Bradyrhizobium neotropicale sp. nov. isolated from effective nodules of *Centrolobium paraense.*

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Title

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Short title

Bradyrhizobium neotropicale sp. nov.

Contents category

New taxa

Subsection

Proteobacteria

Jerri E. Zilli[1], Alexandre C. Baraúna[2], Krisle da Silva[3], Sofie E. De Meyer[4,5], Eliane N. C. Farias[3], Paulo E. Kaminski[3], Ismaele B. da Costa[3], Julie K. Ardley[4], Anne Willems[5], Natália N. Camacho[1], Fernanda dos S. Dourado[1] and Graham O'Hara[4]

[1]Embrapa Agrobiologia, Rodovia BR 465 km 07, Seropédica, Rio de Janeiro 23891-000, Brazil.

[2]Universidade Federal Rural do Rio de Janeiro, Rodovia BR 465 km 07, Seropédica, Rio de Janeiro 23890-000, Brazil.

[3]Embrapa Roraima, Rodovia BR 174 km 08, Boa Vista, Roraima 69301-970, Brazil.

[4]Centre for Rhizobium Studies, Murdoch University, 90 South Street, Murdoch 6150, Western Australia, Australia.

[5]Laboratory of Microbiology, Department of Biochemistry and Microbiology (WE10), Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium.

***Corresponding author**

e-mail: jerri.zilli@embrapa.br

Phone: ++ 55 21 3441-1611

Fax: ++ 55 21 2682-1230

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, ITS 16S-23S rRNA, *dnaK*, *glnII*, *gyrB recA*, *rpoB*, *nodC* and *nifH* gene sequences of *Bradyrhizobium neotropicale* sp. nov. BR 10247^T are KF927051, KJ661686, KJ661693, KJ661700, KJ661707, KJ661714, KF983829, KJ661727 and KJ661728, respectively. The accession numbers for all other strains are listed in Table S2.

Abstract

Root nodule bacteria were isolated from *Centrolobium paraense* Tul. grown in soils from the Amazon region, State of Roraima (Brazil). The 16S rRNA gene sequence analysis of seven strains (BR 10247^T, BR 10296, BR 10297, BR 10298, BR 10299, BR 10300 and BR 10301) placed them into the genus *Bradyrhizobium* with the closest neighbouring species *B. paxllaeri* (98.8%), *B. icense* (98.8%), *B. lablabi* (98.7%), *B. jicamae* (98.6%), *B. elkanii* (98.6%), *B. pachyrhizi* (98.6%) and *B. retamae* (98,3%). This high similarity, however, was not confirmed by the ITS 16S-23S rRNA analysis nor by MLSA. Phylogenetic analyses of five housekeeping genes (*dnaK*, *glnII*, *gyrB*, *recA* and *rpoB*) revealed *Bradyrhizobium iriomotense* strain EK05^T (=LMG 24129^T) to be the closest type strain (95.7% sequence similarity or less). Chemotaxonomic data,

including fatty acid profiles (with majority being $C_{16:0}$ and Summed Feature 8 ($C_{18:1}$ w7c), DNA G+C content (% mol), the slow growth rate and carbon compound utilization patterns supported the placement of our strains in the genus *Bradyrhizobium*. Results of DNA-DNA relatedness and physiological data (especially C source utilization) differentiated our strains from the closest validly named *Bradyrhizobium* species. Symbiosis-related genes for nodulation (*nodC*) and nitrogen fixation (*nifH*) placed the new species in a new branch within the *Bradyrhizobium* genus. Based on the current data, these seven strains represent a novel species for which the name *Bradyrhizobium neotropicale* sp. nov. (BR 10247^T = HAMBI 3599^T) is proposed.

Centrolobium paraense Tul. (Leguminosae, Papilionoideae), tribe Dalbergieae, locally known as "pau-rainha", is a nodulating Neotropical leguminous tree occurring from the northern Brazilian Amazonia to Panama (Pirie *et al*., 2009). It grows in semi-deciduous forest, gallery forest, forest islands in cerrado and transition cerrado/forest, and has several ecological roles including nutrient input through symbiotic nitrogen fixation, protecting soils against erosion, and being a pioneer or early secondary plant (Marques *et al*., 2001; Dahmer *et al*., 2009). This species has also economic and social importance because its wood is used by indigenous communities and in the industry as timber or fuel (Dahmer *et al*., 2009; Pedreira, 2010).

C. paraense is capable of forming nodules with rhizobia native to the soil of Amazonia (Souza *et al.*, 1994; Baraúna, 2013) and bacteria belonging to the genera *Bradyrhizobium* and *Rhizobium* have been reported as symbionts of *Centrolobium* spp. However, previous studies have not identified the bacteria at species level (Moreira *et al.*, 1998; Pagano, 1995, Baraúna *et al.*, 2013).

A recent investigation of the ecology of root-nodulating-bacteria isolated from *C. paraense* grown in soils collected from different areas in Roraima State-Brazil found that about 90% of the 178 isolates exhibit phenotypic characteristics similar to species of *Bradyrhizobium* (Baraúna *et al.*, 2014). Analysis of partial 16S rRNA gene sequences confirmed these results, but placed these isolates in branches different from previously described species. Nine of the new isolates also show high efficiency in nitrogen fixation associated with *C. paraense* (Baraúna *et al.*, 2014).

Here we report the results from a polyphasic taxonomic study of seven isolates (BR 10247^{T}, BR 10296, BR 10297, BR 10298, BR 10299, BR 10300 and BR 10301). This polyphasic study included gene sequence analysis (16S rRNA, ITS, *dnaK*, *glnII*, *gyrB*, *recA*, *rpoB*, *nodC* and *nifH*), DNA-DNA relatedness, fatty acid profiling and phenotypic characterization. The strains were obtained from *C. paraense* grown in soil samples collected in the Mucajaí municipality-State of Roraima (2º 27 12.9 N; 60º 54 11.2 W) (Baraúna *et al.*, 2014). The climate in this region is classified as Aw (Köppen) with average rainfall of 1600 mm year^{-1} and an average temperature of 27°C (Araújo, *et al.*, 2001). The strains were deposited in the Diazothrophic Microbial Culture Collection -CRB-Johanna Döbereiner- (Embrapa Agrobiologia, Rio de Janeiro, Brazil); strain BR 10247^{T}, was also deposited at the Hambi Collection (http://www.helsink.fi/hambi) as HAMBI 3599^{T}. All strains were cultured on YMA medium (Fred & Waksman, 1928) at 28°C and for long-term storage the cultures were lyophilized and maintained at -80°C.

For PCR, genomic DNA was prepared using the Promega genomic DNA purification kit (cat. A1120), according to the manufacturer's instructions. Nearly full length sequences of the 16S rRNA gene (1336bp) were obtained for all strains using the primers and conditions described previously (Radl *et al.*, 2013). The intergenic transcribed spacer (ITS) sequences were also obtained for the new strains following the

99 conditions presented by Menna *et al.*, (2009). Sequence alignment, alignment editing
100 and phylogenetic analyses were performed using the MEGA5 software package
101 (Tamura *et al.*, 2011). Phylogenetic trees were constructed using the Maximum-
102 Likelihood (ML) (Felsenstein, 1981) reconstruction method as recommended by Tindall
103 *et al.* (2010). The strength of each topology was verified using 1000 bootstrap
104 replications and the ML trees are provided (Fig. 1 and Fig. 2).

105 The 16S rRNA phylogenetic analysis showed that the seven strains shared more
106 than 99.5% sequence similarity with each other and formed a separate branch within the
107 genus *Bradyrhizobium*, with *B. jicamae* PAC68^T as the closest neighbour (Fig. 1). The
108 16S rRNA gene sequence similarity between strain BR 10247^T and other
109 *Bradyrhizobium* type strains was between 96.0-98.8% (Table S1, available in IJSEM
110 online). Thus, even though the 16S rRNA is highly conserved in *Bradyrhizobium*
111 (Menna *et al.*, 2009, Willems *et al.*, 2001b), the analysis showed that our strains form a
112 separate branch compared to other species.

113 ITS phylogenetic analysis (891bp) showed less than 86.6% similarity between
114 our strains and other recognized *Bradyrhizobium* type strains, and the similarity
115 between our strains was greater than 98% (Table S1, available in IJSEM Online). The
116 ITS phylogenetic reconstruction placed the new strains in a different branch with
117 *B. iriomotense* EK05^T as the closest neighbor (Fig. 2). Previous studies have
118 demonstrated that ITS sequences are a suitable marker to separate *Bradyrhizobium*
119 species and 95.5% similarity value or more indicates strains belonging to the same
120 genospecies, corresponding to about 60% DNA: DNA hybridization (Willems *et al.*,
121 2001a, Willems *et al.*, 2003).

122 To confirm the ITS results we performed a Multi Locus Sequence Analysis
123 (MLSA), using housekeeping genes that have previously been used for *Bradyrhizobium*

species delimitation, and produce phylogenies that are supported by ITS sequence and DNA: DNA hybridization data (Rivas *et al.*, 2009; Menna *et al.*, 2009). We obtained sequences and performed the analyses for *dnaK* (238bp), *glnII* (534bp), *gyrB* (591bp), *recA* (418bp) and *rpoB* (408bp) genes following previous reports (Martens *et al.*, 2008; Rivas *et al.*, 2009; Menna *et al.*, 2009; Vinuesa *et al.*, 2005). Congruence between the different gene sequences was firstly checked using the partition homogeneity tests (Farris, *et al.*, 1994) performed with PAUP software v. 4.0b10 (Swofford, 2002). As congruence (p>0.01) was found only between the genes *gyrB*, *recA* and *rpoB*, the concatenation (performed by the software SeaView v. 4.0, Gouy *et al.*, 2010) was done for these three genes, and the other two genes (*dnaK* and *glnII*) were analysed individually.

The phylogenetic tree based on the concatenated sequences of the three genes confirmed our strains belonged to a monophyletic cluster with high bootstrap support (100%) (Fig. 3). Similar relationships were also obtained for the genes *dnaK* and *glnII* when individually analysed (Fig. S1 and S2, available in IJSEM Online). In addition, the sequence similarities between our strains were more than 99% for all investigated genes (Table S1, available in IJSEM Online). The closest type strain in the 16S rRNA analysis, *B. jicamae* PAC68^T, showed less than 90% similarity with strain BR 10247^T in MLSA. However, the closest type strain from the ITS analysis, *B. iriomotense* EK05^T, had a similarity between 92.6-95.7% for all five investigated genes in comparison with strain BR 10247^T.

It is interesting to emphasize that our strains presented a discordance in the 16S rRNA phylogenetic compared to ITS, which was also confirmed by MLSA analysis. While in the 16S rRNA gene analysis the closest strain was *B. jicamae*, closely related to *B. elkanii* (subgroup I), the ITS and concatenated MLSA tree placed our strains

149 together with *B. iriomotense*, belonging to subgroup II (Fig. 1, Fig 2 and Fig. 3). The
150 *Bradyrhizobium* subgroup division (I and II) is based on DNA: DNA hybridisation and
151 was used to separate the *B. japonicum* type strain from the *B. elkanii* type strain (Hollis
152 *et al.*, 1981; Willems *et al.*, 2001). The discordance found for our strains may indicate
153 either a lateral gene transfer or a gene recombination event, leading to a reticulate
154 evolutionary history (van Berkum *et al.*, 2003; van Berkum *et al.*, 2009; Parker *et al.*,
155 2003; Parker *et al.*, 2008).

156 BLAST search (Altschul *et al.*, 1990) revealed, in general, low similarity of the
157 sequences of our strains with deposited sequences. However, high similarity was
158 obtained with gene sequences from strain TUXTLAS-14 (16S rRNA, ITS, *dnaK*, *recA*
159 and *glnII*), isolated from *Vigna unguiculata* in Mexico (Ormeño-Orrillo *et al*., 2012)
160 and strain Cp5-3 (16S rRNA, *recA*), isolated from *Centrosema pubescens*, in Panama
161 (Parker, 2003), and both strains TUXTLAS-14 and Cp5-3 have a phylogenetic pattern
162 similar to our strains with a discordance between 16S rRNA and housekeeping genes
163 (Ormeño-Orrillo *et al*., 2012). This may indicate that strains belonging to this novel
164 *Bradyrhizobium* species are distributed from Northern Brazil to Panama or Mexico.

165 For phenotypic characterization, the strains were Gram stained and were
166 incubated for 7 days on YMA at different temperatures (15, 20, 25, 28, 30, 32, and
167 37°C), pH values (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0) and NaCl concentrations
168 (0.1, 0.3, 0.5, 1.0, 1.5, 2.0 and 2.5% (w/v). Cell motility was observed by light
169 microscopy of wet preparations of the strains grown in YM medium, and cell
170 morphology by transmission and scanning electron microscopy. Oxidase activity was
171 evaluated by touching a colony with a paper impregnated in 1% N,N,N′,N′-tetramethyl-
172 p-phenylenediamine solution and observing the colour change; catalase activity was

173 determined by flooding a colony with 10% (v/v) H_2O_2 and checking for the presence of
174 bubbles.

175 Other biochemical tests were performed by inoculating API 20NE strips
176 (BioMérieux, France) and Biolog GN2 microplates (Biolog Inc, CA, USA) according to
177 the manufacturer's instructions followed by incubation for 8 days at 28°C. The
178 antibiotic susceptibility tests were performed on YMA using the antibiotic Sensi-disc
179 dispenser system (Oxoid) with bio-discs (Oxoid) containing ampicillin (10 µg and 25
180 µg), chloramphenicol (30 µg and 50 µg), erythromycin (30 µg), gentamicin (10 µg),
181 kanamycin (30 µg), neomycin (10 µg), penicillin (10 µg), streptomycin (10 µg and 25
182 µg) and tetracycline (30 µg). The plates were incubated at 28°C and read after 10 days.

183 Discriminating phenotypic characteristics of our strains are given in Table 1 and
184 the details of carbon source utilization are presented in the Supplementary Table S3,
185 available in IJSEM Online. Our strains grew between 15 and 37 °C and in pH range 4.0
186 to 10.0, common characteristics within the *Bradyrhizobium* genus. The optimum growth
187 was verified at 28-32°C and pH 5-7 (Table 1). All strains were resistant to
188 chloramphenicol (50 µg) and sensitive to ampicillin (10 µg), penicillin (10 µg),
189 streptomycin (10 µg and 25 µg) and tetracycline (30 µg), while the closest type strain *B.*
190 *iriomotense* EK05^T showed penicillin and streptomycin resistance (Table 1). Enzymatic
191 reactions were positive for catalase, oxidase, urease, arginine dihydrolase and
192 hydrolysis of esculin, and negative for nitrate reduction, tryptophan deaminase, glucose
193 fermentation, hydrolysis of gelatine and β-galactosidase. The *Centrolobium* strains
194 differed from LMG 24129^T in β-galactosidase, arginine dihydrolase and nitrate
195 reduction (Table 1).

Whole-cell fatty acid methyl esters of strain BR 10247^T were extracted according to the MIDI protocol (http://www.microbialid.com/PDF/TechNote_101.pdf, (Delamuta *et al.*, 2013). Cultures were grown for 5 days at 28°C on YMA prior to extraction. The profiles were generated using a chromatograph Agilent model 6850 and identified using the TSBA database version 6.10 (Microbial Identification System - MIDI Inc.). The most abundant cellular fatty acids detected were $C_{16:0}$ (13.56%) and Summed Feature (SF) 8 ($C_{18:1}$ w7c) (66.52%). Moderate amounts of $C_{18:1}$ w7c 11-methyl (11.77%) and $C_{19:0}$ cyclo w8c (7.14%) and $C_{18:0}$ (1.04%) were also found. The presence of $C_{16:0}$ and SF 8 supports the placement of these strain in the genus *Bradyrhizobium* (Tighe *et al.*, 2000) and revealed some differences between BR 10247^T and *B. iriomotense* EK05^T, especially the higher abundance of $C_{16:0}$ (14.7%) and lower $C_{18:1}$ w7c (80.1%) as was first presented by Islam *et al.* (2008).

For DNA-DNA hybridization and determination of DNA G+C content, high-molecular weight DNA was prepared as described by Pitcher *et al.* (1989). DNA-DNA hybridizations were performed using a microplate method and biotinylated probe DNA (Ezaki *et al.,* 1989). The hybridization temperature was 50°C ± 1°C. Reciprocal reactions (A x B and B x A) were performed in triplicate for each DNA pair and their variation was within the limits of this method (Goris et *al.,* 1998). The DNA-DNA relatedness between BR 10247^T and the closest type strain *B. iriomotense* EK05^T was 63.8%, confirming that our strains belong to a new species, since the threshold recommended is 70% (Lindström & Gyllenberg, 2007; Tindall *et al.*, 2010). The G+C content of DNA was determined by HPLC according to the method of Mesbah *et al.* (1989) using a Waters Breeze HPLC system and XBridge Shield RP18 column thermostabilised at 37°C. The solvent was 0.02M $NH_4H_2PO_4$ (pH 4.0) with 1.5% (v/v) acetonitrile. Non-methylated lambda phage (Sigma) and *E. coli* DNA were used as

221 calibration reference and control, respectively. The DNA G+C content of strain BR
222 10247^T, was 63.9 mol% (Table 1), differentiating it from the closest type strain LMG
223 24129^T for which the G+C mol% was 61.2 (Islam *et al.*, 2008).

224 Nodulation and nitrogen fixation genes are required for effective legume
225 symbiosis, therefore *nodC* and *nifH* genes were analysed according to Sarita *et al.*,
226 (2001) and Ueda *et al.*, (1995), respectively. Phylogenetic trees were constructed as
227 described previously and the results are given in Figs. S3 and S4 (available in IJSEM
228 Online) for *nodC* and *nifH*, respectively. *NodC* analysis placed strain BR 10247^T in a
229 separate branch in relation to other *Bradyrhizobium* species and it was grouped outside
230 the four recognized symbiovars (Cobo-Díaz *et al*., 2014) (similarity less than 80%),
231 possibly indicating a new symbiovar within this genus (Fig. S3, Table S1). *NifH* gene
232 sequence analysis clustered strain BR 10247^T in the same branch as *B. iriomotense*
233 EK05^T (closest strain in the previous analyses), but the similarity observed was lower
234 than 90% between both strains (Fig. S4, Table 1), indicating again that it might be a
235 new symbiovar.

236 To confirm the nodulation ability of the strains investigated in this study, two
237 glasshouse experiments were performed. In the first experiment, the seven strains were
238 tested on their original host *C. paraense* and this was performed using Leonard jars
239 containing N-free nutrient solution according to Radl *et al.* (2013). Secondly, host plant
240 tests with strain BR 10247^T were performed on 14 different legume species using the
241 axenic sand-culture system described previously (Howieson *et al*., 2013). For both
242 experiments the seeds were surface sterilized with H_2O_2 (5%; (v/v); 5 min) and
243 inoculated with 1 mL of YM broth suspension containing approximately 10^9 bacterial
244 cells grown for 5 days at 28°C. All treatments, plus an uninoculated control were
245 replicated four times in a split-plot design (Howieson et al., 2013). Nodulation was

evaluated 70 days and 35 days after inoculation in the first and the second experiment, respectively. The seven strains nodulated *C. paraense* (Table S4, available in IJSEM Online). Strain BR 10247^T formed effective nitrogen fixing nodules on roots of *Arachis hypogaea*, *Acacia ligulata*, *Cajanus cajan*, *Crotalaria juncea*, *Macroptillium atropurpureum*, *Vigna unguiculata*, *V. angularis*, *V. radiata*, and ineffective (non-fixing) root nodules on *Ornithopus compressus* and *Phaseolus vulgaris*. No nodulation was observed for *Glycine max*, *Lupinus angustifolius*, *Pisum sativum* and *Vicia faba*.

The genotypic and phenotypic data presented in this study demonstrate that the strains isolated from *Centrolobium paraense* root nodules collected in the Amazonia represent a novel species, for which the name *Bradyrhizobium neotropicale* sp. nov. is proposed, with BR 10247^T (= HAMBI 3599^T) as the type strain.

Description of *Bradyrhizobium neotropicale* sp. nov.

Bradyrhizobium neotropicale (ne.o.tro.pi.ca'le. Gr. adj. *neos*, new; N.L. adj. *tropicalis* (from L. masc. adj. *tropicus*, tropical), referring to the tropical region; N.L. neut. adj. *neotropicale* of the neotropics)

Cells are motile with polar flagella, Gram-negative rods (approximately 2.4 x 0.6 µm), aerobic, non-spore-forming (Supplementary Fig. S5). Colonies on YMA medium are circular and translucent, and have a diameter of 1 mm within 7–8 days of incubation at 28 °C. The generation time is 10.8 h in YM broth. The pH range for growth on YMA is 4.0 –10.0, with optimum growth at pH 5.0-7.0. Growth occurs between 15°C and 37°C, with optimum growth at 28-30°C. Does not grow in the presence of 1.5% (w/v) NaCl or higher. Positive reactions for carbon source utilization were recorded for L-arabinose,

D-arabitol, D-fructose, L-fucose, D-galactose, D-mannitol, D-mannose, L-rhamnose, acetic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketoglutaric acid, D.L-lactic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, glycerol, methyl pyruvate, mono-methyl-succinnate, D-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-leucine, L-phenylalanine, urocanic acid, succinamic acid, glucuronamide and bromo succinic acid. Oxidase, catalase, arginine dihydrolase, hydrolysis of esculin and urease were also positive, while nitrate reduction, tryptophan deaminase, glucose fermentation, β-galactosidase and hydrolysis of gelatin were negative. The most abundant cellular fatty acids are $C_{16:0}$ and summed feature 8 ($C_{18:1}$ w7c). The DNA G+C content of the type strain BR 10247^T is 63.9 mol%. The type strain BR 10247^T (=HAMBI 3599^T) was isolated from nodules of *Centrolobium paraense* grown in soils of Amazonia, Roraima State-Brazil.

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Table 1. Different features of *Bradyrhizobium neotropicale* sp. nov. strains and closest related strain *Bradyrhizobium iriomotense* EK05^T.

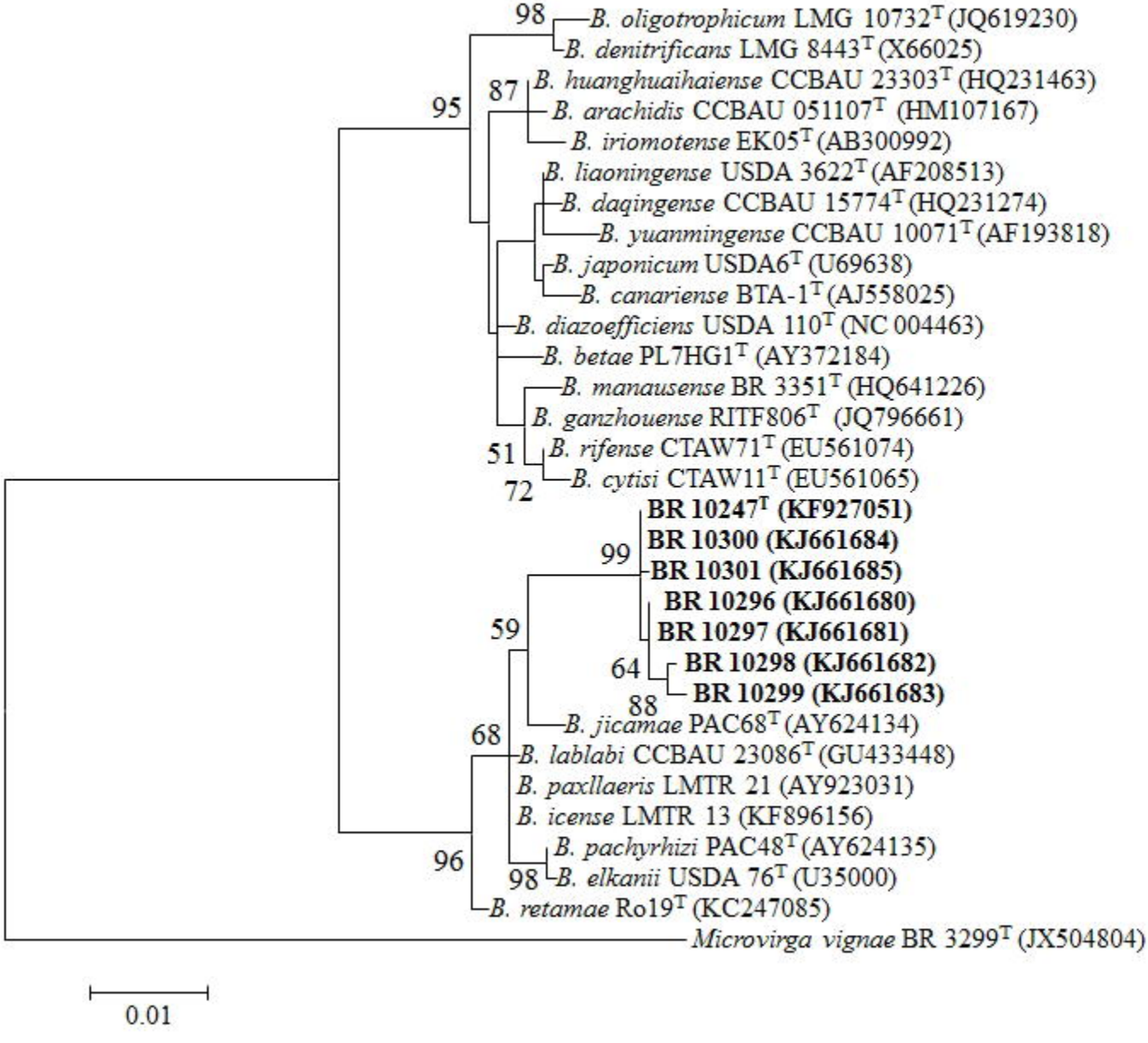
Characteristic	BR 10247^T	BR 10296	BR 10297	BR 10298	BR 10299	BR 10300	BR 10301	EK05$^{T(1)}$
C source utilization								
Adonitol	-	-	-	-	-	-	-	+
D-Cellobiose	-	-	-	-	-	-	-	+
L-Rhamnose	+	+	+	+	+	+	+	-
N-Acetyl-D-glucosamine	-	-	-	-	-	-	-	+
D-Glucuronic acid	-	-	-	-	-	-	-	+
Glycyl-L-aspartic acid	-	-	-	-	-	-	-	+
Glycyl-L-glutamic acid	-	-	-	-	-	-	-	+
L-Proline	-	-	-	-	-	-	-	+
L-Threonine	-	-	-	-	-	-	-	+
γ-Aminobutyric acid	-	-	-	-	-	-	-	+
Enzymatic reaction								
β-galactosidase	-	-	-	-	-	-	-	+
Arginine dihydrolase	+	+	+	+	+	+	+	-
Antibiotic resistance								
Erythromycin (30 μg)	+	+	-	+	+	+	+	+
Gentamicin (10 μg)	-	+	+	-	+	+	+	+
Penicillin (10 μg)	-	-	-	-	-	-	-	+
Streptomycin (10)	-	-	-	-	-	-	-	+
Temperature Growth range (°C)	15-37	15-37	15-37	15-37	15-37	15-37	15-37	15-32$^{(2)}$
pH growth range	4-10	4-10	4-10	4-10	4-10	4-10	4-10	4,5-9$^{(2)}$
Generation Time (h)	10.8	ND	Nd	Nd	Nd	Nd	Nd	7-9$^{(2)}$
NaCl tolerance (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	<1.0$^{(2)}$
DNA G+C content (% mol)	63.9	ND	ND	ND	ND	ND	ND	61.2$^{(2)}$

(1) The strain LMG 24129^T (formal deposit of the strain EK05^T) were obtained from the LMG culture collection.
(2) Data from (Islam *et al*., 2008); ND = not determined

Fig. 1 - Maximum likelihood phylogeny based on 16S rRNA gene sequences showing the relationships between *Bradyrhizobium neotropicale* strains (shown in bold) and other members of the *Bradyrhizobium* genus. The significance of each branch is indicated by a bootstrap value (greater than 50% showed) calculated for 1000 subsets. Bar, 1 substitution per 100 nucleotide positions. Sequence accession numbers of the 16S rRNA genes are presented in parenthesis.

Fig. 2. Maximum likelihood phylogeny based on intergenic transcribed spacer (ITS) sequences showing the relationships between strains from the novel species (shown in bold) and other members of the *Bradyrhizobium* genus. The significance of each branch is indicated by a bootstrap value (greater than 50% showed) calculated for 1000 subsets. Bar, 5 substitutions per 100 nucleotide positions. Sequence accession numbers of the ITS are presented in parenthesis.

Fig. 3. Maximum likelihood phylogeny based on concatenated *gyrB*, *recA* and *rpoB* gene sequences showing the relationships between strains from the novel species (shown in bold) and other members of the *Bradyrhizobium* genus. The significance of each branch is indicated by a bootstrap value (greater than 50% showed) calculated for 1000 subsets. Bar, 2 substitutions per 100 nucleotide positions.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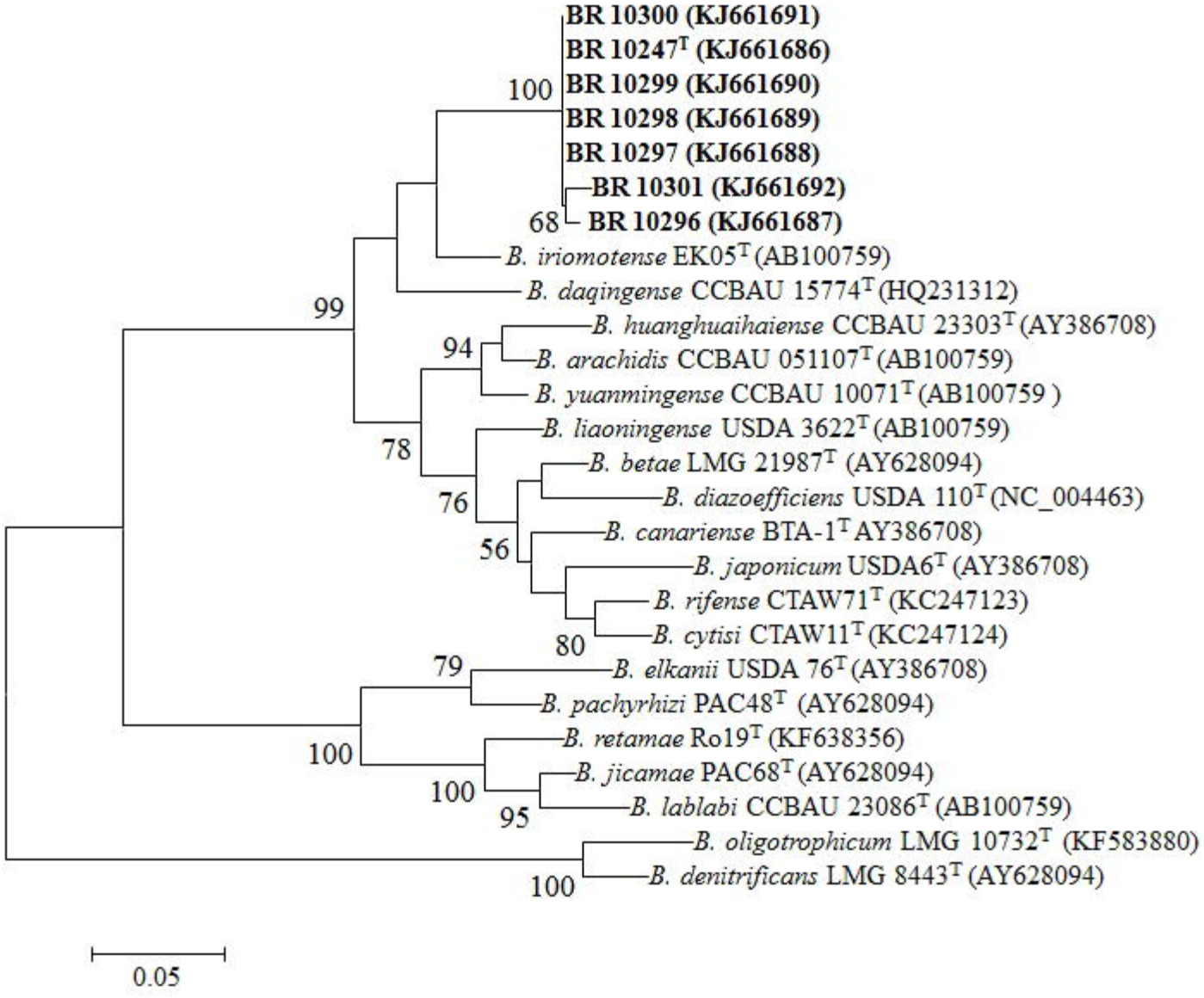
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B. jicamae PAC68T (AY628094)
100
B. lablabi CCBAU 23086T (AB100759)
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B. denitrificans LMG 8443T (AY628094)
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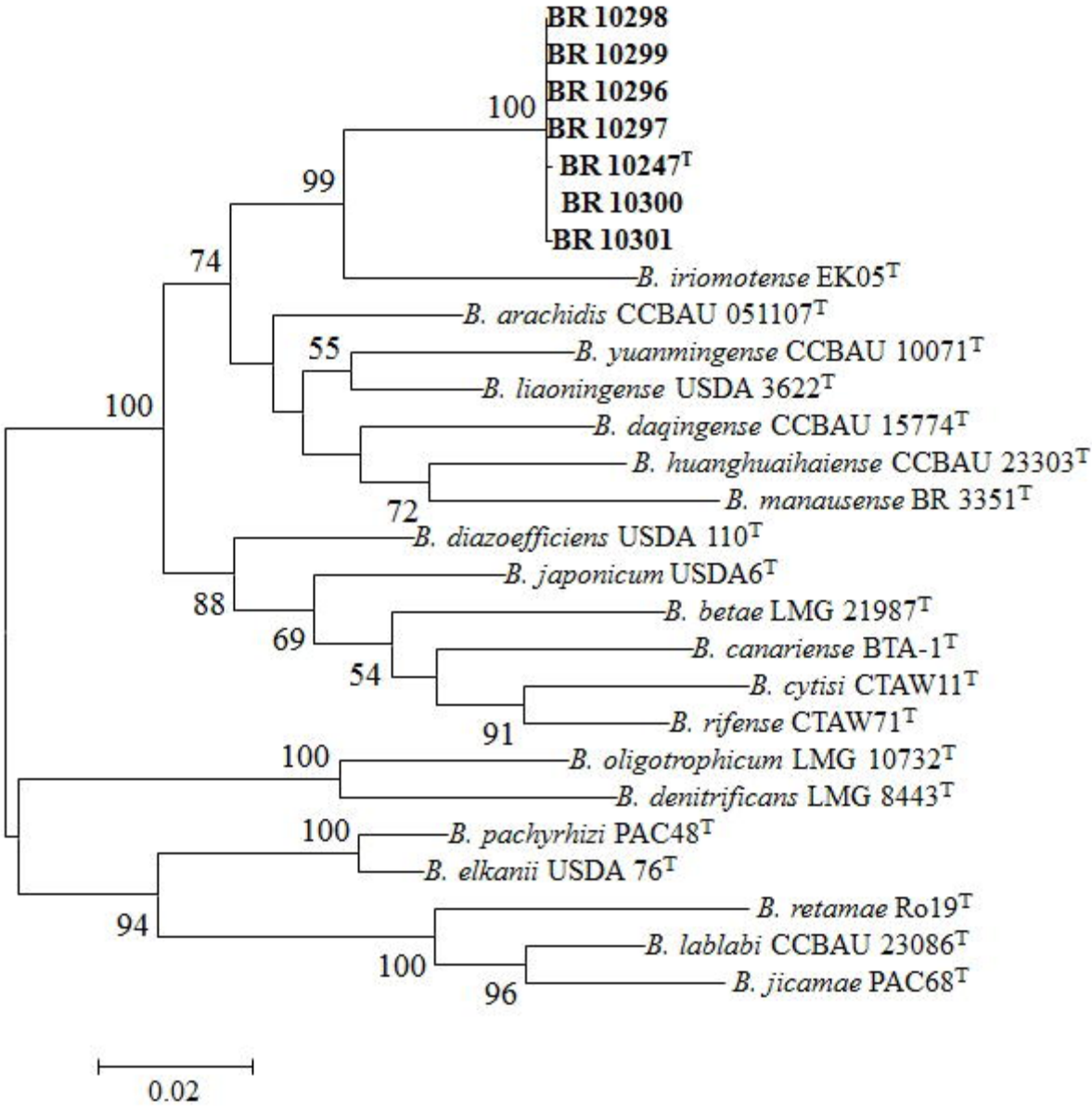
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BR 10297
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B. arachidis CCBAU 051107T
B. yuanmingense CCBAU 10071T
B. liaoningense USDA 3622T
B. daqingense CCBAU 15774T
B. huanghuaihaiense CCBAU 23303T
B. manausense BR 3351T
B. diazoefficiens USDA 110T
B. japonicum USDA6T
B. betae LMG 21987T
B. canariense BTA-1T
B. cytisi CTAW11T
B. rifense CTAW71T
B. oligotrophicum LMG 10732T
B. denitrificans LMG 8443T
B. pachyrhizi PAC48T
B. elkanii USDA 76T
B. retamae Ro19T
B. lablabi CCBAU 23086T
B. jicamae PAC68T
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69
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