F
<i>Glycine max</i> (L.) Merr. 'Peking'	N	N	N	0	0	0	0
<i>Indigofera tinctoria</i> L.	N <sup>s</sup>	N <sup>s</sup> + F	N <sup>s</sup> + F	N	N	N	N
<i>Leucaena leucocephala</i> (Lam.) DeWit 'Cunningham'	F	F	F	F	N	F	F
<i>Lotus corniculatus</i> L.	F	0	F	0	N	0	0
<i>Macropitium atropurpureum</i> Urb.	N <sup>s</sup> + F	N <sup>s</sup> + F	N <sup>s</sup> + F	F	N <sup>s</sup>	N <sup>s</sup> + F	N <sup>s</sup> + F
<i>Phaseolus angularis</i> (Willd.) Wight	N <sup>s</sup>	N <sup>s</sup>	N <sup>s</sup> + F	F	N <sup>s</sup> + F	0	0
<i>Phaseolus vulgaris</i> L.	F	F	F	F	F	F	F
<i>Sesbania exaltata</i> (Raf.) Cory	F	0	F	F	F	F	F
<i>Tephrosia vogelii</i> Hook f.	N	N	N	N	N	N	N
<i>Vigna umbellata</i> (Thunb.) Ohwi & Ohashi	N <sup>s</sup> + F	N <sup>s</sup> + F	N <sup>s</sup> + F	F	F	N	N
<i>Vigna unguiculata</i> (L.) Walp.	N + F	N + F	N + F	N <sup>s</sup> + F	N <sup>s</sup> + F	F	F
<i>Vigna vexillata</i> (L.) A. Rich	N <sup>s</sup> + F	N <sup>s</sup> + F	F	F	N	F	F

<sup>a</sup> F, nodules contained leghemoglobin; N, nodules lacked leghemoglobin; N<sup>s</sup>, nodules lacked leghemoglobin and additionally contained visibly darkened and senescing cells; 0, no nodules. The following species did not form nodules with any of the strains: *Acacia ataxacantha* DC, *Albizia saman* (Jacq.) F. Muell., *Apios americana* Medik., *Arachis hypogaea* L., *Calopogonium caeruleum* Benth. (Hemsl.), *Desmodium uncinatum* (Jacq.) DC, *Erythrina crista-galli* L., *Galactia striata* (Jacq.) Urb., *Glycine soja* Sieb. & Zucc., *Kummerowia stipulacea* (Maxim.) Makino, *Lablab purpureus* (L.) Sweet 'Rongai', *Lotononis bainesii* Bak., *Lupinus albus* L., *Macrotyloma axillare* (E. Mey.) Verdc., *Medicago sativa* L., *Mucuna pruriens* (L.) DC, *Pisum sativum* L., *Psophocarpus tetragonolobus* (L.) DC, *Stylosanthes capitata* Vog., *Trifolium subterraneum* L., and *Vicia benghalensis* L.

*tropici*. All strains failed to nodulate 21 of the 43 diverse legume species used as test plants. Included in this group of legumes are *Medicago sativa*, *Trifolium subterraneum*, and *Pisum sativum*, hosts, respectively, of *R. meliloti*, *R. leguminosarum* bv. trifolii, and *R. leguminosarum* bv. viciae. This category also encompasses *Calopogonium caeruleum*, *Desmodium uncinatum*, *Lablab purpureus*, and *Psophocarpus tetragonolobus*, all of which are hosts of the well-known, broad-host-range strain *Rhizobium* sp. strain NGR234 (19, 34).

Only five legume species responded uniformly to all seven strains. *Canavalia ensiformis*, *Cajanus cajan*, and *Tephrosia vogelii* produced nodules that inevitably failed to fix nitrogen, and *P. vulgaris* and *Gliricidia maculata* always produced fully Fix<sup>+</sup> nodules. The uniformly compatible response of *P. vulgaris* is in full agreement with previous observations (23, 29). *G. maculata* is a tropical American shrub and a member of the tribe Galegae (1). Although this species is not closely related to bean, rhizobia from *Gliricidia* spp. can fix nitrogen in association with *P. vulgaris* (13), and thus there appears to be a reciprocal sharing of nodule organisms between these two species.

The remaining 17 legumes responded differentially to the seven test strains. Interactions were often uniformly Fix<sup>+</sup>, as in combinations of *Sesbania exaltata* with *R. tropici* CIAT899 and *Vigna umbellata* with *Rhizobium* sp. strain F16 (Fig. 3A). Other responses were more complex. Individual plant responses sometimes were distinctly heterogeneous, consisting of large, deeply pigmented Fix<sup>+</sup> nodules intermingled with smaller nodules that lacked leghemoglobin and often were visibly abnormal (Fig. 3B). Combinations yielding such aberrant nodules are designated Nod<sup>s</sup> in Table 1. In one pairing, that of *Crotalaria sericia* and *R. tropici*, nodules possessed dark, spherical,

senescing interiors surrounded by a translucent cortical layer. Abnormal nodules in other combinations were characterized by rust-colored necrotic cells that appeared to ensheath the infection thread. Thus, although Fix<sup>+</sup> nodules appeared in many combinations, the interactions were in fact incompatible to varying extents, as indicated in Table 1.

Our nodulation data allow a number of important general conclusions to be drawn. It is apparent, for example, that none of the strains is symbiotically restricted to the two legumes that have been examined in the past, *P. vulgaris* and *L. leucocephala*. Nodulating abilities, although sometimes strain specific, extend well beyond these two legumes to encompass a diversity of other species. Thus, *R. etli* is not a narrow-host-range symbiont, as has been assumed previously (23, 29), and the unclassified organisms, too, seem to be broadly adapted to symbiosis.

Our experiments with *R. tropici* greatly expand previous preliminary observations that species other than *P. vulgaris* and *L. leucocephala* are nodulated (23), and they indicate that this organism, too, has a broad host range. There is distinct strain heterogeneity: host reactions to CIAT899 and CFN299 are virtually identical and distinctly broader than that of the third strain, UMR1173, a unique strain that cannot be classified as either type A or type B (23). Four legume species formed Fix<sup>+</sup> nodules with the first two strains but were unreactive with UMR1173. It is also evident that almost all of the aberrant Nod<sup>s</sup> responses involved *R. tropici*.

The symbiotic phenotypes of the two *R. etli* strains and the two *Rhizobium* strains from *L. leucocephala* are not readily distinguishable from one another, and in fact, they are not greatly different from those of *R. tropici*. Although there are strain-specific differences on a number of hosts (Table 1), *P.*

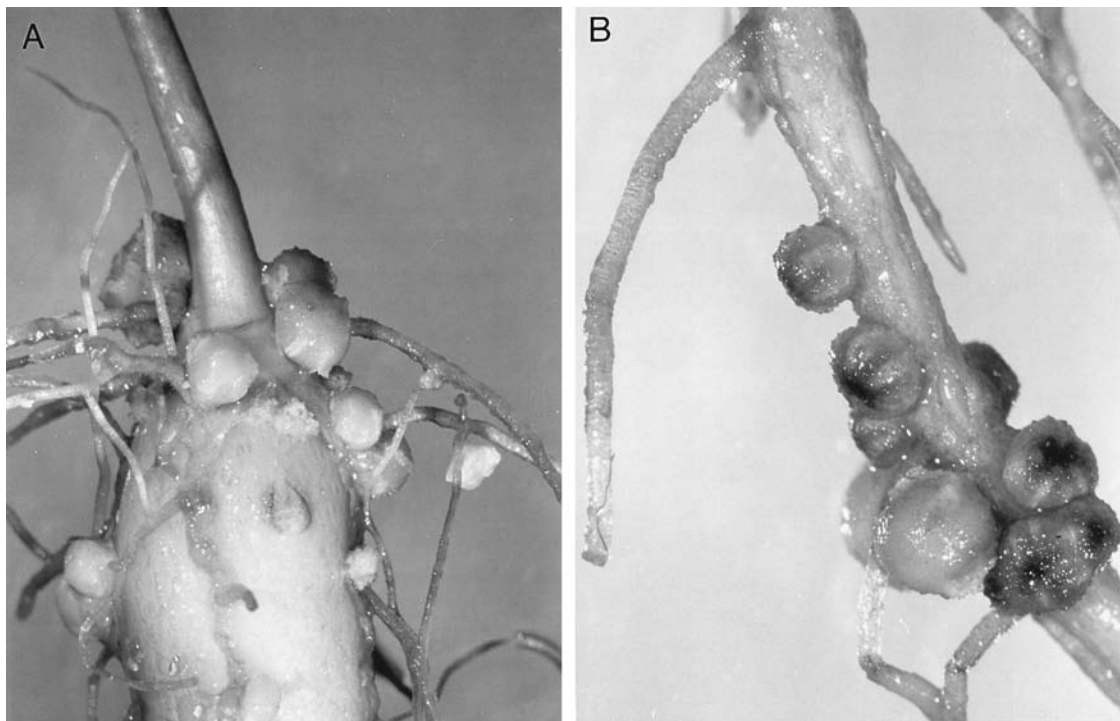


FIG. 3. Nitrogen-fixing nodules produced by (A) *Rhizobium* sp. strain F16 on the swollen tap root of *Vigna umbellata* and (B) *R. tropici* CIAT899 on the root crown of *Macroptilium atropurpureum*.

*angularis* is the only legume yielding a  $\text{Fix}^+$  response with *R. etli* versus no response to the leucaena strains. *V. umbellata* also can discriminate *R. etli* from the leucaena strains, but the distinction is that of  $\text{Fix}^+$  versus  $\text{Nod}^+$ .

The response of *L. leucocephala* to the tested strains warrants special note in light of previous conclusions that strains of *R. tropici*, but not those of *R. etli*, can nodulate this species (9, 23, 29, 36, 39). Although we found that the type strain of *R. etli* forms only small nodules on *L. leucocephala*, strain F16 elicited a fully wild-type response that was not obviously different from that to *R. tropici* (Table 1). Thus, *R. etli* and *R. tropici* both should be viewed as symbionts of this legume tree, an observation that has significant implications for interpretation of data on responses of nodulation genes to signals from roots of *L. leucocephala* (36, 37, 39). As nodulators of *L. leucocephala*, these organisms also share a common host with rhizobia originally isolated from a diverse group of legumes: *Astragalus onobrychis*, *Calliandra* spp., *Coronilla varia*, *G. maculata*, *Lablab purpureus*, *Lotus divaricatus*, *Onobrychis viciifolia*, and others (3, 14, 34, 35).

When viewed collectively, our results reinforce the utility of bean as a host to sort out the nodulation strategies of phylogenetically diverse rhizobia (29, 35–37). We have identified additional, readily available hosts that may be broadly useful for these experiments (*G. maculata*, *Macroptilium atropurpureum*, and three species of *Vigna*), and we have discovered aberrant nodule responses that may prove to be useful models for studying legume-*Rhizobium* incompatibility.

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#### REFERENCES

- Allen, O. N., and E. K. Allen. 1981. The Leguminosae. A source book of characteristics, uses, and nodulation. University of Wisconsin Press, Madison.
- Bal, A. K., S. Shantharam, and P. P. Wong. 1982. Nodulation of pole bean (*Phaseolus vulgaris* L.) by *Rhizobium* species of two cross-inoculation groups. *Appl. Environ. Microbiol.* **44**:965–971.
- Bromfield, E. S. P., and L. R. Barran. 1990. Promiscuous nodulation of *Phaseolus vulgaris*, *Macroptilium atropurpureum* and *Leucaena leucocephala* by indigenous *Rhizobium meliloti*. *Can. J. Microbiol.* **36**:369–372.
- de Lajudie, P., A. Willems, B. Pot, D. Dewettinck, G. Maestrojuan, M. Neyra, M. D. Collins, B. Dreyfus, K. Kersters, and M. Gillis. 1994. Polyphasic taxonomy of rhizobia: emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. *Int. J. Syst. Bacteriol.* **44**:715–733.
- Devereux, J., P. Haerberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12**:387–395.
- Eardly, B. D., F.-S. Wang, T. S. Whittam, and R. K. Selander. 1995. Species limits in *Rhizobium* populations that nodulate the common bean (*Phaseolus vulgaris*). *Appl. Environ. Microbiol.* **61**:507–512.
- Eardly, B. D., J. P. W. Young, and R. K. Selander. 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.
- Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. Root nodule bacteria and leguminous plants. University of Wisconsin, Madison.
- George, M. L. C., J. P. W. Young, and D. Borthakur. 1994. Genetic characterization of *Rhizobium* sp. strain TAL1145 that nodulates tree legumes. *Can. J. Microbiol.* **40**:208–215.
- Graham, P. H., K. J. Draeger, M. L. Ferrey, M. J. Conroy, B. E. Hammer, E. Martínez, S. R. Aarons, and C. Quinto. 1994. Acid pH tolerance in strains of *Rhizobium* and *Bradyrhizobium*, and initial studies on the basis for acid tolerance of *Rhizobium tropici* UMR1899. *Can. J. Microbiol.* **40**:198–207.
- Graham, P. H., M. J. Sadowsky, H. H. Keyser, Y. M. Barnett, R. S. Bradley, J. E. Cooper, D. J. de Ley, B. D. W. Jarvis, E. B. Roslycky, B. W. Strijdom, and J. P. W. Young. 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.
- Herrera, M. A., E. J. Bedmar, and J. Olivares. 1985. Host specificity of

- Rhizobium* strains isolated from nitrogen-fixing trees and nitrogenase activities of strain GRH2 in symbiosis with *Prosopis chilensis*. *Plant Sci.* **42**:177–182.
13. **Hungria, M., A. A. Franco, and J. I. Sprent.** 1993. New sources of high-temperature tolerant rhizobia for *Phaseolus vulgaris* L. *Plant Soil* **149**:103–109.
  14. **Jarvis, B. D. W.** 1983. Genetic diversity of *Rhizobium* strains which nodulate *Leucaena leucocephala*. *Curr. Microbiol.* **8**:153–158.
  15. **Jukes, T. H., and C. R. Cantor.** 1969. Evolution of protein molecules, p. 21–132. In H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, New York.
  16. **Krishnan, H. B., and S. G. Pueppke.** 1991. Sequence and analysis of the *nodABC* region of *Rhizobium fredii* USDA257, a nitrogen-fixing symbiont of soybean and other legumes. *Mol. Plant Microbe Interact.* **4**:512–520.
  17. **Laguerre, G., M. P. Fernandez, V. Edel, P. Normand, and N. Amarger.** 1993. Genomic heterogeneity among French *Rhizobium* strains isolated from *Phaseolus vulgaris* L. *Int. J. Syst. Bacteriol.* **43**:761–767.
  18. **Lange, R. T.** 1961. Nodule bacteria associated with the indigenous Leguminosae of south-western Australia. *J. Gen. Microbiol.* **26**:351–359.
  19. **Lewin, A., C. Rosenberg, H. Meyer, C. H. Wong, L. Nelson, J.-F. Manen, J. Stanley, D. N. Dowling, J. Dénarié, and W. J. Broughton.** 1987. Multiple host-specificity loci of the broad host-range *Rhizobium* sp. NGR234 selected using the widely compatible legume *Vigna unguiculata*. *Plant Mol. Biol.* **8**:447–459.
  20. **Martínez, E., R. Palacios, and F. Sánchez.** 1987. Nitrogen-fixing nodules induced by *Agrobacterium tumefaciens* harboring *Rhizobium phaseoli* plasmids. *J. Bacteriol.* **169**:2828–2834.
  21. **Martínez, E., M. A. Pardo, R. Palacios, and M. A. Cevallos.** 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.
  22. **Martínez, E., D. Romero, and R. Palacios.** 1990. The *Rhizobium* genome. *Rev. Plant Sci.* **9**:59–93.
  23. **Martínez-Romero, E., L. Segovia, F. M. Mercante, A. A. Franco, P. Graham, and M. A. Pardo.** 1991. *Rhizobium tropici*: a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. *Int. J. Syst. Bacteriol.* **41**:417–426.
  24. **Nei, M., J. C. Stephens, and N. Saitou.** 1985. Methods for computing the standard errors of branching points in an evolutionary tree and their application to molecular data from humans and apes. *Mol. Biol. Evol.* **2**:66–85.
  25. **Piñero, D., E. Martínez, and R. K. Selander.** 1988. Genetic diversity and relationships among isolates of *Rhizobium leguminosarum* biovar *phaseoli*. *Appl. Environ. Microbiol.* **54**:2825–2832.
  26. **Quinto, C., H. de la Vega, M. Flores, L. Fernández, T. Ballado, G. Soberón, and R. Palacios.** 1982. Reiteration of nitrogen fixation gene sequences in *Rhizobium phaseoli*. *Nature (London)* **299**:724–726.
  27. **Sadowsky, M. J., P. B. Cregan, and H. H. Keyser.** 1988. Nodulation and nitrogen fixation efficacy of *Rhizobium fredii* with *Phaseolus vulgaris* genotypes. *Appl. Environ. Microbiol.* **54**:1907–1910.
  28. **Sawada, H., H. Leki, H. Oyaizu, and S. Matsumoto.** 1993. Proposal for rejection of *Agrobacterium tumefaciens* and revised descriptions for the genus *Agrobacterium* and for *Agrobacterium radiobacter* and *Agrobacterium rhizogenes*. *Int. J. Syst. Bacteriol.* **43**:694–702.
  29. **Segovia, L., J. P. W. Young, and E. Martínez-Romero.** 1993. Reclassification of American *Rhizobium leguminosarum* biovar *phaseoli* type I strains as *Rhizobium etli* sp. nov. *Int. J. Syst. Bacteriol.* **43**:374–377.
  30. **Sousa, C., L. J. Folch, P. Boloix, M. Megías, N. Nava, and C. Quinto.** 1993. A *Rhizobium tropici* DNA region carrying the amino-terminal half of a *nodD* gene and a *nod*-box-like sequence confers host-range extension. *Mol. Microbiol.* **9**:1157–1168.
  31. **Taboada, H., S. Encarnación, C. Vargas, N. Narváez, Y. Mora, E. Martínez, and J. Mora.** 1993. Glutamine synthetase II as a biological marker of the Rhizobiaceae family, p. 657. In R. Palacios, J. Mora, and W. E. Newton (ed.), *New horizons in nitrogen fixation*. Kluwers, Dordrecht, The Netherlands.
  32. **Taboada, H., and J. Mora.** Unpublished data.
  33. **Thomas, P. M., K. F. Golly, J. W. Zyskind, and R. A. Virginia.** 1994. Variation of clonal, mesquite-associated rhizobial and bradyrhizobial populations from surface and deep soils by symbiotic gene region restriction fragment length polymorphism and plasmid profile analysis. *Appl. Environ. Microbiol.* **60**:1146–1153.
  34. **Trinick, M. J.** 1980. Relationships amongst the fast-growing rhizobia of *Lablab purpureus*, *Leucaena leucocephala*, *Mimosa* spp., *Acacia farnesiana* and *Sesbania grandiflora* and their affinities with other rhizobial groups. *J. Appl. Bacteriol.* **49**:39–53.
  35. **Turk, D., and H. H. Keyser.** 1992. Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. *Can. J. Microbiol.* **38**:451–460.
  36. **van Rhijn, P., J. Desair, K. Vlassak, and J. Vanderleyden.** 1994. Functional analysis of *nodD* genes of *Rhizobium tropici* CIAT899. *Mol. Plant Microbe Interact.* **7**:666–677.
  37. **van Rhijn, P., J. Desair, K. Vlassak, and J. Vanderleyden.** 1994. The NodD proteins of *Rhizobium* sp. strain BR816 differ in their interactions with coinducers and in their activities for nodulation of different host plants. *Appl. Environ. Microbiol.* **60**:3615–3623.
  38. **van Rhijn, P., J. S., B. Feys, C. Verreth, and J. Vanderleyden.** 1993. Multiple copies of *nodD* in *Rhizobium tropici* CIAT899 and BR816. *J. Bacteriol.* **175**:438–447.
  39. **Waelkens, F., T. Voets, K. Vlassak, J. Vanderleyden, and P. van Rhijn.** 1995. The *nodS* gene of *Rhizobium tropici* strain CIAT899 is necessary for nodulation on *Phaseolus vulgaris* and on *Leucaena leucocephala*. *Mol. Plant Microbe Interact.* **8**:147–154.
  40. **Willems, A., and M. D. Collins.** 1993. Phylogenetic analysis of rhizobia and agrobacteria based on 16S rRNA gene sequences. *Int. J. Syst. Bacteriol.* **43**:305–313.
  41. **Wilson, J. K.** 1939. Leguminous plants and their associated organisms. Cornell University Agricultural Experiment Station, Ithaca, N.Y.
  42. **Woese, C. R.** 1987. Bacterial evolution. *Microbiol. Rev.* **51**:221–271.
  43. **Yanagi, M., and K. Yamasato.** 1993. Phylogenetic analysis of the family Rhizobiaceae and related bacteria by sequencing of 16S rRNA gene using PCR and DNA sequencer. *FEMS Microbiol. Lett.* **107**:115–120.
  44. **Young, J. P. W., H. L. Downer, and B. D. Eardly.** 1991. Phylogeny of the phototropic *Rhizobium* strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment. *J. Bacteriol.* **173**:2271–2277.
  45. **Young, J. P. W., and A. W. B. Johnston.** 1989. The evolution of specificity in legume-*Rhizobium* symbiosis. *Trends Ecol. Evol.* **4**:341–349.