RHIZOBIA AND OTHER LEGUME NODULE BACTERIA RICHNESS IN BRAZILIAN *Araucaria angustifolia* FOREST

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ABSTRACT: The Araucaria Forest is a sub-type of the Atlantic Forest, dominated by *Araucaria angustifolia*, which is considered an endangered species. The understory has a high diversity of plant species, including several legumes. Many leguminous plants nodulate with rhizobia and fix atmospheric nitrogen, contributing to forest sustainability. This work aimed at bacteria isolation and phenotypic characterization from the root nodules of legumes occurring in Araucaria Forests, at Campos do Jordão State Park, Brazil. Nodule bacteria were isolated in YMA growth media and the obtained colonies were classified according to their growth characteristics (growth rate, color, extra cellular polysaccharide production and pH change of the medium). Data were analyzed by cluster and principal components analysis (PCA). From a total of eleven collected legume species, nine presented nodules, and this is the first report on nodulation of five of these legume species. Two hundred and twelve bacterial strains were isolated from the nodules, whose nodule shapes varied widely and there was a great phenotypic richness among isolates. This richness was found among legume species, individuals of the same species, different nodule shapes and even among isolates of the same nodule. These isolates could be classified into several groups, two up to six according to each legume, most of them different from the used growth standards *Rhizobium tropici*, *Bradyrhizobium elkanii* and *Burkholderia* sp. There is some evidence that these distinct groups may be related to the presence of *Burkholderia* spp. in the nodules of these legumes.
Key words: N fixation, ombrophilous mixed forest, symbiotic bacteria, phenotypical characterization

RIQUEZA DE RIZÓBIOS E DE OUTRAS BACTÉRIAS DE NÓDULOS DE LEGUMINOSAS EM FLORESTA DE *Araucaria angustifolia*

RESUMO: A Floresta de Araucária é um sub-tipo da Mata Atlântica, cujo dossel é dominado por *Araucaria angustifolia*, uma espécie ameaçada de extinção. O sub-bosque dessa floresta tem alta diversidade, incluindo muitas espécies de leguminosas. Estas plantas podem formar nódulos e fixar nitrogênio atmosférico, contribuindo para a sustentabilidade da floresta. Efetuou-se o levantamento de leguminosas no Parque Estadual de Campos do Jordão e o isolamento de bactérias dos nódulos radiculares destas plantas, seguido da caracterização fenotípica dos isolados. As bactérias dos nódulos foram isoladas em meio de cultura YMA, sendo classificadas de acordo com suas características de crescimento (velocidade de crescimento, cor, produção de polissacarídeo extracelular e mudança de pH do meio). Os resultados foram analisados por análise de agrupamento e análise de componentes principais (PCA). De um total de onze espécies de leguminosas, nove apresentaram nódulos, sendo seis espécies descritas como nodulantes pela primeira vez. Duzentas e doze estirpes de bactérias foram isoladas, havendo variação no formato de nódulos e alta riqueza fenotípica das bactérias isoladas. Essa riqueza ocorreu entre as espécies de leguminosas, entre indivíduos da mesma espécie, entre diferentes formatos de nódulos e, inclusive, entre bactérias isoladas de um mesmo nódulo. As bactérias puderam ser classificadas em vários grupos, de dois a seis de acordo com cada leguminosa, a maioria deles diferentes de *Rhizobium tropici*, *Bradyrhizobium elkanii* e *Burkholderia* sp., que foram utilizados como padrões de crescimento. Existem algumas evidências de que estes grupos distintos podem estar relacionados à presença de *Burkholderia* spp. nos nódulos destas leguminosas.
Palavras-chave: fixação de nitrogênio, floresta ombrófila mista, bactérias simbióticas, caracterização fenotípica

INTRODUCTION

The Araucaria Forest, which is characterized by the presence of the Brazilian Pine (*Araucaria angustifolia* Bert. O. Kuntze), a Gymnosperm, is distributed along the regions with subtropical climate, mainly in the southern states of Brazil. It is a sub-type of the Atlantic Forest, classified as ombrophilous mixed forest (IBGE, 1992).

Being a subtropical forest, quite different from other forests situated in tropical areas, it contains many endemic plant species, including legumes (Robim et al., 1990; Roderjan et al., 1998), which tolerate temperatures down to below 0°C in the coldest months. This specific vegetation and the climatic features may have also contributed to the development of a specific microbial community. A better understanding of this microbiota will be essential for a more adequate management of this type of forest, since *Araucaria angustifolia* has been considered an endangered species (Brasil, 1992).

Rhizobia are bacteria that can fix nitrogen in symbiosis with legumes, supplying part of the nitrogen input to the forests (Franco & Faria, 1997; Sprent, 2001). Rhizobia may be associated with other bacteria in legume nodules and all of them may play a special role in the ecological balance of Araucaria Forests. A detailed study may support more adequate management practices.

Campos do Jordão State Park has one of the last remnants of well preserved Araucaria Forests, including mature native forests, planted forests and secondary forests regenerating. Little research has been devoted to the survey of the floristic richness of these forest stands (Robim et al., 1990; Mattos & Mattos, 1982). Moreira et al. (2006) reported on mycorriza in the same araucaria forests, but nothing is known about occurrence of rhizobia or other nodule bacteria in this state park.

The objectives of this study were to search for legumes and nodules, to isolate bacteria from these nodule, to test the strain phenotypic richness, and to group the bacteria using phenotypic characteristics and multivariate approaches.

MATERIAL AND METHODS

Sampling

Legume and nodule samples were collected in July 2006, in the Campos do Jordão State Park, in Campos do Jordão, SP, Brazil, located in the Mantiqueira mountain range (22º45' S and 45º30' W). The average altitude is 1450 m, although some regions are as high as 2000 m. The climate is classified as Cfb (Seibert et al., 1975), i.e., subtropical of altitude, mesothermal and humid, without a dry period. The mean annual precipitation is 2000 mm, which is relatively well distributed during the year. July is the coldest month, the absolute minimal temperature recorded being -7.2°C. The warmest month is February, with a mean temperature of 17.7°C (Seibert et al., 1975).

Collections were carried out in ecologically diverse sites where *Araucaria angustifolia* trees were present, legume species being randomly searched throughout the forest. When they were found, vegetative and, when available, reproductive plant samples were separated for further identification and root samples (containing nodules) were obtained by digging around the plant, taking care to check if the roots were connected to the plants. Along the survey, root and nodule samples were placed in plastic bags, and kept inside a thermo-box with ice until they were sent to the laboratory, where they were kept in a refrigerator at 4°C. The nodules were separated from the roots in the laboratory, where the isolation and phenotypic characterization of indigenous rhizobia was performed. Nodule shapes were recorded using a digital camera connected to a stereomicroscope.

Bacteria isolation and phenotypic characterization

The nodules were disinfected for thirty seconds in ethanol (95%) and for one minute in sodium hypochlorite (6%), adapted from Barrett & Parker (2006). They were then washed four times in sterilized water and finally crushed with a flame-sterilized glass rod. A loopful of the crushed nodule was then streaked across the surface of a Petri dish containing yeast mannitol agar (YMA; Vincent, 1970), and incubated at 28°C in the dark. Typical well-isolated colonies were re-isolated on diagnostic media, adapted from Odee et al. (1997): test tubes containing liquid YM (YMA without agar) with 25 mg kg^{-1} (w/v) brome thymol blue (BTB) as pH indicator; Petri dishes containing YMA with 25 mg kg^{-1} (w/v) congo red.

The bacteria in test tubes were incubated at 28°C on a shaker (220 rpm) and classified according to their ability to change the pH of the growth medium (alkaline, neutral or acid).

Petri dishes were incubated at 28°C in the dark, until the colonies were evaluated. They were characterized according to color (white, pink, translucid, yellow or white with a pink center), to the amount of extracellular polysaccharides (EPS) production (none to moderate or moderate to copious) and to colony size (the colony diameter measured with a ruler, after 3, 6 and 8 days of incubation).

Three replicates for each isolate were analyzed, and the mean growth rate was used to separate dif-

ferent categories (adapted from Odee et al., 1997): very fast – colonies ≥ 5 mm in diameter after 3 days of incubation; fast – colonies ≥ 3 mm diameter after 3 days of incubation; intermediate – colonies ≥ 3 mm diameter after 6 days of incubation; slow – colonies ≥ 3 mm diameter after 8 days of incubation; very slow – colonies ≤ 3 mm diameter after 8 days of incubation.

Color and EPS were only evaluated when the colonies reached the minimum diameter of 3 mm, except for the very slow growing ones, which did not reach this diameter until the eighth day.

Bradyrhizobium elkanii SEMIA 5019, *Rhizobium tropici* SEMIA 4077, and *Burkholderia* sp. Br 3407 (Chen et al., 2005; Faria & Franco, 2002) were used as standards, evaluated under the same conditions.

Statistical analysis

Bacterial richness was analyzed by the principal component biplot method (Leps & Smilauer, 2003) and the bacteria were grouped by a cluster analysis using the average linkage method and Euclidian distance, adapted from Melloni et al. (2006). The data used to perform the analysis consisted of: growth rate (1-very fast, 2-fast, 3-intermediate, 4-slow and 5-very slow), pH change of the medium (1-acid, 2-neutral and 3-alkaline), color (1- translucid, 2-white, 3-white with pink center, 4- pink, 5-yellow) and EPS (1-none to moderate, 2-moderate to copious). Principal component analysis (PCA) was performed using the software CANOCO and the biplot using the software CANODRAW (Leps & Smilauer, 2003). Cluster analyses was performed using the software SAS (SAS, 2002).

RESULTS AND DISCUSSION

Legumes, nodules and isolated bacteria

Two Fabaceae-Faboideae and seven Fabaceae-Mimosoideae legume species containing nodules were found during sampling showing different nodule shapes. Two other Fabaceae-Mimosoideae legumes were collected, however without nodules (Table 1). Mattos & Mattos (1982) did not find any legumes in this same park. Robim et al. (1990) found nineteen legumes while studying the floristic composition of the Campos do Jordão State Park, although only eight of them were collected in habitats associated with Araucaria. Based on Sprent (2001), this is the first report of nodulation in *Galactia crassifolia*, *Collaea speciosa*, *Mimosa furfuracea*, *Mimosa pilulifera* and *Mimosa filipetiola*.

Nodule shapes were recorded for each legume species (Figure 1) and, therefore, it was decided to perform bacterial isolation from individual nodules. For each kind of nodule, two were randomly chosen (nodule A and B), for the isolation of bacteria. A third isolation was performed using all other nodules together (group C), and this made it possible to estimate the total richness occurring in the nodules from the same sample. Thus, the bacteria were isolated from nodules of different shapes, which were obtained from different individuals, and from different legume species, resulting in a total of 212 isolates.

Table 1 - Legumes with different growth habits collected in ecologically diverse sites in the Campos do Jordão State Park and their nodule shapes.

Legume species	Growth habits	Collection site	Nodule shapes
Fabaceae-Faboideae			
Galactia crassifolia (Benth.) Taub.	subshrub	mountain grassland	spherical
Collaea speciosa (Loisel.) DC.	subshrub	border of Araucaria Forest in regeneration	spherical, small rod
Fabaceae-Mimosoideae			
Acacia dealbata (Link) F.J.Muell	tree	planted riparian forest	spherical, rod
Mimosa dolens Vell. var. acerba (Benth.) Barneby	shrub	Araucaria Forest in regeneration	spherical, rod
Mimosa filipetiola Burkart	creeper	border of Araucaria Forest in regeneration	rod, coralloid
Mimosa furfuracea Benth.	shrub	riparian forest	amorphous, rod
Mimosa pilulifera Benth. var. *pseudincana* (Burkart) Barneby	shrub	Araucaria Forest in regeneration	fan shaped, agglomerate
Mimosa monticola Dusen.	shrub	Araucaria Forest in regeneration	no nodules
Mimosa scabrella Benth.	tree	preserved Araucaria Forest	spherical, rod, fan-shaped
Mimosa sp. 1	shrub	Araucaria Forest in regeneration	spherical
Mimosa sp.2	creeper	preserved Araucaria Forest	no nodules

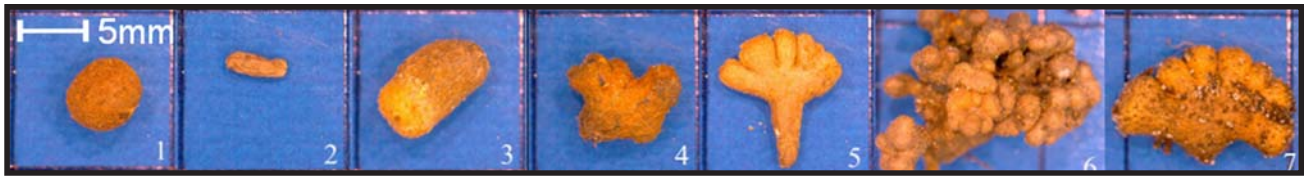

Figure 1 - Nodule shapes found in legumes collected in the Campos do Jordão State Park (1 – spherical, 2 – small rod, 3 – large rod, 4 – amorphous, 5 – fan-shaped, 6 – agglomerate and 7 – coralloid).

Table 2 - Phenotypic richness (%) of bacterial isolates from each legume plant.

Bacteria characteristics	Legume species								
	G. crassifolia	*C.speciosa*	*A. dealbata*	*M. dolens*	*M. filipetiola*	*M. furfuracea*	*M. pilulifera*	*M. scabrella*	*Mimosa* sp. 1
Number of isolates	23	24	24	13	19	22	10	60	17
Growth rate									
very slow	**52.2**	29.2	4.2	**61.5**	5.3	9.1	30.0	10.0	15.8
slow	0	8.3	20.9	30.8	0	0	10.0	3.3	15.8
intermediate	26.1	8.3	**58.3**	7.7	26.3	**50.0**	**40.0**	15.0	10.5
fast	8.7	**45.9**	8.3	0	26.3	13.6	20.0	26.7	**31.6**
very fast	13.0	8.3	8.3	0	**42.1**	27.3	0.0	**45.0**	26.3
Color									
white	8.7	**45.8**	0	0	47.4	27.3	10.0	18.3	**68.4**
pink	21.7	20.8	**45.8**	0	0	4.5	10.0	1.7	5.3
translucid	26.1	16.7	41.7	0	26.3	22.7	20.0	**45.0**	10.5
yellow	0	0	4.2	0	5.3	0	10.0	0	0
white with pink center	**43.5**	16.7	8.3	**100**	21.0	**45.5**	**50.0**	35.0	15.8
pH Change									
alkali	**60.9**	**45.8**	29.2	46.2	**42.1**	**45.4**	30.0	8.3	21.1
neutral	8.7	37.5	**37.5**	**53.8**	26.3	18.2	**40.0**	1.7	10.5
acid	30.4	16.7	33.3	0	31.6	36.4	30.0	**90.0**	**68.4**
EPS									
none to moderate	47.8	8.3	8.3	0	0	0	**90.0**	**100**	10.5
moderate to copious	**52.2**	**91.7**	**91.7**	**100**	**100**	**100**	10.0	0	**89.5**

Some authors use plant taxonomy as a tool for the classification of legume nodules. It is necessary to know some of the developmental history of the nodule to use this criterion (Sprent, 2001). Different shapes may be related to different developmental phases of the nodule. In our study, there was no possibility to find out nodule phenology, and therefore it would not be adequate to try to include our findings in this system. Although accepting the idea that nodule shape is related to plant taxonomy, it was intended to test the hypothesis that the kind of the bacterial isolate might also influence nodule shape. Nevertheless, so far, no correlation was found between the phenotypic classification of the bacteria and nodule shape.

Phenotypic bacterial richness

A great phenotypic bacterial richness was found, and different legumes showed different percentages of strain phenotypes in the nodule bacteria community (Table 2). Cluster analysis was performed for all isolated bacteria together, resulting in six groups, separated by each plant (Table 3). The

Table 3 - Bacterial groups formed for each legume species.

Bacterial groups	Isolated Bacteria*
Galactia crassifolia	
Group I	Gc11A150,Gc11A151,Gc21A135,Gc21A136,Gc21A137,Gc21B138,Gc21B139 and Gc21B140
Group II	Gc11C141, Gc11C142,Gc11C143 and Gc21C147
Group III	*B. elkanii*, Gc21C148
Group IV	Gc11B149, Gc21C145 and Gc21C146
Group V	*R. tropici*, Gc11A170, Gc11B174 and Gc11C144
Group VI	*Burkholderia* sp., Gc11A169, Gc11B173, Gc11C171 and Gc11C172
Collaea speciosa	
Group I	Cs31A160, Cs31A161, Cs31B162, Cs31B163, Cs31C164, Cs41A158, Cs41A159, Cs41C154 and Cs41C155
Group III	*B. elkanii*, Cs12A132, Cs22A122, Cs22A123, Cs41B156 and Cs41B157
Group V	*R. tropici*, Cs12A131, Cs12B130, Cs12C126, Cs12C127, Cs22B124, Cs22B125, Cs22C133, Cs22C134, Cs31C217 and Cs31C218
**Burkholderia* sp. does not form a group with any of these strains.	
Acacia dealbata	
Group I	Ad11C165, Ad13A244, Ad13A245, Ad13C241, Ad13C242 and Ad13C243
Group III	*B. elkanii*, Ad13B247, Ad21C262, Ad21C263, Ad21C264 and Ad23A271
Group IV	Ad21A256, Ad21A257, Ad23B254, Ad23B255 and Ad23C258
Group V	*R. tropici*, Ad11C166, Ad13B246, Ad21B260, Ad23A272, Ad23A273
Group VI	*Burkholderia* sp., Ad13B219, Ad21B261 and Ad23C259
Mimosa dolens	
Group I	Md13A210, Md13A211, Md13B212, Md13B213, Md13C214, Md13C215, Md13C216, Md21A208, Md21A209, Md21C205, Md21C206 and Md21C207
Group IV	Md21C204
**R. tropici*, *B. elkanii* and *Burkholderia* sp. do not form groups with any of these strains.	
Mimosa filipetiola	
Group II	Mi17A281
Group III	*B. elkanii*, Mi17C091, Mi17C092, Mi17C094 and Mi23C152
Group IV	Mi17A279, Mi23A201, Mi23A202 and Mi23A203
Group V	*R. tropici*, Mi17B081, Mi17B082, Mi23A073, Mi23A074, Mi23A075, Mi23A076, Mi23B199, Mi23B200 and Mi23C153
Group VI	*Burkholderia* sp., Mi17A280
Mimosa furfuracea	
Group I	Mf13A250

Continue...

Table 3 - Continuation.

Group II	Mf13A251
Group III	*B. elkanii*, Mf13C252, Mf13C253, Mf24B235 and Mf24B236
Group IV	Mf23A265, Mf23A266, Mf23A268, Mf23B239, Mf23B240, Mf23C269 and Mf23C270
Group V	*R. tropici*, Mf13B093, Mf13B095, Mf13B096, Mf23A267, Mf24A238 and Mf24C234
Group VI	*Burkholderia* sp., Mf24A237, Mf24C232 and Mf24C233
Mimosa pilulifera	
Group I	Mp15A274 and Mp15A275
Group II	Mp26A282 and Mp26A283
Group IV	Mp15A276, Mp26A284, Mp26A286 and Mp26A287
Group V	*R. tropici*, Mp26A288
Group VI	*Burkholderia* sp., Mp26A285
**B. elkanii* does not form a group with any of these strains.	
Mimosa scabrella	
Group I	Ms53B226, Ms53B227, Ms63A231, Ms81A188, Ms81C077, Ms81C079 and Ms81C080
Group II	Ms81A189
Group IV	Ms63B228 and Ms63B229
Group V	*R. tropici*, Ms33A120, Ms33A121, Ms33C116, Ms43A085, Ms43A086, Ms43A087, Ms43B088, Ms43B089, Ms43B090, Ms53C224, Ms63C103, Ms63C104, Ms63C105, Ms63C106, Ms71B179, Ms71B180, Ms71B181, Ms71C184, Ms71C185, Ms75A182, Ms75A183, Ms75B097, Ms75B098, Ms75B099, Ms75B100, Ms75C186, Ms81C078, Ms83A177, Ms83A178, Ms83B107, Ms83B108, Ms83C175, Ms83C176, Ms85A197, Ms85A198, Ms85B196, Ms85C193 and Ms85C194
Group VI	*Burkholderia* sp., Ms33B118, Ms33B119, Ms33C117, Ms43C222, Ms43C223, Ms53C225, Ms63A230, Ms71C187, Ms81B190, Ms81B191, Ms81B192 and Ms85B195
**B. elkanii* does not form a group with any of these strains.	
Mimosa sp. 1	
Group I	M111B221, M123C112
Group II	M111C248
Group III	*B. elkanii*, M111B220, M111C249
Group IV	M111C278
Group V	*R. tropici*, M111A083, M111A084, M111A102, M111B110, M111B111, M123A101, M123C113, M123C114 and M123C115
Group VI	*Burkholderia* sp., M111B109 and M111C277

*Isolated bacteria codes correspond to the initials of the name of the legume species (Gc - *Galactia crassifolia*, Cs - *Collaea speciosa*, Ad - *Acacia dealbata*, Mf - *Mimosa furfuracea*, Mp -*Mimosa pilulifera*, Ms - *Mimosa scabrella*, Mi - *Mimosa filipetiola*, Md - *Mimosa dolens*, M1 - *Mimosa* sp.1); number of the individual legume; nodule shape (1 – spherical, 2 – small rod, 3 – large rod, 4 – amorphous, 5 – fan-shaped, 6 – agglomerate and 7 – coralloid); nodule extraction (A – strain obtained from one single nodule, B – strain obtained from one single nodule different from "A", C – strain obtained from all the other nodules crushed together), and storage number (the last three numbers).

growth standards *Bradyrhizobium elkanii*, *Rhizobium tropici*, and *Burkholderia* sp. are included in groups III, V and VI, respectively, while three groups are quite different from these standards.

PCA analysis was performed for all bacterial isolates and all leguminous species together, but the results are presented separately for each legume species (Figure 2). The interpretation for the axes of the figures is as follows: PCR1 is a combination of "growth" (0.59), "pH change" (0.55) and "color" (0.49), while PCR2 is predominantly formed by "EPS" (0.87). PCR1 explains 47.63% and PCR2 explains 24.2% of the total variation. The variables, represented as vectors, which stand for "growth rate" and "pH reaction" were closely related to each other in the PCA. This had been expected, since, in general, slow growth is related to alkali production and fast growth is related to acid producers (Odee et. al., 1997). Based on the cluster analysis the groups of bacteria were separated for each legume in the PCA.

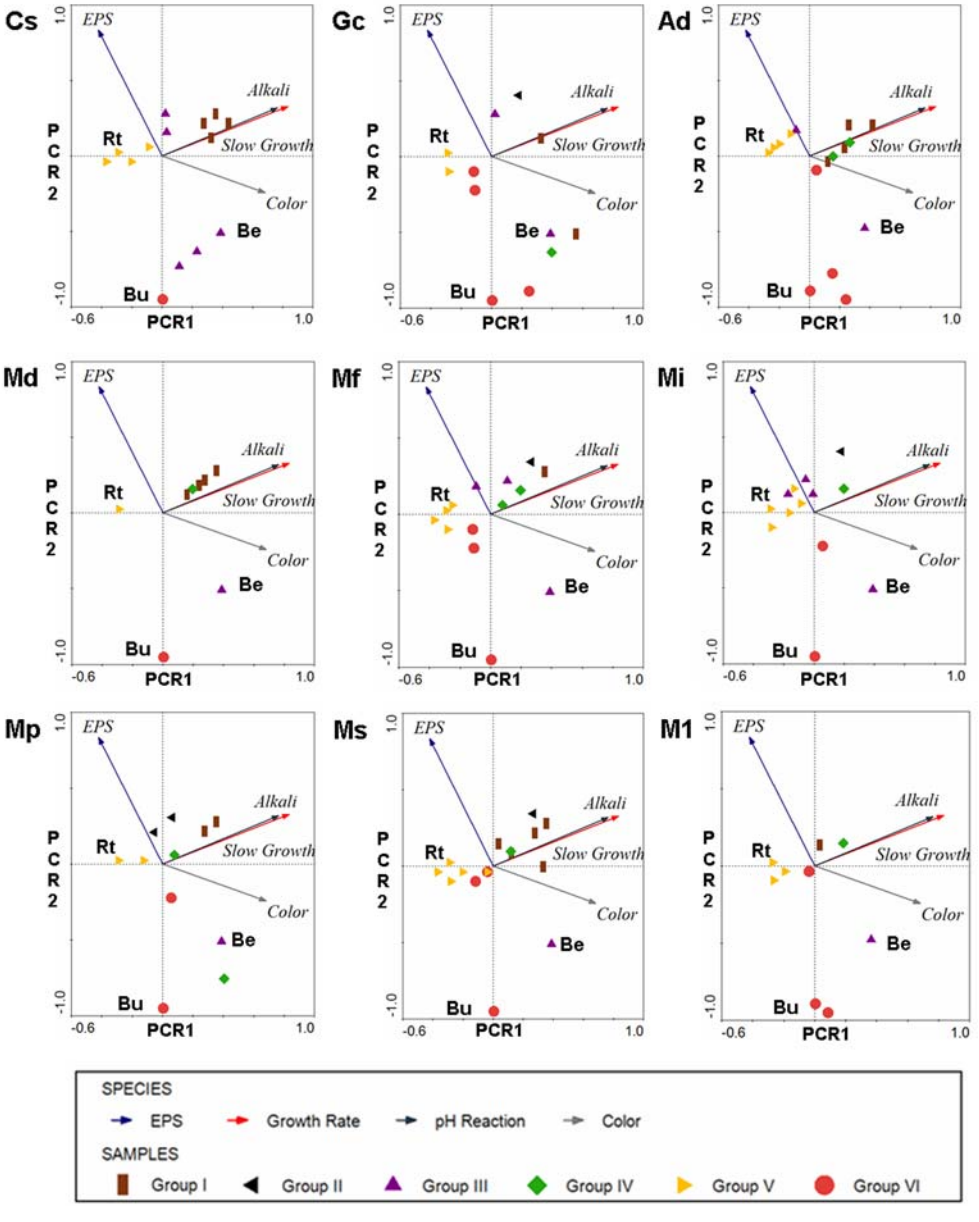

Figure 2 - PCA correlating each leguminous plant (Cs - *Collaea speciosa*, Gc - *Galactia* crassifolia, Ad - *Acacia dealbata*, Md - *Mimosa dolens*, Mi - *Mimosa filipetiola*, Mf - *Mimosa furfuracea*, Mp -*Mimosa pilulifera*, Ms - *Mimosa scabrella*, M1 - *Mimosa* sp.1) with its respective phenotypic bacterial groups. The bacterial standards are *Rhizobium tropici* (Rt), *Bradyrhizobium elkanii* (Be) and *Burkholderia* sp. (Bu).

Since the cluster analysis did not provide characteristics about the groups formed, we used this information together with the PCA analysis (Figure 2) to characterize the predominance of bacterial phenotypes in each group. Group I may be characterized by the predominance of bacteria of slow growth, alkali production, white colonies with pink center, and none to moderate EPS production. Group II has similar characteristics of the previous group, except for the intermediate growth rate and moderate to copious EPS production of its predominant bacteria. Group III has predominance of bacteria with a slow growth rate, alkali producers and white colonies. Group IV has the predominance of bacteria with an intermediate growth, and neutral or alkaline pH reaction. Group V presents predominance of fast growing bacteria, acid producers, with translucid colonies. In group VI there is predominance of bacteria with a fast to intermediate growth rate, with acid or neutral reaction, and white colonies.

Silva & Döbereiner (1982) reported the presence or absence of nodules in Brazilian legumes, including some samples of legumes growing in Araucaria Forests. The isolated bacteria were classified as *Rhizobium* sp., but the bacterial richness or diversity was not studied. Similar studies report richness of rhizobia obtained from several legumes from Kenya, where three phenotypic groups were formed, which the authors called "phena", and from Libyan forests, where five distinct clusters were formed (Odee et al., 1997; Mohamed et al., 2000).

Phenotypic bacterial richness in each legume

The richness varied from two groups in *Mimosa dolens* to six groups in *Galactia crassifolia, Mimosa* sp.1 *and M. furfuracea* (Table 3). In general, each legume harbored more than one bacterial group in its nodules. Also, different individuals of the same species generally presented different bacterial communities. In the same manner, most of the nodules were occupied by more than one bacterial group. Using trap plants, Melloni et al. (2006) found a high diversity of rhizobia in Brazilian areas associated to the presence of *Mimosa scabrella*. In their study six groups were characterized using *Phaseolus vulgaris* L. as trap plant and eight groups using *Vigna unguiculata* (L.) Walp.

Since data here shown indicated a great richness of bacterial isolates, many of them quite different from the standards (*Rhizobium tropici, Bradyrhizobium elkanii* and *Burkholderia* sp.), we partially sequenced the 16S rRNA gene of some of the strains (unpublished data). Based on this technique it was possible to find out that isolates often present great similarity to *Burkholderia*, when submitted to the BLAST software in GenBank (http://www.ncbi.nlm.nih.gov/BLAST).

Moulin et al. (2001) isolated *Burkholderia* spp. from legume nodules and recently it was demonstrated that some *Burkholderia* species form nodules with *Mimosa* spp., including *M. scabrella* and other tropical species from Brazil (Chen et. al., 2005; Elliott et al., 2007). In a similar study, Barrett & Parker (2006) suggested that *Burkholderia* spp. and other β-proteobacterial species are more ubiquitous as root nodule symbionts than previously believed. Thus, *Burkholderia* spp. might also be present in legume nodules from the Araucaria Forest.

CONCLUSIONS

For the first time, nodulation of five legumes (*Galactia crassifolia, Colllaea speciosa, Mimosa furfuracea, M. pilulifera* and *M. filipetiola*) is reported. A high phenotypic richness of nodule shapes and bacterial isolates was found in leguminous plants that grow in an Araucaria forest in the Campos do Jordão State Park, Brazil. Several of the isolated bacteria are significantly different in phenotypic characteristics from the bacterial standards.

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