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Diversity of Bradyrhizobia from 27 Tropical *Leguminosae* Species Native of Senegal

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Summary

We isolated 71 slow-growing bacterial strains from nodules of 27 native leguminous plants species in Senegal (West-Africa) belonging to the genera *Abrus*, *Alysicarpus*, *Bryaspis*, *Chamaecrista*, *Cassia*, *Crotalaria*, *Desmodium*, *Eriosema*, *Indigofera*, *Moghania*, *Rhynchosia*, *Sesbania*, *Tephrosia*, and *Zornia* playing an ecological role and having agronomic potential in arid regions. The isolates were characterised by restriction fragment length polymorphism (RFLP) analysis of PCR-amplified 16S rDNA and comparative SDS-PAGE of whole-cell proteins; reference strains of the different known rhizobial species and groups were included as references. We conclude that these nodule isolates are diverse, and form several phylogenetic subgroups inside *Bradyrhizobium*. Nodulation tests performed on 5 plant species demonstrated host specificity among the strains studied.

Key words : *Bradyrhizobium* – Tropical legumes – SDS-PAGE – 16S-ARDRA

Introduction

Rhizobia, a general term referring to nitrogen-fixing bacteria capable of living in symbiosis with legume plants, are taxonomically very diverse; they have been studied intensively the last years and their classification has changed and several new species and genera have been created (YOUNG and HAUKKA, 1996; JARVIS et al., 1997; AMARGER et al., 1997; CHEN et al., 1997; VAN BERKUM et al., 1998; DE LAJUDIE et al., 1998a,b; WANG et al., 1998; 1999).

Among the 19000 described legume species in the world, only a small proportion has been studied for isolation of symbiotic bacteria and until recently, most studies concerned cultivated plants. During the last ten years, several authors reported isolation of rhizobia from previously uninvestigated wild legumes in different parts of the world, namely in Brazil (MOREIRA et al., 1993), Canada (PREVOST et al., 1987) Russia (NOVIKOVA et al., 1994; VAN BERKUM et al., 1998), China (CHEN et al., 1991; 1995; 1997; GAO et al., 1994; WANG et al., 1999), Sudan (ZHANG et al., 1991, NICK et al., 1999, HAUKKA et al., 1996), Senegal (DREYFUS et al., 1988; LORQUIN et al., 1993, DUPUY et al., 1994, DE LAJUDIE et al, 1994; 1998a,b; 1999; MOLOUBA et al., 1999), South Africa (DAGUTAT and STEYN, 1994), Canary Islands (VINUESA et al., 1998) and several new rhizobial groups were described.

In Senegal, legume plants have ecological, agronomic and economical importance: they constitute 16% of the total plant species and 11% of the genera of the total vegetation (DIEDHIOU, 1994). A number of them are indigenous, annual or perennial, herbaceous or shrub legumes, well adapted to local arid climatic conditions and mineral- (especially nitrogen-) deficient soils; 85% of the legume plants in the arid and semi-arid regions are spontaneous plants potentially important for programs of soil fertility regeneration, sustainable agriculture, forestry and forage. As a result of prospecting in different arid regions of Senegal we found a number of nodulated legume species, belonging to 14 genera, that could be of importance for soil sustainability.

We report here the isolation and the characterization of 71 slow-growing bacterial nodule isolates from 27 plant species sampled in different places in Senegal: several species of the genera *Abrus*, *Alysicarpus*, *Bryaspis*, *Chamaecrista*, *Cassia*, *Crotalaria*, *Desmodium*, *Eriosema*, *Indigofera*, *Moghania*, *Rhynchosia*, *Sesbania*,

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Tephrosia, *Zornia*. We performed Restriction Fragment Length Polymorphism (RFLP) analysis of the PCR-amplified 16S rRNA gene (ARDRA), comparative Sodium Dodecyl Sulphate - Polyacrylamide gel electrophoresis (SDS-PAGE) analysis of whole cell protein patterns and nodulation tests. Reference strains representing the different rhizobial species and groups were included for comparison.

Materials and Methods

Bacterial strains and isolation procedures: The strains studied are listed in Table 1.

Rhizobial strains were isolated either from naturally occurring root nodules collected *in natura* or from nodules obtained after cultivation of plants (either *in vitro* or in pots containing sterilised soil) inoculated with either crushed nodules or soil sample suspensions. Nodules collected *in natura* were either directly used for rhizobial isolation or stored dried in tubes containing $CaCl_2$ (DATE, 1982). Upon utilisation, nodules were rehydrated in sterile water and surface sterilised by immersion in $HgCl_2$ (0.1%) for 5 min. From this stage the nodules were manipulated aseptically. The nodules were rinsed 8 times in sterile water. Big nodules were aseptically cut and samples were directly picked out from the centre of the nodule and streaked onto Yeast extract Mannitol medium agar (YMA) plates (VINCENT, 1970). Smaller nodules were crushed in a drop of sterile water and the suspension was streaked on YMA. Colonies appeared after several days of incubation at 30 °C in aerobic conditions. They were checked for purity by repeated streaking on YMA and by microscopic examination of living cells. Isolates were checked for nodulation on their original host plants, and stored at –80 °C in YM adjusted to 20% (v/v) glycerol.

In other cases, nodules were obtained *in vitro* by cultivating plants in the presence of either crushed nodules sampled *in natura* or soil suspensions when no nodules could be found *in natura*. Soil samples were collected at 5–20 cm depth in the vicinity of spontaneous legumes in various regions. They were stored in plastic bags at 4 °C and used as soon as possible to minimise possible changes of their internal bacterial population (BROCKWELL, 1982). Four seedlings of the plant species present at the sampling site, grown aseptically for 3–5 days in Jensen slant agar tubes (GIBSON, 1963) were inoculated with one ml of a 60 min-magnetic-stirred soil suspension in 0.8% NaCl (10% w/v). Two to three week-old nodules were collected, washed, surface sterilised and treated as above for rhizobium isolation. ORS 515 was isolated from a nodule formed on *Indigofera senegalensis* inoculated with crushed nodules harvested on *Desmodium setigenum in natura* (no seed of *D. setigenum* was available).

Growth and culture conditions: All *Rhizobium* and *Bradyrhizobium* strains were maintained on YMA medium (VINCENT, 1970) containing (g/l): mannitol, 10; sodium glutamate, 0.5; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.05; $CaCl_2$, 0.04; $FeCl_3$, 0.004; yeast extract (Difco), 1; pH 6.8; agar, 20. *Azorhizobium* strains were maintained on YEB medium containing (in g/l of 0.01 M phosphate buffer, pH 7.2): peptone (Oxoid), 5; yeast extract (Oxoid), 1; beef extract (Oxoid), 5; sucrose, 5 and $MgSO_4 \cdot 7H_2O$, 0.592. All strains were stored at –80 °C on the same medium adjusted to 20% (vol/vol) glycerol; some strains were kept also in lyophilised form.

Plant cultivation and nodulation tests: The seeds were scarified and surface-sterilised with concentrated sulphuric acid. The minutes of treatment with H_2SO_4 for the different plant species were as follows: *Indigofera astragalina* and *Indigofera senegalensis*, 15; *Indigofera tinctoria*, 20; *Rhynchosia minima*, 40; *Tephrosia purpurea*, 60. After treatment, the seeds were washed with water until all traces of acid were removed. The seeds were incubated to germinate in sterile Petri dishes on 1% water agar for 24 to 48 hours and then transferred to tubes containing Jensen seedling slant agar (VINCENT, 1970) for root nodulation trials (4-6 plants were routinely tested with each strain). Plants were grown under continuous light (20W/m^2) at 28°C. Plants were observed for nodule formation during 6 to 8 weeks.

Nitrogen fixation activity: Nitrogen-fixing potential was estimated by visual observation of plant vigour and foliage colour of ca. 30 day old plants, and by measurement of Acetylene reducing activity according to HARDY et al. (1973).

Polyacrylamide gel electrophoresis of total bacterial proteins: All strains were grown at 28 °C for 48 h (fast-growing rhizobia) or 72 h (slow-growing rhizobia) in Roux flasks on TY medium containing (g/l): tryptone (Oxoid), 5; yeast extract (Oxoid), 0.75; KH_2PO_4, 0.454; $Na_2HPO_4 \cdot 12H_2O$, 2.388; $CaCl_2$, 1; agar (Lab M), 20; pH 6.8–7. Whole-cell protein extracts were prepared from 80 mg cells and SDS-PAGE was performed using small modifications of the procedure of LAEMMLI (1970), as described previously (DE LAJUDIE et al., 1994). The normalised densitometric traces of the protein electrophoretic patterns were grouped by numerical analysis, using the GelCompar 4.2 software package (VAUTERIN & VAUTERIN, 1992). The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient (r) converted for convenience to a percent value (POT et al., 1989; 1994).

16S ARDRA: Strains were grown at 28 °C for 5 days on YMA. Total DNA was purified by a slightly modified technique from PITCHER et al. (1989). Cells were suspended in RS buffer (0.15 M NaCl, 0.01 M EDTA, pH 8.0). The deproteinisation step was performed with chloroform:iso-amyl alcohol (24:1). After precipitation, purified DNA was washed three times in ethanol, dried, dissolved overnight at 4 °C in TE buffer, and treated (60 min, 37 °C) with RNAse (50 µg/ml final concentration). The control of the DNA quality and the calculation of DNA concentration were performed as described by HEYNDRICKX et al. (1996). The oligonucleotides used as primers for PCR amplification of the 16S rRNA genes were purchased from Pharmacia Biotech (Amersham Pharmacia Biotech Benelux, Roosendaal, The Netherlands). The sequences of the forward and reverse primers were 5'-AG AGT TTG ATC ATG GCT CAG-3' and 5'-TAC CTT GTT ACG ACT TCA CCC CA-3' respectively (HEYNDRICKX et al., 1996). PCR amplification was carried out in a 50 µl reaction volume containing template DNA (5 µl, 250 ng), reaction buffer (Eurogentec Bel SA, Seraing, Belgium), 10 mM of each dNTP (Pharmacia Biotech), 20 µM of each of the primers and 1 unit of *Taq* polymerase (Eurogentec Bel SA). A negative control containing 5 µl of Milli-Q water instead of DNA was included in every PCR run. Amplifications were carried out in a Gene Amp PCR System 9600 (Perkin Elmer, Nieuwerkerk a/d Ijssel, The Netherlands) using the following program: initial denaturation for 7 min at 95 °C, 50 cycles of denaturation (45 sec at 95 °C), annealing (30 sec at 57 °C) and extension (2 min at 72 °C), and a final extension (10 min at 72 °C). PCR amplified DNAs were visualised by Hoefer HE 33 Mini Submarine electrophoresis (Amersham Pharmacia Biotech) at 70 V during 30 minutes of 5 µl of the amplified mixture on 2% w/v 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/ml of ethidium bromide.

Aliquots of 10 µl of PCR products were digested with restriction endonucleases during 2 hours in a 20 µl final volume as specified by the manufacturer but with an excess of enzyme (5 units per reaction). Five restriction enzymes were selected on

the basis of simulated digests of the 16S rDNA sequences as described by HEYNDRICKX et al. (1996): *Hinf I* (New England Biolabs Inc., Leusden, The Netherlands), *Dde I* (Pharmacia Biotech Benelux), *Mwo I* (Biolabs), *Alu I* (Boehringer Mannheim Biochemica, Bruxelles, Belgium), *Hha I* (New England Biolabs Inc.). Restricted DNA was analysed by horizontal agarose gel electrophoresis as described by HEYNDRICKX et al. (1996) except that we used LSI MP agarose (Life Sciences International, Zellik, Belgium) and a DNA Sub Cell unit (15 × 10 cm tray, 20 wells, Biorad). The scanned gel images were digitised and stored in a computer as described by HEYNDRICKX et al. (1996). Clustering was obtained from the combined profiles using the GelCompar 4.2 software package (VAUTERIN & VAUTERIN, 1992). Dice coefficient (Tol 0.3%, Opt 0.50%, Min. area 0.0%) and UPGMA clustering were used.

Results

Collection of nodule bacterial isolates from spontaneous legumes

We performed large prospectios in arid and semi-arid regions of Senegal and focused on 34 spontaneous nodulated legume plant species (annual or perennial), most likely to be important for the protection of the environment, for soil fertility (in fallow lands) or as forage. Among these, we did bacterial isolations from nodules collected on 27 plant species of 13 genera (Table 1) from 19 different places in Senegal. The majority of the isolates were slow-growing bacteria, but a few fast-growing ones were also obtained from *Rhynchosia minima*, *Tephrosia villosa*, *Tephrosia bracteolata*, *Indigofera senegalensis*, *Indigofera hirsuta*, *Indigofera astragalina*, *Indigofera tinctoria*, *Alysicarpus ovalifolius*. A total of 87 bacterial nodule isolates were obtained, among which 71 slow-growing ones were chosen for further studies. Ten strains were obtained by direct isolation from naturally occurring nodules; 61 isolates were obtained from nodules formed on plants in laboratory conditions by using crushed nodules or soil samples. Nodules and soils originated from 21 different places in Senegal. 24 nodule isolates originate from the North region (isohyets 250–500 mm), 17 from the Central region (isohyets 500–900 mm) and 28 from the South region (isohyets 900–1200 mm).

Tephrosia purpurea and *Rynchosia minima* were only found in the North region. In South Senegal we found several species of *Desmodium* and *Crotalaria*, *Abrus stictosperma*, *Eriosema glomeratum*, *Indigofera microcarpa*, *Alysicarpus glumaceus*, *Alysicarpus rugosus*, *Zornia glochidiata*, *Cassia absus*, *Tephrosia bracteolata* and *Indigofera stenophylla*. *Indigofera tinctoria*, *Indigofera astragalina*, *Indigofera senegalensis* and *Sesbania rostrata* were found in both the North and Central regions, *Indigofera hirsuta*, *Alysicarpus ovalifolius* and *Tephrosia villosa* in both the Central and the South regions.

16S ARDRA

We studied 59 new isolates, including *Bradyrhizobium japonicum*, *Bradyrhizobium elkanii* and *Bradyrhizobium* sp. reference strains representative for ARDRA groups A, B, C and D of our previous report (MOLOUBA et al., 1999). In addition, we included 6 more strains of *B. japonicum*, 2 strains of *B. liaoningense* and several partially characterized *Bradyrhizobium* sp. strains (DUPUY et al., 1994; MOREIRA et al, 1993; 1998; LAGUERRE et al., 1994). 16S ribosomal DNA was PCR-amplified and all strains produced a single band of the expected size of approximately 1500 bp. Amplified DNA was hydrolysed with five endonucleases, and the combined agarose gel electrophoretic patterns were compared numerically using GelCompar version 4.2. The results are shown as a dendrogram in Fig. 1. The results for strain LMG 6134^T (included twice) illustrate the reproducibility of the method. At a mean Dice similarity coefficient (S_D) of 87%, seven main clusters (A to G) could be distinguished, and strains LMG 4265, ORS 30, ORS 1819 occupied separate positions. As four of the clusters corresponded to the previously identified clusters A, B, C and D by MOLOUBA et al. (1999), we named them accordingly.

Cluster C (internal grouping at a S_D of 88%) included 14 new isolates from diverse plants and regions and the 13 strains of the original cluster C of MOLOUBA et al. (1999), including the reference strains of *B. elkanii* (LMG 6134^T and LMG 6135). This cluster included also *Bradyrhizobium* sp. strains LMG 9959 and ORS 133. The strain LMG 4262, so far classified as *B. japonicum*, also grouped in cluster C.

Cluster G (internal grouping at a S_D of 92%) consisted of 8 new isolates from *Indigofera senegalensis* sampled in different places in North Senegal.

In addition to the 10 strains representing ARDRA cluster B of MOLOUBA et al. (1999), the present cluster B (internal grouping at a S_D of 85%) included 24 new isolates, the majority of *B. japonicum* reference strains, among which LMG 6138^T and LMG 6136 belong to DNA-DNA hybridisation group I (HOLLIS et al., 1981) and both strains of *B. liaoningense* included in this study.

Cluster E (internal grouping at a S_D of 82%) consisted of five new isolates and *B. japonicum* strain USDA 110, representative for the DNA hybridisation group Ia (HOLLIS et al., 1981).

Cluster F (internal grouping at a S_D of 85%) consisted of 6 new isolates from diverse plant species originating from Central and South Senegal.

Clusters A and D were unchanged compared to our previous report (MOLOUBA et al., 1999), and no new isolates grouped in them.

SDS-Polyacrylamide gel electrophoresis of total bacterial proteins

The SDS-PAGE whole-cell protein patterns of 64 new rhizobial isolates from Senegal were scanned and analysed numerically together with those of 33 reference strains representing different fast- and slow-growing rhizobial species and 28 other partially characterised strains (MOREIRA et al., 1993; 1998; DUPUY et al., 1994; MOLOUBA et al., 1999), several of which had also been

Table 1. Strains used.

Strain[a]	Other strain designation	Host plant or origin	Mode of isolation[b]	Geographical origin[c]	Reference or source	ARDRA group	SDS-PAGE group
New nodule isolates							
ORS 18	LMG 15159	*Alysicarpus ovalifolius*	NIN	Dakar Bel-Air (CS)	This work	B	nd
ORS 23	LMG 15161	*Tephrosia villosa*	NIN	Dakar Bel-Air (CS)	This work	B	3
ORS 28	LMG 15164	*Indigofera tinctoria*	TIVN	Mbour (CS)	This work	nd	3
ORS 29	LMG 15165	*Indigofera tinctoria*	NIN	Mbour (CS)	This work	B	sep[d]
ORS 30	LMG 15166	*Indigofera hirsuta*	TIVN	Dakar Bel-Air (CS)	This work	sep	3
ORS 31	LMG 15167	*Indigofera tinctoria*	NIN	Pal (NS)	This work	E	3
ORS 84	LMG 15174	*Indigofera astragalina*	TIVS	14° 26' 52" N / 14° 29' 50" W (Payar, CS)	This work	nd[e]	3
ORS 85	LMG 15175	*Indigofera astragalina*	TIVS	14° 26' 52" N / 14° 29' 50" W (Payar, CS)	This work	F	15
ORS 86	LMG 15176	*Tephrosia purpurea*	TIVS	15° 24' 00" N / 14° 24' 00" W (Bourel, Ferlo, NS)	This work	C	19
ORS 87	LMG 15177	*Tephrosia purpurea*	TIVS	15° 24' 00" N / 14° 24' 00" W (Bourel, Ferlo, NS)	This work	C	19
ORS 88	LMG 15178	*Tephrosia purpurea*	TIVS	15° 24' 00" N / 14° 24' 00" W (Bourel, Ferlo, NS)	This work	C	19
ORS 89	LMG 15179	*Tephrosia purpurea*	TIVS	15° 24' 00" N / 14° 24' 00" W (Bourel, Ferlo, NS)	This work	C	19
ORS 90	R-2196	*Tephrosia purpurea*	TIVS	15° 24' 00" N / 14° 24' 00" W (Bourel, Ferlo, NS)	This work	C	19
ORS 91	LMG 15180	*Tephrosia purpurea*	TIVS	15° 24' 00" N / 14° 24' 00" W (Bourel, Ferlo, NS)	This work	nd	19
ORS 515	R-2188	*Desmodium setigenum* /*Indigofera senegalensis*	TIVN	Niaguis (Casamance, SS)	This work	B	nd
ORS 516		*Indigofera senegalensis*	TIPS	Dakar Bel-Air (CS)	This work	nd	nd
ORS 518		*Indigofera senegalensis*	TIPS	Dakar Bel-Air (CS)	This work	nd	nd
ORS 519	R-2191	*Indigofera senegalensis*	TIPS	Dakar Bel-Air (CS)	This work	nd	6
ORS 520	R-2192	*Indigofera senegalensis*	TIPS	Dakar Bel-Air (CS)	This work	B	sep
ORS 521	R-2193	*Indigofera senegalensis*	TIPN	Dakar Bel-Air (CS)	This work	nd	6
ORS 522		*Indigofera senegalensis*	TIVN	Dakar Bel-Air (CS)	This work	nd	3
ORS 523	R-2194	*Indigofera senegalensis*	TIPN	Dakar Bel-Air (CS)	This work	B	6
ORS 524	R-2195	*Desmodium asperum*	TIPN	Kolda (Casamance, SS)	This work	nd	14
ORS 935	LMG 15269	*Rhynchosia minima*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	E	3
ORS 936	LMG 15270	*Rhynchosia minima*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	B	3
ORS 937	LMG 15271	*Rhynchosia minima*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	B	3
ORS 938	LMG 15272	*Rhynchosia minima*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	B	3
ORS 976	R-2197	*Indigofera senegalensis*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	B	nd
ORS 977	LMG 15273	*Indigofera senegalensis*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	B	3
ORS 978	LMG 15274	*Indigofera senegalensis*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	B	3
ORS 979	LMG 15275	*Indigofera senegalensis*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	G	10
ORS 980	LMG 15276	*Indigofera senegalensis*	TIVN	15° 40' 52" N / 14° 19' 49" W (Gueye Kadar, Ferlo, NS)	This work	G	10
ORS 984	LMG 15279	*Indigofera senegalensis*	TIVN	16° 10' 17" N / 14° 01' 47" W (Mboumba, Ferlo, NS)	This work	C	8
ORS 986	LMG 15280	*Indigofera senegalensis*	TIVN	16° 10' 17" N / 14° 01' 47" W (Mboumba, Ferlo, NS)	This work	G	10
ORS 987	LMG 15281	*Indigofera senegalensis*	TIVN	16° 10' 17" N / 14° 01' 47" W (Mboumba, Ferlo, NS)	This work	G	10
ORS 1216	LMG 15300	*Indigofera senegalensis*	TIVS	16° 01' 04" N / 14° 12' 50" W (Boki Namadi, Ferlo, NS)	This work	nd	nd
ORS 1217	LMG 15301	*Indigofera senegalensis*	TIVN	16° 01' 04" N / 14° 12' 50" W (Boki Namadi, Ferlo, NS)	This work	G	17
ORS 1218	R-2200	*Indigofera senegalensis*	TIVN	16° 01' 04" N / 14° 12' 50" W (Boki Namadi, Ferlo, NS)	This work	G	5
ORS 1219	LMG 15302	*Indigofera senegalensis*	TIVN	16° 01' 04" N / 14° 12' 50" W (Boki Namadi, Ferlo, NS)	This work	G	10
ORS 1220	LMG 15303	*Indigofera senegalensis*	TIVN	16° 01' 04" N / 14° 12' 50" W (Boki Namadi, Ferlo, NS)	This work	G	17
ORS 1228	LMG 15304	*Indigofera astragalina*	TIVN	14° 26' 52" N / 14° 29' 50" W (Payar, CS)	This work	F	1
ORS 1229	LMG 15305	*Indigofera astragalina*	TIVN	14° 26' 52" N / 14° 29' 50" W (Payar, CS)	This work	nd	3

ORS 1810	LMG 15242	*Crotalaria lathyroïdes*	TIVN	Kabrousse (Casamance, SS)	This work	B	3
ORS 1811	LMG 15243	*Crotalaria goreensis*	TIVN	Kabrousse (Casamance, SS)	This work	B	1
ORS 1812	LMG 15365	*Abrus stictosperma*	TIVN	Fanghote (Casamance, SS)	This work	C	16
ORS 1813	LMG 15244	*Crotalaria hyssopifolia*	TIVN	Kabrousse (Casamance, SS)	This work	C	2
ORS 1814	LMG 15245	*Crotalaria hyssopifolia*	TIVN	Fanghote (Casamance, SS)	This work	C	2
ORS 1815	LMG 15246	*Crotalaria hyssopifolia*	TIVN	Fanghote (Casamance, SS)	This work	B	3
ORS 1816	LMG 15247	*Crotalaria hyssopifolia*	TIVN	Fanghote (Casamance, SS)	This work	B	4
ORS 1817	LMG 15366	*Eriosema glomeratum*	TIVN	Oukout (Casamance, SS)	This work	B	7
ORS 1818	LMG 15248	*Indigofera microcarpa*	TIVN	Oukout (Casamance, SS)	This work	B	8
ORS 1819	LMG 15249	*Crotalaria retusa*	TIVN	Kabrousse (Casamance, SS)	This work	F	4
ORS 1820	LMG 15250	*Indigofera hirsuta*	TIVN	Fanghote (Casamance, SS)	This work	E	4
ORS 1823	LMG 15367	*Indigofera hirsuta*	TIVN	Wouring (Niokolokoba, SS)	This work	C	2
ORS 1824	LMG 15253	*Indigofera hirsuta*	TIVN	Wouring (Niokolokoba, SS)	This work	C	2
ORS 1826	LMG 15255	*Alysicarpus glumaceus*	TIVN	Fanghote (Casamance, SS)	This work	nd	2
ORS 1828	LMG 15368	*Alysicarpus ovalifolius*	TIVN	Niokolokoba (SS)	This work	B	nd
ORS 1831	LMG 15369	*Alysicarpus rugosus*	TIVN	Fanghote (Casamance, SS)	This work	B	5
ORS 1832	LMG 15258	*Bryaspis lupulina*	TIVN	Kagnout (Casamance, SS)	This work	F	3
ORS 1836	LMG 15261	*Crotalaria glaucoïdes*	TIVN	Kaparan (Casamance, SS)	This work	C	11
ORS 1838	LMG 15263	*Sesbania rostrata*	NIN	Kaolack (CS)	This work	F	3
ORS 1844	LMG 15266	*Chamaecrista* sp.	TIVN	Karounate (Casamance, SS)	This work	B	5
ORS 1847	LMG 15268	*Zornia glochidiata*	TIVN	Fanghote (Casamance, SS)	This work	B	5
ORS 1848	LMG 15373	*Indigofera hirsuta*	TIVN	Kaparan (Casamance, SS)	This work	C	15
ORS 1849	LMG 15374	*Indigofera hirsuta*	TIVN	Fanghote (Casamance, SS)	This work	C	2
ORS 1894	LMG 15696	*Indigofera hirsuta*	NIN	Kolda (Casamance, SS)	This work	F	15
ORS 1896	LMG 15697	*Cassia absus*	NIN	Kolda (Casamance, SS)	This work	B	15
ORS 1898	LMG 15699	*Tephrosia bracteolata*	NIN	Kolda (Casamance, SS)	This work	F	1
ORS 1899	LMG 15700	*Indigofera stenophylla*	NIN	Kolda (Casamance, SS)	This work	E	1
ORS 1903	LMG 15702	*Tephrosia villosa*	NIN	Kolda (Casamance, SS)	This work	E	3
ORS 1905	LMG 15703	*Tephrosia bracteolata*	TIVN	Kolda (Casamance, SS)	This work	B	14
• *Bradyrhizobium* sp. (*Faidherbia*)							
ORS 103	LMG 10665	*Faidherbia albida*	TIVS	Dakar Bel-Air (CS)	Dupuy et al., 1994	B	nd
ORS 110	LMG 10666	*Faidherbia albida*	TIVS	Louga (NS)	Dupuy et al., 1994	B	nd
ORS 117	LMG 10673	*Faidherbia albida*	TIVS	Louga (NS)	Dupuy et al., 1994	nd	19
ORS 121	LMG 10677	*Faidherbia albida*	TIVS	Louga (NS)	Dupuy et al., 1994	C	nd
ORS 130	LMG 10686	*Faidherbia albida*	TIVS	Louga (NS)	Dupuy et al., 1994	nd	sep
ORS 133	LMG 10689	*Faidherbia albida*	TIVS	Louga (NS)	Dupuy et al., 1994	C	nd
ORS 162	LMG 10705	*Faidherbia albida*	TIPS	Casamance (SS)	Dupuy et al., 1994	C	nd
ORS 163	LMG 10706	*Faidherbia albida*	TIPS	Oussouye (Casamance, SS)	Dupuy et al., 1994	nd	5
ORS 166	LMG 15183	*Faidherbia albida*	TIPS	Oussouye (Casamance, SS)	Dupuy et al., 1994	nd	11
ORS 169	LMG 10712	*Faidherbia albida*	TIPS	Kabrousse (Casamance, SS)	Dupuy et al., 1994	B	nd
ORS 170	LMG 10713	*Faidherbia albida*	TIPS	Bayotte (Casamance, SS)	Dupuy et al., 1994	nd	18
ORS 174	LMG 10717	*Faidherbia albida*	TIPS	Badiana (Casamance, (SS)	Dupuy et al., 1994	C	7
ORS 175	LMG 10718	*Faidherbia albida*	TIPS	Djinaki (Casamance, SS)	Dupuy et al., 1994	C	nd
ORS 184	LMG 10723	*Faidherbia albida*	TIPS	Keur Momar Sarr (Guiers Lake, NS)	Dupuy et al., 1994	nd	8
ORS 187	LMG 10726	*Faidherbia albida*	TIPS	Dagana (NS)	Dupuy et al., 1994	B	nd
ORS 188	LMG 10727	*Faidherbia albida*	TIPS	Dagana (NS)	Dupuy et al., 1994	nd	1
• *Bradyrhizobium* sp. (*Aeschynomene*)							
ORS 294(f)	LMG 12192	*Aeschynomene sensitiva*	NIN	Fanda Niaguisse(Casamance, SS)	Molouba et al., 1999	A	nd
ORS 296	LMG 12194	*Aeschynomene sensitiva*	NIN	Fanda Niaguisse(Casamance, SS)	Molouba et al., 1999	D	nd

Table 1. (Continued).

Strain[a]	Other strain designation	Host plant or origin	Mode of isolation[b]	Geographical origin[c]	Reference or source	ARDRA group	SDS-PAGE group
ORS 301	LMG 8290	*Aeschynomene americana*	NIN	Bamako (Mali)	MOLOUBA et al., 1999	B	nd
ORS 309	LMG 8070	*Aeschynomene uniflora*	NIN	Tannanarive (Madagascar)	MOLOUBA et al., 1999	C	nd
ORS 324	LMG 8295	*Aeschynomene afraspera*	NIN	Keur Maktar (NS)	MOLOUBA et al., 1999	A	nd
ORS 326	LMG 11798	*Aeschynomene afraspera*	NIN	Dakar Bel-Air (CS)	MOLOUBA et al., 1999	B	nd
ORS 336	LMG 8298	*Aeschynomene afraspera*	NIN	Anambé (Senegal)	MOLOUBA et al., 1999	C	nd
ORS 348	LMG 12200	*Aeschynomene* sp.	NIN	Senegal	MOLOUBA et al., 1999	D	nd
ORS 358	LMG 10303	*Aeschynomene nilotica*	NIN	Dakar Bel-Air (CS)	MOLOUBA et al., 1999	C	nd
ORS 371	LMG 11804	*Aeschynomene indica*	NIN	Kabrousse (Casamance, SS)	MOLOUBA et al., 1999	A	nd
ORS 384	LMG 15390	*Aeschynomene indica*	NIN	Mbodiene (Senegal)	MOLOUBA et al., 1999	A	nd
ORS 386	LMG 11815	*Aeschynomene indica*	NIN	Joal (Sine Saloum, CS)	MOLOUBA et al., 1999	A	nd
BTAi1	LMG 11795	*Aeschynomene indica*	NIN	United States	MOLOUBA et al., 1999	A	nd
• *Bradyrhizobium japonicum*							
Bonnier 3.1	LMG 4252	*Glycine max*				B	nd
Erdman 1BOa2	LMG 4262	*Albizia julibrissin*				C	nd
Erdman 3c3a1	LMG 4265	*Ulex europaeus*				sep	nd
Bonnier 3.14	LMG 4276	*Glycine max*				B	nd
NZP 5533	LMG 6136	*Glycine max*				B	12
NZP 5549^T	LMG 6138^T	*Glycine max*		Japan		B	3
USDA 135	LMG 8321	*Glycine max*		United States		B	nd
USDA 110		*Glycine max*		United States		E	nd
• *Bradyrhizobium elkanii*							
NZP 5531^T	LMG 6134^T	*Glycine max*		United States	KUYKENDALL et al, 1992	C	13
NZP 5532	LMG 6135	*Glycine max*		United States	KUYKENDALL et al, 1992	C	13
• *Bradyrhizobium liaoningense*							
SFI 2281^T	LMG 18230^T	*Glycine max*		Heilongjiang Province (China)	XU et al., 1995	B	nd
SFI 2062	LMG 18231	*Glycine soja*		Liaoning Province (China)	XU et al., 1995	B	nd
• *Bradyrhizobium* sp.							
TAL 309	LMG 8316	*Macrotyloma africanum*		Zimbabwe		nd	12
BR 29	LMG 9520	Unknown			MOREIRA et al., 1993 BARRERA et al., 1997	C	nd
BR3606	LMG 9959	*Acacia mollissima*		Brazil	MOREIRA et al., 1993	C	nd
BR 3617	LMG 9965	*Acacia mangium*		Rio de Janeiro, Brazil	MOREIRA et al., 1993, 1998	nd	18
BR 3621	LMG 9966	*Acacia mangium*		Espirito Santo, Brazil	MOREIRA et al., 1993, 1998 BARRERA et al., 1997	C	11
BR 4406	LMG 9980	*Enterolobium ellipticum* Benth.		Rio de Janeiro, Brazil	MOREIRA et al., 1993, 1998 BARRERA et al., 1997	C	13
BR 5205	LMG 9991	*Erythrina speciosa*		Rio de Janeiro, Brazil	MOREIRA et al., 1993, 1998	nd	5
BR 5609	LMG 9997	*Paraserianthes falcata*		Espirito Santo, Brazil	MOREIRA et al., 1993, 1998	nd	18
BR 5611	LMG 9998	*Paraserianthes falcata*		Espirito Santo, Brazil	MOREIRA et al., 1993, 1998	nd	16
BR 6011	LMG 10003	*Lonchocarpus costatus* Benth		Espirito Santo, Brazil	MOREIRA et al., 1993, 1998 BARRERA et al., 1997	nd	13

BR 6817	LMG 10010	*Pithecellobium* sp.	Espirito Santo, Brazil	MOREIRA et al., 1993, 1998	nd	5
BR 8406	LMG 10018	*Dalbergia nigra* Allem.	Rio de Janeiro, Brazil	MOREIRA et al., 1993, 1998	nd	9
FL 27	LMG 10023	*Melanoxylon* sp.	Espirito Santo, Brazil	MOREIRA et al., 1993, 1998	nd	16
FL 276	LMG 10025	*Abrus* sp.	Brazil	MOREIRA et al., 1993	nd	16
FL 281	LMG 10026	*Abrus* sp.	Rio de Janeiro, Brazil	MOREIRA et al., 1993, 1998	nd	16
INPA 9A	LMG 10029	*Derris* sp.	Brazil	MOREIRA et al., 1993	B	nd
INPA 36B	LMG 10040	*Inga* sp.	Brazil	MOREIRA et al., 1993	nd	5
INPA 46B	LMG 10043	*Clitoria racemosa* G. Don.	Amazonas, Brazil	MOREIRA et al., 1993, 1998	nd	sep
INPA 54B	LMG 10044	*Inga* sp.	Amazonas, Brazil	MOREIRA et al., 1993, 1998	nd	6
INPA 183B	LMG 10075	*Pentaclethra macroloba* (Willd.) Ktze.	Amazonas, Brazil	MOREIRA et al., 1993, 1998	nd	9
INPA 191A	LMG 10078	*Dimorphandra parviflora* Spruce ex Benth	Amazonas, Brazil	MOREIRA et al., 1993, 1998	nd	11
INPA 495B	LMG 10107	*Inga acreanum* Harms	Brazil	MOREIRA et al., 1993	nd	12
INPA 514A	LMG 10112	*Machaerium quinata*	Amazonas, Brazil	MOREIRA et al., 1998	nd	11
MSDJ 718		*Lupinus luteus*	France	LAGUERRE et al., 1994	B	nd
• *Mesorhizobium plurifarium*						
ORS 1030	LMG 11890	*Acacia senegal*	Senegal	DE LAJUDIE et al., 1998		
ORS 1031	LMG 11891	*Acacia senegal*	Senegal	DE LAJUDIE et al., 1998		
ORS 1032^T	LMG 11892^T	*Acacia senegal*	Senegal	DE LAJUDIE et al., 1998		
• *Mesorhizobium loti*						
NZP 2230	LMG 6126	*Lotus maroccanus*	Morocco	JARVIS et al., 1986		
NZP 2213^T	LMG 6125^T	*Lotus corniculatus*	New Zealand	JARVIS et al., 1986		
• *Mesorhizobium ciceri*						
UPM-Ca7^T	LMG 17150^T	*Cicer arietinum* L.	Spain	NOUR et al., 1994		
522	LMG 17149	*Cicer arietinum* L.	Russia	NOUR et al., 1994		
• *Mesorhizobium huakuii*						
IAM 14158^T	LMG 14107^T	*Astragalus sinicus*	Nanjing,China	CHEN et al., 1991		
• *Mesorhizobium tianshanense*						
A-1BS^T	LMG 15767^T	*Glycyrrhiza pallidiflora*	Xinjiang,China	CHEN et al., 1995		
O17A	LMG 15768	*Sophora alopecuroides*	Xinjiang,China	CHEN et al., 1995		
• *Sinorhizobium fredii*						
USDA 205^T	LMG 6217^T	*Glycine max*	Honan,China	JARVIS et al., 1986		
USDA 191	LMG 8317	Soil	Shanghai,China	JARVIS et al., 1986		
• *Sinorhizobium meliloti*						
NZP 4009	LMG 6130	*Medicago sativa*	Australia			
NZP 4027^T	LMG 6133^T	*Medicago sativa*	Virginia, U.S.A.			
• *Sinorhizobium medicae*						
HAMBI 1808	LMG 16579	*Medicago radiata*	Syria	K. LINDSTRÖM		
HAMBI 1809	LMG 16580	*Medicago truncatulata*	Syria	K. LINDSTRÖM		
• *Sinorhizobium terangae*						
ORS 51	LMG 6464	*Sesbania rostrata*	Senegal	DE LAJUDIE et al., 1994		
ORS 1013	LMG 7844t1	*Acacia senegal*	Senegal	DE LAJUDIE et al., 1994		

Table 1. (Continued).

Strain[a]	Other strain designation	Host plant or origin	Mode of isolation[b]	Geographical origin[c]	Reference or source	ARDRA group	SDS-PAGE group
• *Sinorhizobium saheli*							
ORS 609^T	LMG 7837^T	*Sesbania cannabina*		Senegal	DE LAJUDIE et al., 1994		
ORS 600	LMG 11864	*Sesbania pachycarpa*		Senegal	DE LAJUDIE et al., 1994		
• *Rhizobium leguminosarum*							
ATCC 10004^T	LMG 8817^T						
ATCC 14480	LMG 8820						
• *Rhizobium tropici*							
Group a							
CFN 299	LMG 9517	*Phaseolus vulgaris* L.		Brazil			
Group b							
CIAT 899^T	LMG 9503^T	*Phaseolus vulgaris* L.		Columbia	MARTINEZ-ROMERO et al., 1991		
• *Rhizobium galegae*							
HAMBI 540^T	LMG 6214^T	*Galega orientalis*		Finland	LINDSTRÖM, 1989		
HAMBI 1147	LMG 6215	*Galega orientalis*		Russia	LINDSTRÖM, 1989		
• *Azorhizobium caulinodans*							
ORS 478	LMG 11820						
ORS 571^T	LMG 6465^T	*Sesbania rostrata*		Senegal	DREYFUS et al., 1988		
FY12	LMG 11352	*Sesbania rostrata*		Senegal	RINAUDO et al., 1991		

[a] Original strain number, or as received. ATCC, American Type Culture Collection, Rockville, Md.; BR and FL, strains from the CNPBS/EMBRAPA, Centro Nacional de Pesquisa em Biologia do Solo, Seropédica 23851, Rio de Janeiro, Brazil/Emprasa Brasiliera de Pesquisa Agropequaria; CFN, Centro de Investigación sobre Fijación de Nitrógeno, Universidad Nacional Autónoma de México, Cuernavaca, Mexico; CIAT, *Rhizobium* Collection, Centro International de Agricultura Tropical, Cali, Columbia; HAMBI, Culture Collection of the Department of Microbiology, University of Helsinki, Helsinki, Finland; IAM, Institute for Applied Microbiology; BCCM/LMG™, Bacteria Collection, Laboratorium voor Microbiologie, K.L. Ledeganckstraat, 35, B-9000 Ghent, Belgium; MSDJ, Institut National de la Recherche Agronomique (INRA), Microbiologie des Sols, Dijon, France; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden Laboratory, Hertsfordshire, U. K.; NZP, Culture Collection of the Department for Scientific and Industrial Research, Biochemistry Division, Palmerston North, New Zealand; ORS, I.R.D./ORSTOM Collection, Institut de Recherche pour le Développement, BP 1386, Dakar, Senegal; Pan., Panagopoulos, C., Crete, Greece; R-, Research collection of the Laboratorium voor Microbiologie, K.L. Ledeganckstraat, 35, B-9000 Ghent, Belgium; USDA, U. S. Department of Agriculture, Beltsville, Md; UPM, Universidad Politécnica Madrid, Spain.

[b] NIN Direct isolation from a nodule sampled in natura
TIVS trapping in vitro from a soil sample
TIVN trapping in vitro from crushed nodules sampled in natura
TIPN trapping on plants grown in pots inoculated with crushed nodules sampled in natura
TIPS trapping on plants grown in pots containing a soil sampled in natura

[c] NS, North Senegal (250–500 mm rainfall); CS, Central Senegal (500–900 mm rainfall); SS, South Senegal (900–1200 mm rainfall)

[d] sep, separate

[e] nd, not determined group.

[f] the underlined numbers correspond to the photosynthetic strains from Aeschynomene.

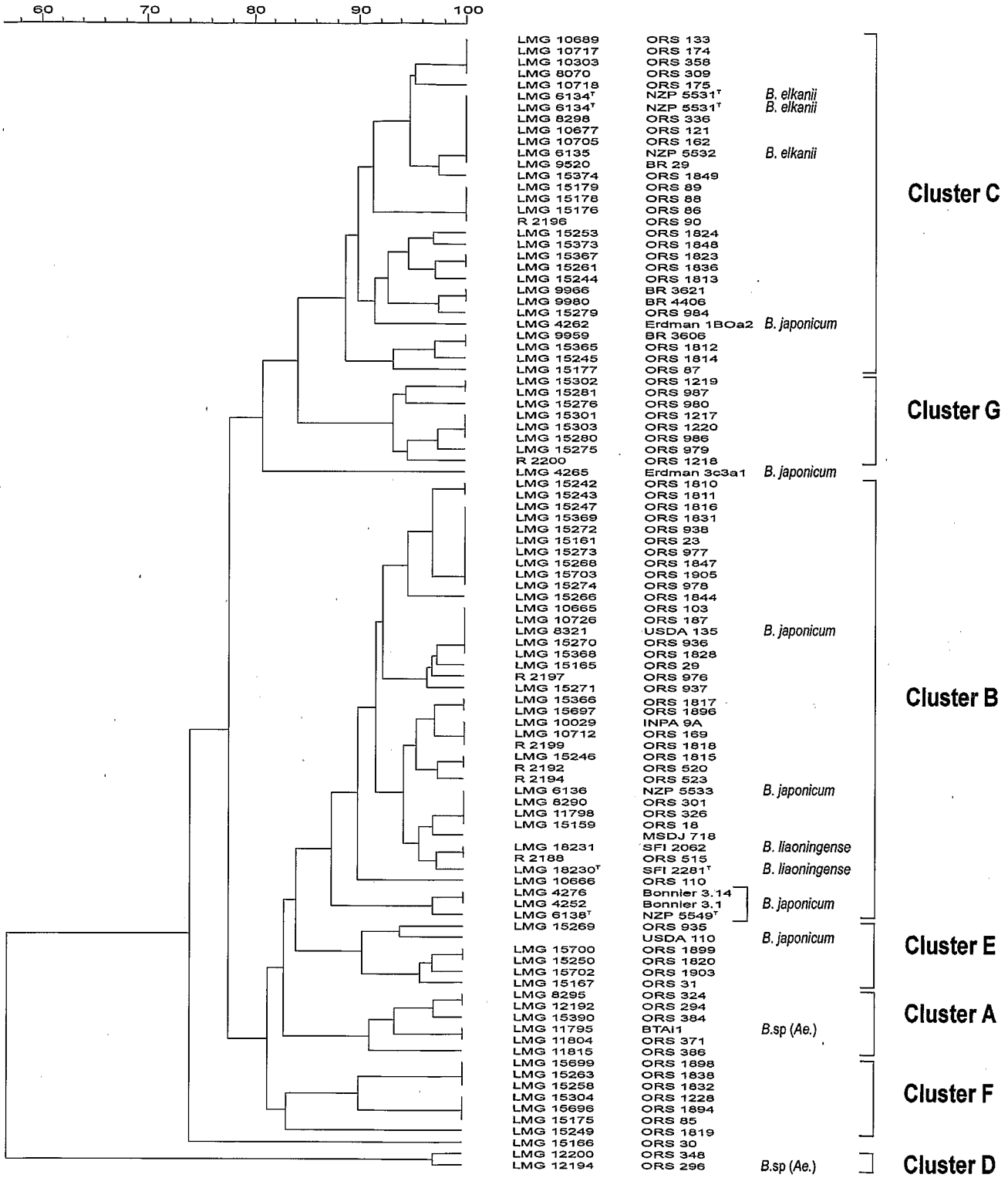

Fig. 1. Dendrogram based on the unweighted pair group method using average linkage (UPGMA) clustering of Dice correlation values (S_D) of normalised and combined ARDRA patterns of Senegalese isolates, some representative strains from Brazil and reference strains of *Bradyrhizobium* obtained with the restriction enzyme combination *Hinf* I, *Dde* I, *Mwo* I, *Alu* I and *Hha* I. *B.* sp (*Ae.*), *Bradyrhizobium* sp. (*Aeschynomene*). The reproducibility is illustrated by the two independent extracts of strain LMG 6134^T.

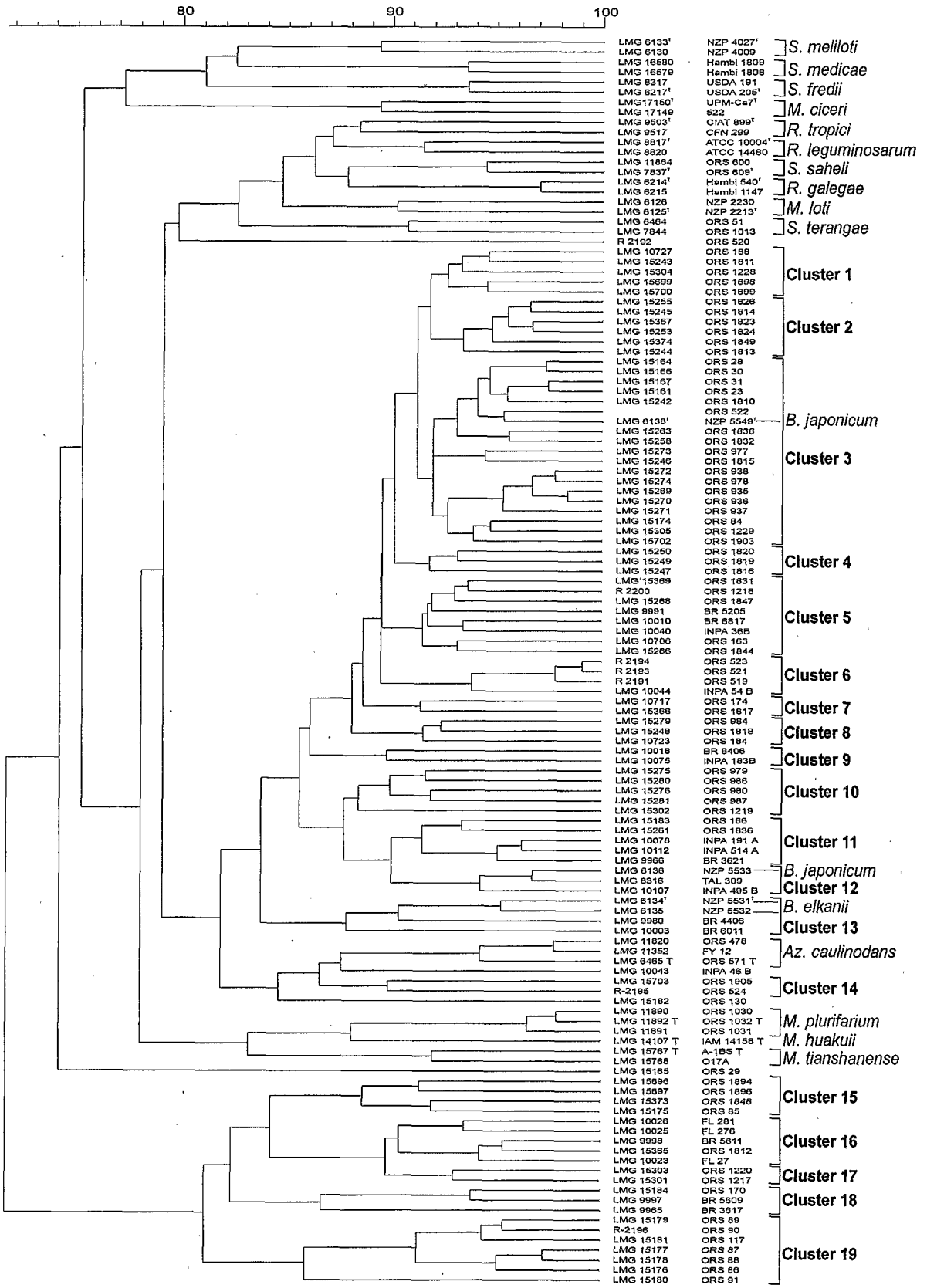

Fig. 2. Dendrogram showing the relationships between the electrophoretic protein patterns from Senegalese, Brazilian and reference strains of *Bradyrhizobium, Rhizobium, Sinorhizobium, Mesorhizobium* and *Azorhizobium*. The mean correlation coefficient (*r*) was represented as a dendrogram and calculated by the unweighted average pairs grouping method (UPGMA). Positions 15 to 320 of the 400 point traces were used for calculation of similarities between individual pair of traces. The scale represents the *r* value converted to percentages.

included in the ARDRA study. The results are presented as a similarity dendrogram in Fig. 2.

Delineation of groups was not evident, and we evaluated the SDS-PAGE results by referring to the ARDRA groups previously identified. At a similarity coefficient of 87% the different fast- and moderately fast- growing rhizobial species formed distinct clusters, and as expected, none of the new isolates grouped with any of them. Majority of the slow-growing strains, new isolates and *Bradyrhizobium* reference strains, formed 19 clusters. Four slow-growing strains had separate positions, namely ORS 130, INPA 46B and two new isolates (ORS 520, ORS 29). New isolates were found in all SDS-PAGE groups except in groups 9, 12 and 18.

SDS-PAGE groups 2, 11, 13, 16 and 19, were formed by strains of ARDRA cluster C; several partially characterised *Bradyrhizobium* sp. strains grouped with them: ORS 166 and ORS 117 (DUPUY et al., 1994), BR 6011, INPA 191 A, INPA 514 A, BR 5611, FL 27, FL 276 and FL 281 (MOREIRA et al., 1994; BARRERA et al., 1997).

SDS-PAGE groups 10 and 17 consisted of the 7 strains of ARDRA cluster G.

SDS PAGE groups 5, 6, 7, 12, 14 contained strains from ARDRA cluster B; *Bradyrhizobium* sp. strains BR 5205, BR 6817, INPA 36B, INPA 54B, INPA 495B (MOREIRA et al.,1998) and ORS 163 (DUPUY et al., 1994) also grouped with them; group 12 included *B. japonicum* strain LMG 6136 and *Bradyrhizobium* sp. strain LMG 8316. Surprisingly, strain ORS 1218 (ARDRA cluster G) and strain ORS 174 (ARDRA cluster C) also grouped with them.

Group 9 consisted of two strains having partial 16S rDNA sequence similarity with *B. japonicum* (MOREIRA et al., 1998); Group 18 consisted of three strains reported to have similarities with *B. elkanii* (DUPUY et al., 1994; MOREIRA et al., 1998).

Several SDS-PAGE groups consisted of strains from different ARDRA clusters. Groups 1 and 3 consisted of strains from clusters B, E and F; strain ORS 188 (DUPUY et al., 1994) grouped in 1, and *B. japonicum* type strain was in group 3, together with ORS 30, ORS 1819, two strains having separate positions in our ARDRA study. Group 4 consisted of strains from clusters B and E. Group 8 consisted of strains from clusters B and C. Group 15 consisted of strains from clusters B, C and F.

Host specificity

Nodulation of 34 strains was tested on 5 plant species: *Indigofera astragalina*, *I. senegalensis*, *I. tinctoria*, *Rhynchosia minima* and *Tephrosia purpurea*. Effectiveness of the symbiosis was estimated by Acetylene Reducing Activity (ARA) test. In addition 8 strains (ORS 515, ORS 516, ORS 518, ORS 519, ORS 520, ORS 521, ORS 523, ORS 524) were tested on *I. senegalensis* only and were found effective on this plant. Results are shown in Table 2, and two groups were distinguished among the new nodule isolates. Group I (20 isolates) nodulated the five host plants tested. However, Acetylene Reducing Activity (ARA) tests revealed a specificity for symbiotic nitrogen fixation: all were effective on *I. astragalina* and *T. purpurea;* only 10 strains were effective on *R. minima*; 13

Table 2. Nodulation tests.

Strain	*Indigofera astragalina*	*Tephrosia purpurea*	*Indigofera tinctoria*	*Indigofera senegalensis*	*Rhynchosia minima*
Group I					
ORS 88, ORS 89, ORS 91, ORS 977, ORS 1229	e*	e	e	e	e
ORS 87, ORS 978	e	e	e	e	I
ORS 90	e	e	e	I	e
ORS 935, ORS 936, ORS 979	e	e	I*	e	e
ORS 86	e	e	I	I	e
ORS 937, ORS 938, ORS 976	e	e	I	e	I
ORS 23, ORS 28, ORS 30, ORS 84,ORS 85	e	e	e	I	I
Group II					
ORS 26, ORS 1228	e	e	e	O*	I
ORS 18	e	e	e	I	O
ORS 29, ORS 31	e	e	e	O	O
ORS 980	e	O	e	e	O
ORS 986	e	e	O	e	O
ORS 987	e	I	e	e	O
ORS 1218, ORS 1219, ORS 1220	e	e	O	e	O
ORS 1217	I	e	e	e	O
ORS 1216	I	O	O	e	O
ORS 984	O	I	O	e	O
ORS 515, ORS 516, ORS 518, ORS 519, ORS 520, ORS 521, ORS 523, ORS 524	nt	nt	nt	e	nt

* I – ineffective nodules; e – effective nodules; O – no nodulation; nt – not tested.

strains were effective on *I. tinctoria*; 13 strains were effective on *I. senegalensis*. The host range of strains of group II (14 isolates) was more or less restricted among the five plants tested. With the exception of 3 strains, all were efficient on *I. astragalina* ; all, except 4 strains, were efficient on *T. purpurea*; only strains ORS 26 and ORS 1228 could induce nodules on *R. minima* (nodules were ineffective); 8 strains were effective on *I. tinctoria*; 9 strains were effective on *I. senegalensis*.

Discussion

Quite a number of diverse nodule isolates from the tropical region have been characterised and described in literature as belonging to the large group of bradyrhizobia (YOUNG, 1991; MOREIRA et al., 1993; LORQUIN et al., 1993; LADHA and SO, 1994; WONG et al., 1994; DUPUY et al., 1994; VAN ROSSUM et al., 1995; VAN BERKUM et al., 1995; BARRERA et al., 1997; MOLOUBA et al., 1999). Here we describe the isolation and characterisation by 16S ARDRA, SDS-PAGE of total proteins and nodulation tests of a collection of 71 new nodule isolates from 27 small indigenous legumes found in different regions of Senegal. In addition to reference strains of the recognised *Bradyrhizobium* species, we included in our study representative strains for groups previously studied by SDS PAGE and/or ARDRA in our research group (MOREIRA et al., 1993; DUPUY et al., 1994; MOLOUBA et al., 1999), and for some of which either partial (MOREIRA et al., 1998) or total (DUPUY et al., 1994; BARRERA et al., 1997) 16S rDNA sequence data were reported. ARDRA and SDS-PAGE techniques were both performed in parallel. Considering SDS-PAGE results alone, grouping of strains was not evident, because the protein profiles were highly similar. Thus we first analysed the ARDRA results, which helped us to then analyse the SDS-PAGE results.

ARDRA on amplified 16S rDNA has been shown to be a powerful and convenient technique to determine the phylogenetic relationships between species and to assign strains to species (LAGUERRE et al., 1994; NICK, 1998; TEREFEWORK et al., 1998). The ARDRA results (figure 1) were consistent with previous phylogenetic reports on bradyrhizobia. We distinguished seven clusters, four of which corresponded to the four main branches described by MOLOUBA et al. (1999), cluster A (consisting of photosynthetic *Bradyrhizobium* sp. (*Aeschynomene*)), cluster B (including *B. japonicum* type strain), cluster C (including *B. elkanii* type strain), cluster D (consisting of *Bradyrhizobium* sp. (*Aeschynomene*)) and three new clusters, cluster G close to cluster C, clusters E and F on the *B. japonicum* branch. Thus clusters C and G can be regarded as constituting the "*B. elkanii* lineage" and clusters A, B, E, F as the "*B. japonicum* lineage". Cluster D is separate. New isolates grouped in clusters B, C, E, F, G.

Cluster B included 24 new isolates, *B. japonicum* strains LMG 6138^T, LMG 8321, LMG 6136, LMG 4276, LMG 4252, and also both *B. liaoningense* strains LMG 18230^T and LMG 18231. This was not surprising since 16S rDNA sequences of *B. japonicum* and *B. liaoningense* are very similar (A. Willems, unpublished results). Two other strains received as *B. japonicum* grouped outside this cluster: LMG 4262 clustered with *B. elkanii* in cluster C, and LMG 4265 had a separate position. LMG 4262 was isolated from *Albizzia julibrissin* and was classified as *B. japonicum* before *B. elkanii* was described.

Cluster C included 14 new isolates, *B. elkanii* strains LMG 6134^T and LMG 6135 together with strains ORS 133, BR 3621, BR 4406 and BR 29 having a 16S rDNA sequence close to that of *B. elkanii* (DUPUY et al., 1994; BARRERA et al. 1997).

Cluster E consisted of 5 new nodule isolates from small legumes and *B. japonicum* USDA 110, representative for the hybridisation group Ia (HOLLIS et al., 1981), and more recently shown to constitute a 16S rDNA subbranch distinct from the *B. japonicum* type strain subbranch representing DNA hybridisation group I (BARRERA et al., 1997). The two other new clusters, clusters G and F (figure 1), exclusively consisted of, respectively, 8 and 6 new nodule isolates.

Diversity of the new isolates inside the ARDRA groups was shown by analysis of total protein profiles by SDS-PAGE. This technique has been extensively used in the past to characterise different groups of rhizobia and bradyrhizobia at the species and intraspecific levels (MOREIRA et al, 1993; DUPUY et al., 1994; DE LAJUDIE et al., 1994; 1998a,b; VAN ROSSUM et al., 1995). In contrast to what we found before in several groups of bacteria studied, Rhizobiaceae (DE LAJUDIE et al., 1994; 1998a,b; 1999) and many others (for a review see VANDAMME et al., 1996), the power of SDS-PAGE of total proteins to delineate groups in the bradyrhizobia is much smaller than in the other studied bacterial groups. Possible reasons for this phenomenon can be: (i) total protein profiles with fewer bands, (ii) the intensity of the bands varies considerably from very weak to rather heavy, (iii) many fuzzy protein bands are found that possibly influence the similarity calculations, (iv) the background is quite high. Consequently the result of the numerical analysis depends on the number of profiles included and the specific quality of each of them. Quite often strains switch from one cluster to another when numerical analyses contain different selections of strains. Moreover, it is possible that the generation times of the various included bradyrhizobia do differ that much that the standardisation to 72 h of growth does not necessarily guarantee comparable start material to extract proteins for all included strains, which can result in less reliable clustering.

When comparing the results of ARDRA with those of SDS-PAGE of proteins, no clear correlation could be found. Because a genomic technique such as ARDRA is less dependent on external growth conditions, it is considered to be quite reproducible, as is indeed illustrated by the fact that in this study we found basically the same groups as described by MOLOUBA et al. (1999). Although for most bacterial groups SDS-PAGE has a finer resolution than ARDRA, we could not localise the SDS-PAGE

clusters within the bigger ARDRA clusters, demonstrating that the *Bradyrhizobium* protein groups should be regarded with reservation. A fine genomic technique is definitely required to further unravel the finer relationships within the ARDRA clusters.

No clear relationship could be evidenced between origin (plant, location) and ARDRA or SDS-PAGE groupings of the isolates, except that all the strains of ARDRA cluster G were isolated from *Indigofera senegalensis* in North Senegal.

By nodulation tests on 5 different plant species, we evidenced some degree of plant specificity for nodulation and symbiotic nitrogen fixation. Interestingly, some relation could be observed between nodulation and SDS-PAGE groups. Except ORS 979 (SDS-PAGE group 10) and ORS 85 (SDS-PAGE group 15) nodulation group I consisted of strains belonging to SDS-PAGE groups 3 and 19. Except ORS 979, all strains of SDS-PAGE groups 10 and 17 (corresponding to ARDRA cluster G) belonged to nodulation group II, together with strains from other SDS-PAGE groups.

In SDS-PAGE, we included strains whose partial and sometimes full length 16S rDNA sequences were published. By full length sequencing DUPUY et al. (1994) and BARRERA et al. (1997) assigned BR 4406, BR 29 and BR 3621 to the *B. elkanii* branch; these results correspond with our ARDRA data because these strains belong in cluster C. However according to MOREIRA et al (1998) strains BR 4406 and BR 3621 have partial 16S rDNA sequences closer to those of the *B. japonicum* type strain. This emphasises again that the evaluation of partial sequences should be done with care. MOREIRA et al. (1998) also conclude that a combination of SDS-PAGE and partial sequencing can be used for complementary taxonomic purposes; we cannot confirm from our results that SDS-PAGE is complementary to ARDRA, another rRNA-based characterisation technique. Therefore we conclude that a genomic technique with a fine discriminatory power is required to further study the relationships within the rRNA clusters found in the bradyrhizobia.

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References

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

BARRERA, L. L., TRUJILLO, M. E., GOODFELLOW, M., GARCIA, F. J., HERNANDEZ-LUCAS, I., DAVILA, G., VAN BERKUM, P., MARTINEZ-ROMERO, E.: Biodiversity of Bradyrhizobia nodulating *Lupinus* spp. Int. J. Syst. Bacteriol. 47, 1086–1091 (1997).

BROCKWELL, J.: Plant-infection counts of rhizobia in soils, pp. 41–58. In: Nitrogen fixation in legumes (J. M. VINCENT, eds.), Academic Press Publishers 1982.

CHEN, W. X., LI, G. S., QI, Y. L., WANG, E. T., YUAN, H. L., LI, J. L.: *Rhizobium huakuii* sp. nov. isolated from the root nodules of *Astralagus sinicus*. Int. J. Syst. Bacteriol. 41, 275–280 (1991).

CHEN, W. X., WANG, E. T., WANG, S. Y., LI, Y. B., CHEN, X. Q., LI, Y.: Characteristics of *Rhizobium tianshanense* sp. nov., a moderately and slowly growing root nodule bacterium isolated from an arid saline environment in Xinjiang, People's Republic of China. Int. J. Syst. Bacteriol. *45*, 153–159 (1995).

CHEN, W. X., TAN, Z. Y., GAO, J. L., LI, Y., WANG, E. T.: *Rhizobium hainanense* sp. nov., isolated from tropical legumes. Int. J. Syst. Bacteriol. 47, 870–873 (1997).

DAGUTAT, H., STEYN, P. L.: Taxonomy and distribution of rhizobia indigenous to South African soils, p. 683. In: Nitrogen fixation: fundamentals and applications (I. A. TIKHONOVICH, N. A. PROVOROV, V. I. ROMANOV, W. E. NEWTON, eds.), Dordrecht, Boston, Kluwer Academic Publishers, 1994

DATE, R. A.: Collection, isolation, characterization and conservation of *Rhizobium*, pp. 95–109. In: Nitrogen fixation in legumes (J. M. VINCENT, ed.), Academic Press Publishers 1982.

DE LAJUDIE, P., WILLEMS, A., POT, B., DEWETTINCK, D., MAESTROJUAN, G., NEYRA, M., COLLINS, M. D., DREYFUS, B., KERSTERS, K., GILLIS, M.: Polyphasic taxonomy of rhizobia: emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. Int. J. Syst. Bacteriol. 44, 715–733 (1994).

DE LAJUDIE, P., LAURENT-FULELE, E., WILLEMS, A., TORCK, U., COOPMAN, R., COLLINS, M. D., KERSTERS, K., DREYFUS, B. L., GILLIS, M.: *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. Int. J. Syst. Bacteriol. 48, 1277–1290 (1998a).

DE LAJUDIE, P., WILLEMS, A., NICK, G., MOREIRA, F., MOLOUBA, F., HOSTE, B., TORCK, U., NEYRA, M., COLLINS, M. D., LINDSTRÖM, K., DREYFUS, B., GILLIS, M.: Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarium* sp. nov. Int. J. Syst. Bacteriol. 48, 369–382 (1998b).

DE LAJUDIE, P., WILLEMS, A., NICK, G., MOHAMMED, S. H., TORCK, U., COOPMAN, R., FILALI-MALTOUF, A., KERSTERS, K., DREYFUS, B., LINDSTRÖM, K., GILLIS, M.: *Agrobacterium* bv. 1 strains isolated from nodules of tropical legumes. Syst. & Appl. Microbiol. 21, 119–132 (1999).

DIEDHIOU, I.: Importance des légumineuses dans les systèmes écologiques arides et semi-arides du Sénégal. D.E.A Sciences de l'environnement (Master in Sciences), Université Cheikh Anta Diop- Dakar, Senegal 1994.

DREYFUS, B., GARCIA, J. L., GILLIS, M.: Characterization of *Azorhizobium caulinodans* gen. nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*. Int. J. Syst. Bacteriol. 38, 89–98 (1988).

DUPUY, N., WILLEMS, A., POT, B., DEWETTINCK, D., VANDENBRUAENE, I., MAESTROJUAN, G., DREYFUS, B. L., KERSTERS, K., COLLINS, M. D., GILLIS, M.: Phenotypic and genotypic characterization of bradyrhizobia nodulating the leguminous tree *Acacia albida*. Int. J. Syst. Bacteriol. 44, 461–473 (1994).

GAO, J. L., SUN, J. G., LI, Y., WANG, E. T., CHEN, W. X.: Numerical taxonomy and DNA relatedness of tropical rhizobia isolated from Hainan province, China. Int. J. Syst. Bacteriol. 44, 151–158 (1994).

GIBSON, A. H.: Physical environment and symbiotic nitrogen fixation. I. The effect of temperature on recently nodulated *Trifolium subterraneum* (L.) plants. Aust. J. Biol. Sci. 16, 28–42 (1963).

HARDY, R. W. F., BURN, R. C., HOLSTEN, R. D.: Application of the acetylene-ethylene assay for measurement of N_2 fixation. Soil. Biol. Biochem. 43, 47–87 (1973).

HAUKKA, K., LINDSTRÖM, K., YOUNG, J. P. W.: Diversity of partial 16S rRNA sequences among and within strains of African rhizobia isolated from Acacia and Prosopis. System. Appl. Microbiol. 19, 352–359 (1996).

HEYNDRICKX, M., VAUTERIN, L., VANDAMME, P., KERSTERS, K., DE VOS, P.: Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns in bacterial phylogeny and taxonomy. J. Microbiol. Method. 26, 247–259 (1996).

HOLLIS, A. B, KLOOS, W. E, ELKAN, G. H.: DNA:DNA hybridization studies of *Rhizobium japonicum* and related *Rhizobiaceae*. J. Gen. Microbiol. 123, 215–222 (1981).

JARVIS, B. D. W., GILLIS, M., DE LEY, J.: Intra- and intergeneric similarities between the ribosomal ribonucleic acid cistrons of *Rhizobium* and *Bradyrhizobium* species and some related bacteria. Int. J. Syst. Bacteriol. 36, 129–138 (1986).

JARVIS, B. D. W., VAN BERKUM, P., CHEN, W. X., NOUR, S. M., FERNANDEZ, M. P., CLEYET-MAREL, J.-C., GILLIS, M.: Transfer of *Rhizobium loti, Rhizobium huakuii, Rhizobium ciceri, Rhizobium mediterraneum,* and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int. J. Syst. Bacteriol. 47, 895–898 (1997).

KUYKENDALL, L. M. , SAXENA, B., DEVINE, T. E., UDELL, S. E.: Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. Can. J. Microbiol. 38, 501–503 (1992).

LADHA, J. K., SO, R. B.: Numerical taxonomy of photosynthetic rhizobia nodulating *Aeschynomene* species. Int. J. Syst. Bacteriol. 44, 62–73 (1994).

LAEMMLI, U. K.: Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London). 227, 680–685 (1970).

LAGUERRE, G., ALLARD, M. R., REVOY, F., AMARGER, N.: Rapid identification of rhizobia by Restriction Fragment Length Polymorphism analysis of PCR-Amplified 16S rRNA genes. Appl. Environ. Microbiol. 60, 56–63 (1994).

LINDSTRÖM, K.: *Rhizobium galegae*, a new species of legume root nodule bacteria. Int. J. Syst. Bacteriol. 39, 365–367 (1989).

LORQUIN, J., MOLOUBA, F., DUPUY, N., N'DIAYE, S., ALAZARD, D., GILLIS, M., DREYFUS, B.L.: Diversity of photosynthetic *Bradyrhizobium* strains from stem nodules of *Aeschynomene* species, pp. 683–689. In: New Horizons in Nitrogen Fixation (R. PALACIOS J. MORA, W. E. NEWTON, eds.), Dordrecht, Boston, Kluwer Academic Publishers 1993.

MARTINEZ-ROMERO, E., SEGOVIA, L., MERCANTE, F. M., FRANCO, A. A., GRAHAM, P., PARDO, M. A.: *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. Int. J. Syst. Bacteriol. 41, 417–426 (1991).

MOLOUBA, F., LORQUIN, J., WILLEMS, A., HOSTE, B., GIRAUD, E., DREYFUS, B., GILLIS, M., DE LAJUDIE, P., MASSON-BOIVIN, C.: Photosynthetic Bradyrhizobia from *Aeschynomene* are specific to stem nodulated species and form a separate 16S rDNA RFLP group. Appl. Environ. Microbiol. 65, 3084–3094 (1999).

MOREIRA, F. M. S., GILLIS, M., POT, B., KERSTERS, K., FRANCO, A. A.: Characterization of rhizobia isolated from different divergence groups of tropical *Leguminosae* by comparative polyacrylamide gel electrophoresis of their total proteins. System. Appl. Microbiol. 16, 135–146 (1993).

MOREIRA, F. M. S., HAUKKA, K., YOUNG, J. P. W.: Biodiversity of rhizobia isolated from a wide range of forest legumes in Brazil. Mol. Ecol. 7, 889–895 (1998).

NICK, G.: Polyphasic taxonomy of rhizobia isolated from tropical tree legumes. *Dissertationes Biocentri Viikki Universitatis Helsingiensis*, Department of Applied Chemistry and Microbiology, University of Helsinki, Finland, 1998.

NICK, G., DE LAJUDIE, P., EARDLY, B., SUOMALAINEN, S., PAULIN, L., ZHANG, X., GILLIS, M., LINDSTRÖM, K.: *Sinorhizobium arboris* sp. nov. and *Sinorhizobium kostiense* sp. nov. isolated from leguminous trees in Sudan and Kenya. Int. J. Syst. Bacteriol. 49, 1359–1368 (1999).

NOUR, S. M., FERNANDEZ, M. P., NORMAND, P., CLEYET-MAREL, J.-C.: *Rhizobium ciceri* sp. nov., consisting of strains that nodulate chickpeas (*Cicer arietinum* L.). Int. J. Syst. Bacteriol. 44, 511–522 (1994).

NOVIKOVA, N. I., PAVLOVA, E. A., VOROBJEV, N. I., LIMESCHENKO, E. V.: Numerical taxonomy of *Rhizobium* strains from legumes of the temperate zone. Int.J. Syst.Bacteriol. 44, 734–742 (1994).

PITCHER, D. G., SAUNDERS, N. A., OWEN, R. J.: Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. 8, 151–156 (1989).

POT, B., GILLIS, M., HOSTE, B., VAN DE VELDE, A., BEKAERT, F., KERSTERS, K., DE LEY, J.: Intra- and intergeneric relationships of the genus *Oceanospirillum*. Int. J. Syst. Bacteriol. 39, 23–34 (1989).

POT, B., VANDAMME, P., KERSTERS, K.: Analysis of electrophoretic whole organism protein fingerprints, pp. 493–521. In: Modern Microbial Methods. Chemical Methods in Procaryotic Systematics (M. GOODFELLOW, A.G. O'DONNELL, eds.), Chichester, England, John Wiley & Sons 1994.

PREVOST, D., BORDELEAU, L. M., CAUDRY-REZNICK, S., SCHULMAN, H. M., ANTOUN, H.: Characteristics of rhizobia isolated from three legumes indigenous to the high arctic: *Astragalus alpinus*, *Oxytropis maydelliana*, and *Oxytropis arctobia*. Plant and Soil. 98, 313–324 (1987).

RINAUDO, G., ORENGA, S., FERNANDEZ, M. P., MEUGNIER, H., BARDIN, R.: DNA homologies among members of the genus *Azorhizobium* and other stem- and root-nodulating bacteria isolated from the tropical legume *Sesbania rostrata*. Int. J. Syst. Bacteriol. 41, 114–120 (1991).

ROME, S., FERNANDEZ, M. P., BRUNEL, B., NORMAND, P., CLEYET-MAREL, J.-C.: *Sinorhizobium medicae* sp. nov., isolated from annual *Medicago* spp. Int. J. Syst. Bacteriol. 46, 972–980 (1996).

TEREFEWORK, Z., NICK, G., SUOMALAINEN, S., PAULIN, L., LINDSTRÖM, K.: Phylogeny of *Rhizobium galegae* with respect to other rhizobia and agrobacteria. Int. J. Syst. Bacteriol. 48, 349–356 (1998).

VAN BERKUM, P., BEYENE, D., BAO, G., CAMPBELL, T. A., EARDLY, B. D.: *Rhizobium mongolense* sp. nov., is one of three rhizobial genotypes identified which nodulate and form nitrogen-fixing symbioses with *Medicago ruthenica* ([L.] Ledebour). Int. J. Syst. Bacteriol. 48, 13–22 (1998).

VAN BERKUM, P., TULLY, R. E., KEISTER, D. L.: Nonpigmented and bacteriochlorophyll-containing *Bradyrhizobia* isolated from *Aeschynomene indica*. Appl. Environ. Microbiol. 61, 623–629 (1995).

VAN ROSSUM, D., SCHUURMANS, F. P., GILLIS, M., MUYOTCHA, A., VAN VERSEVELD, H. W., STOUTHAMER, A. H., BOOGERD, F. C.: Genetic and phenotypic analyses of *Bradyrhizobium* strains nodulating Peanut (*Arachis hypogaea* L.) roots. Appl. Environ. Microbiol. 61, 1599–1609 (1995).

VANDAMME, P., POT, B., GILLIS, M., DE VOS, P., KERSTERS, K., SWINGS, J.: Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. 60, 407–438 (1996).

VAUTERIN, L., VAUTERIN, P.: Computer-aided objective comparison of electrophoresis patterns for grouping and identification of microorganisms. Eur. Microbiol. 1, 37–41 (1992).

VINCENT, J. M.: A manual for the practical study of root-nodule bacteria, pp. 73–97. In: International Biological Programme Handbook no. 15. Oxford, Blackwell Scientific Publications Ltd. 1970.

VINUESA, P., RADEMAKER, J. L. W., de BRUIJN, F. J., WERNER, D.: Genotypic characterization of *Bradyrhizobium* strains nodulating endemic woody legumes of the Canary Islands by PCR-Restriction Fragment Length Polymorphism analysis of genes encoding 16S rRNA (16S rDNA) and 16S-23S rDNA intergenic spacers, repetitive extragenic palindromic PCR genomic fingerprinting, and partial 16S rDNA sequencing. Appl. Environ. Microbiol. 64, 2096–2104 (1998).

WANG, E. T., VAN BERKUM, P., SUI, X. H., BEYENE, D., CHEN, W. X., MARTINEZ-ROMERO, E.: Diversity of rhizobia associated with *Amorpha fructicosa* isolated from chinese soils and description of *Mesorhizobium amorphae* sp. nov. Int. J. Syst. Bacteriol. 49, 51–65 (1999).

WANG, E. T., VAN BERKUM, P., BEYENE, D., SUI, X. H., DORADO, O., CHEN, W. X., MARTINEZ-ROMERO, E.: *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* that has a close phylogenetic relationship with *Rhizobium galegae*. Int. J. Syst. Bacteriol. 48, 687–699 (1998).

WONG, F. Y. K., STACKEBRANDT, E., LADHA, J. K., FLEISCHMAN, D. E., DATE, A. R., FUERST, J. A.: Phylogenetic analysis of *Bradyrhizobium japonicum* and photosynthetic stem-nodulating bacteria from *Aeschynomene* species grown in separated geographical regions. Appl. Environ. Microbiol. 60, 940–946 (1994).

XU, L. M., GE, C., CUI, Z., LI, J., FAN, H.: *Bradyrhizobium liaoningensis* sp. nov. isolated from the root nodules of soybean. Int. J. Syst. Bacteriol. 45, 706–711 (1995).

YOUNG, J. P. W.: Phylogenetic classification of nitrogen-fixing organisms, pp. 43–86. In: Biological Nitrogen Fixation (G. STACEY, R. H. BURRIS, H. J. EVANS, eds.), New York, Chapman & Hall 1991.

YOUNG, J. P. W., HAUKKA, K. E.: Diversity and phylogeny of rhizobia. New Phytol. 133, 87–94 (1996).

ZHANG, X., HARPER, R., KARISTO, M., LINDSTRÖM, K.: Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees. Int.J.Syst.Bacteriol. 41, 104–113 (1991).

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SYSTEMATIC AND APPLIED MICROBIOLOGY

In vitro Susceptibilities of *Aeromonas* Genomic Species to 69 Antimicrobial Agents

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Summary

A total of 217 strains representing all 14 currently described genomic species in the genus *Aeromonas* were tested for susceptibility to 69 antimicrobial agents by a microdilution method. All species were susceptible to tetracyclines, quinolones, chloramphenicol, and most of the aminoglycosides and the cephalosporins, but were resistant to lincosamides, vancomycin, teicoplanin and some penicillins. In general, no signicant differences were found that correlated with the taxonomic designation or the origin of the isolates tested. The microdilution method proved to be easy to perform allowing susceptibility testing of extensive strain collections for a large number of antimicrobial agents.

Key words: *Aeromonas* – genomic species – antibiotic resistance

Introduction

Mesophilic aeromonads occur in almost all kinds of aquatic environments (HOLMES et al., 1996). Some species have been shown to be pathogenic for a wide range of mammals (including cats, dogs, horses, rabbits, guinea-pigs, monkeys, and mice) and avians (including chickens, turkeys and companion birds) (GOSLING, 1996). However, most of the reported infections occur only sporadically and are localized at the most diverse sites within the host. Two major categories of *Aeromonas*-associated infections have been encountered in humans. Extra-intestinal infections can be caused by a broad spectrum of *Aeromonas* taxa, and often have severe or fatal consequences for adult individuals. Gastroenteritis, on the other hand, seems to have a narrower species spectrum and mainly occurs in young children or aging people (JANDA and ABOTT, 1996). Many reported clinical studies have demonstrated that there exists a strong correlation between high numbers of aeromonads in the gastrointestinal tract and human diarrhoeal disease. However, none of these investigations have been able to identify *Aeromonas* as the true causal agent of gastroenteritis (JOSEPH, 1996). Drinking water and food are considered as the major sources of gastrointestinal contamination with *Aeromonas*. Most of the cases reported in literature show that *A. caviae* was the major species isolated, followed by *A. hydrophila* and *A. veronii* biogroups *sobria* and *veronii*.

Since the description of *Aeromonas* in Bergey's Manual of Systematic Bacteriology (POPOFF, 1984) taxonomy of the genus has undergone a large number of structural and nomenclatural improvements. Despite these changes, there remains a striking lack of congruence between phenotypic groups and genomic species or DNA-DNA hybridization groups (HGs). At present 14 *Aeromonas* species have been described, mainly on the basis of DNA similarity studies (ALTWEGG, 1990; CARNAHAN and ALTWEGG, 1996; HUYS et al., 1997a, b). Most of the antimicrobial susceptibility studies conducted so far on aeromonads include isolates belonging to the phenotypic groups *A. hydrophila, A. caviae,* and *A. sobria* using various methodologies ((FAINSTEIN et al., 1982; RICHARDSON et al., 1982; CHANG and BOLTON, 1987; KOEHLER and ASHDOWN, 1993; URBÀSKOVÀ et al., 1993; MORITA et al., 1994; KO et al., 1996). To our knowledge, a comprehensive comparison of antibiotic susceptibility data including all *Aeromonas* genomic species has not yet been published. Therefore, we investigated a large collection of well-characterized representatives of all currently described *Aeromonas* taxa to determine the taxonomic relevance of antibiotic resistance patterns in the genus *Aeromonas*.

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