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Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov.

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Reasons are advanced for removal of *Rhizobium ciceri*, *Rhizobium huakuii*, *Rhizobium loti*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* from the genus *Rhizobium* and for establishment of *Mesorhizobium* gen. nov. for these species. A description of the genus *Mesorhizobium* and amended descriptions of *Mesorhizobium ciceri*, *Mesorhizobium huakuii*, *Mesorhizobium loti*, *Mesorhizobium mediterraneum*, and *Mezorhizobium tianshanense* are provided.

In a review of root nodule symbioses by Vincent (22), the fast-growing rhizobia associated with *Lotus corniculatus* and *Lupinus densiflorus* were recognized as a separate group which merited a specific designation, and the name *Rhizobium loti* was tentatively proposed for it. Approval for the designation of *R. loti* as a new species was also voiced at a roundtable discussion on *Rhizobium* taxonomy associated with the 4th International Congress on Nitrogen Fixation at Canberra, Australia, in 1980. The subsequent publication of the new species in 1982 (9) enabled it to be included in a revised taxonomy of the *Rhizobiaceae* presented by Jordan in *Bergey's Manual of Systematic Bacteriology* (12).

At that time *R. loti* was distinguished from other fast-growing rhizobia on the basis of flagellation (1), esterase patterns (14), response to isoflavonoids (18), plant nodulation (11, 12), internal antigens (23), electrophoresis of soluble cellular proteins (19, 20), and DNA relatedness (4, 8). More recently, cellular fatty acid analysis was used to reveal differences, useful for identification purposes, between strains of *R. loti* and strains of the genus *Agrobacterium* and other *Rhizobium* or *Sinorhizobium* species (10). All of these methods differentiate species but give little indication of the relationships between species and genera.

An early indication of the relationship among *R. loti*, other *Rhizobium* species, and *Sinorhizobium* and *Agrobacterium* species was obtained by studying the intergeneric similarities of rRNA cistrons (7). The results of this analysis indicated that *Rhizobium leguminosarum*, *Rhizobium galegae*, *Sinorhizobium meliloti*, *Sinorhizobium fredii*, *Agrobacterium tumefaciens* (biovar 1), and *Agrobacterium rhizogenes* (biovar 2) were all more closely related to one another than they were to *R. loti*. Subsequent analyses of 16S rRNA gene sequences of species in these genera have confirmed, refined, and extended this observation (21, 24, 25); the levels of 16S ribosomal DNA sequence similarity between *R. loti* and other *Rhizobium* and *Agrobacterium* species are around 93.5%. Consequently, there has been considerable support for the establishment of a separate genus for *R. loti* and related root nodule bacteria (5, 13, 27).

The original description of *R. loti* (9) clearly indicated that fast-growing *Lotus* rhizobia formed part of an extensive plant cross-inoculation group involving plant species in the genera *Lupinus*, *Ornithopus*, *Lotus*, *Anthyllis*, *Caragena*, *Astragalus*, *Ononis*, *Genista*, and *Mimosa*. Jarvis et al. also indicated that the fast-growing *Lotus* rhizobia were related to rhizobia obtained from several plant species in these genera and also to rhizobia obtained from *Cicer arietinum* and *Leucaena leucocephala*. This finding was based on the work of Crow et al. (4), who found a group of rhizobia, identified as "group 4," which exhibited relatively high mean levels of DNA relatedness (50 to 51%) with two fast-growing *Lotus* rhizobia used as reference strains but little relatedness (levels of DNA relatedness, 7 to 10%) with reference strains derived from clover, bean, lucerne, or crown vetch (*Coronilla varia*). The group was genetically diverse since the levels of DNA relatedness between individual strains and the reference strains ranged from 36 to 88%. These values suggested that group 4 probably included other species in addition to *R. loti*.

Since that time several other species of bacteria related to *R. loti* have been described. These species include *Rhizobium huakuii* from *Astragalus sinicus* (3), *Rhizobium ciceri* from *Cicer arietinum* (chickpea) (17), *Rhizobium mediterraneum* from chickpea (16), and *Rhizobium tianshanense* from an arid saline environment in northern People's Republic of China (2). In addition, De Lajudie et al. (5) identified "cluster U" as a group of strains from *Acacia* sp., Brazilian legumes, and *Lotus* sp. which is related to *R. loti* but exhibits considerable genetic heterogeneity. At least three new species can be recognized within cluster U. At present, these species and related strains can be said to constitute the "*R. loti* group."

The use of the genus *Rhizobium* for all of the fast-growing rhizobia, including *R. leguminosarum*, *Rhizobium etli*, *Rhizobium tropici* types A and B, *Rhizobium meliloti*, *Rhizobium fredii*, *R. galegae*, and the *R. loti* group defined above, has resulted in the formation of a polyphyletic genus which ob-

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TABLE 1. Some characteristics of *Mesorhizobium* species[a]

Characteristic	*M. ciceri*	*M. huakuii*	*M. loti*	*M. mediterraneum*	*M. tianshanense*
Morphological characteristics					
Flagellation		One polar or subpolar flagellum	One polar or subpolar flagellum		Peritrichous flagella
Cell shape	Rods	Rods	Rods	Rods	Rods
Colony characteristics					
Opacity	Opaque	Semitranslucent	Opaque	Opaque	Opaque
Diam (mm)	2-4	2-4	>1	2	1-2
Incubation time at 28°C (days)	3-5	5-6	7	4-5	5-7
Color	Colorless	Colorless	Colorless	Colorless	Creamy
Cultural characteristics					
Maximum temp (°C)	40	37-39	<39-40	40	ND[b]
Maximum NaCl concn for growth (%, wt/vol)	2.0	ND	<2.0	2.0	1.0
pH range for growth	5.0-10.0	5.0-9.5	4.0, <10.0	>5.0, <10.0	ND
G+C content (mol%)	63-64	59-64	59-64	63-64	59-63
Substrates used as sole carbon sources					
D- and L-Arabinose	+[c]	+	+	+	d
D-Fructose	+	+	+	+	d
D- and L-Fucose	+	−	+	+	+
Fumarate	+	+	+	+	−
Inositol	+	−	+	+	−
D- and L-Malate	+	+	+	+	−
Maltose	+	+	+	+	d
D-Raffinose	−	+	d	−	d
Sucrose	+	+	+	+	d

[a] For the most part, the table includes only characteristics for which data are available for all five species. The only exception is flagellation; flagellation data are available for only three species. All species are gram-negative, non-spore-forming, rod-shaped bacteria which form circular, convex colonies on solid media under aerobic conditions and utilize D-glucose and rhamnose as sole carbon sources.

[b] ND, not determined.

[c] +, used by all strains; d, used by some strains; −, not used by any strain.

scures the phenetic and phylogenetic differences among these species. The recent transfer of two species, *R. meliloti* and *R. fredii*, to the new genus *Sinorhizobium* (5) and the creation of two new species, *Sinorhizobium saheli* and *Sinorhizobium teranga*, have contributed to the resolution of this problem, but the remaining *Rhizobium* species still constitute a polyphyletic group. Further subdivision of the genus *Rhizobium* was recommended at a roundtable discussion on *Rhizobium* taxonomy associated with the 10th International Congress on Nitrogen Fixation at St. Petersburg, Russia, in 1995 (13), and a proposal was presented at the 15th North American Conference on Symbiotic Nitrogen Fixation (26). Participants at both of these meetings recognized that, based on 16S rRNA sequence data, the species included in the *R. loti* group are monophyletic but are less closely related to other *Rhizobium* species (*R. leguminosarum*, *R. tropici* types A and B, and *R. galegae*) than are species already classified in related genera, such as the genera *Sinorhizobium* and *Agrobacterium*. The *R. loti* group is also phenetically and phylogenetically distinct from the other genera of nitrogen-fixing plant symbionts, the genera *Azorhizobium* and *Bradyrhizobium*. The purpose of this paper is to present the view that the species in the *R. loti* group should be reclassified in a new genus, the genus *Mesorhizobium* (5, 13, 27).

The name *Mesorhizobium* is thought to have been originally proposed by W. X. Chen and coworkers. It was used by Peter Young at the International Symposium on Diversity and Taxonomy of Rhizobia at Wuhan, People's Republic of China, in 1994 for a phylogenetic group corresponding to the *R. loti* group defined above. Subsequently, Chen et al. (2) used the term "meso-growing rhizobia" when they described a new species, *R. tianshanense*, that is closely related to *R. loti* and *R. huakuii*. Thus, the name *Mesorhizobium* has been used to denote both phylogenetic position and growth rate. The former meaning was emphasized at the workshop on *Rhizobium* taxonomy associated with the 10th International Congress on Nitrogen Fixation in St. Petersburg, Russia, (13), where it was used to indicate the intermediate position of the *R. loti* group between the *Agrobacterium-Rhizobium-Sinorhizobium* complex and the genera *Azorhizobium* and *Bradyrhizobium* in the alpha subdivision of the *Proteobacteria*. The meaning referring to the growth rate was emphasized by Young (26) at the 15th North American Conference on Symbiotic Nitrogen Fixation.

Characteristics of *Mesorhizobium* species described by Chen et al. (2, 3), De Lajudie et al. (5), Jarvis et al. (9), and Nour et al. (16, 17) are shown in Table 1. For the most part Table 1 shows only characteristics available for all five species considered. An exception was made for flagellation data, which were available for only three species, because of the differences among *Mesorhizobium huakuii*, *Mesorhizobium loti*, and *Mesorhizobium tianshanense*. Additional information is available in the references mentioned above. Data for *R. loti* recorded by Nour et al. (16) and De Lajudie et al. (5) are included under this species. However, it should be recognized that the additional data are data only for the type strain (16) or the type strain and one other strain (5).

De Lajudie et al. (5) described a group of strains designated cluster U, but since different phenotypic and genotypic groups were found among these bacteria, we concluded that although cluster U strains belong in the new genus *Mesorhizobium*, further study will be required before species can be described.

An examination of the comparative data available for the putative *Mesorhizobium* species revealed that *R. ciceri*, *R. huakuii*, *R. loti*, and *R. mediterraneum* have been compared previously by a number of different methods, including numerical taxonomy of phenotypic characteristics (3, 15, 16), multilocus

enzyme electrophoresis (16, 17), fatty acid analysis (10), restriction fragment length polymorphism of amplified 16S rRNA sequences, including intergenic spacer sequences (16, 17), DNA-DNA hybridization (3, 16, 17), and 16S rRNA gene sequencing (3, 16, 17). These techniques combined to give a good indication of the relationships among these four species. However, the relationship between these species and *R. tianshanense* is less clear. Previously published data for *R. tianshanense* include numerical taxonomy, DNA-DNA hybridization, and partial 16S rRNA gene sequence data (2). DNA-DNA hybridization data indicate that *R. huakuii* CCBAU 2609^T and *R. loti* ATCC 33669^T exhibit 32 and 64 or 34 and 40% DNA relatedness respectively, with two strains of *R. tianshanense*. A partial 16S rRNA gene sequence of *R. tianshanense* A-1BS^T was identical to that of *R. ciceri*. Subsequent work (23a) revealed that the complete 16S rRNA gene sequences of *R. tianshanense* A-1BS^T and *R. huakuii* CCBAU 2609^T differ by 2.1%, while the sequences of *R. ciceri* and *R. mediterraneum* both differ from the *R. tianshanense* sequence by 3.6%. However, a complete 16S rRNA gene sequence for A-1BS^T obtained by one of us (21b) revealed a closer relationship among *R. tianshanense*, *R. ciceri*, *R. hukauii*, and *R. mediterraneum*. We concluded that *R. tianshanense* strains should be classified in the genus *Mesorhizobium*, but further work is needed to establish the relationship of these organisms with other species in the genus.

The phylogenetic relationships among some generic type strains belonging to the alpha subdivision of the *Proteobacteria* are shown in Fig. 1. This tree clearly illustrates the unique position of the new genus *Mesorhizobium* and the phylogenetic distance between the genus *Mesorhizobium* and the remaining members of the genus *Rhizobium*.

Description of *Mesorhizobium* gen. nov. *Mesorhizobium* (Me. so.rhi.zo'bi.um. Gr. adj. *mesos*, middle; M. L. neut. n. *Rhizobium*, bacterial generic name; M. L. neut. n. *Mesorhizobium*, rhizobia phylogenetically intermediate between the genera *Bradyrhizobium* and *Rhizobium*). Cells are gram-negative, aerobic, non-spore-forming rods, motile, usually with one polar or subpolar flagellum. Cells may contain polybetahydroxybutyrate inclusion bodies. Growth on yeast mannitol agar produces colonies that are 2 to 4 mm in diameter after incubation for 3 to 7 days at 28°C. All species assimilate glucose, rhamnose, and sucrose with the production of acidic end products. Strains generally form nitrogen-fixing nodules on the roots of a restricted range of leguminous plants, and cross-inoculation between the strains of one species of this genus and the plant hosts associated with another species is not known. The guanine-plus-cytosine contents of the DNAs are 59 to 64 mol% (as determined by the thermal denaturation method). The type species is *Mesorhizobium loti* (Jarvis et al. 1982) comb. nov.

At the molecular level members of this genus can be recognized by their fatty acid profiles (21a) and their 16S rRNA gene sequences.

Description of *Mesorhizobium ciceri* (Nour et al. 1994) comb. nov. The phenotypic description of *Mesorhizobium ciceri* is the same as that given by Nour et al. (17). In addition, this species can be differentiated from other *Mesorhizobium* species by the results of comparative fatty acid analysis, DNA-DNA hybridization, and 16S rRNA gene sequence analysis.

Description of *Mesorhizobium huakuii* (Chen et al. 1991) comb. nov. The phenotypic description of *Mesorhizobium huakuii* is the same as that given by Chen et al. (3). In addition, this species can be differentiated from other *Mesorhizobium* species by the results of comparative fatty acid analysis, DNA-DNA hybridization, and 16S rRNA gene sequence analysis.

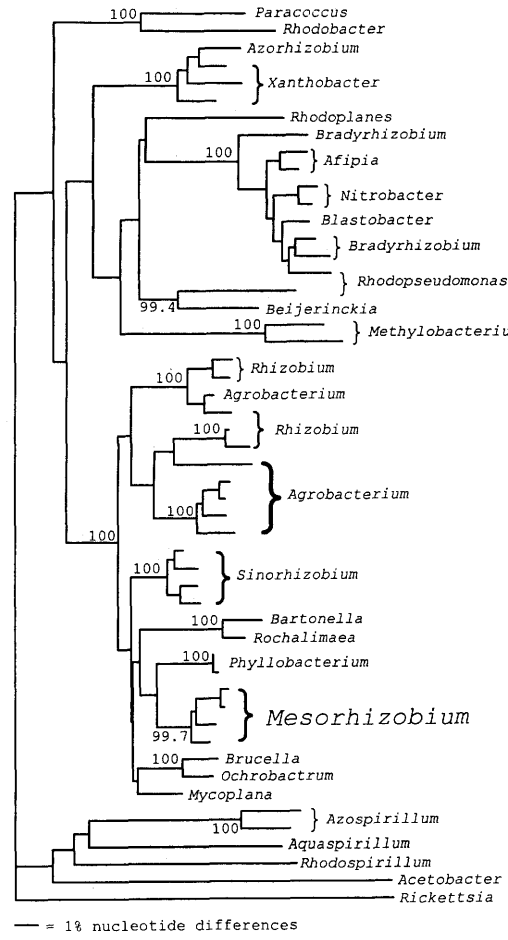

FIG. 1. Phylogenetic relationship of the genus *Mesorhizobium* within the α subdivision of the class *Proteobacteria* based on aligned sequences of the small-subunit rRNA genes. Jukes-Cantor distances were derived from the aligned sequences and used to construct an optimal unrooted tree by the neighbor-joining method. A total of 1,000 replicate trees were generated in a bootstrap analysis to derive a majority rule consensus tree. The levels of support that exceeded 99% are indicated at the nodes on the neighbor-joining tree. The sequences representing the genera indicated on the tree which were used in the phylogenetic analysis were the sequences of *Paracoccus denitrificans* (accession no. X69159), *Rhodobacter sphaeroides* (D16425), *Azorhizobium caulinodans* (X94200), *Xanthobacter flavus* (X94199), *Xanthobacter autotrophicus* (X94201), *Xanthobacter agilis* (X94198), *Rhodoplanes roseus* (D25313), *Bradyrhizobium elkanii* (U35000), *Bradyrhizobium japonicum* (U69638 and Z35330), *Afipia clevelandensis* (M69186), *Afipia felis* (M65248), *Nitrobacter hamburgensis* (L35502), *Nitrobacter winogradskyi* (L35507), *Blastobacter denitrificans* (S46917), *Rhodopseudomonas palustris* (D25312), *Rhodopseudomonas acidophyla* (M34128), *Beijerinckia indica* (M59060), *Methylobacterium* sp. strain BF10 (Z23156), *Methylobacterium extorquens* (D32224), *Rhizobium leguminosarum* bv. viciae (U29386), *Rhizobium etli* (U28916), *Rhizobium tropici* (D11344), *Rhizobium* sp. strain OK 55 (D14510), *Rhizobium galegae* (X67226), *Agrobacterium tumefaciens* bv. 2 strain NCPPB2991 (D14501), *Agrobacterium vitis* (D14502), *Agrobacterium rubi* (D14503), *Agrobacterium tumefaciens* NCPPB1650 (D14506), *Agrobacterium tumefaciens* bv. 1 strain NCPPB2437 (D14500), *Agrobacterium* sp. strain 3-10 (Z30542), *Sinorhizobium fredii* (X67231), *Sinorhizobium meliloti* (X67222), *Sinorhizobium saheli* (X68390), *Sinorhizobium teranga* (X68387), *Bartonella bacilliformis* (M65249), *Rochalimaea henselae* (M73229), *Phyllobacterium myrsinacearum* (D127890), *Phyllobacterium rubiacearum* (D12790), *Mesorhizobium ciceri* (U07934), *Mesorhizobium loti* (X67229), *Mesorhizobium mediterraneum* (L38825), *Mesorhizobium huakuii* (D13431), *Brucella neotomae* (L26167), *Ochrobactrum anthropi* (D12794), *Mycoplana dimorpha* (D12786), *Azospirillum brasilense* (Z29617), *Azospirillum lipoferum* (Z29619), *Aquaspirillum magnetotacticum* (M58171), *Rhodospirillum rubrum* (D30778), *Acetobacter diazotrophicus* (X75618), and *Rickettsia rickettsii* (M21293). The sequences were aligned by using the PILEUP program in the Wisconsin package of the Genetics Computer Group (Madison, Wis.). The aligned sequences were analyzed by using the SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE programs of the PHYLIP, version 3.5c software package (6), and the tree was constructed by using DRAWGRAM.

Description of *Mesorhizobium loti* (Jarvis et al. 1982) comb. nov. The phenotypic description of *Mesorhizobium loti* is the same as that given by Jarvis et al. (9) and supplemented by Nour et al. (16) and De Lajudie et al. (5). In addition, this species can be differentiated from other *Mesorhizobium* species by the results of comparative fatty acid analysis, DNA-DNA hybridization, and 16S rRNA gene sequence analysis.

Description of *Mesorhizobium mediterraneum* (Nour et al. 1995) comb. nov. The phenotypic description of *Mesorhizobium mediterraneum* is the same as that given by Nour et al. (16). In addition, this species can be differentiated from other *Mesorhizobium* species by the results of comparative fatty acid analysis, DNA-DNA hybridization, and 16S rRNA gene sequence analysis.

Description of *Mesorhizobium tianshanense* (Chen et al. 1995) comb. nov. The phenotypic description of *Mesorhizobium tianshanense* is the same as that given by Chen et al. (2). In addition, this species can be differentiated from other *Mesorhizobium* species by the results of 16S rRNA gene sequence analysis.

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