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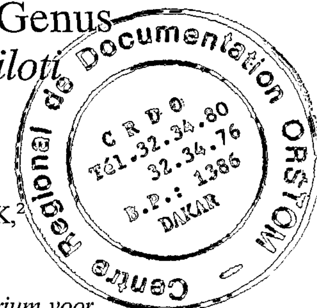
Polyphasic Taxonomy of Rhizobia: Emendation of the Genus *Sinorhizobium* and Description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov.



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A total of 80 bacterial strains isolated from different *Sesbania* and *Acacia* species growing in various sites in Senegal (West Africa) were compared with 35 reference strains of *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Agrobacterium* species and with 33 representative strains of the different groups of Brazilian isolates described on the basis of the results of a numerical analysis of the whole-cell protein patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fifty-two strains could be placed in three protein electrophoretic clusters, two of which were different from the clusters containing various reference or representative strains, while 30 other strains could not be placed in any group. The strains belonging to the three clusters were studied by determining their nodulation host ranges and their morphological, physiological, and auxanographic characteristics. Representative strains of the three clusters were also genotypically characterized by determining their DNA base compositions, by performing DNA-DNA and DNA-rRNA hybridization experiments, and by determining their 16S rRNA gene sequences. Our results showed that two of the clusters identified on the basis of SDS-PAGE data are genotypically and phenotypically distinct groups that belong on the *Rhizobium meliloti-Rhizobium fredii* rRNA branch. The third cluster is localized on the *Rhizobium loti* rRNA branch in the vicinity of *Rhizobium huakuii* and contains strains isolated in Africa, in Brazil, and in New Zealand from different leguminous species. On the basis of the results of the present study, we propose to emend the genus *Sinorhizobium* and to reclassify *R. meliloti* as *Sinorhizobium meliloti* comb. nov. In addition, two new species, *Sinorhizobium saheli* and *Sinorhizobium teranga*, are proposed for isolates from Senegal.

Classification of the legume root-nodulating bacteria has undergone major revisions and improvements in recent years (20). In particular, polyphasic taxonomy, which involves techniques that have various powers of discrimination, has been used, and the use of this method has resulted in a greater understanding of the complex intra- and intergeneric relationships of *Rhizobium* and *Bradyrhizobium* species (for a review see reference 17). In addition to the genera *Rhizobium* and *Bradyrhizobium*, a third genus, *Azorhizobium*, with the single species *Azorhizobium caulinodans*, was created by Dreyfus et al. (14) for stem-nodulating strains isolated from *Sesbania rostrata*; a second genotypic group in this genus was subsequently described (35, 36).

Within the genus *Rhizobium* the following three species were described by Jordan in 1984 (20): *Rhizobium meliloti*, *Rhizobium loti*, and type species *Rhizobium leguminosarum*, containing three biovars. Since then five other species have been created: *Rhizobium galegae* for isolates obtained from *Galega officinalis* and *Galega orientalis* (26), *Rhizobium huakuii* for strains obtained from *Astragalus sinicus* (4), *Rhizobium tropici* for the former *R. leguminosarum* biovar phaseoli type II strains (30), *Rhizobium etli* for the *R. leguminosarum* biovar phaseoli type I strains (39), and *Rhizobium fredii* for the fast-growing soybean-nodulating strains (38). *R. fredii* has been assigned to the genus *Sinorhizobium* (5), and a second species, *Sinorhizobium xinjiangensis*, has been proposed for fast-growing soybean-nodulating isolates obtained from the Xinjiang region in the People's Republic of China. The phylogenetic distinctness of the genus *Sinorhizobium* and the species status of *Sinorhizobium xinjiangensis* have been questioned on the basis of the results of rRNA studies (18, 19). All *Rhizobium* species belong to the alpha subclass of the *Proteobacteria*, where they constitute a single rRNA cluster together with *Agrobacterium*, *Brucella*, and *Rochalimaea* spp. (14, 19, 44, 46, 47). Within this rRNA cluster different groups can be differentiated; one of these groups is the *Agrobacterium-Rhizobium* group. Within this group *R. leguminosarum*, *R. tropici*, *R. etli*, and *Agrobacterium* biovar 2 constitute one subgroup (39, 44, 47). *R. galegae* constitutes a second subgroup together with *Agrobacterium* biovar 1, *Agrobacterium vitis*, and *Agrobacterium rubi*. *R. meliloti* and *R. fredii* are the members of a third subgroup, while *R. loti* and *R. huakuii* form a fourth sublineage (44, 46, 48). In the near future revision of the classification and nomenclature of these genera will be unavoidable. Recently, Sawada et al. (37) proposed that *Agrobacterium* biovars 1 and 2 should have species status; they proposed that the name *Agrobacterium tumefaciens* should be rejected and provided revised descriptions for *Agrobacterium radiobacter* (for the biovar 1 strains) and *Agrobacterium rhizogenes* (for the biovar 2

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strains). In this proposal *Agrobacterium radiobacter* became the type species of the genus *Agrobacterium*. In a request for a Judicial Opinion concerning the type species of the genus *Agrobacterium*, Bouzar (2) emphasized that Sawada et al. (37) did not take into account key judicial elements in *Agrobacterium* nomenclature, and Sawada et al. agreed with this request (2). As long as this request has not been considered, we shall use the *Agrobacterium* nomenclature used by Kersters and De Ley (21).

Most taxonomic work performed to date has been focused on strains that nodulate agriculturally important legumes. Other tropical rhizobia have been studied only sparsely, especially tropical rhizobia isolated from leguminous trees. Previous studies revealed that *Acacia* species are nodulated by *Rhizobium* and/or *Bradyrhizobium* strains (15), while *Sesbania* species are nodulated by *Rhizobium* and/or *Azorhizobium* strains (14). Isolates obtained from 36 *Sesbania* and *Acacia* species were grouped into three phenotypic clusters by Dreyfus et al. (14). By performing a numerical phenotypic analysis, Zhang et al. (49) showed that rhizobia isolated from root nodules of *Acacia senegal* and *Prosopis chiliensis* in Sudan are very diverse and can be placed in at least eight clusters that are distinct from previously described *Rhizobium* species. Moreira et al. (31) compared 180 slowly growing and fast-growing isolates obtained from nodules of tropical leguminous species in the Amazonian region and the Atlantic forests of Brazil with representatives of the different *Bradyrhizobium* species, *Rhizobium* species (except *R. etli* and *R. huakuii*), and *Azorhizobium* species by performing a sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis of their total proteins. Within the fast-growing isolates Moreira et al. identified several clusters which differed from the previously described species.

In this paper we describe the results of a polyphasic study (which included SDS-PAGE of cellular proteins, auxanographic tests, host specificity tests, DNA-DNA hybridization and DNA-rRNA hybridization experiments, and 16S rRNA gene sequencing) of 52 strains isolated from *Acacia* spp. and *Sesbania* spp. in Senegal, West Africa. Reference strains of *Azorhizobium caulinodans*, *Bradyrhizobium japonicum*, and different *Rhizobium* species were also included, together with representative strains of the different clusters of Brazilian rhizobia (31). On the basis of our results we propose that *R. meliloti* be reclassified in the genus *Sinorhizobium* as *Sinorhizobium meliloti* comb. nov. In addition, two new species, *Sinorhizobium saheli* and *Sinorhizobium teranga*, are created for two taxa comprising the new Senegalese isolates.

MATERIALS AND METHODS

Bacterial strains. Rhizobial strains were isolated from naturally occurring *Sesbania* root nodules or from root nodules harvested from young seedlings of different *Acacia* species grown in tubes in the presence of a soil suspension as follows. Soil samples collected at depths of 5 to 20 cm in various regions of Senegal in the neighborhood of a particular *Acacia* sp. were screened for the presence of rhizobial strains by inoculating 5- to 8-day-old *Acacia* seedlings of the same species grown in Jensen slant agar tubes containing 1 ml of a soil suspension (10%, wt/vol) that had been magnetically stirred for 30 min. Root nodules appeared after 1 to 3 weeks, and 2-week-old nodules were collected. The nodules were washed and immersed in 0.1% $HgCl_2$ for 5 min; after this the nodules were manipulated aseptically. Each nodule was rinsed eight times in sterile water and crushed in 1 drop of sterile water. The resulting suspension was streaked onto yeast mannitol agar (YMA) (see below), and isolated colonies appeared after incubation for 2 or 3 days at 33°C. Pure cultures were obtained after single colonies were streaked two or three times.

All of the strains which we used are shown in Table 1. These strains were checked for purity by repeatedly streaking them on YMA and by examining living and Gram-stained cells with a microscope. When two stable colony morphology variants were obtained, both were included in the SDS-PAGE analysis, and these variants were designated t1 and t2. The identities of the nodulating strains were checked by performing plant infection tests with the original host plants.

Type or representative strains of most *Rhizobium* species, *B. japonicum*, *Bradyrhizobium elkanii*, *Azorhizobium caulinodans*, and the various clusters of Brazilian rhizobia described by Moreira et al. (31) were included in this polyphasic study. *R. huakuii* was included only in the auxanographic tests because we obtained the type strain only recently. *R. etli* and *S. xinjiangensis* were not included.

Growth and culture conditions. All *Rhizobium* and *Bradyrhizobium* strains were maintained on YMA, which contained (per liter) 10 g of mannitol, 0.5 g of sodium glutamate, 0.5 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of NaCl, 0.04 g of $CaCl_2$, 0.004 g of $FeCl_3$, 1 g of yeast extract (Difco), and 20 g of agar; the pH of this medium was 6.8. *Azorhizobium* and *Agrobacterium* strains were maintained on yeast extract-peptone-glucose medium, which contained (per liter of 0.01 M phosphate buffer [pH 7.2]) 5 g of peptone (Oxoid), 1 g of yeast extract (Oxoid), 5 g of beef extract (Oxoid), 5 g of sucrose, and 0.592 g of $MgSO_4 \cdot 7H_2O$. All strains were stored at −80°C on the same medium on which they were maintained, except that the medium contained 20% (vol/vol) glycerol. *Mycoplana*, *Ochrobactrum*, and *Phyllobacterium* strains were maintained on nutrient agar, which contained (per liter) 1 g of beef extract (Oxoid), 2 g of yeast extract (Oxoid), 5 g of peptone (Oxoid), 5 g of NaCl, and 20 g of agar; the pH of this medium was 7.4.

Morphological and physiological tests. Cell dimensions and morphology were determined by phase-contrast microscopy. Cells of two or three representative strains of each group were negatively stained with phosphotungstic acid, and the type of flagellation was determined by transmission electron microscopy (25).

Four to eight representatives of each group were used to determine the maximum growth temperature of the taxon by inoculating them onto YMA plates and incubating the resulting cultures at various temperatures.

PAGE of total bacterial proteins. Most strains were grown at 28°C for 48 h (the exceptions were the bradyrhizobia, which were grown for 72 h) in Roux flasks on TY medium, which contained (per liter) 5 g of tryptone (Oxoid), 0.75 g of yeast extract (Oxoid), 0.454 g of KH_2PO_4, 2.388 g of $Na_2HPO_4 \cdot 12H_2O$, 1 g of $CaCl_2$, and 20 g of agar (Lab M) (pH 6.8 to 7). Whole-cell protein extracts were prepared, and SDS-PAGE was performed by using the procedure of Laemmli (24) with slight modifications, as described previously (23). The normalized densitometric traces of the protein electrophoretic patterns were grouped by performing a numerical analysis, using the GelCompar 2.2 software package (41). The level of similarity between each pair of traces was expressed by the Pearson product moment correlation coefficient (r), which for convenience was converted to a percentage (33, 34).

Plant infection tests. Seeds were scarified and surface sterilized with concentrated sulfuric acid. The lengths of time that the seeds of the different plant species were treated with H_2SO_4 were as follows: *Acacia senegal*, 14 min; *Acacia albida*, 30 min; *Acacia seyal*, 30 min; *Acacia raddiana*, 150 min; *Sesbania rostrata*, 30 to 60 min; *Sesbania pubescens*, 60 min;

TABLE 1. Strains used in this investigation

Strain[a]	LMG no.	Host plant or source[b]	Geographical origin[b]	Reference[b]	PAGE group[c]
New isolates					
Sinorhizobium teranga (cluster T)					
ORS 22	6463	*Sesbania rostrata*	Senegal	15	
ORS 51	6464,7843	*Sesbania rostrata*	Senegal	15	
ORS 15	7833	*Sesbania* sp.	Senegal	This study	
ORS 1009[T]	7834[T]	*Acacia laeta*	Senegal	1	
ORS 19	7841t1	*Sesbania cannabina*	Senegal	32	
ORS 20	7841t2	Nonmucous derivative of ORS 19		This study	
ORS 1013	7844	*Acacia senegal*	Senegal	This study	
ORS 1007	7847	*Acacia laeta*	Senegal	This study	
ORS 1016	7851t1	*Acacia laeta*	Senegal	This study	
ORS 1079	7851t2	Nonmucous derivative of ORS 1016		This study	
ORS 929	8313t1	*Acacia* sp.	Senegal	14	
ORS 52	11859	*Sesbania rostrata*	Senegal	15	
ORS 53	11860	*Sesbania rostrata*	Senegal	15	
ORS 604	11865	*Sesbania aculeata*	Senegal	32	
ORS 613	11866	*Sesbania sesban*	Senegal	32	
ORS 8	11870	*Sesbania rostrata*	Senegal	This study	
ORS 1045	11901	*Acacia raddiana*	Senegal	This study	
ORS 1047	11903	*Acacia horrida*	Senegal	This study	
ORS 1057	11911	*Acacia mollissima*	Senegal	This study	
ORS 1058	11912	*Acacia mollissima*	Senegal	This study	
ORS 1071	11924	*Acacia senegal*	Senegal	This study	
ORS 1072	11925	*Acacia senegal*	Senegal	This study	
ORS 1073	11926	*Acacia senegal*	Senegal	This study	
Sinorhizobium saheli (cluster S)					
ORS 609[Td]	7837[T]	*Sesbania cannabina*	Senegal	32	
ORS 609t1	8309t1	*Sesbania cannabina*	Senegal	32	
ORS 609t2	8309t2	*Sesbania cannabina*	Senegal	32	
ORS 611	7842,8310	*Sesbania grandiflora*	Senegal	32	
ORS 10	11858	*Sesbania rostrata*	Senegal	32	
ORS 600	11864	*Sesbania pachycarpa*	Senegal	32	
Cluster U					
NZP 2037	6123	*Lotus divaricatus*	New Zealand		2
NZP 2014	6124	*Lotus corniculatus*			
ORS 1001	7836	*Acacia senegal*	Senegal	1	
ORS 1015	7839	*Acacia senegal*	Senegal	1	
ORS 1005	7845t1	*Acacia* sp.	Senegal	This study	
ORS 1004	7848	*Acacia senegal*	Senegal	1	
ORS 1014t1	7849t1	*Acacia senegal*	Senegal	This study	
ORS 1014t2	7849t2	*Acacia senegal*	Senegal	This study	
ORS 1010	7853	*Acacia senegal*	Senegal	This study	
ORS 1002	7854	*Acacia senegal*	Senegal	1	
ORS 13	7921	*Acacia* sp.	Senegal	This study	
ORS 1088	11880	*Acacia seyal*	Senegal	This study	
ORS 1018	11881	*Acacia senegal*	Senegal	This study	
ORS 1020	11883	*Acacia senegal*	Senegal	This study	
ORS 1024	11884	*Acacia senegal*	Senegal	This study	
ORS 1029	11889	*Acacia senegal*	Senegal	This study	
ORS 1030	11890	*Acacia senegal*	Senegal	This study	
ORS 1031	11891	*Acacia senegal*	Senegal	This study	
ORS 1032	11892	*Acacia senegal*	Senegal	This study	
ORS 1035	11893	*Acacia senegal*	Senegal	This study	
ORS 1036	11894	*Acacia senegal*	Senegal	This study	
ORS 1037	11895	*Acacia senegal*	Senegal	This study	
ORS 1038	11896	*Acacia senegal*	Senegal	This study	
ORS 1040	11898	*Acacia senegal*	Senegal	This study	
ORS 1093		*Acacia senegal*	Senegal	This study	
BR3804	9970	*Chamaecrista ensiformis*	Brazil	31	2
INPA 12A	10031	*Leucaena leucocephala*	Brazil	31	2
INPA 78B	10056	*Leucaena diversifolia*	Brazil	31	2
INPA 118A	10059	*Leucaena pulvurulenta*	Brazil	31	2
INPA 129A	10061	*Leucaena pulvurulenta*	Brazil	31	2
INPA 338A	10093	*Leucaena diversifolia*	Brazil	31	2

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TABLE 1—*Continued*

Strain[a]	LMG no.	Host plant or source[b]	Geographical origin[b]	Reference[b]	PAGE group[c]
ORS 1096	12019	*Acacia* sp.	Senegal	This study	
Bradyrhizobium japonicum					
NZP 5533	6136	*Glycine max*			12E
NZP 5549[T]	6138[T]	*Glycine hispida*	Japan		12B
USDA 135	8321	*Glycine max*	United States		
Bradyrhizobium elkanii					
USDA 76[T]	6134[T]	*Glycine max*			12E
USDA 31	6135	*Glycine max*	United States		12E
Bradyrhizobium sp.					
BR 809	9950	*Leucaena leucocephala*	Brazil	31	5
INPA 522B	10114	*Swartzia polyphylla*	Brazil	31	5
INPA 523B	10115	*Swartzia polyphylla*		31	5
NZP 2309	6128	*Lotus pedunculatus*	Australia		
NZP 2314	6129	*Lotus uliginosus*	Australia		
BR 5202	9990	*Erythrina speciosa*		31	17
BR 5205	9991	*Erythrina speciosa*	Brazil	31	17
BR 5609	9997	*Albizia falcata*	Brazil	31	16
BR 5611	9998	*Albizia falcata*	Brazil	31	15
FL 27	10023	*Melanoxylon* sp.	Brazil	31	15
FL 276	10025	*Abrus* sp.	Brazil	31	15
FL 281	10026	*Abrus* sp.	Brazil	31	15
INPA 198A	10080	*Ormosia macrocalyx*	Brazil	31	22
INPA 223A	10085	*Pentaclethra macroloba*	Brazil	31	22
INPA 306A	10092	*Swartzia schomburgkii*	Brazil	31	20
INPA 549A	10118	*Tachigalia paniculata*	Brazil	31	23
INPA 553A	10119	*Tachigalia paniculata*	Brazil	31	23
INPA 589A	10139	*Clathrotropis nitida*	Brazil	31	20
Rhizobium sp.					
BR 811	9951	*Leucaena leucocephala*	Brazil	31	18
BR 814	9952	*Leucaena leucocephala*	Brazil	31	7
BR 817	9953	*Leucaena leucocephala*	Brazil	31	18
BR 819	9954	*Leucaena leucocephala*	Brazil	31	3
BR 827	9956	*Leucaena leucocephala*	Brazil	31	18
BR 3614	9964	*Acacia decurrens*	Brazil	31	21
BR 4301	9978	*Calliandra callothirsus*	Brazil	31	7
BR 5401	9993	*Sesbania marginata*	Brazil	31	14
BR 5404	9994	*Sesbania marginata*	Brazil	31	14
BR 6001	10000	*Lonchocarpus* sp.	Brazil	31	7
BR 6806	10007	*Pithecellobium dulce*	Brazil	31	3
BR 8005	10012	*Clitoria racemosa*	Brazil	31	11
BR 8006	10013	*Clitoria racemosa*	Brazil	31	11
BR 8802	10020	*Gliricidia sepium*	Brazil	31	7
BR 8803	10022	*Gliricidia sepium*	Brazil	31	7
INPA 133B	10062	*Leucaena leucocephala*	Brazil	31	15
BR 3459a	10131	*Mimosa foliculosa*	Brazil	31	21
BR 8801	10132	*Gliricidia sepium*	Brazil	31	7
INPA 95A	10134	*Leucaena pulvurulenta*	Brazil	31	15
Sinorhizobium fredii					
USDA 205[T]	6217[T]	*Glycine max*	People's Republic of China		18
USDA 191	8317	*Soil*	People's Republic of China		
Rhizobium huakuii IAM 14158[T]	14107[T]	*Astragalus sinicus*	People's Republic of China	4	
Rhizobium galegae					
HAMBI 540[T]	6214[T]	*Galega orientalis*	Finland		8
HAMBI 1147	6215	*Galega orientalis*	USSR		8
Rhizobium leguminosarum biovar trifolii					
NZP 1	6119	*Trifolium repens*			10
ATCC 14480	8820	*Trifolium pratens*			10
Rhizobium leguminosarum biovar viciae ATCC 10004[T]	8817[T]	*Pisum sativum*			10

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TABLE 1—*Continued*

Strain[a]	LMG no.	Host plant or source[b]	Geographical origin[b]	Reference[b]	PAGE group[c]
Rhizobium leguminosarum biovar phaseoli Erd. 316C10a	4285	*Phaseolus vulgaris*			10
Rhizobium loti					
3F6g2	4284	*Caragana arborescens*	Colorado		4
NZP 2230	6126	*Lotus maroccanus*	Morocco		
NZP 2213^T	6125^T	*Lotus corniculatus*	New Zealand		4
Sinorhizobium meliloti					
NZP 4009	6130	*Medicago sativa*	Australia		19
NZP 4027^T	6133^T	*Medicago sativa*			19
3DOa30	4266	*Medicago sativa*			
Rhizobium tropici group a					
CNPAF 119	9502	*Phaseolus vulgaris*	Brazil	31	6
CFN 299	9517	*Phaseolus vulgaris*		30	6
T2A10	10336	*Phaseolus vulgaris*			6
Rhizobium tropici group b					
C-O5	9518	*Phaseolus vulgaris*			6
BR 2611	9519	*Phaseolus vulgaris*			6
CIAT 899^T	9503^T	*Phaseolus vulgaris*		30	6
Azorhizobium caulinodans					
ORS 571^T	6465^T	*Sesbania rostrata*	Senegal	14	9
FY12	11352	*Sesbania rostrata*	Senegal	36	
ORS 470	11818	*Sesbania rostrata*	Senegal	This study	
ORS 478	11820	*Sesbania rostrata*	Senegal	This study	
ORS 491	11823	*Sesbania rostrata*	Senegal	This study	
Azorhizobium sp. strain SG05	11355	*Sesbania rostrata*	Senegal	36	
Agrobacterium biovar 1					
Agrobacterium tumefaciens					
B6^T	187^T				
ICPB TT111	196				
B2a	268				
IIChrysanthemum	303				
Agrobacterium radiobacter					
ATCC 19358^T	140^T				
Bernaerts M2/1	147				
Agrobacterium biovar 2					
Agrobacterium sp. strain Kerr 38	161				
Agrobacterium tumefaciens Apple 185	219				
Agrobacterium rhizogenes ATCC 11325^T	150^T				
Agrobacterium rubi ATCC 13335^T	156^T				
Agrobacterium vitis					
Pan. AG61	257				
Pan. AG63	258				
Agrobacterium (separate taxa)					
Agrobacterium tumefaciens					
NCPPB 1771	233				
Zutra 3I/A	198				
Agrobacterium radiobacter					
CDC A6597	383				
CDC C7258	385				
Mycoplana dimorpha NCIB 9439^T	4061^T				
Mycoplana ramosa NCIB 9440^T	3026^T				

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TABLE 1—*Continued*

Strain[a]	LMG no.	Host plant or source[b]	Geographical origin[b]	Reference[b]	PAGE group[c]
Ochrobactrum anthropi					
AB 940	33				
AB 1196	34				
AB 1293	35				
CCUG 7349	2136				
CNS 2.75	3301				
CNS 23.76	3306				
CIP 8174	3329				
CIP 110.77	3330				
CIP 14970^T	3331^T				
CIP 353.75	3333				
Phyllobacterium myrcinacearum	2(t1)				
NCIB 12127	3				
Phyllobacterium rubiacearum	1(t1)^T				
NCIB 12128^T					
Phyllobacterium sp.					
PGSB 6270	8227				
PGSB 3714	8231				

[a] ATCC, American Type Culture Collection, Rockville, Md.; BR and FL, strains from the CNPBS/EMBRAPA, Centro Nacional de Pesquisa em Biologia do Solo, Seropedica, Rio de Janeiro, Brazil, and the Emprasa Brasiliera de Pesquisa Agropequaria; CFN, Centrode Investigacion sobre Fijacion de Nitrogeno, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico; CIAT, Rhizobium Collection, Centro International de Agricultura Tropical, Cali, Colombia; HAMBI, Culture Collection of the Department of Microbiology, University of Helsinki, Helsinki, Finland; INPA, National Institute of Amazonia Research, Manaus, Brazil; LMG, Collection of Bacteria of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; NZP, Culture Collection of the Department for Scientific and Industrial Research, Biochemistry Division, Palmerston North, New Zealand; ORS, ORSTOM Collection, Institut Français de Recherche Scientifique pour le Développement en Coopération, Dakar, Senegal; Pan., C. Panagopoulos, Crete, Greece; USDA, U.S. Department of Agriculture, Beltsville, Md.

[b] Information is given only when it is known and/or meaningful.

[c] PAGE group of Moreira et al. (31).

[d] In a second subculture of strain ORS 609^T (= LMG 7837^T), two stable colony types were isolated. The protein profiles of these colony types were identical.

Sesbania grandiflora, 60 min; *Neptunia oleracea*, 30 min; and *Leucaena leucocephala*, 30 min. After acid treatment, the seeds were washed with water until all traces of acid were removed. The seeds were incubated so that they would germinate in sterile petri dishes on 1% water agar for 24 to 48 h and then were transferred to tubes containing Jensen seedling slant agar (42) for root nodulation trials (8 to 10 plants were routinely tested with each strain). Root nodules appeared ca. 10 to 20 days after inoculation, and 3 weeks later the nodules were fully developed.

Auxanographic tests. API galleries (API 50CH, API 50AO and API 50AA; bioMérieux, Montalieu-Vercieu, France) were used to determine whether 147 organic compounds were utilized as sole carbon sources, as described previously (22). Inocula were obtained from 36-h YMA slant cultures. After inoculation, the galleries were incubated at 30°C, and results were determined after 1, 2, 4, and 7 days. About 20 strains were included in duplicate on separate occasions to verify the reproducibility of the tests.

The results of the auxanographic tests were scored as described previously (22). The levels of interstrain similarity (S) were calculated by using a similarity distance coefficient derived from the Canberra metric coefficient (d_{Canb}) (40), as follows: $S = 100 \times (1 - d_{Canb})$. A cluster analysis was performed by using the unweighted average pair group method (41), the Clustan 2.1 program of Wishart (45), and the Siemens model 7570-C computer of the Centraal Digitaal Rekencentrum, Universiteit Gent, Ghent, Belgium.

DNA base composition. Cells were grown for 2 to 3 days in Roux flasks on TY medium. High-molecular-weight DNA was prepared by the method of Marmur (28). The guanine-plus-cytosine (G+C) content of the DNA was determined by the thermal denaturation method (12) and was calculated by using the equation of Marmur and Doty (29), as modified by De Ley (8). DNA from *Escherichia coli* LMG 2093 was used as a reference.

DNA-rRNA hybridization. High-molecular-weight DNA was purified by CsCl gradient centrifugation, denatured, and fixed on cellulose nitrate filters (type SM 11358; Sartorius, Göttingen, Germany) as described previously (11). A ^{3}H-labeled 23S rRNA probe was prepared from *Sinorhizobium teranga* ORS 22 by in vivo labeling with [5,6-^{3}H]uracil in medium C containing (per liter) 30 g of glucose, 3 g of peptone, 3 g of $(NH_4)_2SO_4$, 0.2 g of KH_2PO_4, 0.8 g of K_2HPO_4, 0.1 g of $CaSO_4 \cdot 2H_2O$, 10^{-4} g of $FeCl_3 \cdot 6H_2O$, and 5×10^{-3} g of $Na_2MoO_4 \cdot 2H_2O$. The specific activity of the probe was 160,000 dpm. The 23S rRNA probe was prepared and purified as described previously (19). The following other rRNA probes were available from members of our research group: ^{3}H-labeled 23S rRNA from *R. meliloti* LMG 6130 and ^{3}H-labeled 23S rRNA from strain LMG 6123. Hybridizations were performed as described by De Ley and De Smedt (11). The temperature at which 50% of the DNA-rRNA hybrid was denatured under standard conditions [$T_{m(e)}$] was the decisive taxonomic parameter.

Analysis of 16S rRNA genes. The 16S rRNA gene sequences of the following five strains were determined: *Sinorhizobium teranga* ORS 22 and ORS 1009^T (T = type strain), strains ORS 1001 and ORS 1002, and *Sinorhizobium saheli* ORS 609^T. A large fragment of the 16S rRNA gene (positions 21 to 1521; *E. coli* numbering system [3]) was amplified by a PCR and sequenced directly as described previously (43, 44). The sequences determined, together with reference sequences obtained from the EMBL data library, were aligned by using the PILEUP, PRETTY, and UGLY programs in the Genetics Computer Group sequence analysis package (13). The align-

ment was verified and corrected manually. In all, a continuous stretch of 1,401 positions (including gaps) was used for further analysis. Distances were calculated by using the DNADIST program of the Phylogeny Inference Package (16). The programs DNABOOT, NEIGHBOR, and CONSENCE of the same package were used to produce an unrooted phylogenetic tree. Similarity values were calculated by using the GAP program in the Genetics Computer Group package.

DNA-DNA hybridization. DNA-DNA hybridizations were performed by the initial renaturation rate method (10). Renaturations experiments in which approximately 50 μg of DNA per ml was used were carried out at 79.8°C, which is the optimal renaturation temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7).

Nucleotide sequence accession numbers. The newly determined 16S rRNA sequences were deposited in the EMBL Data Library under accession numbers X68387 to X68391.

RESULTS

Isolation of rhizobia from root nodules of *Sesbania* spp. and *Acacia* spp. About 80 isolates were obtained from different ecological areas of Senegal either by directly isolating them from naturally occurring nodules or by trapping them on young plants grown in the presence of soil samples. A total of 52 strains, which we placed in three clusters (as determined by SDS-PAGE and auxanography [see below]), were studied further. These strains are listed in Table 1. Fifteen of these strains originated from different *Sesbania* species (*Sesbania rostrata*, *Sesbania cannabina*, *Sesbania aculeata*, *Sesbania sesban*, *Sesbania pachycarpa*, *Sesbania grandiflora*), and 37 originated from different *Acacia* species (*Acacia senegal*, *Acacia seyal*, *Acacia raddiana*, *Acacia horrida*, *Acacia mollissima*, *Acacia laeta*).

SDS-PAGE of total bacterial proteins. The SDS-PAGE whole-cell protein patterns of the 80 rhizobial isolates obtained from Senegal were scanned and analyzed numerically, together with the patterns of 67 reference strains available in our database, which represented the different *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* groups and the new protein electrophoretic clusters identified among the fast growers by Moreira et al. (31). Representative strains belonging to phenotypic cluster 3 described by Dreyfus et al. (14) (strains ORS 22, ORS 52, and ORS 53) were also included, as were ORS 609^T and ORS 611 [the $T_{m(e)}$ values of the DNA-rRNA hybrids between strains ORS 609^T and ORS 611 and *R. meliloti* LMG 6130 have been determined previously (14)]. The reproducibility of the SDS-PAGE technique was checked by including different subcultures of the same strain and different protein extracts of one strain. In all cases such profiles were very similar (the *r* values were between 93 and 97%). The protein profiles of different colony morphology variants of the same strain (designated t1 and t2) were in most cases nearly identical or very similar (e.g., strains ORS 1014t1 and ORS 1014t2) (Fig. 1) (see below).

At or above a mean correlation coefficient (*r* value) of 87.5% the different *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* species constitute separate clusters. In addition to the members of the *R. loti* protein electrophoretic cluster containing the type strain, two other *R. loti* strains (LMG 6123 and LMG 6124) belong in cluster U (see below). Also at this level most of the clusters of Moreira et al. (31) (designated FM followed by the original cluster numbers) were recovered; the exceptions were (i) cluster FM2, which grouped with one of our clusters (see below); (ii) cluster FM15, which was split up (two strains now belong to a new cluster, while four other strains group together with one representative strain of cluster FM16); and (iii) representative strains of clusters FM17 and FM20, which now belong to a single cluster. *Azorhizobium* sp. strain LMG 11355 produced a unique protein profile.

A total of 52 of the Senegalese isolates could be placed in three protein electrophoretic clusters (clusters T, S, and U), while the remaining 28 strains were not members of any group. Our results are presented as a similarity dendrogram in Fig. 1. For the sake of clarity, most of the 28 ungrouped Senegalese isolates are not included in Fig. 1; the only exception is strain ORS 1096, which is the closest relative of cluster U as determined by its protein profile. Representative profiles are shown in Fig. 2.

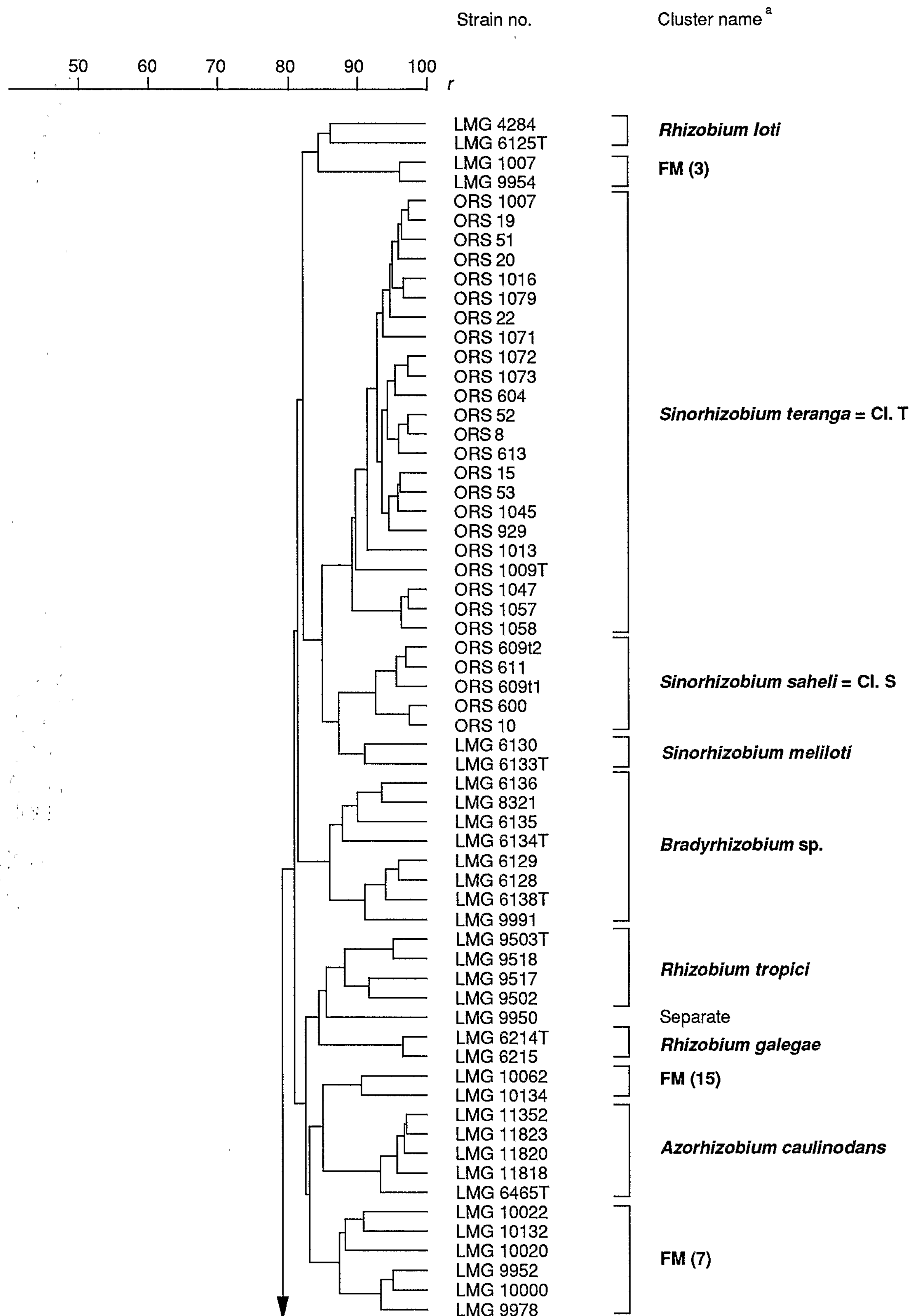
Clusters S and T exhibit high levels of similarity with each other and with the *R. meliloti* protein gel electrophoretic cluster. Cluster T is composed of 10 isolates originating from *Sesbania* spp. and 13 isolates originating from *Acacia* spp. Cluster S is composed of five isolates originating from *Sesbania* spp.

Cluster U consists of 24 Senegalese isolates that originated from *Acacia* spp. (including 20 strains obtained from *Acacia senegal*) and contains four subclusters (subclusters U1 to U4) and one separate strain (ORS 1002). Cluster FM2 of Moreira et al. (31), which contained five strains of Brazilian rhizobia and *R. loti* LMG 6123 and LMG 6124, belongs in cluster U (subclusters U3 and U4, respectively).

Morphological and physiological characteristics. All of the cluster S, T, and U isolates were fast growers and grew at temperatures up to 40°C. Cluster U strains grew at temperatures up to 42°C, and most of the cluster S and T strains grew at temperatures up to 44°C. Most of the strains had one or several polar or subpolar flagella.

Host specificity. The host range of cluster U strains is more or less restricted to *Acacia*, *Leucanea*, and *Neptunia* species, while the S and T strains are more promiscuous and are found in *Sesbania*, *Acacia*, *Leucaena*, and *Neptunia* species (27).

Numerical analysis of auxanographic results. All of the new isolates (except strains ORS 20, ORS 1073, ORS 10, and ORS 13) were tested for utilization of 147 organic compounds as sole carbon sources by using the API 50 system. The reproducibility of the tests was good. The average interstrain similarity values for strains tested in duplicate were between 88 and 92%. Subcultures of the same strain obtained on different dates (and consequently with different Collection of Bacteria of the Laboratorium voor Microbiologie numbers) gave reproducible results (89 to 95%). The results obtained for representative strains belonging to other *Rhizobium* species and related groups (including representatives of the three *Agrobacterium* biovars and the genera *Ochrobactrum*, *Phyllobacterium*, and *Mycoplana*) were available in the database of our research group and were included in the numerical analysis. The results are shown in Table 2 and in Fig. 3. At a similarity coefficient of 85%, different clusters could be distinguished, and several of these clusters corresponded to clusters identified by SDS-PAGE pattern analysis. The subgroups of *R. tropici* (subgroups a and b) did not group together. Representative strains of the two biovars of the genus *Agrobacterium*, *Agrobacterium rubi*, and *Agrobacterium vitis* were members of separate auxanographic groups. All *Ochrobactrum anthropi* strains investigated clustered together, as did the members of the genera *Phyllobacterium* and *Mycoplana*. The correspondence between gel electrophoretic clusters T and S and the auxanographic groups was excellent; gel electrophoretic cluster U was more difficult to recognize because the auxanographic results obtained for the Brazilian isolates (LMG 10093, LMG 10056, LMG 10061) belonging to protein cluster FM2 were quite different from the


Strain no.
Cluster name [a]
50
60
70
80
90
100
r
LMG 4284
LMG 6125T
Rhizobium loti
LMG 1007
LMG 9954
FM (3)
ORS 1007
ORS 19
ORS 51
ORS 20
ORS 1016
ORS 1079
ORS 22
ORS 1071
ORS 1072
ORS 1073
ORS 604
ORS 52
ORS 8
ORS 613
ORS 15
ORS 53
ORS 1045
ORS 929
ORS 1013
ORS 1009T
ORS 1047
ORS 1057
ORS 1058
Sinorhizobium teranga = Cl. T
ORS 609t2
ORS 611
ORS 609t1
ORS 600
ORS 10
Sinorhizobium saheli = Cl. S
LMG 6130
LMG 6133T
Sinorhizobium meliloti
LMG 6136
LMG 8321
LMG 6135
LMG 6134T
LMG 6129
LMG 6128
LMG 6138T
LMG 9991
Bradyrhizobium sp.
LMG 9503T
LMG 9518
LMG 9517
LMG 9502
Rhizobium tropici
LMG 9950
Separate
LMG 6214T
LMG 6215
Rhizobium galegae
LMG 10062
LMG 10134
FM (15)
LMG 11352
LMG 11823
LMG 11820
LMG 11818
LMG 6465T
Azorhizobium caulinodans
LMG 10022
LMG 10132
LMG 10020
LMG 9952
LMG 10000
LMG 9978
FM (7)

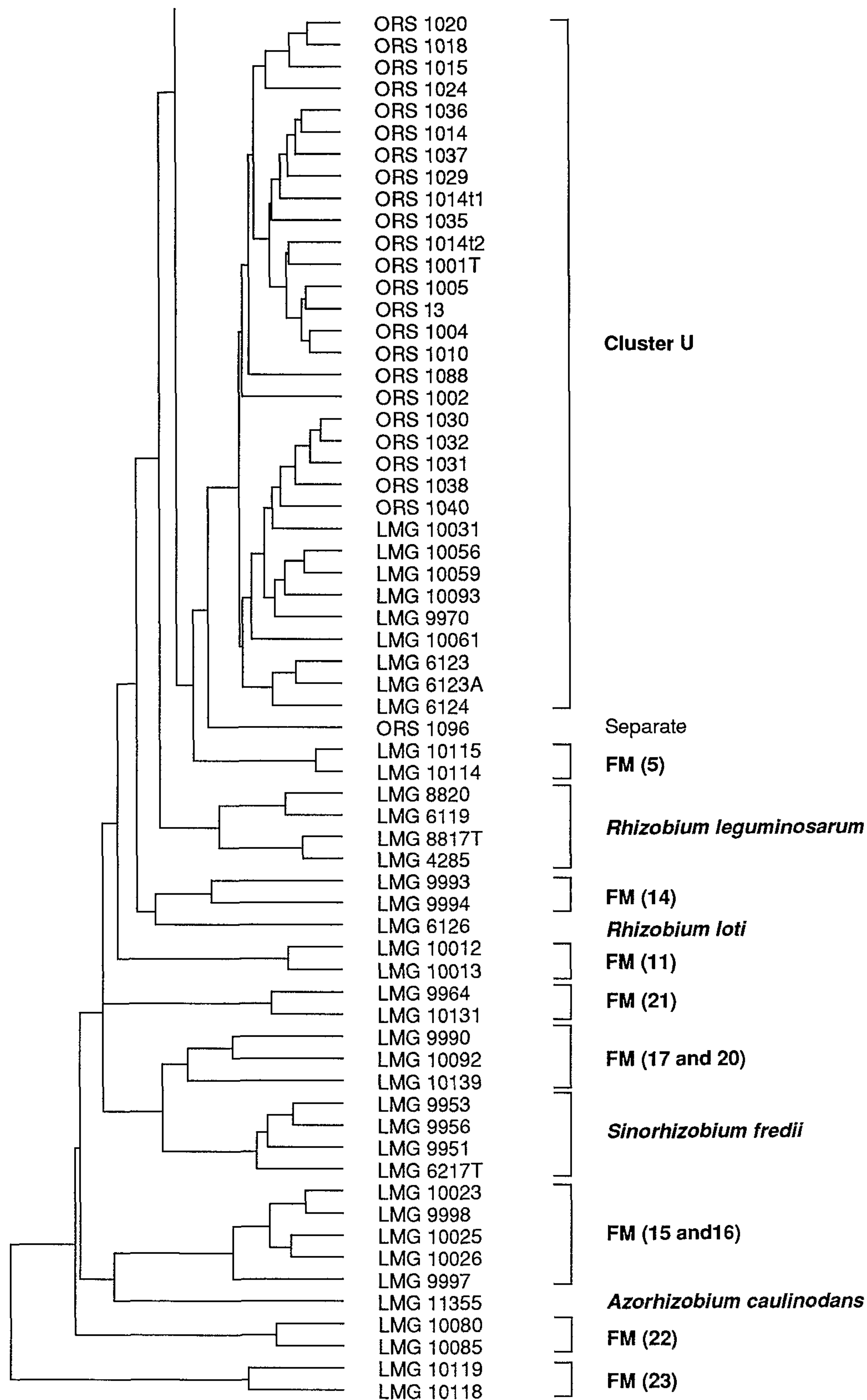
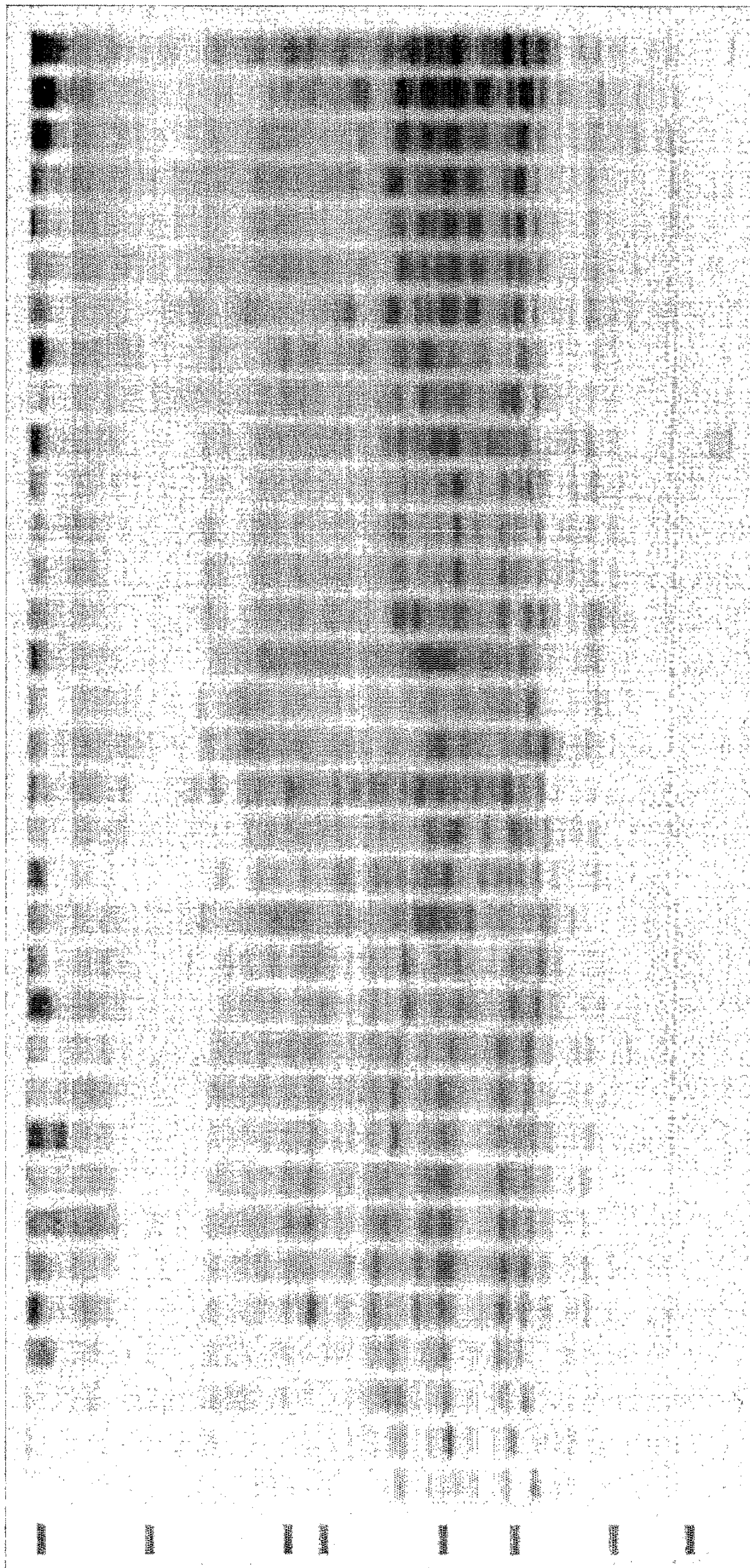


FIG. 1. Dendrogram showing the relationships among the electrophoretic protein patterns of Senegalese, Brazilian, and reference strains of *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Sinorhizobium* species. The dendrogram is based on mean correlation coefficient (r) values, which were grouped by the unweighted average pair group method. Positions 10 to 320 of the 400-point traces were used to calculate the levels of similarity between individual pairs of traces. The reproducibility of the technique is illustrated by results obtained for two independent extracts of strain LMG 6123. The bar indicates r values converted to percentages. Cl., cluster.



LMG 6125T
ORS 1007
ORS 1016
ORS 1072
ORS 52
ORS 1045
ORS 1013
ORS 1009T
ORS 1047
ORS 609t1
ORS 611
ORS 600
ORS 10
LMG 6133T
LMG 6138T
LMG 6135
LMG 6134
LMG 9502
LMG 9503T
LMG 6214T
LMG 6465T
ORS 1020
ORS 1024
ORS 1037
ORS 1001T
ORS 1004
ORS 1032
ORS 1031
LMG 10056
LMG 10061
LMG 6123
LMG 6124
LMG 8817T
LMG 6217T
MWM

results obtained for the other members of cluster U. A single Senegalese isolate (ORS 1029) was auxanographically the most aberrant strain in gel electrophoretic cluster U. *R. loti* LMG 6123, another member of gel electrophoretic cluster FM2, was similar to the Senegalese isolates. Two other strains of *R. loti*, including the type strain, were auxanographically more similar to the Senegalese members of cluster U than to the Brazilian members. Of the 147 carbon sources tested, 35 were utilized by all of the strains belonging to the three groups, while 59 were utilized by none. We found that xylitol, glycolate, and L-citrulline could be used to discriminate among clusters S, T, and U (Table 2). We compared our results with the data in the database available in our research group, and features that distinguish clusters T, S, and U and other *Rhizobium* species and related genera are shown in Table 2. We found several features that distinguish clusters T and S and their closest neighbors, *R. meliloti* and *R. fredii* (utilization of erythritol, methyl-xyloside, L-sorbose, dulcitol, methyl-D-glycoside, xylitol, D-lyxose, D-tagatose, L-arabitol, 2-ketogluconate, acetate, propionate, isobutyrate, glycolate, DL-hydroxybutyrate, aconitate, *p*-hydroxybenzoate, L-valine, L-serine, L-threonine, trigonelline, L-aspartate, L-lysine, L-citrulline, β-alanine, and DL-3-aminobutyrate). *R. loti* and cluster U could not be distinguished by the results of the auxanographic tests used in this study. On the basis of previously published data and our results obtained with the type strain (Table 2), *R. huakuii* differs from *R. loti* and other members of cluster U by utilizing D-melibiose, D-fucose, L-fucose, and oxalate.

G+C contents of DNAs. The G+C content ranges for clusters T and U were 60.8 to 61.6 and 62.6 to 63.9 mol%, respectively (Table 3). Two representative strains of cluster S had a G+C content of 65.7 mol% (Table 3).

DNA-rRNA hybridization. In order to determine the genetic relationships among clusters S, T, and U and the different *Rhizobium* species, we performed DNA-rRNA hybridization experiments with an rRNA probe from cluster T strain ORS 22 and other rRNA probes available from members of our research group (one from *R. meliloti* LMG 6130 and one from strain LMG 6123, representing cluster U). The results are shown in Table 3.

Cluster U could not be differentiated from *R. loti* on the basis of DNA-rRNA hybridization data. In fact, strain LMG 6123 has been described as a member of *R. loti* by Crow et al. (6) and has been used previously (19) to prepare a labeled rRNA probe to differentiate the *R. loti* rRNA branch that is the most divergent branch (9, 14) in the *Rhizobium-Agrobacterium* rRNA cluster. As expected from their similar protein profiles, cluster T strains had indistinguishable $T_{m(e)}$ values when rRNA from strain ORS 22 was used. Both cluster S and cluster T are rather closely related to *R. meliloti.*

16S rRNA gene sequencing. The 16S rRNA sequences which we determined were compared with the 16S rRNA sequences of other members of the alpha 2 subclass of the *Proteobacteria* available from the EMBL Data Library. Figure 4 is a tree showing the phylogenetic positions of the new isolates within the alpha 2 subclass of the *Proteobacteria*. Representatives of cluster T (strains ORS 22 and ORS 1009^T) and cluster S (strain ORS 609^T) were found to be closely related but nevertheless members of distinct species within the *R. fredii-R. meliloti* lineage (level of sequence similarity, 98.9%, corresponding to 17 differences in a comparison of 1,436 bases). The levels of sequence similarity between cluster S and *R. meliloti* and between cluster S and *R. fredii* were 98.3% (24 differences in a comparison of 1,436 bases) and 99.2% (11 differences in a comparison of 1,436 bases), respectively; and the levels of sequence similarity between cluster T and *R. meliloti* and between cluster T and *R. fredii* were 97.6% (34 differences in a comparison of 1,436 bases) and 98.4% (24 differences in a comparison of 1,437 bases), respectively. For comparison, the level of sequence similarity between *R. meliloti* and *R. fredii* was 99.0% (15 differences in a comparison of 1,437 bases). Cluster U strains ORS 1001 (subcluster U1) and ORS 1002 (subcluster U2) exhibited high levels of sequence similarity with *R. huakuii* (99.6%, corresponding to 7 differences in 1,430 bases) and *R. loti* (98.0%, corresponding to 25 differences in 1,436 bases). The level of sequence similarity between *R. loti* and *R. huakuii* was 98.3% (24 differences in a comparison of 1,428 bases). The partial sequences (positions 350 to 850) of two representative strains of subclusters U3 (LMG 6123) and U4 (LMG 10056) were also determined, and the close phylogenetic relatedness of these organisms to strains ORS 1001 and ORS 1002 was confirmed (0 or 1 differences in a comparison of 500 nucleotides).

DNA-DNA hybridization. As Table 4 shows, we found high levels of DNA-DNA binding (79 to 100%) within clusters T and S. Representative DNAs of members of clusters T and S did not hybridize to significant degrees with each other or with the DNAs of *R. meliloti* and *R. fredii* (levels of hybridization, less than 25%), which are their closest phylogenetic relatives (Fig. 4). However, considerable genetic heterogeneity was evident within cluster U. Our hybridization results indicated that strains belonging to subclusters U1 and U2 certainly belong to a single species and that members of subcluster U3 exhibit significant levels of DNA binding (mean, 37%) with members of clusters U1 and U2. For strain LMG 6123 (subcluster U4) the mean level of DNA binding with members of the other subclusters (23%) was not significant (values less than 25% are not significant when the initial renaturation method is used). Representative members of cluster U exhibited no significant level of DNA binding with the type strain of *R. loti*, which is their closest phylogenetic relative (Fig. 4). Because the other *Rhizobium* species are further removed from the *R. loti* subcluster (Fig. 4), we presumed that there would be no significant levels of DNA-DNA binding between cluster U strains and strains belonging to the other *Rhizobium* species; consequently, such hybridization experiments were not performed.

DISCUSSION

We used a polyphasic approach to study the taxonomic relationships of new rhizobial isolates obtained from Senegal. SDS-PAGE of total proteins was a fast method which was used to identify groups and to compare new results with data obtained from our large database. In order to describe and differentiate the new groups, we performed auxanographic

FIG. 2. Computer-processed print-out of positions 0 (top of gel, right side of the pattern) to 350 (bottom of gel, left side of the pattern) of the digitized and normalized protein patterns obtained for representative strains of the different clusters. Lane MWM contained mixture of molecular weight markers that included (from right to left) β-galactosidase (molecular weight, 116,000), bovine albumin (66,000), egg albumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), trypsin inhibitor (20,100), and lysozyme (14,500).

TABLE 2. Results of carbon assimilation tests performed with *Sinorhizobium teranga*, *Sinorhizobium saheli*, cluster U strains, and *Agrobacterium*, *Azorhizobium*, *Mycoplana*, *Ochrobactrum*, *Phyllobacterium*, *Sinorhizobium*, and *Rhizobium* reference strains

Substrate[a]	Utilization by:																
	Sinorhizobium teranga (cluster T) (*n* = 20)[b]	*Sinorhizobium saheli* (cluster S) (*n* = 4)	*Sinorhizobium meliloti* (*n* = 3)	*Sinorhizobium fredii* (*n* = 2)	*R. tropici* (*n* = 3)	*R. leguminosarum* LMG 4285	*Agrobacterium* biovar 2 (*n* = 3)	*R. galegae* (*n* = 2)	*Agrobacterium* biovar 1 (*n* = 7)	*Agrobacterium vitis* (*n* = 2)	*R. huakuii* LMG 14107^T	Cluster U (*n* = 27)	*R. loti* (*n* = 2)	*Azorhizobium caulinodans* LMG 6465^T	*Phyllobacterium* (*n* = 5)	*Mycoplana* (*n* = 2)	*Ochrobactrum anthropi* (*n* = 10)
Glycerol	+[c]	+	+	+	+	+	66	+	+	+	+	+	+	+	+	+	+
Erythritol[d,e]	−	−	+	50	+	−	+	−	−	−	−	−	50	−	−	−	+
D-Arabinose[d]	+	+	+	50	+	+	+	+	+	50	+	+	+	−	+	−	+
Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	50	+
L-Xylose[d]	75(+)[f]	25(−)	33	−	+	+	+	−	50	50	+	+	+	−	−	−	−
Methyl-D-xyloside[d,e]	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
L-Sorbose[d,e]	−	−	+	−	−	−	+	−	75	−	−	33	50	−	−	50	−
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
Dulcitol[d,e]	−	−	+	−	−	+	66	−	+	−	+	63	−	−	+	−	+
Inositol[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	50	+
Mannitol	+	+	+	50	+	+	+	+	+	+	+	+	+	−	+	+	+
Sorbitol	+	+	+	50	66	+	+	+	+	+	+	+	+	−	+	+	+
Methyl-D-mannoside[d]	−	−	−	−	−	+	−	−	−	50	−	−	−	−	−	−	−
Methyl-D-glucoside[d,e]	35(+)	−	+	−	33	−	+	−	+	−	−	33	50	−	+	−	65
N-acetylglucosamine[d]	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+
Amygdalin[d]	40(−)	−	−	−	−	−	+	−	+	50	−	−	−	−	−	−	−
Arbutin[d]	+	+	+	+	+	+	+	−	+	+	+	15	50	−	−	−	−
Salicin[d]	50(+)	25(−)	+	+	−	−	33	−	+	+	−	15	−	−	−	−	−
D-Cellobiose[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
Maltose[d]	+	+	+	+	66	+	+	+	+	+	+	+	+	−	+	−	+
Lactose[d]	+	+	+	+	66	+	+	+	+	+	+	+	+	−	−	−	−
D-Melibiose[d]	+	+	+	+	+	+	+	+	+	+	−	+	50	−	−	−	−
Sucrose[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
Trehalose[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
D-Melezitose[d]	35(+)	50(−)	+	50	−	−	−	−	+	−	−	−	−	−	40	−	−
D-Raffinose[d]	+	+	+	+	+	+	+	+	+	+	+	44	−	−	−	−	−
Xylitol[d,e]	+	−	+	−	+	+	+	+	+	−	+	+	50	−	+	−	−
β-Gentiobiose[d]	35(+)	50(−)	+	+	66	+	33	+	+	50	+	63	50	−	60	−	80
D-Turanose[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
D-Lyxose[d,e]	70(w)	50(−)	+	−	+	+	+	+	+	+	+	+	+	−	40	+	+
D-Tagatose[d,e]	−	−	+	−	−	+	+	−	+	−	−	74	−	−	+	−	+
D-Fucose[d]	−	−	66	50	33	−	+	+	+	+	−	67	+	−	+	−	+
L-Arabitol[d,e]	−	−	+	+	+	+	+	−	+	−	+	26	+	−	60	−	−
Gluconate[d]	50(+)	25(+)	−	−	+	+	+	+	+	+	−	11	−	+	+	−	+
2-Ketogluconate[d,e]	+	25(+)	+	−	−	−	+	+	+	50	−	15	50	+	+	50	+
5-Ketogluconate[d]	−	−	−	−	−	−	+	−	+	−	−	−	−	+	80	−	80
Acetate[d,e]	+	+	33	−	33	−	−	+	+	+	−	81	+	+	+	+	+
Propionate[d,e]	40(−)	+	33	−	−	−	−	−	+	−	−	15	−	+	60	−	+
Butyrate[d]	15(−)	−	−	−	−	−	−	50	−	+	−	18	−	+	+	50	+
Isobutyrate[d,e]	70(+)	+	66	−	−	−	−	−	−	−	−	22	−	+	+	−	+
n-Valerate[d]	−	−	−	−	−	−	−	−	−	−	−	11	−	+	+	−	+
Isovalerate[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Oxalate[d]	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Caprate[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Malonate	−	−	−	−	−	−	33	−	−	−	−	−	−	−	−	−	−
Succinate[d]	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+
Maleate	−	−	33	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fumarate[d]	+	+	66	+	+	−	+	+	+	+	+	+	+	+	+	−	+
Glutarate	−	−	−	−	66	−	−	−	50	+	−	−	−	−	−	−	+
Glycolate[d,e]	−	+	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+
DL-Lactate[d]	+	+	+	50	+	−	+	+	+	+	+	+	+	+	60	+	+
DL-Glycerate[d]	75(+)	+	66	50	66	−	+	+	+	+	+	70	+	+	+	+	+
DL-3-Hydroxybutyrate[d,e]	65(+)	+	33	−	66	−	33	+	+	+	+	85	50	+	+	50	+
D-Malate[d]	+	50(+)	66	+	+	+	66	+	+	+	+	+	50	+	+	−	+
L-Malate[d]	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+
D-Tartrate[d]	15(−)	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
L-Tartrate[d]	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−

Continued on following page

TABLE 2—*Continued*

Substrate[a]	Utilization by:																
	Sinorhizobium teranga (cluster T) (*n* = 20)[b]	*Sinorhizobium saheli* (cluster 5) (*n* = 4)	*Sinorhizobium meliloti* (*n* = 3)	*Sinorhizobium fredii* (*n* = 2)	*R. tropici* (*n* = 3)	*R. leguminosarum* LMG 4285	*Agrobacterium* biovar 2 (*n* = 3)	*R. galegae* (*n* = 2)	*Agrobacterium biovar 1* (*n* = 7)	*Agrobacterium vitis* (*n* = 2)	*R. huakuii* LMG 14107[T]	Cluster U (*n* = 27)	*R. loti* (*n* = 2)	*Azorhizobium caulinodans* LMG 6465[T]	*Phyllobacterium* (*n* = 5)	*Mycoplana* (*n* = 2)	*Ochrobactrum anthropi* (*n* = 10)
Meso-Tartrate[d]	15(−)	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Pyruvate[d]	+	+	+	50	66	−	+	+	75	+	+	+	+	+	+	+	30
2-Ketoglutarate[d]	−	−	−	50	66	−	+	−	50	−	+	−	−	+	−	−	70
Aconitate[d,e]	85(−)	+	−	50	+	−	−	−	+	−	−	11	−	+	+	−	−
Citrate[d]	−	−	−	−	+	−	+	−	−	+	−	85	−	+	+	−	+
Phenylacetate[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
m-Hydroxybenzoate[d]	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
p-Hydroxybenzoate[d,e]	75(+)	+	−	−	+	+	+	+	75	+	−	−	−	+	+	−	40
D-Mandelate[d]	−	−	33	−	−	−	+	−	−	−	−	−	−	−	−	−	−
L-Mandelate[d]	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Glycine[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
D-(−)-Alanine[d]	−	−	−	−	33	−	−	−	50	−	−	−	−	−	40	−	+
L-(−)-Alanine	80(+)	+	66	−	+	+	+	+	+	+	+	81	50	+	+	+	+
L-Leucine[d]	+	75(w)	66	50	+	−	66	50	25	−	+	85	50	−	+	−	+
L-Isoleucine[d]	50(+)	−	66	−	−	+	−	−	25	−	+	55	50	−	+	+	+
L-Norleucine[d]	−	−	−	−	−	−	−	−	50	−	−	−	−	−	−	−	+
L-Valine[d,e]	45(+)	+	33	−	−	−	−	−	25	−	−	30	−	−	80	50	+
DL-Norvaline[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
L-Serine[d,e]	35(−)	+	33	−	+	−	66	−	+	50	−	−	−	−	80	+	+
L-Threonine[d,e]	70(+)	+	66	−	66	−	33	−	+	50	+	33	+	−	+	+	+
L-Cysteine	−	−	−	−	−	−	−	−	75	−	−	−	−	−	−	−	40
L-Phenylalanine[d]	−	75(+)	−	50	+	−	−	−	−	−	−	30	50	−	−	+	−
L-Tyrosine[d]	15(−)	75(−)	66	−	+	+	−	−	−	−	+	89	+	−	20	+	−
L-Histidine[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	−	20	+	+
L-Tryptophan	−	−	−	−	−	−	−	−	−	−	+	63	+	−	60	−	−
Trigonelline[d,e]	−	75(+)	+	50	−	−	+	−	25	+	−	44	50	−	80	−	−
L-Aspartate[d,e]	65(+)	+	+	−	+	+	+	−	+	+	−	30	−	−	+	+	+
L-Glutamate[d]	+	+	+	+	+	+	66	−	+	50	+	+	+	+	+	+	+
L-Ornithine[d]	+	+	+	+	66	+	66	−	+	−	+	+	50	−	20	+	+
L-Lysine[d,e]	+	+	+	−	66	−	−	−	+	−	−	55	50	−	60	+	+
L-Citrulline[d,e]	−	+	−	50	−	−	66	−	+	−	−	−	−	−	−	−	+
L-Arginine[d]	80(+)	+	+	50	66	−	−	−	+	−	+	+	50	−	20	+	+
L-Proline[d]	+	+	+	50	+	+	66	+	+	+	−	+	+	−	+	+	+
Betaine[d]	+	+	+	50	+	+	+	+	+	50	+	+	+	−	+	50	+
Creatine[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
β-Alanine[d,e]	−	50(w)	+	−	−	−	−	−	50	−	−	48	50	−	40	+	+
DL-3-Aminobutyrate[d,e]	−	−	+	−	−	−	−	−	−	−	−	18	−	−	20	−	+
DL-4-Aminobutyrate[d]	+	75(−)	+	+	33	+	−	−	75	+	−	+	+	−	+	+	+
DL-5-Aminovalerate[d]	20(+)	25(−)	66	−	−	−	−	−	25	−	−	41	+	−	20	+	+
Sarcosine[d]	−	−	−	−	−	−	33	−	+	−	−	74	−	−	20	+	+
Ethanolamine[d]	65(+)	+	+	+	66	+	−	−	+	−	+	22	+	−	+	+	80
Diaminobutane[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Spermine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	50	−
Histamine	−	−	−	−	−	−	−	−	−	−	−	67	−	−	−	−	−
Glucosamine[d]	+	+	+	+	+	+	66	+	75	+	+	+	50	−	80	50	80

[a] As determined by API 50 tests, all strains except *Azorhizobium caulinodans* LMG 6465[T] grew on L-arabinose, D-xylose, adonitol, D-galactose, D-glucose, D-fructose, and D-arabitol and did not grow on esculin, inulin, starch, glycogen, *n*-caproate, heptanoate, caprylate, pelargonate, adipate, pimelate, suberate, azelate, sebacate, levulinate, citraconate, itaconate, mesaconate, benzoate, *o*-hydroxybenzoate, phthalate, isophthalate, terephthalate, DL-2-aminobutyrate, L-methionine, D-tryptophan, DL-kynurenine, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, urea, benzylamine, sarcosine, ethylamine, butylamine, amylamine, and tryptamine. *Azorhizobium caulinodans* LMG 6465[T] grew on suberate, azelate, and sebacate and did not grow on L-arabinose, D-xylose, adonitol, D-galactose, D-glucose, D-fructose, D-arabitol, esculin, inulin, starch, glycogen, *n*-caproate, heptanoate, caprylate, pelargonate, adipate, pimelate, levulinate, citraconate, itaconate, mesaconate, benzoate, *o*-hydroxybenzoate, phthalate, isophthalate, terephthalate, DL-2-aminobutyrate, L-methionine, D-tryptophan, DL-kynurenine, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, urea, benzylamine, sarcosine, ethylamine, butylamine, amylamine, and tryptamine.

[b] *n* is the number of strains studied.

[c] +, all strains are positive; −, all strains are negative; d, less than 95% but more than 5% of the strains are positive. The values are the percentages of positive strains.

[d] Carbon source that gave different results for different groups.

[e] Carbon source that could be used to distinguish *Sinorhizobium saheli*, *Sinorhizobium teranga*, *Sinorhizobium fredii*, and *Sinorhizobium meliloti.*

[f] The reactions in parentheses are the reactions of the type strains. w, weakly positive.

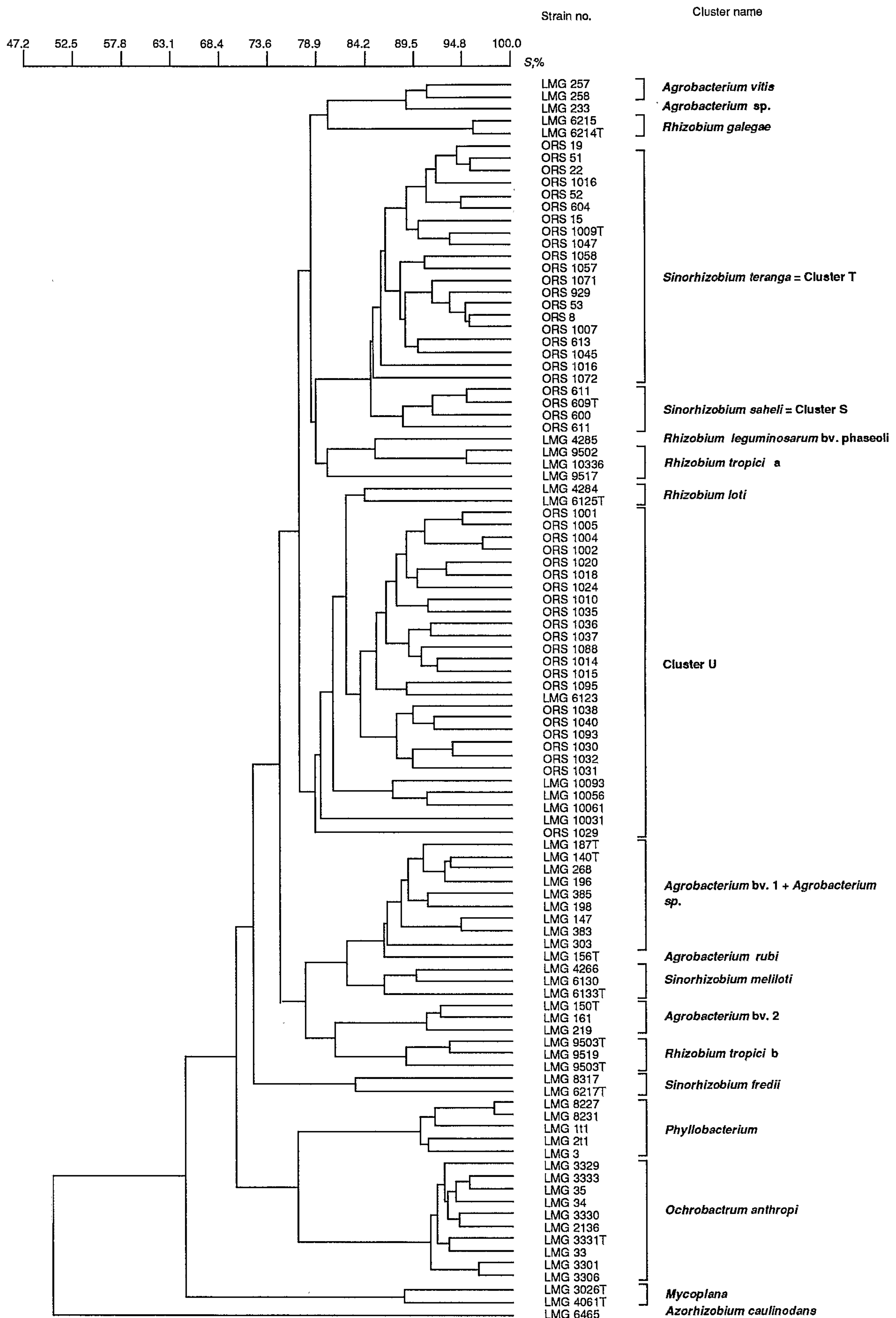


FIG. 3. Dendrogram obtained from an unweighted average pair group cluster analysis of Canberra metric similarity coefficients based on 147 auxanographic characteristics.

TABLE 3. G+C contents and $T_{m(e)}$ values of DNA-rRNA hybrids determined by using labeled rRNAs from *Sinorhizobium teranga* ORS 22, *Sinorhizobium meliloti* LMG 6130, and cluster U strain LMG 6123

Source of DNA		G+C content (mol%)	$T_{m(e)}$ (°C) with [^{3}H]rRNA from:		
Taxon	Strain		*Sinorhizobium teranga* ORS 22	Cluster U strain LMG 6123	*Sinorhizobium meliloti* LMG 6130
Sinorhizobium meliloti	LMG 6133^T			76.0	81.8[a]
R. leguminosarum biovar trifolii	LMG 6119	76.0[a]	79.7[a]		
R. tropici	LMG 9503^T	59.7	77.3		77.3
Sinorhizobium fredii	LMG 6219				81.3
R. loti	LMG 6125^T			80.6[a]	76.4[a]
R. galegae	LMG 6214^T			74.5[a]	78.1
Sinorhizobium teranga	ORS 51	60.8			76.9 (77.8)[a]
	ORS 1009^T	61.6	81.4		77.1
	ORS 22		81.3		79.8[a]
Sinorhizobium saheli	ORS 609^T	65.7			79.7[b]
	ORS 611	65.7			
Cluster U	ORS 1001^T			78.3	73.0
	ORS 1002	62.6		79.3	
	ORS 1024	63.1			
	ORS 1030	64.0			
	ORS 1037	63.3			
	LMG 6123	63.9		81.2	
	LMG 10056	63.0			
Agrobacterium biovar 1	LMG 196				78.1[a]
Agrobacterium biovar 2	LMG 150^T			75.1[a]	79.5[a]

[a] Data from reference 19.
[b] The value in parentheses is from reference 14.

tests with API 50CH, API 50AO, and API 50AA galleries. We identified three groups among the Senegalese isolates, clusters S, T, and U. In our genotypic studies we used DNA-rRNA hybridization and 16S ribosomal DNA sequencing to determine the phylogenetic relationships and DNA-DNA hybridization to determine the species status of the groups. Consistent with the results of reports on tropical rhizobia isolated in Brazil (31) and Sudan (49), we found considerable heterogeneity in the SDS-PAGE protein profiles and phenotypic features of fast-growing rhizobia isolated in Senegal. Except for the members of cluster U (containing cluster FM2 [31]), the Senegalese isolates were electrophoretically distinct from the protein electrophoretic clusters described by Moreira et al. (31). A detailed auxanographic characterization of electrophoretic clusters S, T, and U was performed, and the results provided some distinguishing characteristics (Table 2). Generally, we obtained good correlations with previous carbon source utilization test results (14, 30), with the following exceptions. With L-phenylalanine, L-threonine, L-alanine, and L-tryptophan our results for *R. tropici* contradicted the results of Martinez-Romero et al. (30); and for *Rhizobium* strains isolated from *Sesbania* spp. the data for *p*-hydroxybenzoate contradicted data in a previous report (14). For *Azorhizobium* spp. we observed more differences with previous results (14) since the organisms did not grow on malonate, maleate, adipate, pimelate, citraconate, L-aspartate, L-lysine, *m*-hydroxybenzoate, and glutarate, but did grow on DL-lactate and *p*-hydroxybenzoate. Also, in contrast to the results of Dreyfus et al. (14), we found that most of the cluster T and S strains could grow at 44°C. The reasons for these discrepancies are probably that the previously described results were obtained by classical phenotypic techniques and that we used many more rhizobial isolates obtained from *Acacia* and *Sesbania* species but fewer *R. fredii* strains than were used in the previous studies. Only the type strain of *R. huakuii* was included in this study, and the results obtained for this organism agree well with the results described by Chen et al. (4), except that we observed growth on dulcitol and inositol.

On the basis of 16S rRNA gene sequencing and DNA-DNA hybridization results, clusters S and T were also shown to be genotypically distinct from each other and from the reference organisms examined. Strains belonging to these two clusters were shown to represent two branches of the *R. meliloti-R. fredii* subgroup (Fig. 4). Because internally both cluster S and cluster T exhibited high degrees of DNA binding and because no significant levels of DNA binding were detected between clusters S and T or between either cluster and *R. meliloti* or *R. fredii* (Table 4), we concluded that these clusters represent new genospecies. These taxa can be differentiated phenotypically (Table 2) from each other, from the other members of the *R. meliloti-R. fredii* subgroup, and from the members of the other subgroups of the *Agrobacterium-Rhizobium* group (37, 44, 46), justifying species status for both groups. As explained previously (46), profound revision of the genus and species classification of the *Agrobacterium-Rhizobium* group is inevitable, because some *Rhizobium* species are phylogenetically more closely related to *Agrobacterium* subgroups than they are to other *Rhizobium* species. The results of polyphasic taxonomic studies which are now available (37; this study) should allow us to propose a general revision of the genera *Rhizobium* and *Agrobacterium*, as well as two new species for clusters T and S. As discussed previously (46), revision of the classification of the *Agrobacterium-Rhizobium* group could include the description of a separate genus for the *R. meliloti-R. fredii* subgroup (including two new species for clusters S and T); revision of the genus *Agrobacterium* so that it contains the biovar 1 strains, *Agrobacterium vitis*, *Agrobacterium rubi*, and [*Rhizobium*] *galegae*; and revision of the genus *Rhizobium* so that it contains *R. leguminosarum* (the type species), *R. tropici*, *R. etli*, and the [*Agrobacterium*] biovar 2 strains that constitute a separate new species. *R. loti*, *R. huakuii*, and our cluster U constitute another lineage that also deserves separate genus status. However,

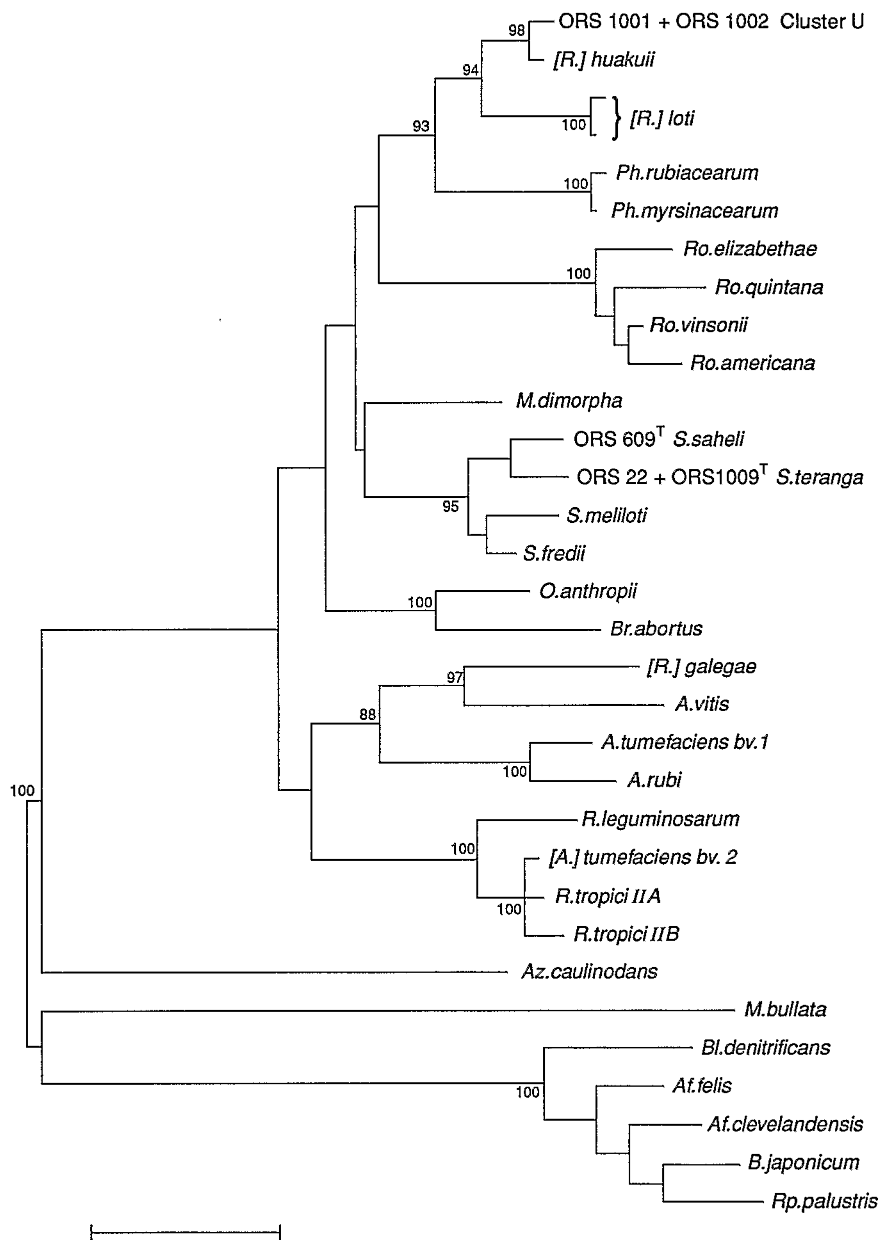


FIG. 4. Dendrogram obtained by the neighbor-joining method, showing the phylogenetic positions of *Sinorhizobium saheli* ORS 609^T, *Sinorhizobium teranga* ORS 22 and ORS 1009^T, and cluster U strains ORS 1001 and ORS 1002 within the alpha subclass of the *Proteobacteria*. Significant bootstrap probability values are indicated at the branching points. The bootstrap value for *Sinorhizobium saheli* and *Sinorhizobium teranga* was 67, and the bootstrap value for *Sinorhizobium meliloti* and *Sinorhizobium fredii* was 58. Bar = 2% nucleotide differences. Abbreviations: *R.*, *Rhizobium*; *Ph.*, *Phyllobacterium*; *Ro.*, *Rochalimaea*; *M.*, *Mycoplana*; *S.*, *Sinorhizobium*; *O.*, *Ochrobactrum*; *Br.*, *Brucella*; *A.*, *Agrobacterium*; *Az.*, *Azorhizobium*; *Bl.*, *Blastobacter*; *Af.*, *Afipia*; *B.*, *Bradyrhizobium*; *Rp.*, *Rhodopseudomonas*.

because of nomenclatural controversy concerning the rearrangement proposed for the genus *Agrobacterium* (2, 37), it is not possible to start a thorough revision of the genera *Rhizobium* and *Agrobacterium* before it has been decided whether *Agrobacterium tumefaciens* or *Agrobacterium radiobacter* should be the type species of the revised genus *Agrobacterium* and whether the epithet "*rhizogenes*" will be retained for the species containing the biovar 2 strains. In the *R. meliloti-R. fredii* sublineage the nomenclatural changes are less complicated. On the basis of mainly phenotypic criteria, *R. fredii* has recently been designated the type species of a new genus, *Sinorhizobium* (5). Although the proposal of *Sinorhizobium* by Chen et al. (5) has been questioned (18), the results of the present study demonstrate that *R. fredii* and *R. meliloti*, together with clusters S and T, merit a distinct genus. Therefore, we formally propose to emend the genus *Sinorhizobium* and to reclassify *R. meliloti* as *Sinorhizobium meliloti* comb. nov. In addition, we propose two new species, *Sinorhizobium saheli* for cluster S strains and *Sinorhizobium teranga* for cluster T strains. The taxonomic position of *S. xinjiangensis* (5) remains to be determined. We realize the reference to China in the name *Sinorhizobium* does not apply to all the species proposed here,

TABLE 4. Levels of DNA-DNA binding at 79.8°C between DNAs from *Rhizobium*, *Sinorhizobium*, and cluster U strains

DNA from:		% Binding with DNA from:													
Taxon	Strain	*Sinorhizobium teranga* ORS 51	*Sinorhizobium teranga* ORS 1009^T	*Sinorhizobium saheli* ORS 611	*Sinorhizobium saheli* ORS 609t1	*Sinorhizobium meliloti* LMG 6133^T	*Sinorhizobium fredii* LMG 6217^T	*Rhizobium loti* LMG 6125^T	Subcluster U1 strain ORS 1024	Subcluster U2 strain ORS 1037	Subcluster U2 strain ORS 1001	Cluster U strain ORS 1002	Subcluster U3 strain ORS 1030	Subcluster U3 strain LMG 10056	Subcluster U4 strain LMG 6123
Sinorhizobium teranga	ORS 51	100													
Sinorhizobium teranga	ORS 1009^T	79	100												
Sinorhizobium saheli	ORS 611	20		100											
Sinorhizobium saheli	ORS 609t1			89	100										
Sinorhizobium meliloti	LMG 6133^T	22		23		100									
Sinorhizobium fredii	LMG 6217^T	22		26		23	100								
Rhizobium loti	LMG 6125^T							100							
Rhizobium sp. cluster U subcluster U1	ORS 1024								100						
Rhizobium sp. cluster U subcluster U2	ORS 1037								83	100					
Rhizobium sp. cluster U subcluster U2	ORS 1001							10			100				
Rhizobium sp. cluster U	ORS 1002							13	77	88	80	100			
Rhizobium sp. cluster U subcluster U3	ORS 1030								37	38		42	100		
Rhizobium sp. cluster U subcluster U3	LMG 10056								28	29	40	47	32	100	
Rhizobium sp. cluster U subcluster U4	LMG 6123							23	16	24		14	33	25	100

but we are bound by the rules of nomenclatural priority to use this name.

The taxonomic situation of cluster U is more complex because there is considerable genotypic heterogeneity among the strains belonging to this cluster. Preliminary DNA-DNA hybridization data indicated that there are at least two genomic species in cluster U, one containing subclusters U1, U2, and U3 and strain ORS 1002 and one containing subcluster U4. Comparative 16S rRNA gene sequencing data revealed that cluster U strains are phylogenetically closely related. These organisms belong to the *R. loti-R. huakuii* lineage, and *R. loti* is a close relative but is nevertheless distinct. At the present time we consider it unwise to assign species status to cluster U because of the absence of distinguishing phenotypic traits. In addition, the 16S rRNA sequence data revealed that this taxon may be closely related to *R. huakuii*. Additional chromosomal DNA-DNA pairing studies performed with representative strains of cluster U and *R. huakuii* strains will be necessary to clarify the taxonomic relationships of these organisms.

Cluster U contains strains isolated from diverse plants (different *Acacia* species, *Lotus divariaticus*, different *Leucaena* species, and *Chamaecrista ensiformis*) in various countries (Senegal, Brazil, and New Zealand). In the study of Chen et al. (4) rather high levels of DNA binding between *R. huakuii* and isolates obtained from *Leucaena leucocephala* were found, and it is striking that four of the five Brazilian cluster U isolates were also isolated from *Leucaena* species.

Emended description of *Sinorhizobium* (Chen, Yan and Li 1988). Rods that are 0.5 to 1 by 1.2 to 3 μm. Commonly pleiomorphic under adverse growth conditions. Cells usually contain poly-β-hydroxybutyrate granules which are refractile as determined by phase-contrast microscopy. Non-spore forming. Gram negative. Motile by means of one polar or subpolar flagellum or two to six peritrichous flagella. Fimbriae occur in a few strains. Aerobic, having a respiratory type of metabolism with oxygen as the terminal electron acceptor. Often able to grow well under oxygen tensions less than 1.0 Pa. Optimum temperature, 25 to 33°C. Optimum pH, 6 to 7. Colonies are circular, convex, semitranslucent, raised, and mucilaginous and usually are 2 to 4 mm in diameter within 3 to 5 days on yeast-mannitol-mineral salts agar. Pronounced turbidity develops after 2 to 3 days in agitated broth media. Chemoorganotrophic, utilizing a wide range of carbohydrates (but not cellulose and starch) and salts of organic acids as carbon sources (5) (Table 2). Cells produce an acid reaction in mineral salts media containing several carbohydrates. Peptone is poorly utilized. 3-Ketolactose is not produced from lactose. Growth on carbohydrate media is usually accompanied by copious extracellular polysaccharide slime production.

The organisms are typically able to invade the root hairs of temperate zone and tropical zone leguminous plants (family Leguminosae) and incite the production of root nodules, where the bacteria occur as intracellular symbionts. All strains exhibit host range activities ("host specificity"). The bacteria are present in root nodules as pleiomorphic forms (bacteriods) which are normally involved in fixing atmospheric nitrogen into a combined form (ammonia) that can be utilized by the host plant. The G+C content of the DNA is 57 to 66 mol% (as determined by the melting method). The type species is *Sinorhizobium fredii*.

At the molecular level the genus can be recognized by the sequence of the 16S rRNA genes.

Description of *Sinorhizobium meliloti* (Dangeard 1926) comb. nov. The description of the species is the description given by Jordan (20). In addition, this species can be differentiated from the other *Sinorhizobium* species by its auxanographic characteristics (5) (Table 2). At the molecular level it can be differentiated from other *Sinorhizobium* species by its gel electrophoretic protein profiles, by DNA-DNA hybridization data, and by the sequence of its 16S rRNA genes.

Additional description of *Sinorhizobium fredii* (Scholla and Elkan 1984) (Chen, Yan and Li 1988). Descriptions of the species are given by Scholla and Elkan (38) and Chen et al. (5). In addition, this species can be differentiated from the other *Sinorhizobium* species by its auxanographic characteristics (5) (Table 2), and on the molecular level it can be differentiated by its protein electrophoretic profiles, by the results of DNA-DNA hybridization experiments, and by the sequence of its 16S rRNA genes.

Description of *Sinorhizobium teranga* sp. nov. *Sinorhizobium teranga* (te'ran.ga. local Wolof [a language of the West African Wolof people] n. *teranga*, hospitality; N. L. n. *teranga*, hospitality, referring to the fact that this species contains strains isolated from different host plants). Aerobic, gram-negative, non-spore-forming rods that are 0.5 to 0.7 μm wide by 1.5 to 2 μm long. Motile by means of one or several polar or subpolar flagella in liquid medium. The cells grow on yeast mannitol medium at temperatures as high as 44°C. Colonies of most strains on YMA are circular, cream colored, semitranslucent, and mucilaginous and sometimes spread over an entire plate within 2 to 4 days. Old colonies turn brown. The exceptions are strains ORS 22 and ORS 613, which produce nonmucilaginous colonies. In some strains nonmucilaginous mutants arise spontaneously during subculturing. A wide range of carbohydrates, organic acids, and amino acids are utilized as sole carbon sources for growth. Features that distinguish this species from other species and related genera are shown in Table 2. Grows on xylitol but not on L-citrulline and glycolate.

Strains can nodulate *Sesbania* and *Acacia* spp., *Leucaena leucocephala*, and *N. oleracea.*

At the molecular level this species can be differentiated by the results of DNA-rRNA hybridization experiments, by the SDS-PAGE patterns of proteins, by the results of total DNA-DNA hybridization experiments, and by the sequence of the 16S ribosomal DNA.

The G+C content is 60.8 to 61.6 mol%.

Well-studied strain ORS 1009 (= LMG 7854), which was isolated from *Acacia laeta*, is the type strain, and the characteristics of this strain are shown in Table 2. All *Sinorhizobium teranga* strains have been deposited in the Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium, and in the Culture Collection of the Laboratory of Soil Microbiology, ORSTOM, Dakar, Senegal.

Description of *Sinorhizobium saheli* sp. nov. *Sinorhizobium saheli* (sa'hel.i. N.L. gen. n. *saheli*, of the Sahel, the region in Africa where the organisms were isolated). Aerobic, gram-negative, non-spore-forming rods that are 0.5 to 0.7 μm wide by 1.5 to 2 μm long. Motile by means of one or several polar or subpolar flagella in liquid medium. Grows on YMA at temperatures up to 44°C. On YMA the colonies are circular, white, semitranslucent, and convex. When there is confluent growth, the white centers of the original colonies have a marbled appearance.

A wide range of carbohydrates, organic acids, and amino acids are utilized as sole carbon sources for growth. Distinguishing features are shown in Table 2. Grows on L-citrulline and glycolate but not on xylitol.

Strains have been isolated from *Sesbania* species in the Sahel area and can nodulate different *Sesbania* species, *Acacia seyal*, *Leucaena leucocephala*, and *N. oleracea.*

At the molecular level this species can be differentiated from other *Sinorhizobium* species and related genera by the results of DNA-DNA hybridization and DNA-rRNA hybridization experiments, by the SDS-PAGE patterns of proteins, and by the sequence of the 16S rRNA genes.

The G+C content of the DNA is 65 to 66 mol%.

Strain ORS 609 (= LMG 7837) is the type strain; the phenotypic characteristics of this organism are shown in Table 2. All *Sinorhizobium saheli* strains have been deposited in the Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium, and in the Culture Collection of the Laboratory of Soil Microbiology, ORSTOM, Dakar, Senegal.

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