Rhizobium paranaense sp. nov., an effective N_2-fixing symbiont of common bean (*Phaseolus vulgaris* L.) with broad geographical distribution in Brazil

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Nitrogen (N), the nutrient most required for plant growth, is key for good yield of agriculturally important crops. Common bean (*Phaseolus vulgaris* L.) can benefit from bacteria collectively called rhizobia, which are capable of fixing atmospheric nitrogen (N_2) in root nodules and supplying it to the plant. Common bean is amongst the most promiscuous legume hosts; several described species, in addition to putative novel ones have been reported as able to nodulate this legume, although not always effectively in terms of fixing N_2. In this study, we present data indicating that Brazilian strains PRF 35^T, PRF 54, CPAO 1135 and H 52, currently classified as *Rhizobium tropici*, represent a novel species symbiont of common bean. Morphological, physiological and biochemical properties differentiate these strains from other species of the genus *Rhizobium*, as do BOX-PCR profiles (less than 60 % similarity), multilocus sequence analysis with *recA*, *gyrB* and *rpoA* (less than 96.4 % sequence similarity), DNA–DNA hybridization (less than 50 % DNA–DNA relatedness), and average nucleotide identity of whole genomes (less than 92.8.%). The novel species is effective in nodulating and fixing N_2 with *P. vulgaris*, *Leucaena leucocephala* and *Leucaena esculenta*. We propose the name *Rhizobium paranaense* sp. nov. for this novel taxon, with strain PRF 35^T (=CNPSo 120^T=LMG 27577^T=IPR-*Pv* 1249^T) as the type strain.

Agricultural soils are frequently damaged by intense management and stressful climate conditions, leading to nutrient impoverishment and loss of biological activity. Since nitrogen (N) is an essential nutrient for crops, its deficiency in soil can negatively affect crop growth. Symbiotic diazotrophic bacteria known as rhizobia can fix atmospheric N_2 in the root nodules of legumes, supplying a large proportion of the host plant's nitrogen needs. Besides environmental benefits, the application of these bacteria as inoculants can promote significant economy in N-fertilizer use (Ormeño-Orrillo *et al.*, 2013). Important commercial crops such as common bean (*Phaseolus vulgaris* L.) possess the ability to associate with a variety of diazotrophic bacteria within the genus *Rhizobium*, including species effective in fixing N_2 (Fix^+) (*Rhizobium leguminosarum* sv. phaseoli, *Rhizobium phaseoli*, *Rhizobium tropici*, *Rhizobium*

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Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession number of the sequences obtained in this work are in supplementary table S1.

Three supplementary figures and six supplementary tables are available with the online version of this paper.

etli, *Rhizobium leucaenae*, *Rhizobium giardinii* sv. phaseoli, *Rhizobium gallicum*, *Rhizobium lusitanum*, *Rhizobium pisi*, *Rhizobium freirei*, *Rhizobium mesoamericanum*), as well as Fix$^-$ species (*R. giardinii* sv. giardinii and *Rhizobium miluonense*).

All strains used in commercial inoculants in Brazil with common bean belong to *R. tropici* [SEMIA 4077^T (=CIAT 899^T) and SEMIA 4088 (=H 12)] or *R. freirei* [SEMIA 4080^T (=PRF 81^T)], and show genetic stability and high tolerance of stressful environmental conditions (Hungria *et al.*, 2000, 2003; Dall'Agnol *et al.*, 2013). In our studies, we have identified other strains, including PRF 35^T, PRF 54, CPAO 1135 and H 52, which show high efficiency in N_2 fixation with common bean, and are classified as *R. tropici* (Hungria *et al.*, 2000, 2003; Mostasso *et al.*, 2002; Pinto *et al.*, 2007). However, our research group has accumulated evidence to indicate that these strains represent a distinct species (Mostasso *et al.*, 2002; Chueire *et al.*, 2003; Pinto *et al.*, 2007; Ribeiro *et al.*, 2009).

The four above-mentioned strains composed a group by cluster analysis of BOX-PCR genomic fingerprints, with less than 60 % similarity with other *R. tropici*-related species (Fig. S1, available in the online Supplementary Material). The four strains have been isolated from disparate regions of Brazil: PRF 35^T and PRF 54 are from Paraná State in the south (Hungria *et al.*, 2000; Cardoso *et al.*, 2012); H 52 is from the Cerrados region in central-western Brazil (Mostasso *et al.*, 2002), and CPAO 1135 is from Mato Grosso do Sul State, in the western region (Pinto *et al.*, 2007) (Table 1). Strains from this group are able to nodulate and establish effective N_2-fixing symbioses with *P. vulgaris*, *Leucaena leucocephala* and *Leucaena esculenta*; form pseudonodules on *Calopogonium mucunoides* and *Macroptilium atropurpureum*; and are unable to nodulate *Centrosema pubescens*, *Lupinus albus*, *Medicago sativa* and *Vigna unguiculata* (Hungria *et al.*, 2000 and this study). Due to their potential agronomic and environmental importance, we performed a polyphasic analysis to demonstrate that these four strains show considerable genetic and phenotypic

Table 1. Strains used in this study

Strain	Host species	Geographical origin	Reference
Rhizobium paranaense sp. nov.			
PRF 35^T (=CNPSo 120^T=LMG 27577^T=IPR-*Pv* 1249^T)	*Phaseolus vulgaris*	Paraná, Brazil	Hungria *et al.* (2000); Cardoso *et al.* (2012)
PRF 54 (=CNPSo 121)	*P. vulgaris*	Paraná, Brazil	Hungria *et al.* (2000)
CPAO 1135 (=CNPSo 234)	*P.vulgaris*	Mato Grosso do Sul, Brazil	Pinto *et al.* (2007)
H 52 (=CNPSo 731)	*P.vulgaris*	Goiás, Brazil	Mostasso *et al.* (2002)
Rhizobium leucaenae CFN 299^T (=USDA 9039^T=LMG 9517^T =UMR1026^T=CENA 183^T=SEMIA 4083^T=CNPSo 141^T)	*P. vulgaris*	Brazil	Ribeiro *et al.* (2012)
Rhizobium tropici CIAT 899^T (=USDA 9030^T=ATCC 49672^T=UMR 1899^T=TAL 1797^T=HAMBI 1163^T=CM0^T=SEMIA 4077^T=DSM 11418^T=BR 322^T =CNPSo 142^T)	*P. vulgaris*	Colombia	Martínez-Romero *et al.* (1991)
Rhizobium rhizogenes ATCC 11325^T (=DSM 30148^T=LMG 150^T=NBRC 13257^T=IAM 13570^T=CNPSo 1976^T)			Velázquez *et al.* (2010)
Rhizobium hainanense CCBAU 57015^T(=I66^T=BCRC 15793^T=CCRC 15793^T=CECT 4658^T=DSM 11917^T=ICMP 13690^T=LMG 18074^T=USDA 3588^T=CNPSo 2450^T)	*Desmodium sinuatum*	Hainan, China	Chen *et al.* (1997)
Rhizobium lusitanum P1-7^T (=CECT 7016^T=CIP 109524^T =LMG 22705^T=CNPSo 2055^T)	*P. vulgaris*	Arcos de Valdevez, Portugal	Valverde *et al.* (2006)
Rhizobium multihospitium CCBAU 83401^T (=HAMBI 2975^T =LMG 23946^T=LMG 24298^T=CNPSo 2054^T)	*Halimodendron halodendron*	Xinjiang, China	Han *et al.* (2008)
Rhizobium miluonense CCBAU 41251^T (=HAMBI 2971^T =LMG 24208^T=CNPSo 2056^T)	*Lespedeza chinensis*	Hunan, China	Gu *et al.* (2008)
Rhizobium calliandrae CCGE524^T (=ATCC BAA-2435^T =CIP 110456^T=LBP2-1^T=CNPSo 2466^T)	*Calliandra grandiflora*	Chiapas, Mexico	Rincón-Rosales *et al.* (2013)
Rhizobium mayense CCGE526^T (=CIP 110454^T =NSJP1-1^T=CNPSo 2464^T)	*C. grandiflora*	Chiapas, Mexico	Rincón-Rosales *et al.* (2013)
Rhizobium jaguaris CCGE525^T (=ATCC BAA-2445^T =CIP 110453^T=SJP1-2^T=CNPSo 2465^T)	*C. grandiflora*	Chiapas, Mexico	Rincón-Rosales *et al.* (2013)
Rhizobium freirei PRF 81^T(=SEMIA 4080^T=IPR-*Pv*81^T; WDCM 440^T=CNPSo 122^T)	*P. vulgaris*	Paraná, Brazil	Dall'Agnol *et al.* (2013)

dissimilarities with known species of the genus *Rhizobium*, and therefore should be considered a distinct taxon.

16S rRNA gene sequences were retrieved from the GenBank database, or obtained at Embrapa's Soil Biotechnology Laboratory (Table S1) with primers and conditions specified in Table S2. Alignments and phylogenies were obtained with MEGA 5.1 software, using the maximum-likelihood (Felsenstein, 1981) algorithm, as suggested by Tindall *et al.* (2010), as well as the neighbour-joining (Saitou & Nei, 1987) algorithm and Kimura two-parameter (K2P) distances (Kimura, 1980). The 16S rRNA gene phylogeny based on the maximum-likelihood algorithm showed that strains PRF 35^T, PRF 54, CPAO 1135 and H 52 fall within a clade formed by eleven species of the '*Rhizobium tropici* group' (*R. tropici*, *R. leucaenae*, *R. lusitanum*, *R. multihospitium*, *R. miluonense*, *R. hainanense*, *R. calliandrae*, *R. mayense*, *R. jaguaris*, *R. rhizogenes* and *R. freirei*) (Ribeiro *et al.*, 2012; Dall'Agnol *et al.*, 2013) (Fig. 1). The phylogeny was confirmed with the neighbour-joining algorithm (Fig. S2). The levels of 16S rRNA gene sequence similarity among these four strains ranged from 99.3 % to 99.9 %, overlapping the range between distinct species within this group (99.1–99.8 %) (Table S3). As many authors have verified, phylogenetic analysis based exclusively on the 16S rRNA gene may not reflect the correct taxonomic position of a strain at the species level (Wang & Martínez-Romero, 2000; Coenye *et al.*, 2001; Martens *et al.*, 2007; Menna *et al.*, 2009). For example, we have recently reported that *R. freirei* and *R. multihospitium* have identical 16S rRNA gene sequences (Dall'Agnol *et al.*, 2013). According to Vandamme *et al.* (1996), different species should show less than 97 % of nucleotide identity in the 16S rRNA gene, which does not occur within any of the type strains used in our study.

To clarify the relationships among strains PRF 35^T, PRF 54, CPAO 1135 and H 52 and other species of the genus *Rhizobium*, a multilocus sequence analysis (MLSA) was performed. Sequences of three housekeeping genes, *gyrB*, *recA* and *rpoA*, were obtained from the GenBank database or at Embrapa's Biotechnology Laboratory using previously described primers and conditions (Table S2). The phylogenetic tree was reconstructed as described for the 16S rRNA gene, and congruence between the three housekeeping genes was confirmed before they were concatenated into a unique sequence. Fig. 2 shows the result of the three-gene

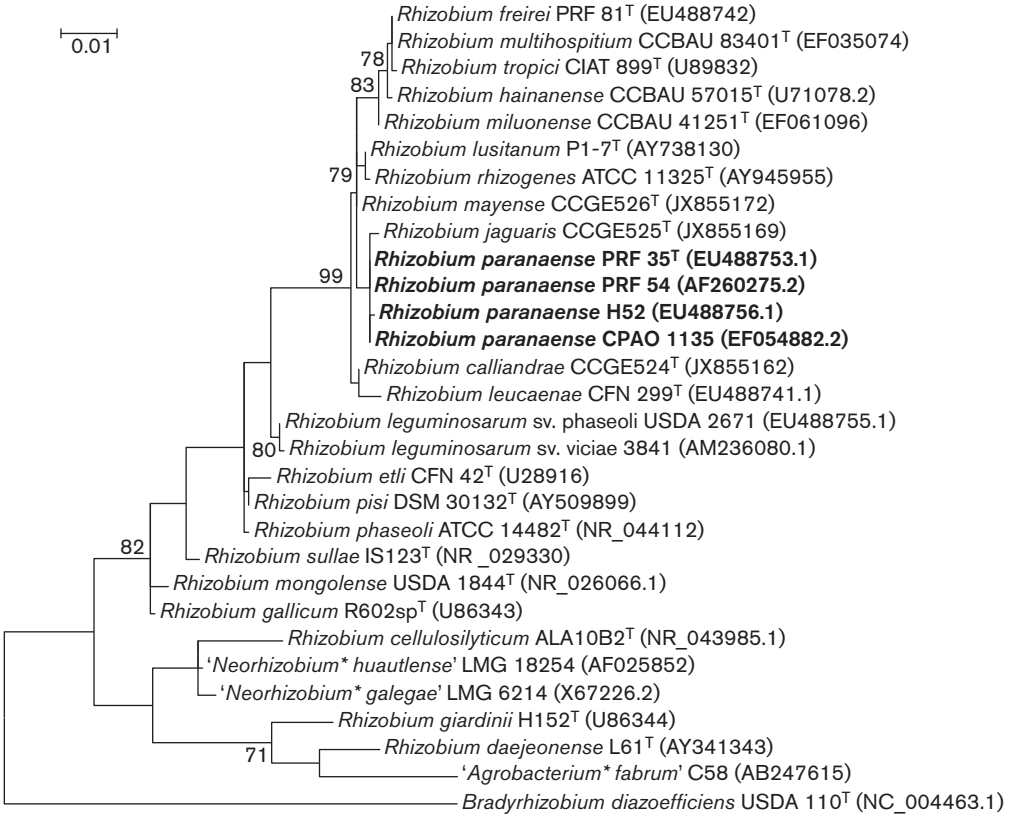

Fig. 1. Maximum-likelihood 16S rRNA gene phylogeny based on gene sequences from *Rhizobium paranaense* sp. nov. and other species of the genus *Rhizobium*. Bootstrap support values based on 1000 resamplings are shown at nodes only when they were ⩾70 %. Bar, percentage of nucleotide substitutions. Asterisks indicate that according to the recent proposal by Mousavi *et al.* (2014), *R. galegae* and *R. huautlense* should be reclassified in the new genus '*Neorhizobium*' and some species should return to the genus *Agrobacterium*.

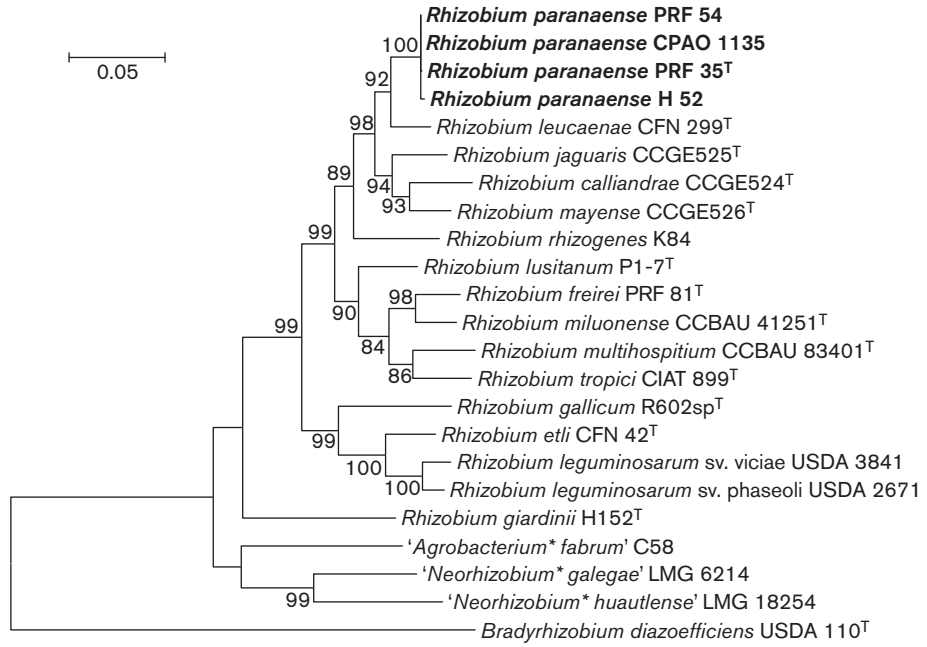

Fig. 2. Maximum-likelihood phylogeny based on concatenated alignment of *gyrB*, *recA* and *rpoA* gene sequences from *R. paranaense* sp. nov. and closely related species of the genus *Rhizobium*. Bootstrap support values based on 1000 resamplings are shown at nodes only when they were ⩾70 %. Bar, percentage of nucleotide substitutions. Asterisks indicate that according to the recent proposal by Mousavi *et al.* (2014), *R. galegae* and *R. huautlense* should be reclassified in the new genus '*Neorhizobium*' and some species should return to the genus *Agrobacterium*.

concatenated phylogenetic analysis, where strains PRF 35^T, PRF 54, CPAO 1135 and H 52 were placed into a single, highly supported group in the tree built with the maximum-likelihood algorithm; a similar tree was obtained with the neighbour-joining algorithm (Fig. S3). Nucleotide identities of the concatenated sequences among the strains ranged from 99.7–99.9 % (Table S3). Strains PRF 35^T, PRF 54, CPAO 1135 and H 52 were part of a subclade including *R. leucaenae*, *R. jaguaris*, *R. mayense* and *R. calliandrae*, all belonging to the '*R. tropici* group'. *R. hainanense* was not included in the MLSA, but similarities of *gyrB* and *recA* sequences were lower than with species closer to strain PRF 35^T (Table S3). *R. leucaenae* was the closest species to strains PRF 35^T, PRF 54, CPAO 1135 and H 52 (Fig. 2), with a nucleotide identity of 96.4 % with strain PRF 35^T (Table S3). The concatenated tree is also supportive of the recent proposal of reclassification of *R. huautlense* and *R. galegae* in the new genus '*Neorhizobium*', and of *R. radiobacter* as a member of the genus *Agrobacterium*; it is also indicative that *R. giardinii* might represent a new genus (Mousavi *et al.*, 2014).

To support the distinctiveness of the group of four strains from other related species, DNA–DNA hybridization (DDH) experiments (Martínez-Romero *et al.*, 1991) were performed between strain PRF 35^T and all type strains of the '*R. tropici* group'. The DNA–DNA relatedness value obtained was less than 50 % with all type strains evaluated (Table S4), confirming that the group of four investigated strains correspond to a novel species of the genus *Rhizobium*.

Average nucleotide identity (ANI) of genome sequences has been proposed as an alternative to DDH in prokaryotic taxonomy with an ANI of 95–96 % corresponding to 70 % DDH (Richter & Rosselló-Móra, 2009; Konstantinidis *et al.*, 2006). The genome sequences of strain PRF 35^T (a 20-fold coverage, our unpublished results), *R. tropici* CIAT 899^T, *R. freirei* PRF 81^T (Ormeño-Orrillo *et al.*, 2012), *R. rhizogenes* K84 (Slater *et al.*, 2009) and *R. leucaenae* CFN 299^T (a 20-fold coverage, our unpublished results) were used to calculate ANI values using JSpecies (Richter & Rosselló-Móra, 2009) and mummer for sequence alignment. These species represent the diversity found within the '*R. tropici* group' (Fig. 2). Strain PRF 35^T had ANI values of 92.7 %, 86.7 %, 86.5 % and 86 % with *R. leucaenae* CFN 299^T, *R. freirei* PRF 81^T, *R. rhizogenes* K84 and *R. tropici* CIAT 899^T, respectively, all below the species circumscription threshold.

To determine the DNA G+C content of strain PRF 35^T, genome contigs were concatenated and the proportion of G+C bases was calculated with BioEdit (Hall, 1999). The genomic DNA G+C content of strain PRF 35^T was 59.1 mol%, which is within the range reported for species of the genus *Rhizobium* (Jordan, 1984).

Phenotypic characterization of the four investigated strains was performed as described previously (Hungria *et al.*, 2001; Ribeiro *et al.*, 2012). The morpho-physiological tests

Table 2. Distinctive phenotypic features of *Rhizobium paranaense* sp. nov. and phylogenetically related species of the '*Rhizobium tropici* group'

Taxa: 1, PRF 35^T; 2, *R. leucaenae*; 3, *R. tropici*; 4, *R. lusitanum*; 5, *R. miluonense*; 6, *R. multihospitium*; 7, *R. hainanense*; 8, *R. rhizogenes*; 9, *R. calliandrae*; 10, *R. mayense*; 11, *R. jaguaris*; 12, *R. freirei*. +, Growth, −, no growth; W, weak growth*; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Colony characteristics												
Morphology on YMA	Opaque, gummy	Opaque, dry	Translucent, gummy	Opaque, gummy	Opaque, gummy	Opaque, gummy	Translucent, gummy	Translucent, gummy	Translucent, gummy	Opaque, gummy	Opaque, gummy	Opaque, gummy
Colour on YMA	White	Rose	White	Light rose	White	White	White	Rose	Light rose	Rose	Rose	Rose
Elevation	Convex	Flat	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
Growth in/at:												
PY without Ca	−	−	+	+	+	+	+	+	−	−	−	W
LB	−	−	+	+	+	−	−	+	−	−	−	W
TY 40 °C	+	W	+	−	−	−	−	+	−	−	−	+
TY pH 4	+	W	+	−	+	−	ND	−	−	−	−	+
Carbon source utilization												
Glycerol	+	+	+	+	+	+	+	W	+	W	+	+
Erythritol	+	−	+	+	+	+	+	+	+	+	+	+
D-Arabinose	+	+	+	W	+	+	+	+	W	W	W	W
D-Xylose	+	+	+	+	+	+	+	W	W	W	W	W
L-Xylose	+	+	−	+	+	+	+	+	W	W	W	+
D-Adonitol	+	+	+	+	W	+	+	+	W	W	W	+
Methyl β-D-xylopyranoside	W	+	+	+	+	+	+	+	W	+	W	W
D-Glucose	+	+	+	+	+	+	+	W	+	W	W	+
D-Fructose	+	+	+	+	+	+	+	W	+	W	W	W
D-Mannose	+	+	+	+	+	+	+	W	+	W	W	+
L-Sorbose	−	−	−	W	+	W	W	W	−	−	−	W
L-Rhamnose	+	+	+	+	+	+	+	W	W	+	+	W
Dulcitol	−	W	−	−	W	W	W	W	W	−	−	−
Inositol	+	+	+	+	+	+	W	W	W	W	W	W
D-Mannitol	+	+	+	W	+	+	+	W	+	+	W	W
D-Sorbitol	−	−	−	W	+	W	+	W	−	W	W	W
Methyl α-D-mannopyranoside	−	−	−	W	−	W	−	−	−	−	−	−
Methyl α-D-glucopyranoside	W	−	+	W	+	+	+	+	W	W	W	W
N-Acetylglucosamine	W	−	−	−	+	+	W	+	−	W	−	+
Amygdalin	−	−	−	W	W	W	+	+	−	W	−	−
Arbutin	W	−	W	+	+	+	+	+	W	W	W	+
Salicin	+	−	W	+	+	+	W	+	W	W	W	+

Table 2. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cellobiose	+	W	+	+	+	+	+	+	+	+	W	W
Maltose	+	W	+	+	+	+	+	W	W	W	W	W
Lactose	+	W	W	+	+	+	W	W	W	W	W	W
Melibiose	W	W	W	+	+	+	W	W	W	W	W	W
Sucrose	W	W	+	+	+	+	W	+	W	W	W	W
Trehalose	W	W	+	+	+	+	+	+	W	W	W	W
Melezitose	+	−	−	−	−	−	W	−	−	−	−	−
Raffinose	W	W	W	+	+	+	W	W	W	W	W	W
Glycogen	+	−	−	+	+	+	+	+	+	+	+	+
Xylitol	W	−	−	W	+	W	+	W	−	W	−	W
Gentiobiose	+	W	W	+	+	+	+	+	W	W	W	W
Turanose	W	W	W	W	+	+	+	W	W	W	W	W
D-Tagatose	−	W	−	W	+	W	W	+	W	−	−	W
D-Arabitol	+	+	+	W	+	+	+	W	W	W	W	W
L-Arabitol	−	−	W	W	W	W	+	+	−	−	−	+
Resistance to:												
Nalidixic acid (30 μg)	+	+	+	+	+	+	−	W	+	+	+	+
Ampicillin (10 μg)	−	−	+	−	+	+	−	−	−	−	−	W
Chloramphenicol (30 μg)	−	−	+	−	+	−	W	+	W	−	−	−
Cefuroxime (30 μg)	−	−	+	+	+	+	−	−	−	−	−	+
Erythromycin (15 μg)	+	+	+	+	+	+	+	+	+	+	−	+
Streptomycin (10 μg)	−	−	+	−	W	W	W	+	−	−	−	−
Neomycin (30 μg)	−	−	−	W	−	W	−	W	W	+	W	W
Penicillin (10U)	+	−	+	+	+	+	+	+	+	W	W	+
Tetracycline (30 μg)	−	−	−	−	−	−	−	−	−	−	−	−
Enzyme activity												
Urease	+	W	ND	ND	ND	ND	ND	ND	-	W	+	+

*Growth was considered weak when optical density was between 20 and 59 % of the full growth in liquid medium, or with the light green colour in the API 50CH kit.

included colony morphology, growth characteristics, utilization of different carbon sources, tolerance of antibiotics and urease activity in YM medium with red phenol (Vincent, 1970). The strains used as comparisons are shown in Table 1. The phenotypes of strain PRF 35^T were compared with those of strains from the '*R. tropici* group', including *R. leucaenae*, *R. tropici*, *R. lusitanum*, *R. miluonense*, *R. multihospitium*, *R. hainanense*, *R. rhizogenes*, *R. calliandrae*, *R. mayense*, *R. jaguaris* and *R. freirei* (Table 2) and to other common-bean *Rhizobium* symbionts (Table S5). The results showed that strain PRF 35^T can be distinguished from *R. leucaenae*, the closest species by MLSA, in mucus production, colony colour on YMA medium, tolerance of penicillin, growth at 40 °C and at pH 4, and in 15 out of the 49 carbon sources tested with the API 50CH kit (Table 2). It also differs from *R. tropici*, the species it is usually classified as representing, in colony morphology, growth in LB medium and PY medium without Ca, tolerance of ampicillin, chloramphenicol, cefuroxime and streptomycin, and the pattern of utilization of 13 carbon sources. In relation to *R. jaguaris*, also a close species in the 16S rRNA gene phylogeny, it differs in colony morphology, tolerance of erythromycin, neomycin and penicillin, and in the pattern of utilization of 19 carbon sources (Table 2).

Fatty acid profiles were obtained with the MIDI system using FAME library TSBA6 from a culture grown for 5 days at 28 °C on YMA plates. Like many species within the genus *Rhizobium*, the four investigated strains presented summed feature 8 ($C_{18:1}\omega7c/\omega6c$) as major fatty acids (Tighe *et al.*, 2000), but at a higher proportion than other species of the '*R. tropici* group', except for, *R. leucaenae* and *R. freirei*. (Table S6). Other fatty acids can also be used to distinguish the group of four investigated strains from other species due to differences in relative amounts. Strains PRF 35^T, PRF 54, CPAO 1135 and H 52 had lower proportions of $C_{16:0}$ than *R. rhizogenes* and *R. hainanense*; they also had higher amounts of $C_{19:0}$ cyclo $\omega8c$ than *R. calliandrae*, *R. jaguaris* and *R. freirei*, but lower amounts than *R. leucaenae*, *R. tropici*, *R. lusitanum*, *R. multihospitium* and *R. rhizogenes* (Table S6).

The genotypic, phenotypic and phylogenetic data presented in this work indicate that strains PRF 35^T, PRF 54, CPAO 1135 and H 52 represent a homogeneous group distinct from all other species described in the genus *Rhizobium*. Hence, we propose the name *Rhizobium paranaense* sp. nov. to accommodate this group of strains.

Description of *Rhizobium paranaense* sp. nov.

Rhizobium paranaense (pa.ra.na.en′se. N.L. neutr. adj. *paranaense* of or belonging to Paraná. Named after Paraná State, where our research group is established and where the type strain was isolated).

Cells are Gram-reaction-negative, aerobic, non-spore-forming rods. Colonies on YMA medium are circular convex, white, opaque, with abundant production of mucus and usually 3–5 mm in diameter within 2–3 days of incubation at 28 °C. Acidifies YMA medium after 3 days. Can tolerate 40 °C. Grows at pH 4, but not in PY medium without Ca and in LB medium. Sensitive to ampicillin (10 μg), neomycin (30 μg), chloramphenicol (30 μg), streptomycin (10 μg), cefuroxime (30 μg) and tetracycline (30 μg), and resistant to nalidixic acid (30 μg), erythromycin (15 μg) and penicillin (10 U). Utilizes glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, D-mannitol, salicin, cellobiose, maltose, lactose, melezitose, glycogen, gentiobiose, D-lyxose, D-fucose, L-fucose and D-arabitol as carbon sources; weakly uses methyl β-D-xylopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, arbutin, melibiose, sucrose, trehalose, raffinose, xylitol and turanose. Induces the formation of root nodules and is effective in fixing N_2 with *Phaseolus vulgaris*, *Leucaena leucocephala* and *Leucaena esculenta*.

The type strain is PRF 35^T (=CNPSo 120^T=LMG 27577^T=IPR-*Pv* 1249^T) isolated from an effective nodule of *P. vulgaris* collected in Paraná State, Brazil. The DNA G+C content of the type strain is 59.1 mol%.

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