
Rhizobial Diversity for Tropical Pulses and Forage and Tree Legumes in Brazil

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Abstract

The current information on Brazilian rhizobial diversity, concentrating especially on the microbial symbionts of tropical pulses, forage legumes, and legume trees, some of which are native to Brazil or of which Brazil is a major producer, is highlighted. These legume species are nodulated by a large number of currently known rhizobial genera, including both alpha and beta rhizobia, with widely varying nitrogen-fixing efficiencies. The rhizobial diversity is strongly affected by soil and climatic factors, as well as genetic variation among pulses. The greater diversity among rhizobia may allow the selection of more effective nitrogen-fixing strains which could be used as inexpensive inoculants to substitute/to reduce the use of nitrogen fertilizers.

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6.1 Introduction

Biological nitrogen fixation (BNF) through the legume-rhizobia symbiosis is the most important nitrogen (N) source for most agroecosystems (Herder et al. 2010). Currently, these bacteria are classified as *Allorhizobium*, *Aminobacter*, *Azorhizobium*, *Bradyrhizobium*, *Devosia*, *Ensifer* (*Sinorhizobium*), *Mesorhizobium*, *Methylobacterium*, *Microvirga*, *Ochrobacterium*, *Phyllobacterium*, *Rhizobium*, and *Shinella* among the α -Proteobacteria and *Burkholderia*, *Cupriavidus*, and *Herbaspirillum* among the β -Proteobacteria (Vinuesa 2015). At least one *Pseudomonas* sp. is also among the γ -Proteobacteria (Shiraishi et al. 2010). This genus-level diversity is repeated on lower taxonomic levels, since a single soil may harbor several species and strains of a single species (Guimarães et al. 2015), while strains from a single species may be found at faraway points (Martins et al. 2015). Phenotypic characteristics are generally used for initial characterization and screening of rhizobia (Oliveira et al. 2011; Rufini et al. 2014), but molecular characterization has mostly replaced the more traditional phenotypic characteristics due to sensitivity of techniques and precision in results. Among molecular tools, fingerprinting techniques using conserved and repetitive DNA oligonucleotides sequences such as BOX (genomic box elements), ERIC (enterobacterial repetitive intergenic consensus), and REP (repetitive extragenic palindromic) are frequently used in rhizobial diversity research (Guimarães et al. 2012; Bianco et al. 2013). For example, a polyphasic approach based on phenotypic characterization and BOX, ERIC, and REP was used to evaluate *Mimosa caesalpinifolia* rhizobial diversity between different regions of the Northeast Brazil (Martins et al. 2015). At the same time, the 16S rRNA housekeeping gene is no longer considered to be sufficiently discriminatory between closely related rhizobial species (Menna et al. 2006; Delamuta et al. 2012) or for intraspecific analysis. This conclusion led to the increased use of the 16S-23S rRNA intergenic transcribed spacer (ITS) (Tessfaye and Holl 1998; van Berkum and Fuhrmann 2000), but the ribosomal gene proximity may lead to erroneous phylogenetic conclusions if horizontal gene transference occurs (van Berkum et al. 2003) as it is relatively common among rhizobial species.

Since no single gene, even among the housekeeping ones, can reliably avoid horizontal gene transfer, nowadays the multilocus sequence analysis (MLSA) of several housekeeping genes is being increasingly used for rhizobial phylogenetic and taxonomic identification (Zilli et al. 2014). This technique is based on the sequencing and linking of several housekeeping genes dispersed over at least 100 kb of the genome and thus should be largely immune to horizontal gene transfer effects (Martens et al. 2008; Ribeiro et al. 2015a). For example, MLSA has found high *Bradyrhizobium* strain diversity from several legume species and land use systems which was not described earlier (Guimarães et al., 2015). Even when just soybean rhizobial diversity was evaluated from soils of different ecological regions of Brazil, new species were found from both the Northeast (tropical) and Southeast (subtropical to tropical) regions, some of which were highly efficient for BNF (Ribeiro et al. 2015a) when 16S rRNA and five housekeeping genes were sequenced. This is a

major breakthrough, since it might lead to more efficient inoculants for this culture, which under Brazilian conditions does not receive any nitrogen fertilizer.

Another toolkit frequently used in rhizobial diversity and phylogeny research is functional gene analysis/sequencing, most frequently *nifH* which is highly conserved among diazotrophs and codes for the Fe-protein of the nitrogenase complex (Coelho et al. 2009). As an example, several unknown species as well as strains from *Bradyrhizobium* and *Sinorhizobium* were found (Roesch et al. 2007) even though the authors used maize colms for DNA extraction while at the same time finding that soil clay content affects diazotroph diversity, while *nifH* pyrosequencing from a gradient of agricultural soils found that both diversity and dynamics of diazotroph communities are affected by soil chemical characteristics (Collavino et al. 2014). Since there is a huge scope for these studies, this review will concentrate its efforts on some legume groups which are somewhat less studied abroad than in Brazil. This differential concentration might be due to any of several equally important reasons, ranging from the lower importance of the crop to the endemic or indigenous nature of the species. As such, we decided to cover *Phaseolus vulgaris* (common or French beans), *P. lunatus* (lima beans), *Vigna unguiculata* (cowpeas), and tropical legume trees and forage species while, at the same time, not including soybean, peas, or any of the temperate climate forage legumes.

6.2 *Phaseolus vulgaris*

Common or French beans (*Phaseolus vulgaris* L.) figure among the main protein sources in Latin America (Torres et al. 2009) and may fix nitrogen with a wide range of rhizobial species (Table 6.1). There are several reports that indicate some promising results from field inoculation in several bean-growing regions in Brazil (Raposeiras et al. 2006; Lombardi et al., 2009; Torres et al. 2009). *R. tropici*, in particular, is usually described as highly efficient, genetically stable, and tolerant to environmental stresses and is commonly found in Brazilian soils (Hungria et al. 2000; Mostasso et al. 2002), while *R. etli* is usually dominant and has been frequently found in Brazil (Mostasso et al. 2002; Soares et al. 2006a; Giongo et al. 2007), including when plants were grown under environmental stresses such as high temperature, aluminum stress, and low pH, together with *R. leguminosarum* strains (Soares et al. 2006a; Grange et al. 2007; Stocco et al. 2008). The prevalence of *R. etli* may be linked to the different centers of origin of *P. vulgaris*, since strains from the Northeast region of Brazil were genetically closer to a Mexican strain than those from the South region, based on 16S rRNA (Grange et al. 2007). This link between bean cultivar and rhizobial diversity has also been found for strains from the Mesoamerican and Andean centers of origin, with higher diversity for the first than the second center (Oliveira et al. 2011). Another study in South Brazil found that 32.5% of the strains were *R. leguminosarum* (Stocco et al. 2008) and this species was also found in several other studies in Brazil (Giongo et al. 2007; Pinto et al. 2007) and Columbia (Eardly et al. 1995). Besides the species-level diversity found in Brazil, high strain-level diversity is also found in Brazilian soils. For example,

Table 6.1 Rhizobial species currently known to nodulate *Phaseolus vulgaris* and their known geographical occurrences

Species	Origin	References
<i>α-Proteobacteria</i>		
<i>Rhizobiales</i>		
<i>Rhizobiaceae</i>		
<i>Rhizobium</i>		
<i>R. leguminosarum/ biovares</i>	Europe, South, Central and North America, Asia	Andrade et al. (2002); Soares et al. (2006a); Giongo et al. (2007); Grange et al. (2007); Pinto et al. (2007)
<i>R. paranaense</i>	South America	Dall'agnol et al. (2014)
<i>R. etli</i>	Europe, South and North America, Asia	Hungria et al. (2003); Aguilar et al. (2004); Grange et al. 2007; Stocco et al. (2008)
<i>R. ecuadorensis</i>	South America	Ribeiro et al. (2015b)
<i>R. tropici</i>	South and North America, Europe, Asia	Mostasso et al. (2002); Lombardi et al. (2009); Torres et al. (2009)
<i>R. giardinii</i>	South America, North Africa, Asia	Mhamdi et al. (2002); Torres et al. (2009)
<i>R. gallicum</i>	North Africa, Europe	Rodriguez-Navarro et al. (2000); Mhamdi et al. (2002)
<i>R. galegae</i>	South America, Europe	Laguerre et al. (2001); Melloni et al. (2006)
<i>R. phaseoli</i>	Europe	Atzorn et al. (1988)
<i>R. lusitanum</i>	Europe	Valverde et al. (2006)
<i>R. freirei</i>	South America	Dall'agnol et al. (2013)
<i>R. mongolense</i>	South America, Africa do Norte	Andrade et al. (2002); Mhamdi et al. (2002)
<i>R. meliloti</i>	Europe	Bromfield and Barran (1990)
<i>Sinorhizobium</i>		
<i>S. americanum</i>	North Africa	Mnasri et al. (2012)
<i>Bradyrhizobiaceae</i>		
<i>Bradyrhizobium</i>		
<i>B. japonicum</i>	South America	Michiels et al. (1998)
<i>B. elkanii</i>	Europe	Laguerre et al. (2001)
<i>Xanthobacteriaceae</i>		
<i>Azorhizobium</i>		
<i>A. caulinodans</i>	South America	Melloni et al. (2006)
<i>Phyllobacteriaceae</i>		
<i>Mesorhizobium</i>		
<i>M. loti</i>	Europe	Laguerre et al. (2001)
<i>M. tianshanense</i>	Asia	Chen et al. (1995)
<i>β-pProteobacteria</i>		
<i>Burkholderiales</i>		
<i>Burkholderiaceae</i>		

(continued)

Table 6.1 (continued)

Species	Origin	References
<i>Burkholderia</i>		
<i>B. tuberum</i>	North Africa	Elliott et al. (2007)
<i>B. phymatum</i>	North Africa	Elliott et al. (2007)
<i>B. caribensis</i>	America do Norte	Estrada-De Los Santos et al. (2012)
<i>B. cepacia</i>	South America	Peix et al. (2001)
<i>Paraburkholderia</i>		
<i>P. nodosa</i>	South America	Dall'agnol et al. (2016)

62 strains from Amazon agricultural soils analyzed by BOX-PCR resulted in 50 genotypes with 70% similarity and 21 genotypes with 30% similarity (Guimarães et al. 2012). Although 16S rRNA sequencing indicated higher prevalence of *Bradyrhizobium*, species from *Rhizobium*, *Burkholderia*, and *Achromobacter* (Guimarães et al. 2012) were also identified.

6.3 *Phaseolus lunatus*

Lima bean (*Phaseolus lunatus*) is an important species of plant for humans in tropical regions. This legume originated in Peru; and archeological evidence supports the hypothesis that lima beans were domesticated in Mesoamerica and South America (Salgado et al. 1995). The lima bean seed is considered a main crop and an important source of protein for people of South America, Africa, and Mexico. The rustic quality of lima bean and its capacity to resist to long, dry periods are important characteristics for the semiarid region of Northeast Brazil (Azevedo et al. 2003). The rhizobia associated with this crop have scarcely been studied. In the old host-based classification scheme, symbionts of *P. lunatus* were included in the same group as rhizobia associated with slow-growing cowpea (*Vigna unguiculata*). This group was a diverse assemblage of strains that were later included in the genus *Bradyrhizobium*. The rhizobial isolates for *P. lunatus* were obtained from areas where this legume is not native, and research has only focused on analysis of symbiotic characteristics, such as infectiveness and effectiveness (Ormeño-Orrillo et al. 2006). Antunes et al. (2011) evaluated the symbiotic effectiveness of 17 rhizobial isolates of lima bean in Northeast Brazil, and they compared the isolates with two reference *Rhizobium* strains CIAT 899 and NGR 234. They found eight isolates with higher N accumulation and N₂-fixation efficiency compared with the reference strains CIAT 899 and NGR 234. The morphological and biochemical characteristics of these isolates revealed that six isolates belonged to genera *Bradyrhizobium* and two isolates to *Rhizobium*.

Interestingly, lima beans are nodulated by both fast- and slow-growing rhizobia (Santos et al. 2011), and some studies found *Bradyrhizobium* and *Rhizobium* as symbionts of this legume (Thies et al. 1991; Santos et al. 2011). A very few studies on the genetic diversity of rhizobia from lima bean have been conducted using

collection from geographic locations including Mexico and Brazil (Ormeño et al. 2007; Lopez-Lopez et al. 2013). Ormeño-Orrillo et al. (2006) evaluated the molecular diversity of rhizobial isolates associated with lima bean in Peru. They found divergent bradyrhizobial lineages (*Bradyrhizobium yuanmingense* and *Bradyrhizobium* sp.) according to PCR-RFLP of the *rpoB* gene and sequence analysis of the 16S rDNA and *dnaK*, *nifH*, and *nodB* genes. Lopez-Lopez et al. (2013) described the nodule bacteria from native lima beans from Mexico. The bacterial diversity of isolates from nitrogen-fixing nodules of *P. lunatus*, using ERIC-PCR and PCR-RFLP of *rpoB* genes and sequencing of *recA*, *nodZ*, and *nifH* genes, shows that nodule bacteria correspond to *Bradyrhizobium*. According to the authors, this is the first report of nodule bacteria from *P. lunatus* in its Mesoamerican site of origin and domestication, and it may confirm that *Bradyrhizobium* is the main nodulating group of lima bean. However, the fast-growing rhizobia that nodulate *Phaseolus* commonly belong to the genus *Rhizobium* and *Sinorhizobium*, and Ormeño et al. (2007) found a strain of *S. meliloti* isolated from lima bean in Peru, and they suggested that this rhizobial species may also nodulate this legume. A study about the genetic diversity of native rhizobia that nodulate lima bean from Brazil shows a broad spectrum of rhizobial groups associated with lima bean (Santos et al. 2011). In this study, rhizobia isolates were obtained and placed into groups based on the differences in their morphological, physiological, and genetic characteristics. The restriction patterns obtained with endonucleases MboI, HaeIII, and NheI showed sufficient variability to discriminate isolates identified as species from the genera *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*. Araujo et al. (2015) further sequenced the 16S rDNA of the above isolates and found that species that nodulate lima bean belonged to the genus *Bradyrhizobium*, *Sinorhizobium*, and *Rhizobium*. These results confirm that lima bean may be nodulated by diverse rhizobia species.

6.4 *Vigna unguiculata*

Cowpea (*Vigna unguiculata* (L.) Walp.) is an African pulse, traditionally grown in the tropical regions of Africa, America, and Asia and is used mainly as a protein source (Carvalho et al. 2012). It has high genetic variability, and some genotypes are tolerant to water deficit (Nascimento et al. 2011) and pests (Torres et al. 2016), as well as they adapt well to other environmental stresses, such as low soil fertility (Ferreira et al. 2013). It fixes nitrogen in association with several genera of rhizobia (Table 6.2) including *Rhizobium* (Jaramillo et al. 2013), *Mesorhizobium* (Moreira 2008), *Microvirga* (Marinho et al. 2014; Radl et al. 2014), *Achromobacter* (Guimarães et al. 2012), *Burkholderia* (Moreira 2008), *Brevibacillus* (Costa et al. 2013), *Sinorhizobium* (Moreira 2008), *Acinetobacter* (Marra et al. 2012), *Azorhizobium* (Moreira 2008), *Ralstonia* (Sarr et al. 2009), and *Allorhizobium* (Moreira 2008), leading to its frequent use as a bait crop to trap the largest diversity of soil rhizobia in diversity studies. *Bradyrhizobium* species have been found to frequently nodulate cowpea in Africa, America, and Asia, with large strain

Table 6.2 Bacterial genera forming symbiosis with *Vigna unguiculata* in Brazil and other regions of the world

Genera	Location	Source
<i>Bradyrhizobium</i>	India	Appunu et al. (2009)
<i>Bradyrhizobium</i>	Europe and Africa	Bejarano et al. (2014)
<i>Bradyrhizobium</i> , <i>Rhizobium</i> , <i>Bacillus</i> , <i>Paenibacillus</i>	Brazil (Cerrado)	Costa et al. (2011)
<i>Bradyrhizobium</i> , <i>Rhizobium</i> , <i>Burkholderia</i> ; <i>Achromobacter</i>	Brazil (Amazon)	Guimarães et al. (2012)
<i>Rhizobium</i> , <i>Ochrobacterium</i> , <i>Paenibacillus</i> , <i>Bosea</i> , <i>Bacillus</i> , <i>Enterobacter</i> , and <i>Stenotrophomonas</i>	Brazil (Amazon)	Jaramillo et al. (2013)
<i>Bacillus</i> , <i>Firmicutes</i> , <i>Acinetobacter</i> , <i>Rhizobium</i> , <i>Microbacterium</i> , and <i>Paenibacillus</i>	Brazil	Marra et al. (2012)
<i>Bradyrhizobium</i>	Botswana Ghana, Africa do Sul	Pule-Meulenberg et al. (2010)
<i>Microvirga</i>	Brazil (Semiarid)	Radl et al. (2014)
<i>Bradyrhizobium</i>	Brazil (Cerrado)	Rufini et al. (2014)
<i>Bradyrhizobium</i> and <i>Ralstonia</i>	Japan	Sarr et al. (2009)
<i>Bradyrhizobium</i>	Japan	Sarr et al. (2011)
<i>Bradyrhizobium</i> , <i>Klebsiella</i> , <i>Rhizobium</i> , and <i>Enterobacter</i>	Brazil (Amazon)	Silva et al. (2012)
<i>Bradyrhizobium</i>	Africa	Steenkamp et al. (2008)

diversity, even where the crop is not traditionally grown. For example, in Japan, cowpea-nodulating *Bradyrhizobium* diversity is geographically diverse, indicating some effect of temperature, vegetation, and soil type and pH on the prevalence of this bacterium (Sarr et al. 2011). In yet another study, a total of 1010 rhizobial strains were recovered from Western Amazon (Nóbrega 2006), of which 148 were obtained from an agroforestry system and which were dominated by *Bradyrhizobium* strains (Jaramillo et al. 2013), whereas another set of 119 was isolated from areas with annual crops and included species of *Bradyrhizobium*, *Rhizobium*, *Burkholderia*, and *Achromobacter* (Guimarães et al. 2012). At the same time, nonsymbiotic endophytic bacteria have been isolated from superficially disinfested nodules. For example, Meyer et al. (2015) found a large diversity of these nonsymbiotic bacteria from unconventional legumes, based on partial 16S rRNA sequencing, including members of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Firmibacteria*, *Flavobacteria*, and *Sphingobacteria*, with close to 18% being *Bacillus* and 16% being *Pseudomonas*. Some of these unconventional endophytic rhizobacteria such as *Pseudomonas* (Li et al. 2008), *Paenibacillus* (Marra et al. 2012), *Bacillus* (Marra et al. 2012; Jaramillo et al. 2013), *Enterobacter* (Costa et al. 2013), and *Pontibacter* (Dastager et al. 2011) were also isolated from cowpeas. The identification of such unconventional endophytic rhizobacteria opens a new area where these bacteria can be tested for their nitrogen-fixing efficiency

using legumes as a host plant. And hence, they can be applied under field environment as microbial inoculants for enhancing legume production.

Under Brazilian law, inoculant producers can use strains suggested only by the Agricultural Ministry, and in accordance to this law, only four *Bradyrhizobium* strains including INPA 3-11B and UFLA 3-84 of *Bradyrhizobium* (Soares et al. 2006b; Moreira 2008) and BR 3267 and BR 3262 belonging to *Bradyrhizobium yuanmingense* and *B. pachyrhizi*, respectively (Simões-Araújo et al. 2016a, b), have been approved for cowpea inoculation (Brasil 2011). These inoculants have resulted into sufficiently higher yields due to sufficient N availability to crops cultivated in several field experiments in different ecosystems ranging from the semiarid to the Amazon, from 2°N to 23°S (Zilli et al. 2009; Chagas Junior et al. 2010; Almeida et al. 2010; Costa et al. 2011; Ferreira et al. 2013; Marinho et al. 2014).

6.5 Legume Trees

There are 147 genera and 1190 species of leguminous tree (Garcia and Fernandes 2015) in Brazil including 65 Faboideae (Papilionoideae), 54 Caesalpinioideae, and 28 Mimosoideae subfamilies. Many surveys conducted on nodulation of legume trees have reported numerous highly efficient rhizobial strains (Franco and Faria 1997; Menna et al. 2009). From these studies, several rhizobial strains were officially recommended as inoculants for 43 native and exotic species of leguminous trees (Brasil 2011). However, the diversity among such rhizobial species is still insufficiently understood. The first study on rhizobial diversity of legume trees in Brazil was conducted by Moreira and coworkers (Moreira et al. 1992, 1993), and a total of 800 rhizobial strains were isolated from the Amazonian and Atlantic forests. After phenotypic characterization, 171 strains were selected for total protein analysis using polyacrylamide gel electrophoresis (PAGE) (Moreira et al. 1993), followed by partial 16S rRNA gene sequence analysis. Using these techniques, *Azorhizobium*, *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*, and *Mesorhizobium* genera were identified (Moreira et al. 1998). One of these species was later described as a new species, *Azorhizobium doebereineriae* (Moreira et al. 2006). Strains already recommended for commercial inoculant production for several legume tree species (*Acacia*; *Albizia*; *Clitoria*; *Dalbergia*; *Enterolobium*; *Falcataria*; *Gliricidia*; *Prosopis*; *Leucaena*; *Mimosa*; *Ormosia*; *Piptadenia*; *Sesbania*; and *Tipuana*) were identified as *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*, *Burkholderia*, and *Azorhizobium* (Menna et al. 2006, 2009) including probable new rhizobial species.

Brazil is a major diversification center for *Mimosa* (Simon and Proença 2000), with 323 species (Garcia and Fernandes 2015), and its rhizobial diversity is well studied (Bontemps et al. 2010; Reis Junior et al. 2010; Bournaud et al. 2013). *Mimosa* genus is mostly nodulated by *Betaproteobacteria* including the genera *Burkholderia* and *Cupriavidus* (Gyaneshwar et al. 2011). However, in Brazil *Mimosa* have a particular association with *Burkholderia*, but *Cupriavidus* was not found (Bontemps et al. 2010; Reis Junior et al. 2010). An evaluation of just 143 bacteria from root nodules of 47 native species of *Mimosa*, evaluating 16S rRNA and

recA gene sequences, found that 98% of isolates were *Burkholderia* grouped in seven clades (Bontemps et al. 2010). The isolates of three of these clades present sequence distant from those of all type strains of the species, indicating a large diversity of *Burkholderia* in Brazil. Later, four (three obtained from *M. cordistipula* and one from *M. misera*) and three isolates (one obtained from *M. candollei*, one from *M. tenuiflora*, and one *M. pudica*) were described as *B. symbiotica* (Sheu et al. 2012) and *B. diazotrophica* (Sheu et al. 2013), respectively. In a study conducted on *M. caesalpiniiifolia* Benth., four geographically distant woodlots were sampled, in regions ranging from tropical semiarid to subhumid, sea level to about 600 m elevation, achieving 47 isolates of *Burkholderia*, some of which presented low similarity in 16S rRNA gene with the type strains, indicating the possibility of new species (Martins et al. 2015). The predominance and high diversity of *Burkholderia* isolates were also observed with *M. scabrella* plants in a subtropical humid forest known as Araucaria forest (Lammel et al. 2013). Actually, at least five *Burkholderia* species, namely, *B. mimosarum* (Chen et al. 2006), *B. nodosa* (Chen et al. 2007), *B. sabiae* (Chen et al. 2008), *B. symbiotica* (Sheu et al. 2012), and *B. diazotrophica* (Sheu et al. 2013), able to nodulate *Mimosa* plants were described from Brazil. Genera of the tribe Mimoseae, such as *Piptadenia*, *Parapiptadenia*, *Pseudopiptadenia*, *Pityrocarpa*, *Anadenanthera*, and *Microlobius*, all of which phylogenetically close to *Mimosa*, were also found in symbiosis with strains of *B. sabiae*, *B. phymatum*, *B. caribensis*, *B. diazotrophica*, *B. nodosa*, *B. phenoliruptrix*, and possible new species large diversity of *Burkholderia* (Bournaud et al. 2013). Phylogenetic analyses of neutral and symbiotic markers showed that symbiotic genes in *Burkholderia* from the tribe Mimoseae have evolved mainly through vertical transfer but also by horizontal transfer in two species (Bournaud et al. 2013). *Inga* (Mimosoideae) is another genus of leguminous tree adapted to acid and low fertility soils that establish symbiosis with rhizobia, of which 131 species are present in Brazil (Garcia and Fernandes 2015). However, very little is known about the diversity of rhizobia associated with this genus in Brazil. While there are two *Bradyrhizobium* spp. which are officially recommended for *Inga marginata* Willd inoculation (Franco and Faria 1997; Menna et al. 2009), 17 strains were obtained from root nodules of *Inga laurina* (Sw.) Willd in 2008, naturally growing in the savannah of Roraima state, in the Amazon region (Silva et al. 2014). Six representative strains were subjected to detailed polyphasic taxonomic studies and were named as *Bradyrhizobium ingae*. Recently, 178 nitrogen-fixing bacteria were isolated from root nodules of *Centrolobium paraense* Tul (Faboideae), a neotropical legume tree from the northern Brazilian Amazon (Baraúna et al. 2014). The most common rhizobia belonged to genus *Bradyrhizobium*, but *Rhizobium* and *Burkholderia* were also found among the isolates. This result was confirmed by the *rpoB* gene sequencing (Baraúna et al. 2014). This new species was later named as *Bradyrhizobium neotropicale* (Zilli et al. 2014). Interestingly, these strains presented a discordance in the 16S rRNA phylogeny compared with the ITS phylogeny, which was also confirmed by MLSA. While the 16S rRNA gene sequence analysis placed the strains in the subgroup I (*B. elkanii*), the ITS and concatenated MLSA trees placed the strains in the subgroup II (*B. japonicum*). This result indicates a high diversity of *Bradyrhizobium*

strains associated with *C. paraense*. Brazilian rhizobial diversity was also evaluated for *Chamaecrista*, *Dimorphandra*, and *Tachigali* (Moreira et al. 1998; Fonseca et al. 2012). *Chamaecrista ensiformis* (Vell.) H.S. Irwin and Barneby has a *Mesorhizobium* strain recommended as inoculant (Moreira et al. 1998). *Tachigali paniculata* Aubl. is associated to *Bradyrhizobium* (Moreira et al. 1998); *Dimorphandra parviflora* Spruce ex Benth., *D. exalata* Schott, and *D. wilsonii* Rizzini are associated to *Bradyrhizobium* (Menna et al. 2009; Fonseca et al. 2012); and *D. mollis* Benth. is associated to *Rhizobium* and *Sinorhizobium* (Moreira et al. 1998).

6.6 Forage Legumes

Although there is a large legume diversity in Brazil, with over 2800 species identified (Garcia and Fernandes 2015), majority of them are not consistently used as forage. However, species of *Stylosanthes*, *Arachis*, *Centrosema*, *Macroptilium*, *Desmodium*, *Desmanthus*, *Leucaena*, and *Calopogonium* are some of the most commonly used forage legumes (Pereira 2001; Valle 2002). Unfortunately, relatively little work is done on rhizobial diversity of these legumes. However, one strain is already recommended for inoculant production (Menna et al. 2006) for 32 different forage species. This paucity of research leads to an unclear picture of the rhizobial diversity. For example, fast-growing strains were isolated from *Arachis*, *Stylosanthes*, and *Aeschynomene* plants grown under tropical humid conditions in the Northeast Brazil (Santos et al. 2007; Guimarães et al. 2012). Species currently recommended for inoculant production for *Stylosanthes* are identified as *Bradyrhizobium* (Menna et al. 2006). Similar results were found for rhizobial isolates from *Calopogonium mucunoides*, in this case from a tropical subhumid area also in the Northeast Brazil. Most of the 1575 isolated strains were fast growers (Calheiros et al. 2013, 2015), while the currently recommended strain is a *B. japonicum* (Menna et al. 2006), although *B. stylosanthis* has been described as a new species which is also used for inoculation of this legume. *Macroptilium atropurpureum* is widely recognized as a very promiscuous legume and is also found in Brazil. For example, a study conducted in the Amazon region revealed species from at least six genera (*Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Burkholderia*, *Mesorhizobium*, and *Sinorhizobium*), based on just 88 strains (Lima et al. 2009), while soil from seasonally dry mountain tops also had *Burkholderia* and *Paenibacillus* strains. Most of the *Burkholderia*, however, did not nodulate, and those which nodulated were ineffective (Araújo 2014) as reported earlier by Moulin et al. (2001).

Conclusion

A large number of new rhizobial species are still being discovered for lesser studied legume species, particularly those found in tropical regions. However, there is greater need to identify and evaluate some novel rhizobia for their potential growth-promoting activities so that they could be used as inoculants to reduce the application of chemical nitrogen fertilizer in agronomic production. Also, efforts should be directed to assess the impact of environmental variables such as

temperature and other stress factors on the survival of rhizobia besides taking into account the genetic variations among legumes before recommending the rhizobial strains for use in farming practices especially in legume cultivation.

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