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Genotypic Characterization of *Bradyrhizobium* Strains Nodulating Small Senegalese Legumes by 16S-23S rRNA Intergenic Gene Spacers and Amplified Fragment Length Polymorphism Fingerprint Analyses

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We examined the genotypic diversity of 64 *Bradyrhizobium* strains isolated from nodules from 27 native leguminous plant species in Senegal (West Africa) belonging to the genera *Abrus*, *Alysicarpus*, *Bryaspis*, *Chamaecrista*, *Cassia*, *Crotalaria*, *Desmodium*, *Eriosema*, *Indigofera*, *Moghania*, *Rhynchosia*, *Sesbania*, *Tephrosia*, and *Zornia*, which play an ecological role and have agronomic potential in arid regions. The strains were characterized by intergenic spacer (between 16S and 23S rRNA genes) PCR and restriction fragment length polymorphism (IGS PCR-RFLP) and amplified fragment length polymorphism (AFLP) fingerprinting analyses. Fifty-three reference strains of the different *Bradyrhizobium* species and described groups were included for comparison. The strains were diverse and formed 27 groups by AFLP and 16 groups by IGS PCR-RFLP. The sizes of the IGS PCR products from the *Bradyrhizobium* strains that were studied varied from 780 to 1,038 bp and were correlated with the IGS PCR-RFLP results. The grouping of strains was consistent by the three methods AFLP, IGS PCR-RFLP, and previously reported 16S amplified ribosomal DNA restriction analysis. For investigating the whole genome, AFLP was the most discriminative technique, thus being of particular interest for future taxonomic studies in *Bradyrhizobium*, for which DNA is difficult to obtain in quantity and quality to perform extensive DNA:DNA hybridizations.

Due to their nitrogen-fixing symbiosis with soil bacteria collectively named rhizobia, legumes play an important role in the nitrogen cycle, especially in the tropics. They are used to restore or increase soil fertility of degraded soils in intercropping systems, as green manure, and to produce medicinal and commercial by-products. In Senegal (West Africa), many native legumes are important plant resources for many purposes, and they are the only alternatives to the costly and pollutive mineral nitrogen fertilizers used in agriculture. Many of these legumes are well adapted to the local arid climatic conditions. As drought and soil erosion progress, they are still present as colonizers. The family *Leguminosae* is considered to be of tropical or subtropical origin, and many of the recently proposed new rhizobial species originate from leguminous plants from these zones that had not been previously investigated.

After several prospections around the country, we focused on 27 native nodulated leguminous plants species belonging to the genera *Abrus*, *Alysicarpus*, *Bryaspis*, *Chamaecrista*, *Cassia*, *Crotalaria*, *Desmodium*, *Eriosema*, *Indigofera*, *Moghania*, *Rhynchosia*, *Sesbania*, *Tephrosia*, *Zornia*, which play an important ecological role and have agronomic potential in arid regions. As very little or no information concerning their associated rhizobia were available so far, we obtained 71 nodule isolates from these legumes in different regions in Senegal and first characterized them by sodium dodecyl sulfate-polyacrylamide gel electrophoresis whole-cell protein profiles and by 16S amplified ribosomal DNA restriction analysis (ARDRA) fingerprint analysis (9). Our main conclusion was that the strains were diverse and belonged to five phylogenetic subgroups inside *Bradyrhizobium*, but further genotypic characterization of these strains was needed to precisely place them in the general classification.

Taxonomically, rhizobia comprise six genera, *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium* (for a review, see reference 44), and *Allorhizobium* (8). They constitute a phylogenetically heterogeneous group, and their taxonomy is being reexamined. Some rhizobia are more closely related to clinical bacteria, like *Afipia*, *Blastobacter*, and other nonsymbiotic bacteria, like *Mycoplana* and *Bartonella*, than to other rhizobia (39, 43, 44). In addition, several new rhizobial groups have been described, but have not yet been classified or named (for reviews, see references 24 and 44). In particular, the precise taxonomical status of many *Bradyrhizobium* sp. strains isolated from various legumes is not clarified (9, 10, 25, 26, 27, 34). For this genus (*Bradyrhizobium*), several authors have reported the lack of consistency between results obtained by different taxonomic techniques (10, 20, 32, 45).

A need for a confident strategy to investigate diversity among *Bradyrhizobium* populations was claimed, specifically including molecular methods (9, 34). 16S-23S rRNA intergenic gene spacer (IGS; corresponding to the spacer between 16S and 23S rRNA genes) sequences exhibit a large variability and are useful to identify genomic groups at the intraspecific level (4, 16, 22). Moreover, PCR-restriction fragment length polymorphism (PCR-RFLP) of IGS has been reported to be a useful fingerprinting method to characterize bacterial strains, with a higher discriminating power than the 16S ARDRA method. It has been applied to known rhizobial species, such as *Rhizobium leguminosarum*, *Rhizobium* "*hedysari*," *Sinorhizobium meliloti*, and *Rhizobium galegae* (6, 22, 31, 33), and also

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FIG. 1. Sites of sampling in Senegal.

tropical rhizobia strains (18) and *Bradyrhizobium* strains from the Canary Islands (37).

The amplification fragment length polymorphism (AFLP) technique (38, 46) is a highly discriminating fingerprinting method, based on the selective PCR amplification of certain restriction fragments from a digest of total genomic DNA. The technique involves three steps: (i) restriction of the DNA with two enzymes and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) polyacrylamide gel electrophoresis of the amplified fragments. Originally developed for plant genome studies, this technique has also been used to characterize various bacterial species (for a review, see reference 5). As this technique investigates the whole genome, and as the results were reported to be in good agreement with those obtained by DNA:DNA hybridization, it could be a good alternative to the latter, which is especially difficult to perform with *Bradyrhizobium*.

Here, 64 *Bradyrhizobium* sp. strains from small Senegalese legumes were further characterized genotypically by PCR-RFLP analysis of the IGS region between 16S and 23S rRNA genes and by AFLP analysis, including representative strains for *Bradyrhizobium* species and groups reported in the past (9, 10, 25, 26, 40). The results are compared with previous 16S ARDRA grouping, and taxonomic resolution levels of the three techniques are discussed.

MATERIALS AND METHODS

Bacterial strains and isolation procedures. The strains used in this study are listed in Table 1, and their places of isolation are indicated on Fig. 1. Bacterial strains were grown as previously described (9, 40).

PCR amplification of IGS (16S-23S rDNA) region. Total DNA was prepared as previously described (9). Alternatively, for some strains, the PCR amplification was done from bacterial cell preparation, prepared as follows: cells were grown on yeast extract-mannitol agar slants (36) for 72 h at 28°C and then washed with sterile distilled water. The cell suspension was adjusted to an optical density (620 nm) of 0.5 by dilution in water. An aliquot of 100 μl of this cell suspension was pelleted, and cells were resuspended in 100 μl of sterile Milli-Q water (Milli-Q plus; Millipore, Saint-Quentin-Yvelines, France) and lysed with 100 μl of 10 mM Tris-HCl (pH 8.2) and 13 μl of proteinase K (1 mg/ml) (Merck-Belgolabo, Overijse, Belgium) during 2 h at 55°C. Then, the cells were boiled for 10 min to denature the enzyme. The PCR was carried out with 250 ng of DNA or with 10 μl of bacterial cell suspension as template DNA.

Primers FGPS1490 (28) and FGPS132′ (29) were used to amplify the IGS regions. FGPS1490 corresponds to conserved sequences in the 3′ part of the 16S rDNA gene right next to the IGS (corresponding to the *Escherichia coli* numbering positions 1525 to 1541), and reverse primer FGPS132′ corresponds to the beginning 5′ part of the 23S rDNA (corresponding to the *E. coli* numbering positions 115 to 132). PCR was performed as described by Laguerre et al. (22).

PCR-amplified DNA was visualized by Hoefer HE 33 Mini Submarine electrophoresis (Amersham Pharmacia Biotech) at 70 V for 30 min with 5 μl of the amplified mixture on 2% (wt/vol) Biozym DNA Agarose (Biozym, Landgraaf, The Netherlands) in TAE buffer (40 mM Tris-acetate, 2 mM EDTA, 20 mM acetic acid, pH 8.0) containing 0.5 mg of ethidium bromide/ml.

RFLP analysis. Aliquots (10 μl) of PCR products were digested with 5 U of restriction endonuclease in 20-μl reaction volumes by using the manufacturer's recommended buffer and incubation conditions. The following restriction enzymes were used: *Alu*I and *Nde*II (Boehringer Mannheim Biochemica, Brussels, Belgium), *Dde*I (Amersham Pharmacia Biotech Benelux, Roosendaal, The Netherlands), and *Hae*III, *Hha*I, *Hin*fI, *Msp*I, and *Rsa*I (New England Biolabs Inc., Leusden, The Netherlands). Restricted DNA was analyzed by horizontal electrophoresis in 4% Nusieve 3:1 or Metaphor agarose (FMC, Rockland, Maine) gels. DNA molecular-weight-marker VIII (Boehringer Mannheim) was used as standard for gel calibration. Electrophoreses were carried out at 80 V for 4 h with standard gels (11 by 14 cm or 10 by 15 cm) on a Bethesda Research Laboratories Horizon 11-14 apparatus or with a DNA Sub Cell unit (15-by-10-cm tray) (20 wells; Bio-Rad Laboratories NV, Nazareth-Eke, Belgium).

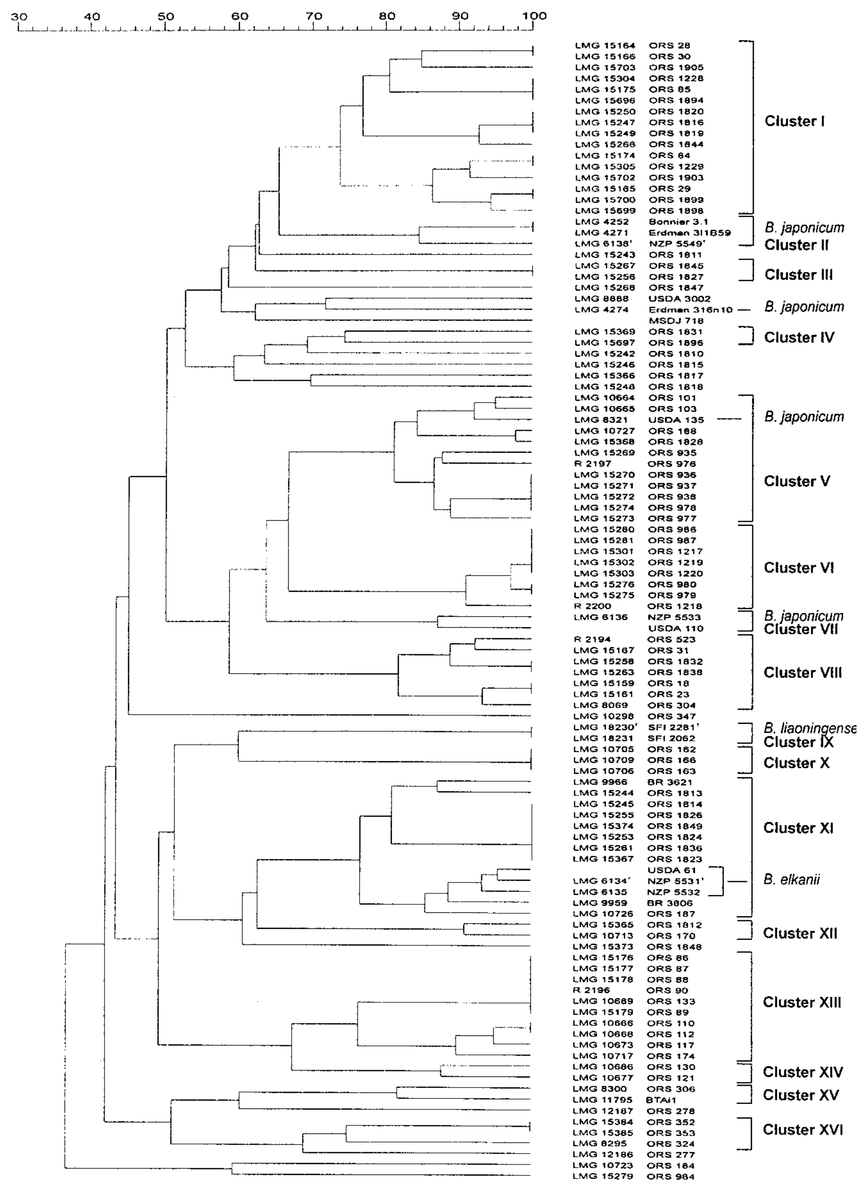

FIG. 2. Dendrogram (UPGMA) obtained by numerical analysis comparison of the normalized and combined IGS patterns by PCR-RFLP analysis. Clusters of strains were delineated above 70% similarity.

The gels were stained and photographed as described by Heyndrickx et al. (12).

The scanned gel images were digitized and stored in a computer as described by Heyndrickx et al. (12).

Pattern analysis was performed using the Gel Compar software (35) version 4.2 (Applied Maths, Kortrijk, Belgium), and an unweighted pair group method with averages (UPGMA) dendrogram was constructed using the Dice similarity coefficient (S_D).

AFLP analysis. The experimental protocol used was modified from that of Vos et al. (38) as described by Huys et al. (14) and was essentially similar to that used in the study of Willems et al. (40). In short, 1 μg of total genomic DNA was digested by two restriction enzymes. Double-stranded oligonucleotide adapters with a single-stranded overhang homologous to the 5′ and 3′ ends generated during restriction were ligated to the DNA fragments. The ligated DNA fragments were amplified by PCR using primers complementary to the adapter and restriction site sequence with additional selective nucleotides at their 3′ end. The amplified products were separated by polyacrylamide gel electrophoresis, and the resulting banding pattern was revealed through autoradiography. *Bordetella holmesii* strain LMG 15945 was used as the reference strain because AFLP analysis of this strain generated a banding pattern displaying evenly distributed and well-separated bands over the entire length of the gel.

The cluster analysis of AFLP patterns was done by analyzing the scanned profiles on a personal computer with the Gel Compar program, version 4.2, using the Dice coefficient and the UPGMA clustering algorithm.

RESULTS

IGS PCR-RFLP analysis. (i) Cluster analysis of IGS RFLP patterns. The 16S-23S rDNA IGS region of 104 *Bradyrhizobium* strains was amplified by PCR and restricted with eight endonucleases. One to six DNA fragments were generated by each restriction enzyme. For each strain, the patterns obtained with the eight enzymes were combined, resulting in 70 different combinations referred to as IGS rDNA types (Table 2).

A dendrogram (Fig. 2) was constructed based on the UPGMA algorithm by analyzing the similarity between the restriction fragments with the software Gel Compar, version 4.2 (35).

TABLE 1. Strains used

Strain[a]	Other strain designation	Host plant or origin	Geographical origin[b]	Reference or source	IGS PCR-RFLP grouping	AFLP grouping
ORS 18	LMG 15159	*Alysicarpus ovalifolius*	Dakar Bel-Air (CS)	9	VIII	17
ORS 23	LMG 15161	*Tephrosia villosa*	Dakar Bel-Air (CS)	9	VIII	17
ORS 28	LMG 15164	*Indigofera tinctoria*	Mbour (CS)	9	I	36
ORS 29	LMG 15165	*Indigofera tinctoria*	Mbour (CS)	9	I	43
ORS 30	LMG 15166	*Indigofera hirsuta*	Dakar Bel-Air (CS)	9	I	36
ORS 31	LMG 15167	*Indigofera tinctoria*	Pal (NS)	9	VIII	17
ORS 84	LMG 15174	*Indigofera astragalina*	Payar (CS)	9	I	37
ORS 85	LMG 15175	*Indigofera astragalina*	Payar (CS)	9	I	47
ORS 86	LMG 15176	*Tephrosia purpurea*	Bourel (Ferlo, NS)	9	XIII	40
ORS 87	LMG 15177	*Tephrosia purpurea*	Bourel (Ferlo, NS)	9	XIII	40
ORS 88	LMG 15178	*Tephrosia purpurea*	Bourel (Ferlo, NS)	9	XIII	40
ORS 89	LMG 15179	*Tephrosia purpurea*	Bourel (Ferlo, NS)	9	XIII	34
ORS 90	R-2196	*Tephrosia sp.*	Bourel (Ferlo, NS)	9	XIII	34
ORS 523	R-2194	*Indigofera senegalensis*	Dakar Bel-Air (CS)	9	VIII	ND[c]
ORS 935	LMG 15269	*Rhynchosia minima*	Gueye Kadar (Ferlo, NS)	9	V	20
ORS 936	LMG 15270	*Rhynchosia minima*	Gueye Kadar (Ferlo, NS)	9	V	20
ORS 937	LMG 15271	*Rhynchosia minima*	Gueye Kadar (Ferlo, NS)	9	V	20
ORS 938	LMG 15272	*Rhynchosia minima*	Gueye Kadar (Ferlo, NS)	9	V	20
ORS 976	R-2197	*Indigofera senegalensis*	Gueye Kadar (Ferlo, NS)	9	V	20
ORS 977	LMG 15273	*Indigofera senegalensis*	Gueye Kadar (Ferlo, NS)	9	V	20
ORS 978	LMG 15274	*Indigofera senegalensis*	Gueye Kadar (Ferlo, NS)	9	V	20
ORS 979	LMG 15275	*Indigofera senegalensis*	Gueye Kadar (Ferlo, NS)	9	VI	46
ORS 980	LMG 15276	*Indigofera senegalensis*	Gueye Kadar (Ferlo, NS)	9	VI	46
ORS 984	LMG 15279	*Indigofera senegalensis*	Mboumba (Ferlo, NS)	9	Sep[d]	Sep
ORS 986	LMG 15280	*Indigofera senegalensis*	Mboumba (Ferlo, NS)	9	VI	46
ORS 987	LMG 15281	*Indigofera senegalensis*	Mboumba (Ferlo, NS)	9	VI	46
ORS 1216	LMG 15300	*Indigofera senegalensis*	Boki Namadi (Ferlo, NS)	9	ND	Sep
ORS 1217	LMG 15301	*Indigofera senegalensis*	Boki Namadi (Ferlo, NS)	9	VI	46
ORS 1218	R-2200	*Indigofera senegalensis*	Boki Namadi (Ferlo, NS)	9	VI	46
ORS 1219	LMG 15302	*Indigofera senegalensis*	Boki Namadi (Ferlo, NS)	9	VI	46
ORS 1220	LMG 15303	*Indigofera senegalensis*	Boki Namadi (Ferlo, NS)	9	VI	46
ORS 1228	LMG 15304	*Indigofera astragalina*	Payar (CS)	9	I	47
ORS 1229	LMG 15305	*Indigofera astragalina*	Payar (CS)	9	I	37
ORS 1810	LMG 15242	*Crotalaria lathyroïdes*	Kabrousse (Casamance, SS)	9	Sep	Sep
ORS 1811	LMG 15243	*Crotalaria goreensis*	Kabrousse (Casamance, SS)	9	Sep	Sep
ORS 1812	LMG 15365	*Abrus stictosperma*	Fanghote (Casamance, SS)	9	XII	42
ORS 1813	LMG 15244	*Crotalaria hyssopifolia*	Kabrousse (Casamance, SS)	9	XI	Sep
ORS 1814	LMG 15245	*Crotalaria hyssopifolia*	Fanghote (Casamance, SS)	9	XI	19
ORS 1815	LMG 15246	*Crotalaria hyssopifolia*	Fanghote (Casamance, SS)	9	Sep	Sep
ORS 1816	LMG 15247	*Crotalaria hyssopifolia*	Fanghote (Casamance, SS)	9	I	38
ORS 1817	LMG 15366	*Eriosema glomeratum*	Oukout (Casamance, SS)	9	Sep	Sep
ORS 1818	LMG 15248	*Indigofera microcarpa*	Oukout (Casamance, SS)	9	Sep	Sep
ORS 1819	LMG 15249	*Crotalaria retusa*	Kabrousse (Casamance, SS)	9	I	38
ORS 1820	LMG 15250	*Indigofera hirsuta*	Fanghote (Casamance, SS)	9	I	38
ORS 1823	LMG 15367	*Indigofera hirsuta*	Wouring (Niokolokoba, SS)	9	XI	19
ORS 1824	LMG 15253	*Indigofera hirsuta*	Wouring (Niokolokoba, SS)	9	XI	19
ORS 1826	LMG 15255	*Alysicarpus glumaceus*	Fanghote (Casamance, SS)	9	XI	19
ORS 1827	LMG 15256	*Alysicarpus glumaceus*	Fanghote (Casamance, SS)	9	III	44
ORS 1828	LMG 15368	*Alysicarpus ovalifolius*	Niokolokoba (SS)	9	V	20
ORS 1831	LMG 15369	*Alysicarpus rugosus*	Fanghote (Casamance, SS)	9	IV	45
ORS 1832	LMG 15258	*Bryaspis lupulina*	Kagnout (Casamance, SS)	9	VIII	41
ORS 1836	LMG 15261	*Crotalaria glaucoïdes*	Kaparang (Casamance, SS)	9	XI	35
ORS 1838	LMG 15263	*Sesbania rostrata*	Kaolack (CS)	9	VIII	41
ORS 1844	LMG 15266	*Chamaecrista* sp.	Karounate (Casamance, SS)	9	I	38
ORS 1845	LMG 15267	*Moghania faginea*	Kaparang (Casamance, SS)	9	III	44
ORS 1847	LMG 15268	*Zornia glochidiata*	Fanghote (Casamance, SS)	9	Sep	Sep
ORS 1848	LMG 15373	*Indigofera hirsuta*	Kaparan (Casamance, SS)	9	Sep	35
ORS 1849	LMG 15374	*Indigofera hirsuta*	Fanghote (Casamance, SS)	9	XI	19
ORS 1894	LMG 15696	*Indigofera hirsuta*	Kolda (Casamance, SS)	9	I	47
ORS 1896	LMG 15697	*Cassia absus*	Kolda (Casamance, SS)	9	IV	45
ORS 1898	LMG 15699	*Tephrosia bracteolata*	Kolda (Casamance, SS)	9	I	37
ORS 1899	LMG 15700	*Indigofera stenophylla*	Kolda (Casamance, SS)	9	I	43
ORS 1903	LMG 15702	*Tephrosia villosa*	Kolda (Casamance, SS)	9	I	37
ORS 1905	LMG 15703	*Tephrosia bracteolata*	Kolda (Casamance, SS)	9	I	33
Bradyrhizobium sp. (*Faidherbia*)						
ORS 101	LMG 10664	*Faidherbia albida*	Senegal	10	V	20
ORS 103	LMG 10665	*Faidherbia albida*	Dakar Bel-Air (CS)	10	V	20
ORS 110	LMG 10666	*Faidherbia albida*	Louga (NS)	10	XIII	Sep
ORS 112	LMG 10668	*Faidherbia albida*	Louga (NS)	10	XIII	33
ORS 117	LMG 10673	*Faidherbia albida*	Louga (NS)	10	XIII	33
ORS 121	LMG 10677	*Faidherbia albida*	Louga (NS)	10	XIV	29

Continued on following page

TABLE 1—*Continued*

Strain[a]	Other strain designation	Host plant or origin	Geographical origin[b]	Reference or source	IGS PCR-RFLP grouping	AFLP grouping
ORS 130	LMG 10686	*Faidherbia albida*	Louga (NS)	10	XIV	29
ORS 133	LMG 10689	*Faidherbia albida*	Louga (NS)	10	XIII	Sep
ORS 156	LMG 11951	*Faidherbia albida*	Djinaki, Senegal	10	ND	19
ORS 166	LMG 10709	*Faidherbia albida*	Casamance (SS)	10	X	31
ORS 162	LMG 10705	*Faidherbia albida*	Casamance (SS)	10	X	31
ORS 163	LMG 10706	*Faidherbia albida*	Oussouye (Casamance, SS)	10	X	31
ORS 170	LMG 10713	*Faidherbia albida*	Bayotte (Casamance, SS)	10	XII	42
ORS 174	LMG 10717	*Faidherbia albida*	Badiana (Casamance, SS)	10	XIII	33
ORS 180	LMG 10719	*Faidherbia albida*	North Senegal	10	ND	37
ORS 184	LMG 10723	*Faidherbia albida*	Keur Momar Sarr (Guiers Lake, NS)	10	Sep	Sep
ORS 187	LMG 10726	*Faidherbia albida*	Dagana (NS)	10	XI	20
ORS 188	LMG 10727	*Faidherbia albida*	Dagana (NS)	10	V	20
Bradyrhizobium sp. (*Aeschynomene*)						
ORS 277	LMG 12186	*Aeschynomene sensitiva*	Elinkine (Casamance, SS)	25	Sep	Sep
ORS 278	LMG 12187	*Aeschynomene sensitiva*	Elinkine (Casamance, SS)	25	Sep	Sep
ORS 304	LMG 8069	*Aeschynomene elaphroxylon*	Guiers Lake (NS)	1	VIII	17
ORS 306	LMG 8300	*Aeschynomene indica*	Guiers Lake (NS)	1	XV	28
ORS 324	LMG 8295	*Aeschynomene afraspera*	Keur Maktar (NS)	25	XVI	6
ORS 347	LMG 10298	*Aeschynomene afraspera*	Tobor (Casamance, SS)	2	Sep	Sep
ORS 352	LMG 15384	*Aeschynomene afraspera*	Senegal	25	XVI	6
ORS 353	LMG 15385	*Aeschynomene afraspera*	Senegal	25	XVI	6
ORS 358	LMG 10303	*Aeschynomene nilotica*		25	ND	34
BTAi1	LMG 11795	*Aeschynomene indica*	United States	25	XV	28
Bradyrhizobium japonicum						
Bonnier 3.1	LMG 4252	*Glycine max*			II	15
Erdman 1BOa2	LMG 4262	*Albizia julibrissin*			ND	Sep
Erdman 3c3a1	LMG 4265	*Ulex europaeus*			ND	Sep
USDA 59	LMG 4271	*Glycine max*	North Carolina		II	15
Erdman 314a8	LMG 4272	*Pueraria lobata*			ND	Sep
Erdman 316n10	LMG 4274	*Vigna unguiculata*			Sep	Sep
NZP 5533	LMG 6136	*Glycine max*	United States		VII	12
NZP 5549[T]	LMG 6138[T]	*Glycine max*	Japan	17	II	15
USDA 135	LMG 8321	*Glycine max*	Iowa		V	20
USDA 110		*Glycine max*	Florida		VII	12
Bradyrhizobium elkanii						
NZP 5531[T]	LMG 6134[T]	*Glycine max*	Maryland	19	XI	32
NZP 5532	LMG 6135	*Glycine max*	Wisconsin	19	XI	32
USDA 61		*Glycine max*	North Carolina		XI	ND
Bradyrhizobium liaoningense						
SFI 2281[T]	LMG 18230[T]	*Glycine max*	China	42	IX	16
SFI 2062	LMG 18231	*Glycine max*	China	42	IX	16
Bradyrhizobium sp.						
NZP 2314	LMG 6129	*Lotus pedunculatus*	Australia	26	ND	39
USDA 3002	LMG 8888	*Acacia decurens*	Brazil	26	Sep	Sep
BR 6011	LMG 9514	*Lonchocarpus costatus*	Brazil	26	ND	Sep
BR 3606	LMG 9959	*Acacia mollissima*	Brazil	26	XI	Sep
BR 3621	LMG 9966	*Acacia mangium*	Espirito Santo, Brazil	26, 27	XI	48
BR 4406	LMG 9980	*Enterolobium ellipticum* Benth.	Rio de Janeiro, Brazil	26, 27	ND	Sep
BR 8406	LMG 10018	*Dalbergia nigra* Allem.	Rio de Janeiro, Brazil	26, 27	ND	48
INPA 9A	LMG 10029	*Derris* sp.	Brazil	26	ND	Sep
TAL 1127	LMG 10295	*Cajanus cajan*			ND	36
ORS 58	LMG 10663	*Dalbergia melanoxylon*	Senegal	9	ND	17
MAR 1505	LMG 14304	*Vigna unguiculata*		34	ND	Sep
MAR 1587	LMG 14311	*Arachis hypogaea*		34	ND	Sep
MSDJ 718		*Lupinus luteus*	France	21	Sep	39

[a] Original strain number, or as received. BR, strains from the CNPBS/EMBRAPA, Centro Nacional de Pesquisa em Biologia do Solo, Seropedica 23851, Rio de Janeiro, Brazil/Emprasa Brasiliera de Pesquisa Agropequaria; BCCM/LMG, Bacteria Collection, Laboratorium voor Microbiologie, Ghent, Belgium; MAR, Soil Productivity Research Laboratory, Marondera, Zimbabwe; MSDJ, Institut National de la Recherche Agronomique (INRA), Microbiologie des Sols, Dijon, France; INPA, National Institute of Amazonia Research, Manaus, Brazil; NZP, Culture Collection of the Department for Scientific and Industrial Research, Biochemistry Division, Palmerston North, New Zealand; ORS, ORSTOM Collection, Institut de Recherche pour le Développement, BP 1386, Dakar, Senegal; R-, Research collection of the Laboratorium voor Microbiologie, Ghent, Belgium; SFI, Soils and Fertilizers Institute, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China; TAL, Nitrogen Fixation in Tropical Agricultural Legumes (NifTAL), University of Hawaii, Paia; USDA, U.S. Department of Agriculture, Beltsville, Md.

[b] CS, central Senegal; NS, north Senegal; SS, south Senegal.

[c] ND, not determined.

[d] Sep, separate.

TABLE 2. Different rDNA IGS types and restriction patterns determined by PCR-RFLP analysis of the rDNA IGS regions

rDNA IGS clusters and their composition	rDNA IGS type[a]	Size[b] of IGS PCR product (bp)	Different restriction patterns types[c] of rDNA IGS digested with:							
			*Alu*I	*Dde*I	*Hae*III	*Hha*I	*Hin*fI	*Msp*I	*Nde*II	*Rsa*I
Cluster I	1	**825**	**2**	**2**	**2**	**31**	**3**	**2**	**2**	**2**
	2	**824**	**2**	**2**	**2**	**3**	**3**	**2**	**2**	**2**
	3	**820**	**2**	**2**	**2**	**4**	**3**	**2**	**3**	**2**
	4	**823**	**2**	**2**	**2**	**2**	**3**	**2**	**2**	**3**
	5	**816**	**2**	**2**	**2**	**3**	**3b**	**2**	**2**	**8**
	6	**802**	**2**	**2**	**2**	**31**	**2**	**2**	**2**	**5**
	7	**820**	**2**	**2**	**2**	**2**	**14**	**2**	**4**	**2**
	8	**825**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	9	**825**	**2**	**2**	**2**	**3**	**2**	**2**	**4**	**2**
Cluster II	10	821	2b	2	2	28	2	9b	27b	17
B. japonicum	11	825	22	23	9	28	2	9b	27b	17
Cluster III	13	**900**	**4**	**22**	**2**	**3**	**16**	**20**	**5**	**6**
Cluster IV	18	**820**	**4**	**22**	**30**	**11**	**21**	**35**	**3**	**5**
	19	**850**	**4**	**22**	**30**	**11**	**2**	**36**	**37**	**5**
Cluster V	24	850	12	6	9	13	6	10	18	7
Bradyrhizobium sp. (*F. albida*)	25	854	12	19	9	2	6	10	18	7
B. japonicum	26	820	12	19	9	2	6	10	16	7
	27	860	12	6	9	11	6	10	18	8
	28	**850**	**12**	**6**	**36**	**11**	**2**	**10**	**18**	**8**
	29	**864**	**12**	**6**	**10**	**2**	**6**	**10**	**18**	**7**
	30	**853**	**12**	**6**	**10**	**32**	**6**	**10**	**18**	**7**
	31	**900**	**12**	**6**	**10**	**2**	**2**	**10**	**18**	**7**
	32	**900**	**12**	**6**	**10**	**39**	**2**	**10**	**18**	**8**
Cluster VI	33	**804**	**11**	**6**	**34**	**40**	**2**	**29**	**15**	**5**
	34	**862**	**11**	**6**	**11**	**12**	**5**	**11**	**15**	**6**
	35	**862**	**29**	**6**	**11**	**5**	**5**	**29**	**31**	**2**
Cluster VII	36	870	10	6	9	11	2	12	17	5
B. japonicum	37	880	10	6	9	11	6	12	17	6
Cluster VIII	38	**900**	**7**	**3**	**5**	**23**	**5**	**6**	**7**	**5**
	39	**920**	**7**	**3**	**5**	**6b**	**6**	**6**	**7**	**6**
	40	**913**	**7**	**3b**	**6**	**6**	**6**	**6**	**7**	**6**
	41	**842**	**7**	**3**	**5**	**23**	**19**	**5b**	**7b**	**4**
Bradyrhizobium sp. (*A. elaphroxylon*)	42	950	8	3	5	7	19	5	7	4
Cluster IX	44	780	5	2	15	45	2	10	4	5
B. liaoningense										
Cluster X	45	845	16	7	12	14	5	13	14	10
Bradyrhizobium sp. (*F. albida*)										
Cluster XI	46	850	15	20	14	20	5	15	13	12
	47	**824**	**13**	**21**	**14**	**34**	**5**	**15**	**15**	**12**
Bradyrhizobium sp. (*A. mangium*)	48	**843**	**13**	**21**	**14**	**18**	**6**	**15**	**12**	**12**
Bradyrhizobium sp. (*A. mollissima*)	49	862	14	10	15	18	6	17	12	11
Bradyrhizobium sp. (*F. albida*)	50	864	14	20	14	18	6	16	12	11
B. elkanii	51	860	14	20	15	8	2	16	12	11
	52	870	15	20	14	18	5	15	13	11
	53	852	23	10	15	18	6	17	12	11
Cluster XII	54	**822**	**19**	**8**	**13**	**16**	**6**	**14**	**22**	**11**
Bradyrhizobium sp. (*F. albida*)	55	800	19	8	13	15	6	14	22	11
Cluster XIII	57	**866**	**21**	**4**	**7**	**8**	**7**	**7**	**9**	**6**
	58	850	20	4	8	9	7	7	10	6
Bradyrhizobium sp. (*F. albida*)	59	840	20	4	8	9	7	8	10	6
	60	840	20	4	8	9	7	8	10	5
Cluster XIV	61	880	21	5	8	9	6	9	11	6
Bradyrhizobium sp. (*F. albida*)	62	862	21	5	8	31	6	9	11	6

Continued on following page

TABLE 2—*Continued*

rDNA IGS clusters and their composition	rDNA IGS type(s)[a]	Size[b] of IGS PCR product (bp)	Different restriction patterns types[c] of rDNA IGS digested with:							
			*Alu*I	*Dde*I	*Hae*III	*Hha*I	*Hin*fI	*Msp*I	*Nde*II	*Rsa*I
Cluster XV	63	1,000	9	14	22	25	12	22	8	15
Bradyrhizobium sp. (*A. indica*)	64	1,000	9	14	23	25	12	23	8	15
Cluster XVI	66	1,038	17	11	19	22	10	19	19	13
Bradyrhizobium sp. (*A. afraspera*)	67	950	17	11	18	21	9	19	20	18
Separate	12	**780**	**2**	**2**	**31**	**2**	**2**	**2**	**34**	**2**
	14	**795**	**3**	**2**	**2**	**37**	**2**	**2**	**4**	**21**
	20	**870**	**31**	**2**	**30**	**23**	**5**	**20**	**26**	**5**
	21	**832**	**4**	**28**	**32**	**31**	**21**	**31**	**3**	**20**
	22	**900**	**34**	**13**	**2**	**7**	**5**	**33**	**37**	**13**
	23	**920**	**32**	**29**	**2**	**23**	**23**	**26**	**35**	**13**
	56	**880**	**13**	**20b**	**30**	**15**	**6**	**35**	**38**	**23**
	70	**923**	**25**	**17**	**26**	**20**	**10**	**26**	**28**	**13**
Bradyrhizobium sp. (*A. decurens*)	15	814	3	2	3	5	4	2	3	3
B. japonicum	16	840	37	33	3	20	4	9	18	2
Bradyrhizobium sp. (*L. luteus*)	17	806	5	23	3	28	4	3	3	5
Bradyrhizobium sp. (*A. afraspera*)	43	913	27	15	24	7	14	24	26	16
Bradyrhizobium sp. (*A. sensitiva*)	65	1,006	28	25	27	30	12	28	30	15
Bradyrhizobium sp. (*A. sensitiva*)	68	934	26	12	21	23	13	21	23	6
Bradyrhizobium sp. (*F. albida*)	69	910	25	9	7	17	8	2	29	13

[a] An rDNA IGS type corresponds to one combination of restriction patterns obtained with eight restriction enzymes.
[b] The size of the PCR product corresponds to the mean size of the undigested PCR product estimated by summing the sizes of the restriction fragments. Results of *Bradyrhizobium* strains from small legumes are in bold.
[c] A restriction pattern type refers to the pattern obtained for one strain with one restriction enzyme.

At a correlation coefficient of about 70%, the different IGS rDNA types formed 16 clusters (I to XVI), 6 of which together contained the majority of the strains studied (clusters I, V, VI, VIII, XI, and XIII). Fifteen strains occupied separate positions, namely eight Senegalese strains from small legumes (ORS 1811, ORS 1847, ORS 1810, ORS 1815, ORS 1817, ORS 1818, ORS 1848, and ORS 984), strain LMG 8888 (from Brazil), *Bradyrhizobium japonicum* strain LMG 4274, *Bradyrhizobium* sp. (*Lupinus luteus*) strain MSDJ 718, two photosynthetic *Bradyrhizobium* sp. (*Aeschynomene sensitiva*) strains (ORS 277 and ORS 278), *Bradyrhizobium* sp. (*Aeschynomene afraspera*) strain ORS 347, and *Bradyrhizobium* sp. (*Faidherbia albida*) strain ORS 184.

The three reference strains of *Bradyrhizobium elkanii* grouped in cluster XI, together with two strains from Brazil, BR 3606 and BR 3621, the latter having a 16S rDNA sequence close to that of *Bradyrhizobium elkanii* (3, 10). Cluster XI also included *Bradyrhizobium* sp. (*F. albida*) strain ORS 187 and seven strains from small legumes.

The two *Bradyrhizobium liaoningense* reference strains formed cluster IX.

Except for LMG 4274 (separate position, see above), *B. japonicum* reference strains were found in three clusters, namely II, V, and VII. Clusters II and VII consisted of only *B. japonicum* strains, the type strain (LMG 6138^{T}, hybridization group I [13]) being in cluster II, and USDA 110 (hybridization group Ia [13]) being in cluster VII. Cluster V included *B. japonicum* strain LMG 8321, three *Bradyrhizobium* sp. (*F. albida*) strains, and eight strains from small legumes.

Clusters X and XIV consisted of only *Bradyrhizobium* sp. (*F. albida*) strains. Clusters XV and XVI consisted of only photosynthetic *Bradyrhizobium* sp. (*Aeschynomene*) strains. Of the two nonphotosynthetic strains from *Aeschynomene* included in the study, one occupied a separate position in the dendrogram (ORS 347, see above) and the other (ORS 304) was grouped in cluster VIII together with strains from small legumes. Clusters I, III, IV, and VI consisted only of strains from small legumes. Half of the strains of cluster I originated from south Senegal and half were from three *Indigofera* species in central Senegal. Clusters III and IV consisted of strains isolated from south Senegal, while all strains of cluster VI were isolated from north Senegal. Other strains from small legumes were found in clusters V (see above), VIII, XI (with *B. elkanii*, see above), XII, and XIII [half *Bradyrhizobium* sp. (*F. albida*) strains and half *Bradyrhizobium* sp. (*Tephrosia purpurea*) strains].

Most of the strains forming each of the clusters V and XI are strains from small legumes of the same geographical origin, north Senegal and south Senegal, respectively.

Consequently, there is some correlation between the clustering of the PCR-RFLP IGS rDNA patterns and the geographic origin of the strains.

In total, no obvious relationship was apparent between the PCR-RFLP IGS rDNA clustering and the host-plant origin or host range (9) of the strains.

(ii) Size of IGS PCR products. We estimated the sizes of IGS PCR products by summing the sizes of the restriction fragments. All strains produced a single PCR product ranging from 780 to 1,038 bp, depending on the strain (Table 2). The differences observed in the size of the PCR products could be in part explained by the presence of several tRNA genes varying in number and type in the IGS regions (16, 22, 23).

PCR products from the strains of *B. japonicum* were quite diverse in size. *B. japonicum* strains USDA 110 and LMG 6136 (cluster VII) each produced an amplification product of around 900 bp, which is in agreement with a report of Laguerre et al. (22). *B. japonicum* strains LMG 4252, LMG 6138^{T}, LMG 4271 (cluster II), and LMG 8321 (cluster V) produced single amplification products of 821 to 825 bp. *B. japonicum* LMG 4274 (Fig. 1, separate position in the dendrogram) produced a PCR product with an intermediate size of 840 bp.

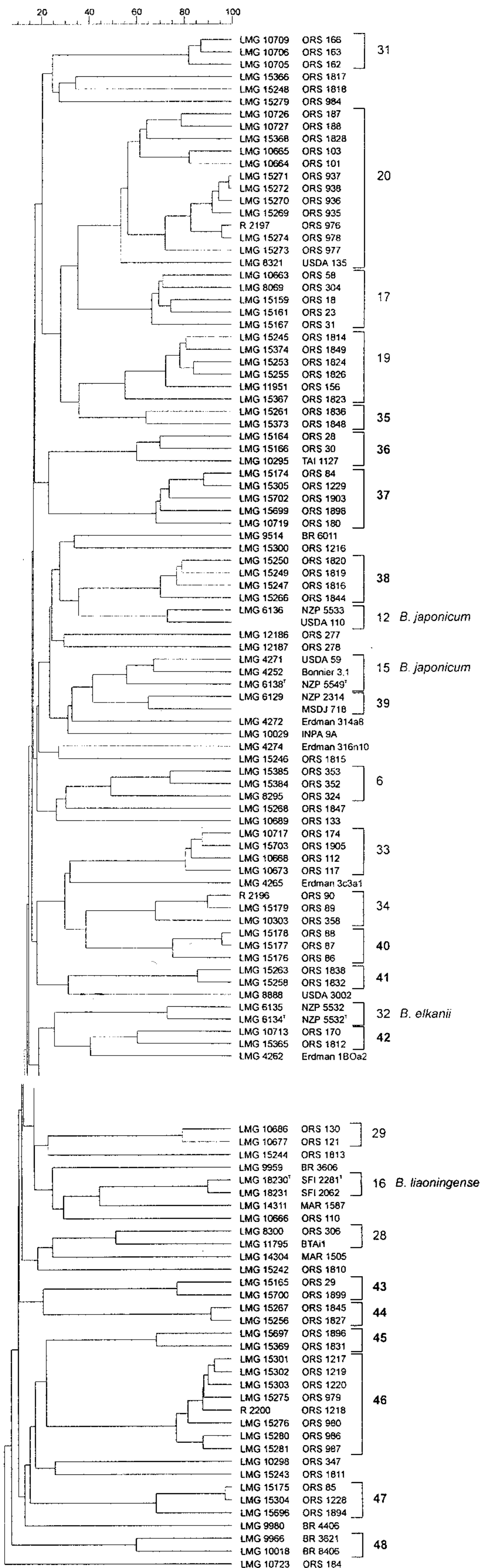

FIG. 3. Dendrogram obtained by UPGMA analysis of AFLP patterns of Senegalese isolates, some representative strains from Brazil, from *F. albida*, and from *Aeschynomene*, and the reference strains of *Bradyrhizobium*. The clusters were delineated at above 50% similarity. The clusters 6, 12, 15, 16, 17, 19, 20, 28, 29, 31, 32, 33, and 34 correspond to previously described AFLP clusters (42). The new groups identified in this study (35 to 48) are in bold.

The three *B. elkanii* strains (cluster XI) studied produced PCR products of almost identical size (860 to 864 bp).

The two strains of *B. liaoningense* (cluster IX) and one strain from a small legume, LMG 15243 (with a separate pattern), showed the smallest PCR product (780 bp).

PCR products of *Aeschynomene* photosynthetic strains were the largest, and sizes ranged between 934 and 1,038 bp. The two nonphotosynthetic strains of *Aeschynomene* included in the study yielded PCR products of 913 and 950 bp.

The sizes of the PCR products for the strains of *F. albida* were also diverse, ranging from 800 to 910 bp, and only four of the strains yielded PCR products of the same size.

The strains from small legumes produced single bands ranging from 780 to 923 bp. In general, the strains from small legumes exhibiting PCR products of approximately the same size grouped in the same cluster; exceptions are strains of cluster VIII (Fig. 2) exhibiting PCR products of heterogeneous sizes (842 to 920 bp).

AFLP analysis. (i) Optimization of the AFLP technique for *Bradyrhizobium* strains. Several enzyme combinations were tested to determine the most suitable one, i.e., one producing a large number of fragments (30 to 50) of many different lengths resulting in an evenly distributed banding pattern. For *Bradyrhizobium* organisms which have a high GC percentage in their genomes, the combination of *Taq*I (T/CGA) and *Apa*I (GGGCC/C) proved the most useful.

In the same way, four combinations of primers with different selective bases were tested, and primers 5′-GACTGCGTACA GGCCC**G**-3′ and 5′-GATGAGTCCTGACCGA**A**-3′ were retained (characters in bold were the selective bases).

(ii) Numerical analysis of *Bradyrhizobium* AFLP patterns. In our AFLP study, we included 63 strains from small legumes, 14 reference strains representative for *B. japonicum*, *B. elkanii*, and *Bradyrhizobium liaoningense*, 18 *Bradyrhizobium* sp. (*F. albida*) and 10 *Bradyrhizobium* sp. (*Aeschynomene*) strains representative for AFLP groups 6, 16, 17, 19, 20, 28, 29, 31, 33, and 34 described elsewhere (40), and 13 other *Bradyrhizobium* sp. strains from various host plants (10, 21, 26, 27, 34).

As 13 of the clusters corresponded to previous AFLP groups 6, 12, 15, 16, 17, 19, 20, 28, 29, 31, 32, 33, and 34 (40), we named them accordingly and we numbered our new clusters from 35 to 48. Similarities between AFLP patterns were calculated using the Dice coefficient, and the strains were then grouped by UPGMA cluster analysis (Fig. 3).

Considerable profile heterogeneity between strains was revealed, and at a similarity coefficient of 50% (which is commonly used to delineate AFLP clusters in other bacterial groups [14, 15] and in *Bradyrhizobium* [40]), 27 groups could be distinguished.

B. japonicum reference strains were found in different clusters. Five *B. japonicum* strains formed groups 12 (including USDA 110) and 15 (including the type strain, LMG 6138). *B. japonicum* strains LMG 4262, LMG 4265, and LMG 4272 had separate positions, and strain LMG 8321 grouped in cluster 20, which mainly consisted of new isolates from *Rynchosia minima* and *Indigofera senegalensis* and of three *Bradyrhizobium* sp. (*F. albida*) strains.

TABLE 3. Comparison of results from 16S ARDRA and AFLP and PCR-RFLP analyses of 16S-23S rDNA IGS region

ARDRA groups[a]	IGS rDNA PCR-RFLP cluster	IGS rDNA PCR-RFLP type(s)	AFLP cluster(s)
A, B, E, F	I	2	33
		3	47
		4, 5	38
		6, 7, 9	37
		8	43
	II	10, 11	15
	IV	18, 19	45
	V	25, 26, 27, 28, 29, 30, 31, 32	20
	VII	36, 37	12
	VIII	38	ND[b]
		39, 41	17
		40	41
	IX	44	16
	XI	53	20
	XIII	58	Sep[c]
	XV	63, 64	28
	XVI	66, 67	6
	Sep	12, 14, 21, 22	Sep
		17	39
		20, 23	Sep
C, G	VI	33, 34, 35	46
	X	45	31
	XI	46	48
		47	Sep
		48	19, 35
		50, 51	32
		52	Sep
	XII	54, 55	42
	XIII	57	34, 40, Sep
		60	33
	XIV	61, 62	29
	Sep	21, 22, 70	Sep
		56	35
Sep	ND	ND	Sep
	I	1	36
ND	I	1	36
	III	13	44
	V	24	20
	VIII	42	17
	XI	49[d]	ND
	XIII	58, 59	33
	Sep	15, 16, 43, 65, 68, 69	Sep

[a] Group designations are according to those of Doignon-Bourcier et al. (9).
[b] ND, not determined.
[c] Sep, separate.
[d] The IGS PCR-RFLP type 49 corresponds to *B. elkanii* reference strain USDA 61.

The two strains of *B. elkanii* formed cluster 32. The two *B. liaoningense* strains formed cluster 16.

Each of the 13 *Bradyrhizobium* sp. strains from various host plants (10, 21, 26, 27, 34) occupied separate positions in the dendrogram, except for the two of them forming cluster 48.

Of strains from small legumes, 19 grouped in previously described groups 17, 19, 20, 33, and 34 (40), 9 occupied separate positions, and 35 formed new clusters 35, 36, 37, 38, 40, 41, 42, 43, 44, 45, 46, and 47. Clusters 37 and 42 contained *Bradyrhizobium* sp. (*F. albida*) strains LMG 10719 and LMG 10713, which were previously described as separate (40). Cluster 36 consisted of two Senegalese strains and one *Bradyrhizobium* sp. (*Cajanus cajan*) strain.

In general, AFLP clusters consisted of strains isolated either from north Senegal or from south Senegal.

Except for one strain, all the strains from small legumes grouping in cluster 20 were isolated from the same place in north Senegal. The strains forming cluster 40 were isolated from the same host plant and had the same geographic origin.

So, a relationship may exist between AFLP grouping and the geographic origin of the strains.

On the other hand, there is no evident relationship between the plant origin or nodulation host range (9) of the strains and AFLP grouping except for cluster 46, which consisted of strains exclusively isolated from the same host plant.

DISCUSSION

For *Bradyrhizobium*, many groups have been identified during recent years, but their taxonomic status has remained unclear due to the inconsistency of the results obtained by different taxonomic methods. There is a need to use several methods, preferably genotypic ones, to draw more reliable taxonomic conclusions.

In a previous report, we isolated and performed initial characterization of 71 nodule isolates from small legumes in Senegal by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total proteins and by 16S ARDRA and compared them to a number of *Bradyrhizobium* reference strains. We concluded that these *Bradyrhizobium* strains were diverse and formed seven phylogenetic groups. Here, we continued the study of these strains by two genomic techniques, PCR-RFLP analysis of the IGS region between 16S and 23S rRNA genes and AFLP analysis. We focused on the applicability and comparison of the taxonomic resolution level of these two techniques to characterize *Bradyrhizobium* strains and help elucidate taxonomic problems encountered in this genus. Both IGS PCR-RFLP and AFLP analyses confirmed that *Bradyrhizobium* strains from small legumes are diverse.

By IGS PCR-RFLP using eight restriction enzymes, the 104 strains studied produced 70 types of combined restriction profiles, forming 16 groups. By AFLP analysis, the strains formed 27 groups. Table 3 shows comparative results obtained with essentially the same strains by the two techniques IGS PCR-RFLP and AFLP analyses and also by previous 16S ARDRA (9). There is very good agreement between results from the three techniques, except for the two *Bradyrhizobium* sp. (*F. albida*) strains ORS 187 and ORS 110. The 16S ARDRA and AFLP results for ORS 187 are consistent, but not with the IGS results; the 16S ARDRA and IGS results for ORS 110 do not match. In most cases, each AFLP cluster contained strains showing the same or very similar rDNA IGS types and belonging to one IGS PCR-RFLP cluster.

The strains belonging to the 16S ARDRA groups A, B, E, and F (*B. japonicum* lineage) belonged to 43 IGS PCR-RFLP types (corresponding to 12 main IGS PCR-RFLP clusters) and to 17 AFLP clusters. Interestingly, strains of 16S ARDRA group A, exclusively consisting of photosynthetic strains, were also separate from nonphotosynthetic strains in AFLP and IGS PCR-RFLP groups. The strains belonging to 16S ARDRA groups C and G (*B. elkanii* lineage) belonged to 20 IGS PCR-RFLP types (corresponding to six IGS PCR-RFLP main clusters) and 10 AFLP clusters.

This confirms, as expected from literature dealing with other bacterial groups, that IGS PCR-RFLP (4, 22) and AFLP (15) analyses are more discriminative than 16S ARDRA for *Bradyrhizobium* and do almost differentiate at the strain level. Moreover, it has been reported that the AFLP method was more efficient for assessing intrapathovar diversity of the genus

Pseudomonas than the rapid amplified polymorphic DNA method (7).

For screening purposes, fine discriminating genotypic techniques such as IGS PCR-RFLP and AFLP are recommended; IGS PCR-RFLP has some advantages because the experimental protocol is sure, simple, and similar to that used for other studies on the 16S rDNA gene and is less laborious than the AFLP technique. Our results agreed with the study of Leblond-Bourget et al. (23) on *Bifidobacterium* species, showing that IGS rDNA regions are useful for rapid identification or intraspecific phylogenetic studies, while 16S rRNA sequences are a good tool to infer interspecific links (11).

As expected from the literature (3, 9, 13, 19, 30, 40, 41, 45), our results show some heterogeneity among *B. japonicum* reference strains. Four of the *B. japonicum* reference strains included, LMG 4262, LMG 4265, LMG 4272, and LMG 4274, were of different plant origins, *Albizzia*, *Ulex*, *Pueroria*, and *Vigna*, respectively. They all occupied separate positions by both IGS PCR-RFLP and AFLP analyses. On the contrary, a majority of the *B. japonicum* reference strains originating from *Glycine max* clustered in two main groups, IGS II/AFLP 15 and IGS VII/AFLP 12. One exception is *B. japonicum* strain LMG 8321 grouping together with isolates from small legumes and from *F. albida* in IGS V/AFLP 20. By the two techniques, and also by 16S ARDRA (9), *B. japonicum* strain USDA 110 grouped separately from the *B. japonicum* type strain LMG 6138. This result corroborated other studies based on DNA: DNA hybridizations (13) and 16S rDNA analysis (3, 37).

By the three techniques, the *B. elkanii* strains grouped together; moreover, by AFLP analysis, they formed a separate group.

By ARDRA, both *B. liaoningense* strains were found in the same group as *B. japonicum* type strain LMG 6138; here, they were distinct from *B. japonicum* and formed a separate group by both IGS PCR-RFLP and AFLP analyses.

Of the eight restriction enzymes used for IGS PCR-RFLP in our study, *Hin*fI and *Rsa*I were the least discriminative. Except for three strains, LMG 15268, LMG 8888, and LMG 4274, all the strains could be differentiated by using the combination of the IGS PCR-RFLP patterns obtained with two enzymes, *Nde*II and *Msp*I.

No clear relationship could be evidenced between plant origin, host range (9), and the grouping of strains by AFLP and IGS PCR-RFLP analyses. On the contrary, we found a certain degree of relationship between AFLP and IGS PCR-RFLP groups and the geographical origins of the strains, especially between north and south Senegal, corresponding to different ecological regions. Senegal is a constrasting country, from its northern part being in the sahelian zone (dry steppe receiving 100 to 300 mm rainfall per year) to the southern part being in the guineo-soudanian zone (deciduous forest receiving 800 to 1,200 mm of rainfall per year). In general, groups mainly consisted of isolates either from north Senegal (IGS groups V, VI, XIII, and XIV corresponding to AFLP groups 20, 29, 33, 34, 40, and 46) or south or central Senegal (IGS groups I, III, IV, X, XI, and XII corresponding to AFLP groups 19, 31, 35, 36, 37, 38, 42, 43, 44, 45, and 47). However, this is not a general rule since some exceptions exist, like IGS group VIII and AFLP group 33 consisting of strains originating from everywhere in Senegal.

Here, we extended the screening of our *Bradyrhizobium* strain collection by AFLP analysis. The AFLP technique has been described as having a similar discriminating power as DNA:DNA hybridizations, making it a potentially useful taxonomic tool for species delineation for a number of different bacterial groups (7, 14, 15). This result is not unexpected since this method studies the whole bacterial genome, resulting in a profile with many bands (15). DNA:DNA hybridizations remain required to fully evaluate the discriminative power of the technique in the case of *Bradyrhizobium*.

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