Characterization of rhizobia from *Sesbania* species native to seasonally wetland areas in Uruguay

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Abstract

Naturally growing *Sesbania* species with tolerance to unfavourable habitats are widely distributed in non-cultivated seasonally wetland areas in Uruguay. We investigated the relative abundance, diversity and symbiotic efficiency of *Sesbania punicea* and *S. virgata* rhizobia in three ecologically different undisturbed and water-logged sites in Uruguay. Numbers of native-soil rhizobia infective on *S. punicea* or *S. virgata* were low, with higher numbers associated with the presence of *S. virgata*. Plants of *S. virgata* inoculated with soil suspension showed aerial and nodule biomass greater than that obtained with *S. punicea*. The rhizobia nodulating *Sesbania* species in water-logged lands in different regions of Uruguay were diverse differing in growth rates, acid production, growth at 39°C and in LB medium, host range and symbiotic efficiency. Seventeen representative strains clustered into four groups on the basis of phenotypic characteristics, ARDRA and DNA fingerprinting (GTG_5-PCR). Partial sequence of 16S rRNA from eight of these strains classified them into at least two genera with four species: *Azorhizobium doebereinerae*, *Rhizobium* sp. related to *R. etli* and two different *Rhizobium* sp.-*Agrobacterium*. Our results confirm the presence of the specie *Azorhizobium doebereinerae* as microsymbionts of *S. virgata* in South America. No strain of *Rhizobium etli* has previously been reported as a microsymbiont of *Sesbania*, though *R. etli* like organisms have also been recovered from *Dalea purpurea* and *Desmanthus illinoensis*. Significant increases in dry matter production were obtained with *S. virgata* plants inoculated with selected rhizobial strains under growth chamber conditions.

Introduction

The genus *Sesbania* contains about 70 widespread tropical and subtropical species, including annuals and perennials, herbaceous shrubs and trees. The genus has attracted interest for its fast growth, high crop yield, flooding tolerance, root and stem nodulation (Dreyfus et al. 1988) and high nitrogen (N_2) fixation activity (Ventura and Watanabe 1993). *Sesbania rostrata* and *S. aculeata* are used as green manure for rice in Asia and Africa (Yadav 2004; Ventura and Watanabe 1993; Becker et al. 1991) and other species grown in paddy fields are used in intercropping or in agroforestry (Becker et al. 1995). Recently, *S. virgata* has been used for revegetation of riparian forests, for controlling soil erosion, rehabilitation of degraded areas and charcoal production (Moreira et al. 2006).

Symbiosis between *Sesbania* and root and stem nodule bacteria has been intensively studied in the warmer latitudes (Vinuesa et al. 2005; Wolde-meskel et al. 2004; Wang et al. 2003; Bala et al. 2002; Odee 1990); species of *Rhizobium, Mesorhizobium, Sinorhizobium, Azorhizobium* and *Agrobacterium,* can nodulate *Sesbania* spp (Moreira et al. 2006; Vinuesa et al. 2005; Giller 2001; Frioni et al. 2001; Wang and Martínez-Romero 2000; de Lajudie et al. 1998; Wang et al. 1998; Odee 1990; Dreyfus et al. 1988). Few reports on this symbiosis exists for temperate areas.

Sesbania virgata (Cav.) Pers. (Sv) and *S. punicea* (Cav.) Benth. (Sp) are perennial shrubs naturally widely distributed in Uruguay, but most often associated with lowland and low fertility soils. The information available on these species and on other legume tree–rhizobia associations in Uruguay is limited (Frioni et al. 2001). Potential exploitation of native *Sesbania* species in Uruguay depends on further studies to identify effective strains and plants capable or fixing N_2.

In the present study, our objectives were to (1) determine the number, type and diversity of rhizobia associated to *Sesbania* species at three seasonally wetland soils sites and (2) evaluate the symbiotic efficiency of rhizobia collected from *S. punicea* and *S. virgata* naturally growing in these areas.

Materials and methods

Sampling sites and soil analyses

In Uruguay, native *Sesbania* species are found in water-logged soils of the Atlantic Ocean coast and of the Rio de la Plata coast. Three sites from different autumn–winter natural wetland habitats were sampled for soils, seeds and *Sesbania* plants: (a) La Coronilla, Rocha (33°53′12″ S and 53°30′20″ W), where *Sesbania punicea* plants were found (b) Valizas, Rocha (34°19′37″ S and 53°47′38″ W), where both *S. punicea* and *S. virgata* were present; both sites are at 10 km from the Atlantic Ocean coast and (c) La Colorada, Montevideo, in the Río de la Plata coast (34°51′10″ S 56°22′50″ W), where only *S. virgata* plants were found. Sites (a) and (b) were next to each other while the other site (c) was more than 300 km far; all three sites had not been disturbed for more than 10 years. The main plant species accompanying *Sesbania* species in the three sampling sites were as follows: *Schizachyrium* sp., *Paspalum notatum* and *Andropogan* sp. Whole *S. virgata* and *S. punicea* plants were carefully collected in summer at flowering or in the early pod development so as to obtain root and nodules. Meanwhile triplicate soil samples were taken to a depth of 20 cm and 5 cm away from tap roots; samples were stored at 6°C until analysis. Determination of chemical and physical properties of soils were carried out on dry subsamples as described in Sparks (1996); the properties are shown in Table 1.

Enumeration and nitrogen fixation of rhizobial population

The plant infection technique (Somasegaran and Hoben 1994) was used to estimate most probable number (MPN) counts of *Sesbania* spp. rhizobia in soil from each site, using seeds of *S. virgata* and *S. punicea* as host. Uniform seeds were selected, surface sterilized and scarified by immersion in concentrated sulphuric acid for 15 min and washed with sterile water. Then they were imbibed for 12 h in sterile water, transferred to Petri dishes containing water–agar (1%) and pregerminated for 3 days at 30°C.

Seedlings were aseptically transferred into 30 × 220 mm glass tubes containing 40 ml of N-free Farhaeus nutrient agar (Somasegaran and Hoben 1994). Moist soil was subjected to a tenfold dilution series so as to have a dilution range from 10^{-1} to 10^{-6}; then 1 ml of each dilution was inoculated into four tubes (Somasegaran and Hoben 1994). The plants were incubated in the growth chamber at 30°C during the day and 25°C during the night with a 14-h photoperiod; the plants were harvested 25 days after inoculation and the presence or absence of nodules determined.

The symbiotic efficiency of the soil rhizobia populations from the three soils was determined using the whole-soil inoculation technique (Brockwell et al. 1988). Sterilized and pregerminated seeds of *S. virgata* and *S. punicea* were sown in plant tests tubes (30 × 220 mm) containing vermiculite and 40 ml N-free solution. The seeds were inoculated with 1 ml of each soil dilution; each soil sample was replicated five times. The experiment included non-inoculated plants as controls. The plants were incubated under the conditions indicated above and harvested 40 days after inoculation. Shoot and nodule dry weight were determined after oven drying the plant material to constant weight (Somasegaran and Hoben 1994).

Isolation and characterization of rhizobia

Root nodules were collected from five randomly selected healthy *Sesbania* species of each site. Both *Sesbania* species were collected at Valizas site, while only *S. punicea* and *S. virgata* plants were found at La Coronilla and at La Colorada sites, respectively. Isolation, purification and authentication of bacteria from root nodules were done according to classic procedures (Somasegaran and Hoben 1994). The isolates were stored on slants (yeast–mannitol–agar YMA) at 5°C.

Ninety rhizobial isolates were obtained from the two *Sesbania* species and due to the cost it was not possible to characterize them. Therefore, we randomly selected 17 isolates for characterization, which included analyses of morphologies, ability to absorb Congo red, capacity to produce acid in YMA plate media after incubation at 28°C for 3 days, tolerance to high temperature after incubation at 39°C for 5 days, growth at pH 5.5 in YM broth (with 20 mM MES) after 3 days incubation at 28 C (Howieson et al. 1992) and growth in LB medium (Miller 1972). To study the capacity of native isolates to form stem nodules in both *Sesbania* species, pre-germinated seeds were placed in sterilized test tubes containing 40 ml of N-free Farhaeus nutrient agar (Somasegaran and Hoben 1994). For stem inoculation, the liquid inoculum (1×10^9 rhizobia ml^{-1}) was rubbed onto the stem with a sterilized cotton tip (Vinuesa et al. 2005). Stem nodulation of the plants was observed after 4 weeks of growth under conditions described above.

Host range

Host range was determined using the procedure with the glass tubes and vermiculite described above and using surface-sterilized seeds of *Sesbania punicea*, *S. virgata*, *Medicago sativa*, *Lotus subiflorus* and *L. corniculatus* as host legumes. Rhizobial cultures were grown on broth-YM media under shaking for 3 days at 28°C, then 2 ml of culture were applied per plant; each host/strain combination was replicated five times. The plants were grown for 3 weeks at 30°C during the day and 25°C during the night with a 14 h photoperiod. Then nodulation was evaluated.

Extraction of total DNA and $(GTG)_5$-fingerprinting

Rhizobial isolates were grown in 5 ml tryptone-yeast (TY) broth medium (Somasegaran and Hoben 1994) at 28°C for 24 h. DNA was extracted as reported by Versalovic et al. (1994).

PCR amplification was performed using the primer $(GTG)_5$ 5′-GTGGTGGTGGTGGTG-3′ (Versalovic et al. 1994). PCR reactions were performed in a 25 µl reaction mixture containing buffer 1X (Fermentas, Canada), the primer (0.5°µM), each deoxynucleoside triphosphate (0.2 µM), $MgCl_2$ (1.5 mM), BSA (5 mg/ml), Taq DNA polymerase (1 U; Fermentas, Canada) and 5 µl of 1/10 dilution of DNA template. PCR conditions were as follows: 1 cycle at 95°C for 5 min, 30 cycles at 94°C for 1 min, at 40°C for 1°min, and at 72°C for 4 min; 1 cycle at 72°C for 15 min, in a Thermolyne, Amplitron I thermal cycler (Thermolyne, USA). The tubes were cooled at 4°C, then PCR products were separated on 3% agarose gels at 5 V/cm for 3 h; then, the gels were stained with 0.5 µg ethidium bromide ml^{-1} and photographed using a digital camera Kodak CX7430.

Amplified 16S rDNA restriction analysis

Primers fD1 5′AGAGTTTGATCCTGGCTCAG-3′ and rD1 5′-aaggaggtgatccagcc-3′ (Weisburg et al. 1991) were used for the amplification of about 1,500 pb fragment of the 16S rRNA gene of the 17 selected isolates. Amplifications were carried out in a 25 µl volume containing buffer 1X (Fermentas, Canada), 2.5 mM $MgCl_2$, 0.2 µM of each deoxinucleotide, 0.2 µM of primer, 5 mg ml^{-1} BSA, 0.5 U *Taq* polymerase (Fermentas, Canada) and 5 µl of 1/10 diluted DNA. Amplifications included 35 cycles of 1 min at 94°C, 1 min at 56°C, 2 min at 72°C, with an initial cycle of 5 min at 95°C and a final extension of 10 min at 72°C. Amplification of DNA was confirmed by gel electrophoresis in 1% agarose with TBE 0.5× buffer at 5 V/cm for 1 h; then gels were stained with ethidium bromide and photographed under UV illumination. Amplicons were digested with the endonuclease enzymes *Taq I*, *Hinf I*, and *MspI* according to the manufacturer (Fermentas, Canada); 30 µl reaction mixture contained 15 µl of PCR products, buffer 1×, 3 U of the enzyme and double distilled water. RFLPs were resolved by electrophoresis in 3% agarose gels with TBE 0.5× buffer at 5 V/cm for 3 h. The gels were stained with ethidium bromide and photographed under UV illumination.

Sequence analysis of 16S rRNA gene fragments

Eight representative rhizobia were chosen for 16S rRNA sequencing; amplifications were performed as described above. Purification of PCR products was performed with Qiaquick PCR kit ™ (QIAGEN, GmbH) and 750 pb were sequenced by Macrogen Corp. S.A. (Korea) using the primers fD1 and rD1. Resulting sequences were compared with related bacteria sequences of the GeneBank and sequence similarities were determined using BLAST Sequence Similarity Search (Altschul et al. 1997).

Symbiotic efficiency

Five of the 17 rhizobia isolates from the two *Sesbania* species and the different experimental sites were selected to evaluate their symbiosis with *S. virgata* and *S. punicea* plants grown in modified Leonard jars as previously described. The experiment was a randomized complete block design with five replications and three seedlings per jar. After 5 days, the plants were thinned to 2 plants per jar. Rhizobial cultures were grown on YM broth media for 5 days at 28°C ($\geq 1 \times 10^8$ rhizobia/ml) and 2 ml of the broth were used to inoculate each plant. The plants were grown in a controlled environment chamber as described above and harvested 45 days after planting at the beginning of flowering. The +N treatment (2.5 mM NH_4N0_3 in the plant nutrient solution) and −N uninoculated control were included for comparison. Nodule and shoot dry weight were determined according to Somasegaran and Hoben (1994).

Data analysis

Data obtained from the growth chamber isolate trials, were analyzed by ANOVA using biostatistical analysis programme (SAS 1996).

Results

At each site, *Sesbania punicea* and *S. virgata* plants had globose or enlarged nodules located throughout the root system. They had a smooth surface, were 3–8 mm long, and were pink or green. Stem nodules were not observed in *Sesbania* species field collected, and they were not induced by inoculation.

Rhizobia nodulating *S. virgata* plants were more abundant than those nodulating *S. punicea* at the three sampling locations; the MPN of the former rhizobia ranged from 10^2 to 10^3 g^{-1} soil and those of the latter ranged from 1.5×10^1 to 10^2 g^{-1} soil. Higher rhizobia number sg^{-1} soil were recovered from La Coronilla and Valizas soils than from La Colorada (Table 2).

Nodule and shoot dry mass of *S. virgata* plants inoculated with soil suspension differed among sampling sites but not for *S. punicea* plants; they were 43 and 39% higher in *S. virgata* than *S. punicea* plants (Table 2). A total of 90 intermediate and fast-growing rhizobia were obtained from root nodules of naturally grown *S. punicea* and *S. virgata* plants collected from the three sites. While rhizobia from *S. punicea* plants all nodulated *S. virgata* plants, only 80% of *S. virgata* isolates nodulated *S. punicea* plants (data not shown).

Eight of 17 isolates obtained from *Sesbania* species formed pseudonodules on *Medicago sativa* (Table 3); most of the inoculated plants showed shoot growth when compared to uninoculated ones. Three isolates formed ineffective collar nodules on *Lotus subiflorus*, but none of the isolates formed root nodules on *Lotus corniculatus* (Table 3) and none of the strains formed stem nodules on *S. rostrata.*

The 17 isolates were classified in three groups according to their morphological and physiological characteristics: (a) group I included 13 isolates forming visible colonies after 2 days in YMA, producing acid and exopolysaccharides and growing at high temperature (39°C) in LB media and in YMA at pH 5.5; (b) group II with Sv4 and Sv83 isolates, which had the same morphological characteristics of colonies formed by rhizobia of group I but were unable to grow at 39°C, in LB media or in YMA at pH 5.5; and (c) group III with Sp2 and Sv80 isolates formed small visible colonies after 4 days in YMA, with scarce exopolysaccharide production; in addition, they were unable to produce acid or to grow in LB media (Table 4). Group I included most of the rhizobial isolates obtained from both *Sesbania* species and from the three sites, while rhizobia of group II and III were isolated from La Coronilla and Valizas areas (Table 4). Five of the 13 isolates of group I absorbed Congo red (data not shown).

Amplificons obtained by PCR amplification with fD1 and rD1 primers gave a single band with an estimated length of about 1,500 bp with all strains. Four 16S rRNA types were obtained among the 17 isolates after digestion with the restriction enzymes (Table 4). *Msp*I was the best discriminatory enzyme, resulting in four restriction patterns (Fig. 1). Strains with 16S rRNA of the type I were found in all sampled soils (Table 4).

The 16S rRNA partial sequence of Sv79, Sp15, Sv80, Sp2, Sp92, Sv72, Sv83 and Sv4 isolates were compared to those of the GenBank database; the assignment was based on a 98–99% similarity (Table 4). Isolates Sp2 and Sv80 (genotype III) were identified as *Azorhizobium doebereinerae* (Moreira et al. 2006), Sv83 and Sv4 (genotype II) appeared closely related to *Rhizobium etli*, while Sp15, Sv72, Sv79 and Sp92 were closely related to *Rhizobium* spp-*Agrobacterium* spp. Sp15 and Sv79 were from genotype I and Sv72 and Sp92 from genotype IV. Similarity between 16S rDNA sequences of genotypes I and IV (strains from group I are 97% similar to strains of group IV), indicating that these strains are probably two different species of *Rhizobium*.

The symbiotic efficiency of Sp15, Sp92, Sv72, Sp2 and Sv4 on *S. punicea* and *S. virgata* plants was evaluated determining shoot and nodule weight (Table 5). Sp92, Sv72 and Sp2 strains were effective with both hosts, and shoot biomass values were higher than those of the +N control. Nodule and plant weight of inoculated *S. virgata* plants were higher that those of *S. punicea*.

Discussion

Nodulation of *S. virgata* and *S. punicea*

Nodulation of *Sesbania* species by natural-soil rhizobia is well known in tropical and subtropical areas (Bala et al. 2002; Mohammed et al. 2001) and has recently been shown in some temperate areas of South America (Montecchia et al. 2007; Moreira et al. 2006). In this study, conducted in Uruguayan soils, numbers of native rhizobia of soil were low, with higher numbers in the presence of *S. virgata*, thus confirming the importance of host genotype affecting the rhizobia number of soil. Both, *S. punicea* and *S. virgata* had nodules at each site sampled, whereas Gris and Moreira (personal communication) found no nodulation of *S. virgata* after inoculation with suspension of different Amazon region, central and south Brazil soils. In addition, Veasey et al. (1997) found that *S. virgata* and *S. punicea* were the only shrubs that did not nodulate with the native population from a red yellow latosol in south Brazil. The small rhizobial populations of most soils probably may explain the response of both herbaceous and woody legumes to inoculation (Turk et al. 1993; Thies et al. 1991).

Rhizobia from different species of *Sesbania* can cross-nodulate (Moreira et al. 2006; de Lajudie et al. 1994). Our results indicated a relatively narrow host range and they are similar to those obtained by Vinuesa et al. (2005), who found that *S. punicea* isolates from Venezuelan soils only nodulated efficiently with their original host and *Vigna unguiculata.*

Our study is the first on the efficiency of native rhizobia nodulating *Sesbania* species grown in Uruguay. *S. virgata* non-inoculated plants produced higher aerial biomass than *S. punicea* plants. We also found that *S. virgata* plants showed higher shoot biomass when inoculated with soil rhizobia than *S. punicea.* These results suggest that soil rhizobia population nodulating *S. virgata* may be more efficient in nitrogen fixation than rhizobia nodulating *S. punicea.* Besides, *S. virgata* appear to have better genetic traits than *S. punicea*. We can confirm that native legume–rhizobia can form successful symbiosis (Vinuesa et al. 2005) improving plant growth.

Identification of rhizobia nodulating *S. virgata* and *S. punicea*

Sesbania species can be effectively nodulated by *Rhizobium gallicum* and *R. tropici* (Zurdo-Pineiro et al. 2004), *R. huautlense* (Wang et al. 1998), *Sinorhizobium saheli* and *S. terangae* (de Lajudie et al. 1994), *Mesorhizobium plurifarium* (de Lajudie et al. 1998), *Azorhizobium caulinodans* (Dreyfus et al. 1988), and *A. doebereinerae* (Moreira et al. 2006). Our strains were from *Rhizobium* and *Azorhizobium* genera. *A. doebereinerae* has been recently described to establish a symbiotic efficient association with *S. virgata* plants from South Brazil (Moreira et al. 2006) and from Argentina (Montecchia et al. 2007). Therefore, our results confirm that *A. dobereinerae* can be found in South America soils while the other species (*A. caulinodans*) is present in tropical Africa associated to *S. rostrata* (Dreyfus et al. 1988). Apart from similar 16S rRNA gene sequence, cultural characteristics of Sp2 and Sv80 strains agree with those of *A. doebereinerae* (Moreira et al. 2006). However, these strains have different symbiotic characteristics than those described by the latter mentioned authors and they can establish an effective symbiosis with *S. punicea* and *S. virgata*; both strains were not able to induce nodulation on *S. rostrata* stem.

The 16S rDNA sequences showed that 2 strains related to *Rhizobium etli* could form nodule on *S. virgata* and *S. punicea* roots. *R. etli* is relatively restricted in host range; *R.etli* bv. phaseoli has been reported to nodulate *Phaseolus vulgaris* (L.) (Segovia et al. 1993), *R. etli* bv. mimosae has been reported to nodulate *Mimosa affinis*, a native tree legume from Central Mexico (Wang et al. 1999) and *R. etli* like organisms have also been recovered from *Dalea purpurea* and *Desmanthus illinoensis* (Martin et al. 2007; Beyhaut et al. 2006).

Strains Sp15, Sv72, Sv79 and Sp92 obtained from nodules of *S. punicea* and *S. virgata* roots were closely related to *Rhizobium–Agrobacterium* lineage. Their exact position on the phylogenetic tree of rhizobia must be confirmed by complete 16S rRNA sequencing (Stackebrandt and Goebel 1994; Graham et al. 1991). The similarities between rhizobia and agrobacteria is well established. The rhizobia nodulating *Sesbania* species identified as *Rhizobium* sp. are perhaps symbiotic partners of other leguminous species native to these soils.

Vinuesa et al. (2005) found that *Mesorhizobium plurifarium* was associated to *S. punicea* in Venezuelan soils, whereas in our soils, this plant was associated with *Rhizobium–Agrobacterium* (strains Sp92, Sp15) or with *A. doebereinerae* (strain Sp2). It may be assumed that *S. punicea* can be nodulated by different rhizobia species according to the habitat where it is naturally growing.

Genetic diversity of rhizobia isolates were studied in this work using ARDRA and DNA fingerprinting $(GTG)_5$-genotyping. This technique has been used for genetic fingerprinting of different bacteria (Gevers et al. 2001; Jussila et al. 2006; Nick et al. 1999). Both GTG-PCR fingerprinting and ARDRA analysis differentiated four genomic profiles in *S. punicea* and *S. virgata*, suggesting probably low rhizobial strains diversity in nodulated *Sesbania* species from Uruguayan wetland areas. Moreira et al. (2006) studying rhizobia associated to *S. virgata* from Brazil found that rep-PCR profiles were 90% similar between isolates form nearby place in the same state and 98% similarity between isolates from the same municipalities. These results agree with our data considering that the three studied sites were very near.

In conclusion, *Sesbania virgata* seems to be a species with potentially higher symbiotic nitrogen fixation characteristics than *S. punicea*, and our results indicate that it is a potentially useful legume to evaluate in field conditions. Natural rhizobia able to nodulate *S. punicea* and *S. virgata* roots belongs at least to *Rhizobium* and *Azorhizobium* generas and include the recently described *A. doebereinerae*.

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Table 1 Chemical and physical properties of soils

Soil properties	La Coronilla	Valizas	La Colorada
pH (1:5 water)	6.8	5.7	5.8
Organic-C percent	2.4	1.4	1.3
K ($cmol.kg^{-1}$)	0.68	0.53	0.72
Na ($cmol.kg^{-1}$)	0.44	0.45	>1.63
Ca ($cmol.kg^{-1}$)	2.12	1.04	1.24
Mg ($cmol.kg^{-1}$)	1.71	1.52	1.32
Sand (%)	22	9	35
Clay (%)	12	11	28
Silt (%)	66	80	37

Table 2. Most probable number (MPN) count and symbiotic efficiency on Sesbania punicea and S. virgata by native rhizobia populations from different sites

Soil site	*S. punicea*[a]			*S. virgata*[a]		
	MPN[b]	Shoot wt.[c]	Nodule wt.[c]	MPN[b]	Shoot wt.[c]	Nodule wt.[c]
La Coronilla	2.1	43.3[a]	4.2[a]	2.7	72.3[a]	6.9[a]
Valizas	1.5	41.6[a]	3.0[a]	2.7	70.4[a]	7.5[a]
La Colorada	1.7	38.7[a]	4.3[a]	2.1	58.5[b]	5.8[b]
Control[d]	–	34.0	–	–	48.5	–

Table 3 Nodulation of rhizobia isolated from *Sesbania punicea* and *S. virgata* grown in different sampling areas of Uruguay

Soil site	Sesbania species	Isolate	Host range			
			Sesbania punicea	*Sesbania virgata*	*Medicago sativa*	*Lotus subiflorus*
La Coronilla	*S. punicea*	Sp 1	E	E	PS	0
		Sp 2	E	E	PS	0
		Sp 3	E	E	PS	0
		Sp 8	E	E	0	Nd
Valizas	*S. virgata*	Sv 1	0	E	0	NE
		Sv 4	E	E	PS	0
		Sv 5	E	E	PS	0
		Sv 12	E	E	0	0
		Sv 80	±	E	0	0
		Sv 83	±	E	0	0
		Sv 84	±	E	PS	0
		Sv 91	0	E	0	Nd
	S. punicea	Sp 13	E	E	0	Nd
		Sp 15	E	E	0	NE
		Sp 92	E	E	PS	0
La Colorada	*S. virgata*	Sv 72	E	E	0	0
		Sv 78	E	E	PS	NE
		Sv 79	E	E	0	0
	S. rostrata	ORS 571	0	E	0	0

Fig. 1. Amplificons obtained by PCR amplification with fD1 and rD1 primers gave a single band with an estimated length of about 1,500 bp with all strains. Four 16S rRNA types were obtained among the 17 isolates after digestion with the restriction enzymes (Table 4). *Msp*I was the best discriminatory enzyme, resulting in four restriction patterns (Fig. 1). Strains with 16S rRNA of the type I were found in all sampled soils (Table 4).

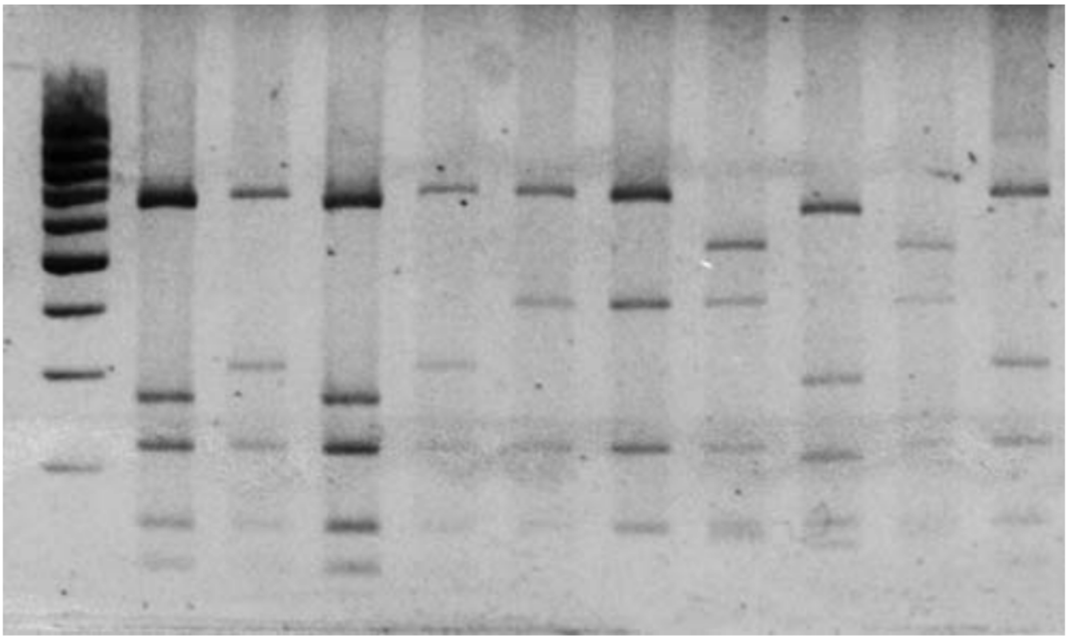

Table 4 Phenotypic and genotypic profiles of rhizobial isolated from naturally nodulated *Sesbania virgata* and *S. punicea* plants collected at different sampling sites

Strain	Phenotypic group[a]	ARDRA profile[b]	$(GTG)_5$ genotype	Site	Taxonomic affiliation[c]	Original host
Sv 1	I	I	I	Valizas		*S. virgata*
Sv 5	I	I	I	Valizas		*S. virgata*
Sp 1	I	I	ND	Coronilla		*S. punicea*
Sp 3	I	I	ND	Coronilla		*S. punicea*
Sp 8	I	I	I	Coronilla		*S. punicea*
Sv 78	I	I	I	Colorada		*S. virgata*
Sv 79	I	I	I	Colorada	*Rhizobium* spp- *Agrobacterium* (99%)	*S. virgata*
Sv 84	I	I	I	Valizas		*S. virgata*
Sv 12	I	I	I	Valizas		*S. virgata*
Sp 13	I	I	I	Valizas		*S. virgata*
Sp 15	I	I	I	Valizas	*Rhizobium* spp- *Agrobacterium* (99%)	*S. punicea*
Sv 72	I	IV	IV	Colorada	*Rhizobium* spp—*Agrobacterium* (99%)	*S. virgata*
Sp 92	I	IV	IV	Valizas	*Rhizobium* spp—*Agrobacterium* (99%)	*S. punicea*
Sv 80	III	III	III	Valizas	*Azorhizobium doebereinerae* (99%)	*S. virgata*
Sp 2	III	III	III	Coronilla	*Azorhizobium doebereinerae* (99%)	*S. punicea*
Sv 4	II	II	II	Valizas	*Rhizobium etli*—*Rhizobium spp* (98%)	*S. virgata*
Sv 83	II	II	II	Valizas	*Rhizobium etli*—*Rhizobium* spp (98%)	*S. virgata*

Fig. 2. $(GTG)_5$ PCR-fingerprinting patterns of DNA from rhizobial isolates. *Lane 1* molecular weight of standard DNA (100 pb), *lanes 2–15* DNA of rhizobial isolates from *S. virgata* and *S. punicea*: Sv72, Sp92, Sv78, Sv84, Sp8, Sv1, Sv12, Sv79, Sp15, Sp13, Sv83, Sv4, Sv80, Sp2

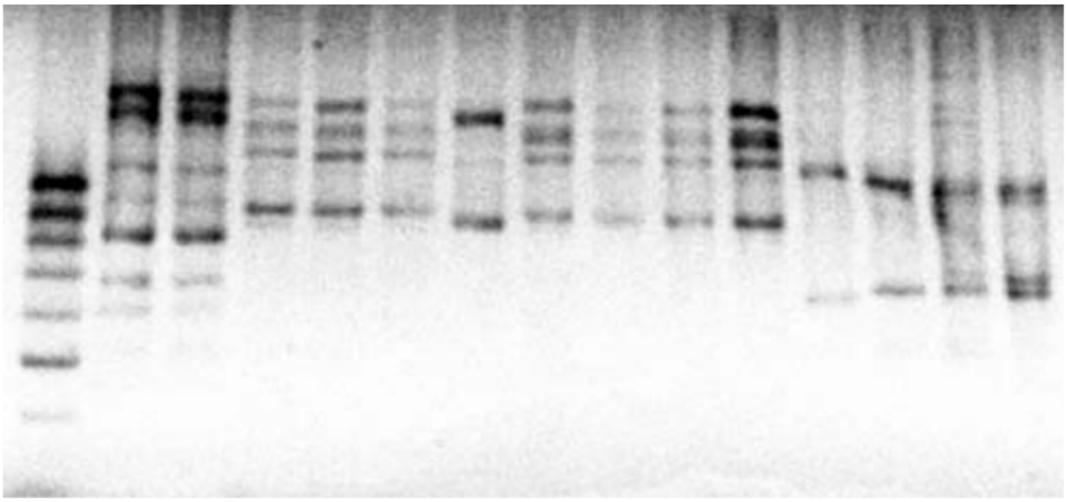

Table 5 Symbiotic efficiency of native rhizobia on *Sesbania virgata* (Sv) and *S. punicea* (Sp) plants[a]

Strains	*Sesbania punicea*[b]		*Sesbania virgata*[b]	
	Nodule biomass[c]	Aerial biomass[d]	Nodule biomass[c]	Aerial biomass[d]
Sp15	148.5[a]	1.07[a]	136.6[ab]	1.23[a]
Sp 92	140.5[a]	1.23[a]	221.7[a]	1.38[a]
Sv 72	113.7[a]	0.94[a]	200.1[a]	1.47[a]
Sp 2	146.4[a]	1.11[a]	195.2[a]	1.28[a]
Sv 4	111.2[a]	0.82[a]	125.7[b]	0.75[b]
Nitrogen	–	1.04[a]	–	1.23[a]
Control	–	0.30	–	0.28