Diversity and nitrogen fixation efficiency of rhizobia isolated from nodules of *Centrolobium paraense*

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Abstract – The objective of this work was to isolate and characterize rhizobia from nodules of *Centrolobium paraense* and to evaluate their symbiotic efficiency. Soil samples collected from four sites of the Roraima Cerrado, Brazil, were used to cultivate *C. paraense* in order to obtain nodules. Isolates (178) were obtained from 334 nodules after cultivation on medium 79. Twenty-five isolates belonging to six morphological groups were authenticated using *Vigna unguiculata* and they were characterized by 16S rRNA. Isolates identified as *Bradyrhizobium* were further characterized using *rpoB* gene sequencing. A greenhouse experiment was carried out with *C. paraense* to test the 18 authenticated isolates. Approximately 90% of the isolates grew slowly in medium 79. The 16S rRNA analysis showed that 14 authenticated isolates belong to the genus *Bradyrhizobium*, and *rpoB* indicated they constitute different groups compared to previously described species. Only four of the 11 fast-growing isolates nodulated *V. unguiculata*, two of which belong to *Rhizobium*, and two to *Pleomorphomonas*, which was not previously reported as a nodulating genus. The *Bradyrhizobium* isolates ERR 326, ERR 399, and ERR 435 had the highest symbiotic efficiency on *C. paraense* and showed a contribution similar to the nitrogen treatment. *Centrolobium paraense* is able to nodulate with different rhizobium species, some of which have not yet been described.

Index terms: *Bradyrhizobium*, *Rhizobium*, bacteria diversity, biological nitrogen fixation.

Diversidade e eficiência na fixação do nitrogênio de rizóbios isolados de nódulos de *Centrolobium paraense*

Resumo – O objetivo deste trabalho foi isolar e caracterizar rizóbios de nódulos de *Centrolobium paraense* e avaliar sua eficiência simbiótica. Amostras de solo, coletadas de quatro locais do Cerrado de Roraima, foram utilizadas para o cultivo de *C. paraense*, para obtenção dos nódulos. Os isolados (178) foram obtidos dos 334 nódulos coletados após o cultivo em meio 79. Vinte e cinco isolados, pertencentes a seis grupos morfológicos, foram autenticados com uso de *Vigna unguiculata* e caracterizados pelo 16S rRNA. Os isolados identificados como *Bradyrhizobium* foram caracterizados depois pelo sequenciamento do gene *rpoB*. Um experimento em casa de vegetação foi realizado com *C. paraense*, para testar os 18 isolados autenticados. Aproximadamente 90% dos isolados mostraram crescimento lento em meio 79. A análise do 16S rRNA mostrou que 14 dos isolados autenticados pertencem ao gênero *Bradyrhizobium*, e o *rpoB* indicou que eles constituem grupos diferentes em comparação às espécies já descritas. Somente quatro dos 11 isolados com crescimento rápido nodularam *V. unguiculata*, dois dos quais pertencentes a *Rhizobium* e dois a *Pleomorphomonas*, que não foi relatado anteriormente como gênero nodulífero. Os isolados de *Bradyrhizobium* ERR 326, ERR 399 e ERR 435 apresentaram a maior eficiência em *C. paraense* e mostraram contribuição similar ao tratamento nitrogenado. *Centrolobium paraense* é capaz de nodular com diferentes espécies de rizóbios, algumas das quais ainda não foram descritas.

Termos para indexação: *Bradyrhizobium*, *Rhizobium*, diversidade bacteriana, fixação biológica de nitrogênio.

Introduction

Centrolobium paraense Tul. (Fabaceae), commonly known as "pau-rainha", is a nodulating Neotropical leguminous tree occurring from the northern Brazilian Amazon to Panama, specifically in Roraima state (Pirie et al., 2009). It is present in semi-deciduous, gallery, and transition forests and is thought to play several

ecological roles, which are essential to environmental sustainability, as increasing nutrient availability through biological nitrogen fixation (BNF), protecting against soil erosion, and acting as pioneer or early secondary species, which have also been reported for other *Centrolobium* species (Marques et al., 2001; Dahmer et al., 2009). In addition to its ecological importance, *C. paraense* has an economic and social importance because its wood is used by indigenous communities and industry (Dahmer et al., 2009; Pedreira, 2011). However, extractive exploration has been causing a gradual decrease in this plant resource, with an estimated decline of approximately 30% of the natural populations over the next years, demanding studies to increase the knowledge about this species and its conservation (Pirie et al., 2009).

Previous studies have indicated that *C. paraense* is capable of forming nodules with rhizobia native to the Amazon soil (Souza et al., 1994), but there are no published studies on the isolation and characterization of these rhizobia. Bacteria belonging to both genera *Bradyrhizobium* and *Rhizobium* have been reported to be able to establish symbioses with other species of *Centrolobium*, although studies have not been performed at the species level (Moreira et al., 1993; Pagano, 2008).

Cultural characteristics have been used for the initial characterization and screening of rhizobia; however, molecular techniques are necessary for a reliable characterization and identification. The 16S rRNA gene is the universal genetic marker most widely used for identification, classification and reconstruction of bacteria phylogeny. However, for identification of different rhizobia, the 16S rRNA gene sequencing is not suitable. For instance, in the genus *Bradyrhizobium,* the gene 16S rRNA presents a high degree of conservation among species, and sequence similarity is relatively high (Willems et al., 2001), limiting the species separation. However, housekeeping genes, which are involved in fundamental cell functions, came to be recommended to differentiate closely related species, including those within *Bradyrhizobium* genus, since they give a better resolution (Rivas et al., 2009).

Considering the ecological and economic importance of *C. paraense*, the isolation, characterization, and the symbiotic efficiency assessment of rhizobia is essential to develop inoculants for this legume.

The objective of this work was to isolate and characterize rhizobia from nodules of *C. paraense* and to evaluate their symbiotic efficiency.

Materials and Methods

Soil samples and seeds of *Centrolobium paraense* were collected in February 2010, which is the regional dry season, as well as *C. paraense* seed maturation season (Kaminski, 2004), at four different sites located on a private property, in four municipalities of the state of Roraima (Table 1). Five simple soil samples were collected at 0-20 cm depth, under the canopy of a *Centrolobium paraense* adult tree, at each site. The samples were combined into a composite sample of approximately 1 kg and were chemically characterized. Seed of the same plant were also collected for sowing as trap plant in the greenhouse. The soil samples were mixed with autoclaved sand (1:1) and placed in pots (approximately 3 L) in triplicate. *Centrolobium paraense* seed were scarified with sulphuric acid (5 min), desinfected in 5% hydrogen peroxide for 5 min, washed five times with sterilized water, and sown (5 seeds per pot), in soil samples of the same origin. Plant thinning was performed leaving two plants per pot. The experiment was carried out for 60 days using a completely randomized experimental design, and plants were watered with distilled water.

All the obtained nodules were disinfected with sodium hypochlorite (5%), individually macerated, inoculated in Petri dishes containing culture medium 79 (Fred & Waksman, 1928), and incubated for 10 days, at 28ºC. The resulting colonies were successively cultured in medium 79 until purification. Isolates were initially characterized in the same culture medium containing 0.05% bromothymol blue. Growth time, pH change of the culture medium, colony colour, shape, size, and mucus production were measured following Vincent (1970). These characteristics were used for clustering the isolates using Bionumerics (Applied Mathematics, Kortrijk, Belgium, v.6.1), with the Jaccard coefficient and UPGMA method.

Twenty-five isolates were selected, based on their distribution within the morphological groups; and considering the representativeness in each collecting sites. The isolates were inoculated in *Vigna unguiculata* because of this species has fast development and wide

nodulation capacity with rhizobia from Amazon soils (Guimarães et al., 2012; Silva et al., 2012).

The experiment was performed in 500 mL recyclable amber glass bottles wrapped in aluminum foil with Hoagland nutrient solution (Hoagland & Arnon, 1950) modified by Guimarães et al. (2012) at ¼ of ionic strength, with low nitrogen concentration (5.25 mg L^{-1}). Inoculants were prepared in liquid culture medium 79 (5 days; 28°C), and 1 mL inoculum, at approximately 10^9 cell mL^{-1} concentration, was applied to each plant. A treatment without inoculation was included as a negative control, and a treatment inoculated with BR 3262 *Bradyrhizobium* commercial strain for *V. unguiculata* was included as a positive one. A completely randomized experimental design was used with three replicates.

All isolates inoculated on *V. unguiculata* were grown in culture medium 79, and genomic DNA was extracted using the RBC kit (catalogue number: YGB 300). Amplification of 16S rRNA gene was performed using the Y1 (Young et al., 1991) and B3 primers (Haukka, 1997), and partial sequencing was performed using the primer Y1. The sequencing was performed using a 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, EUA).

For the 14 isolates that were similar to the genus *Bradyrhizobium* according to the 16S rRNA analysis, the gene *rpoB* was also analyzed. DNA was amplified using the rpoB83F and rpoB1061R primers (Martens et al., 2008), followed by sequencing using the primer rpoB83F. Alignment, editing, and phylogenetic analysis were performed in Mega 5.05 (Tamura et al., 2011), using the neighbour-joining method (Saitou & Nei, 1987). The sequences were deposited in the GenBank (2014), and the accession numbers are given in Table 2.

After authentication, 18 isolates were tested for biological nitrogen fixation efficiency in *C. paraense*. This experiment was performed in a greenhouse (28ºC, 50% reflective shade cloth) using a completely randomized experimental design with four replicates. Plants were grown in pots (7 L) containing autoclaved sand and vermiculite (2:1) as substrate. *Centrolobium paraense* seed collected from site III were scarified, desinfected (as described above), and pre-germinated in Petri dishes containing autoclaved moistened cotton and filter paper. Five days following germination, two seeds with approximately 1 cm long radicles were transplanted into each pot and inoculated with 1 mL bacterial suspension (as described above). In addition, two treatments inoculated with *Bradyrhizobium japonicum* and *B. elkanii* USDA 6^T and USDA 76^T strains, respectively, were included: one of the treatments had the addition of nitrogen (N) as ammonium nitrate (10 mg N per week from 0 to 28 days; 15 mg N per week from 29 to 70 days; 20 mg N per week from 71 to 77 days; and 30 mg N per week from 78 to 91 days); and the other treatment was a negative control, without inoculation, and with a low nitrogen concentration. Each pot received 800 mL per week of nutrient solution adapted from Hoagland & Arnon (1950), as above described, at ¼ of ionic strength for 42 days, ½ of ionic strength for the following 35 days, and full ionic strength until the end of the experiment.

The measured variables were: shoot dry matter; root dry matter; number of nodules; nodule dry matter; total N content in the shoots, measured using the Kjeldahl method (Liao, 1981); leaf area, measured as the area of all leaflets using a LI – 3100 area meter (LI-COR, Lincoln, NE, EUA); plant height, measured from the base of the stem to the plant apex; and number of leaflets. Dry matter (of shoots, roots, and nodules) was

Table 1. Chemical analysis of collected soil samples from different phytophysiognomies at the Cerrado of the state of Roraima, Brazil.

Site	Coordinates	Phytophysiognomies	pH	OM[(1)]	Ca	Mg	K	Al	P
				(g dm^{-3})	---------- ($cmol_c$ dm^{-3}) ----------				(mg dm^{-3})
I	3º25'11" N; 59°56'18" W	Forest island	5.8	19.5	1.57	0.67	0.21	0.01	1.68
II	3º44'25" N; 59º40'9" W	Gallery forest	5.1	20.8	1.69	0.68	0.28	0.15	1.78
III	2º43'40" N; 60º51'36" W	Forest island	4.9	20.8	1.35	1.06	0.15	0.66	1.68
IV	2º27'13" N; 60º54'12" W	Transition forest	6.0	29.2	4.92	0.91	0.21	0.06	26.10

[(1)]OM, organic matter.

determined by drying the material at 65°C for 72 hours. The data were subjected to analysis of variance, and averages were compared using the Scott-Knott test, at 5% probability.

Results and Discussion

Nodules (334) were obtained using *C. paraense* as a trap plant. Nodule totals of the three replicates for each site – I through IV (Table 1) - were 94, 87, 20, and 133, respectively. The soil characteristics and, more specifically, the higher soil organic matter, Ca, and P concentrations, at site IV, and the higher Al concentration, at site III, may have determined the differences in nodulation among sites, since these elements were reported as limiting for nodulation (Hungria & Vargas, 2000). However, because the soil collection was performed during the dry season, and because there is usually higher rainfalls in forest/cerrado transition areas, the water availability may have also influenced soil-rhizobia population and, consequently, plant nodulation (Sadowsky, 2005).

Out of the 334 collected nodules, 178 isolates were obtained, 41 originating from the site I, 63 from site II, 10 from site III, and 64 from site IV. Therefore, isolates were obtained from 44 to 70% of the nodules from each soil sample. This variation

Table 2. Morphological groups, sampling areas, nodulation in *Vigna unguiculata*, and identification using 16S rRNA of rhizobia isolates obtained from *Centrolobium paraense* nodules from the Roraima Cerrado, Brazil.

Morphological group (1)	Sampling area	N° of isolates	Isolates authenticated	*V. unguiculata* nodulation	16S Accession nº.	*rpoB* Accession nº.	Most similar 16S rRNA sequence in GenBank		
							Species	Accession nº.	Similarity (%)
1 (FAI)	I, II	5	ERR 333	-	KF983821	NA	*Enterobacter* sp.	HM107175	99
			ERR 366	-	KF983823	NA	*E. sacchari*	HQ204281	99
			ERR 376	-	KF983824	NA	*Pantoea agglomerans*	JQ312027	99
			ERR 378	-	KF983825	NA	*P. agglomerans*	JQ312027	99
2 (FAC)	I, II	7	ERR 308	+	KF983816	NA	*Pleomorphomonas oryzae*	AB681744	100
			ERR 344	+	KF983817	NA	*P. oryzae*	AB681745	98
			ERR 361	-	KF983822	NA	Enterobacteriaceae bacterium	EU887701	99
			ERR 469	-	KF983826	NA	*Burkholderia kururiensis*	FJ608710	100
3 (FNC)	III	3	ERR 377	+	KF983818	NA	*Rhizobium miluonense*	KF515658	100
			ERR 380	+	KF983819	NA	*R. miiluonense*	KF515658	99
4 (SNC)	I, II	3	ERR 312	-	KF983820	NA	*B. kururiensis*	FJ608710	100
5 (SALI)	I, III, IV	25	ERR 309	+	KF983808	KF983833	*Bradyrhizobium* sp.	KF596702	99
			ERR 412	+	KF983812	KF983837	*Bradyrhizobium* sp.	KF357630	99
			ERR 326	+	KF927050	KF983828	*Bradyrhizobium* sp.	JQ419543	100
			ERR 329	+	KF983809	KF983834	*Bradyrhizobium* sp.	KF596702	100
6 (SALC)	I, II, III, IV	135	ERR 298	+	KF983805	KF983830	*Bradyrhizobium* sp.	KC113617	100
			ERR 299	+	KF983806	KF983831	*Bradyrhizobium* sp.	JQ419543	100
			ERR 305	+	KF983807	KF983832	*Bradyrhizobium* sp.	JQ419544	100
			ERR 314	+	KF927049	KF983827	*Bradyrhizobium* sp.	FJ193313	99
			ERR 396	+	KF983810	KF983835	*Bradyrhizobium* sp.	KF357630	99
			ERR 399	+	KF983811	KF983836	*Bradyrhizobium* sp.	KF357630	99
			ERR 417	+	KF983813	KF983838	*Bradyrhizobium* sp.	KF596702	100
			ERR 421	+	KF983814	KF983839	*Bradyrhizobium* sp.	KC113617	100
			ERR 430	+	KF983815	KF983840	*Bradyrhizobium* sp.	KC113617	100
			ERR 435	+	KF927051	KF983829	*Bradyrhizobium* sp.	KC113617	100

FAI, fast-growing, medium acidification, and irregular colonies; FAC, fast-growing, medium acidification, and circular colonies; FNC, fast-growing, no pH medium alteration, and circular colonies; SNC, slow-growing, no pH medium alteration, and circular colonies; SALI, slow-growing, medium alkalinization, and irregular colonies; SALC, slow-growing, medium alkalinization, and circular colonies. NA, nonanalyzed.

can be explained by the presence of many small nodules, apparently still in formation. Cultivation times longer than the tested 60 days may result in more fully formed nodules and in the recovery of a higher number of isolates. Characterization of the isolates, based on the morphology of colonies formed in culture medium 79, showed that 90% of the isolates have characteristics which are typical of the genus *Bradyrhizobium*, such as slow growth (longer than five days), alkalinization and white colour of the colony and mucus (Jordan, 1982). Six morphological groups were obtained with 55% similarity, and groups V and VI comprised 160 isolates, all with slow growth (Table 2).

Twenty-five of the 178 rhizobia isolates (15% of the total) were authenticated in *V. unguiculata*. Isolate selection was based on the morphological grouping and on the representativeness of the different sites in each group. Ten, eight, two, and five isolates were tested from sites I, II, III, and IV, respectively. From the 25 tested isolates, seven belonging to the morphological groups with only fast-growing isolates were not capable to promote *V. unguiculata* nodulation. In contrast, representatives of all groups containing slow-growing bacteria (14 isolates) and four fast-growing isolates formed nodules in *V. unguiculata*, indicating that more than 90% of the isolates were rhizobia.

Comparison of the partial 16S rDNA sequences, obtained for this study with the NCBI database (GenBank, 2014), showed that isolates with slow growing in the medium 79 and capable to form nodules on *V. unguiculata* belong to the genus *Bradyrhizobium* (Table 2). The isolates ERR 377 and ERR 380, highly similar to *Rhizobium*, and the ERR 308 and ERR 344, similar to *Pleomorphomonas*, were also capable to nodulate. From the remaining fast-growing isolates, three were grouped with Enterobacteriaceae, two with *Burkholderia*, and two with *Pantoea*, and none formed nodules on *V. unguiculata*. Isolates belonging to Enterobacteriaceae, *Pantoea* and *Burkholderia* have been reported as capable of colonizing nodules of different leguminous plants without, however, induce nodule formation (Saidi et al., 2011), although there are several *Burkholderia* nodulating species (De Meyer et al., 2013). This indicates that the non nodulating, cultured bacteria in the present study most likely grew in the culture medium before the rhizobia and were thus isolated. Strains ERR 378 and ERR 380 were not included in the phylogenetic analysis using 16S rDNA sequence (509 bp) because fewer than 500 bp of the 16S rDNA were obtained. The ERR 308 and ERR 344 fast-growing isolates were grouped with the type strains of *Pleomorphomonas oryzae* and *P. diazotrophica*, with high similarity, and strains ERR 377 and ERR 380 were grouped with *R. tropici* and *R. miluonense*, confirming that these strains belong to the genera *Pleomorphomonas* and *Rhizobium*, respectively (Figure 1). There are no previous reports on bacteria from the genus *Pleomorphomonas* forming nodules, although the three species described for this genus were indicated as capable to fix N in vitro (Xie & Yokota, 2005; Im et al., 2006; Madhaiyan et al., 2013).

Fast-growing bacteria with similarity to *Rhizobium* have been previously found in nodules of *Centrolobium* plants (Moreira et al., 1993; Pagano, 2008), but symbionts of tropical leguminous plants predominantly belong to the genus *Bradyrhizobium* (Moreira et al., 1998). Bacteria of the genus *Bradyrhizobium* are reported as tolerant to low pH and fertility of tropical soils (Menna & Hungria, 2011). The 14 slow-growing isolates were grouped into four different phylogenetic branches (Figure 1). Two isolates were grouped together with *Bradyrhizobium* group I type strains, which includes the traditional species *B. japonicum* (Delamuta et al., 2013), four strains with *Bradyrhizobium* group II, which includes *B. elkanii*, and two formed a group intermediate to *Bradyrhizobium* groups I and II.

The gene *rpoB* analysis showed high genotypic diversity and polyphyletic distribution of the *Bradyrhizobium* isolates (Figure 2). Ten of the isolates were grouped with the *B. iriomotense* type strain EK05^T, but with four subgroups, three of them with at least two isolates. The remaining four bacteria were grouped with *B. elkanii* and *B. pachyrhizi*, in agreement with which was observed for the 16S rDNA gene. Other works using *V. unguiculata* as a trap plant have shown the existence of *Bradyrhizobium* isolate groups from different sites in the Amazon region that are phylogenetically distant from species already described (Guimarães et al., 2012; Silva et al., 2012). *Centrolobium paraense* probably establishes symbioses preferentially with *Bradyrhizobium* species from Amazon soils, many of which have not yet been described, likely because

Centrolobium is exclusively a Neotropical genus (Dahmer et al., 2009; Pirie et al., 2009). The analysis of 16S rDNA and *rpoB* genes have confirmed that the slow-growing isolates, obtained in the present study, belonged to the genus *Bradyrhizobium*, but that they constitute phylogenetic branches different from those of previously described species. Further taxonomic studies are needed for the confirmation of this hypothesis.

The strains USDA 6^T and 76^T formed only few nodules and did not promote plant growth compared with the negative control (Table 3). Similarly, inoculation with isolates ERR 308, ERR 309, ERR 421, ERR 430, ERR 377, ERR 305, ERR 299, ERR 344, and ERR 380 did not increase root or shoot dry matter compared with the control, although some nodulation and significant effects on some of the measured variables were observed. In contrast, isolates ERR 326, ERR 399, ERR 435, ERR 396, ERR 329, ERR 298, ERR 417, ERR 314, and ERR 412 significantly promoted accumulation of plant biomass which was similar to the treatment

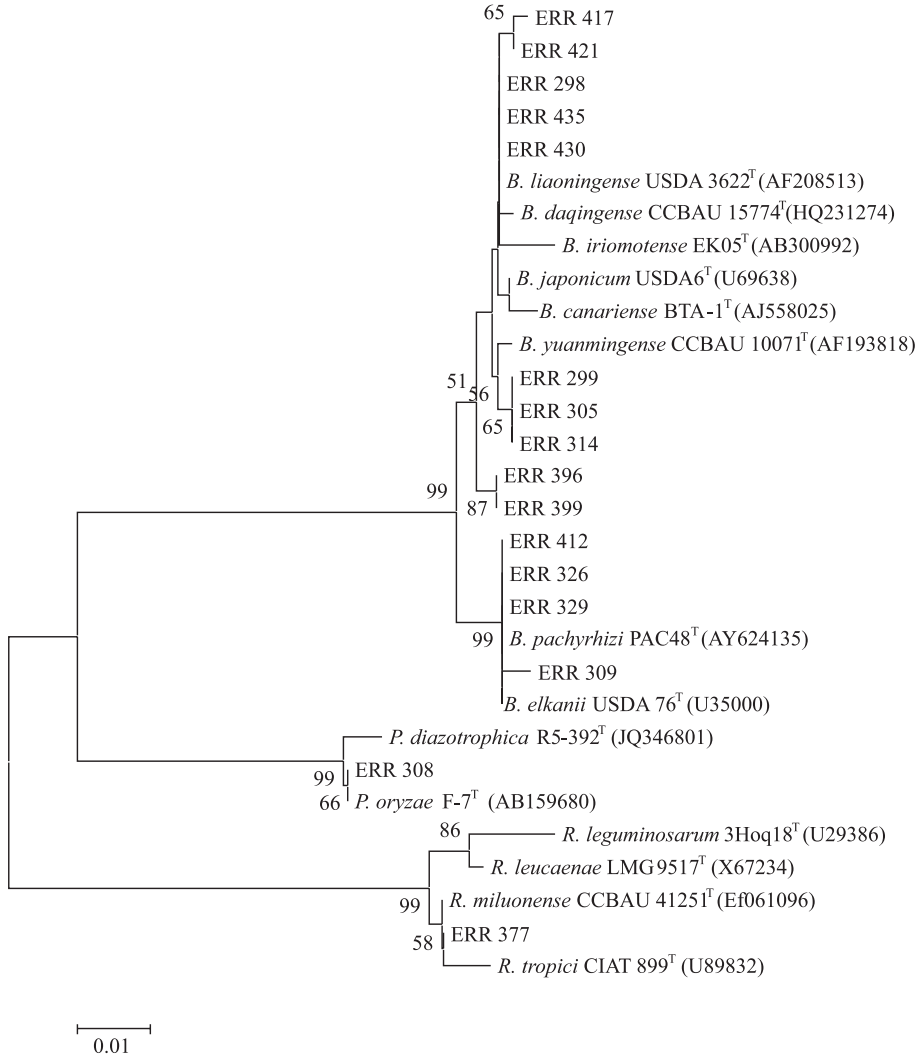

Figure 1. Neighbour-joining phylogeny, based on 16S rRNA gene sequences showing the relationships among rhizobium isolates obtained from *Centrolobium paraense* nodules (shown in bold) and other rhizobium type strains. Bootstrap values greater than 50% are indicated at the nodes. Bar indicates one substitution per 100 nucleotide positions.

receiving mineral nitrogen. By nodulation and plant development results, it can be inferred a wide functional variability among rhizobia isolates for growth promotion in *C. paraense* plants, with the occurrence of high and low efficiency, irrespectively of the sample site (Table 2). There is an established population of rhizobia in the soils of the Roraima Cerrado, with efficient isolates that can contribute significantly to the development of *C. paraense*. Similarly, studies conducted with *C. tomentosum* have also shown variability in the symbiotic efficiency of native strains, and selected strains improved seedling development and plant establishment (Marques et al., 2001; Pagano, 2008).

Among the efficient isolates, ERR 326, ERR 399, and ERR 435 had more pronounced growth promotion effects on *C. paraense* because in addition to higher nodulation (number of nodules higher than 180, and nodule dry matter higher than 320 mg per plant) and increasing biomass production (more than 7 g total dry matter, in 100 days), they also increase total N content in the shoots, leaf area, plant height and number of leaflets (Table 3). Therefore, as these three isolates belong to the genus *Bradyrhizobium* (indicated by 16S rDNA and *rpoB* gene), there is an indication that *Bradyrhizobium* strains are more efficient in BNF than fast-growing strains for *C. paraense*. These three isolates show high potential for use as inoculants for this host.

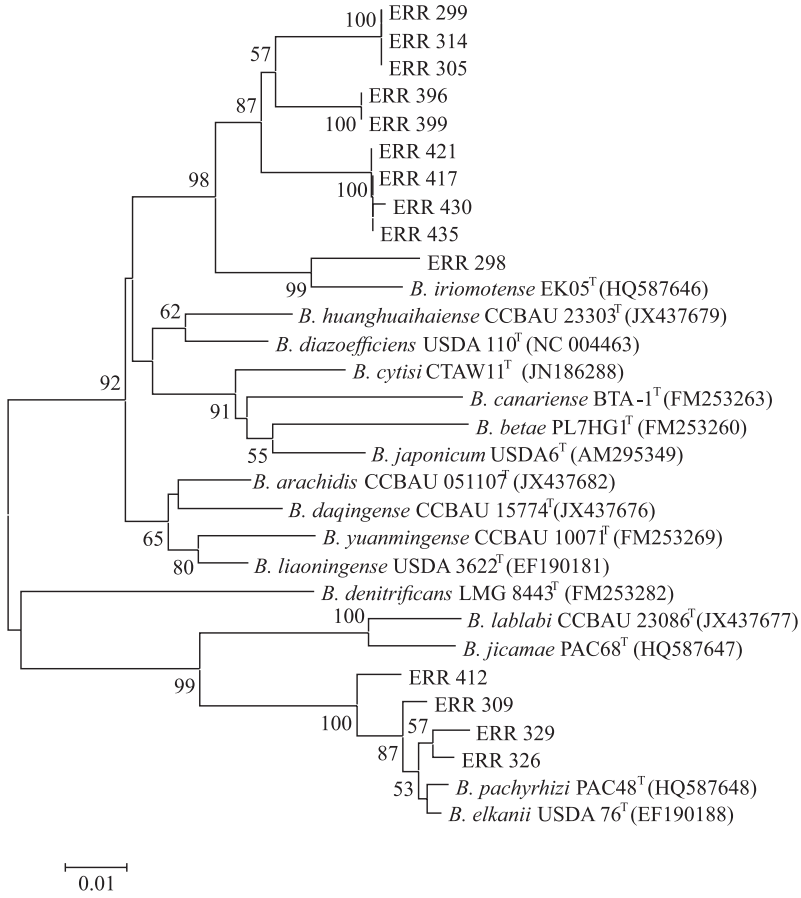

Figure 2. Neighbour-joining phylogeny, based on *rpoB* gene sequences showing the relationships among *Bradyrhizobium* sp. isolates obtained from *Centrolobium paraense* nodules (shown in bold) and other *Bradyrhizobium* type strains. Bootstrap values greater than 50% are indicated at the nodes. Bar indicates one substitution per 100 nucleotide positions.

Table 3. Shoot dry matter (SDM), root dry matter (RDM), number of nodules (NN), nodule dry matter (NDM), total N content in shoots (TNC), leaf area (LA), plant height (PH), and number of leaflets (NL) of pau-rainha (*Centrolobium paraense* Tul.) inoculated with soil-rhizobium isolates from the Roraima Cerrado, Brazil[(1)].

Site	Strain	SDM (g plant^{-1})	RDM (g plant^{-1})	NN (N° plant^{-1})	NDM (mg plant^{-1})	TNC (mg g^{-1})	LA (cm^2)	PH (cm)	NL (N° plant^{-1})
I	ERR 326	4.9a	2.7a	191a	326.3a	111.6a	1408.3a	17.3a	21.8a
III	ERR 399	4.7a	2.5a	212a	448.3a	110.7a	1268.6a	18.1a	21.7a
IV	ERR 435	4.7a	2.4a	187a	320.2a	105.8a	1224.1a	17.0a	19.5a
III	ERR 396	4.4a	2.2a	126b	359.0a	94.5b	1144.9b	19.5a	20.7a
I	ERR 329	4.3a	2.0a	159b	264.0b	98.4b	1196.6a	21.2a	21.8a
II	ERR 298	4.1a	2.1a	74c	225.4b	86.8b	1034.5b	18.9a	19.8a
IV	ERR 417	3.9a	1.9a	197a	265.0b	87.6b	1064.9b	19.2a	22.8a
I	ERR 314	3.8a	1.9a	238a	342.0a	82.0b	1135.9b	20.3a	18.2b
II	ERR 412	3.7a	1.8b	191a	295.1b	73.7c	1032.8b	17.0a	18.0b
I	ERR 308	3.4b	1.6b	133b	229.7b	82.9b	1035.2b	15.6b	20.5a
I	ERR 309	3.1b	1.5b	215a	350.8a	67.7c	1004.0b	17.7a	21.3a
IV	ERR 421	2.9b	1.4b	97c	153.1c	69.6c	832.3c	15.5b	16.0b
IV	ERR 430	2.9b	1.2b	97c	132.1c	68.3c	935.7b	15.1b	19.3a
III	ERR 380	2.7b	1.8b	67c	106.0c	37.1d	726.1c	16.2a	14.6b
II	ERR 344	2.8b	1.6b	26d	104.0c	51.1c	799.0c	13.9b	17.3b
III	ERR 377	2.5b	1.5b	3d	15.3d	37.5d	670.7c	11.8b	15.1b
I	ERR 305	2.3b	1.0b	122b	155.3c	56.3c	808.0c	15.3b	18.2b
I	ERR 299	2.3b	1.3b	113b	168.1c	52.2d	658.0c	12.9b	16.8b
-	USDA 6^T	2.4b	1.5b	7d	32.1d	36.9d	654.9c	11.4b	15.2b
-	USDA 76^T	2.0b	1.2b	2d	8.9d	30.8d	571.3c	9.8b	14.7b
-	Control	2.4b	1.4b	-	-	38.9d	614.0c	12.3b	13.1b
-	Nitrogen	5.3a	2.6a	-	-	129.9a	1348.2a	20.8a	23.3a
CV (%)		20.69	22.39	25.89	29.70	23.31	15.87	18.00	17.44

[(1)]Means followed by equal letters within columns do not differ, by Scott-Knott test, at 5% probability.

Conclusions

1. *Centrolobium paraense* is able to nodulate with different rhizobia species, including some not yet described.

2. Rhizobia belonging to the genus *Bradyrhizobium* are the most common root-nodulating-bacteria for *C. paraense*.

3. Some rhizobia isolated from *C. paraense* exhibit high biological nitrogen fixation efficiency for this host.

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