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Analysis of a bacterial community structure and the diversity of *phz*F gene in samples of the Amazonian Dark Earths cultivated with cowpea [*Vigna unguiculata* (L.) Wald]

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It is important to understand the Amazonian Dark Earth (ADE) diversified microbial communities which colonize agricultural soils and interact with plants, allowing a more sustainable way of soil utilization. Genomic prospection of biotechnological interest, such as the phenazine biosynthesis genes, was carried out by the characterization of the bacterial community structure through the analysis of the 16S rRNA gene in ADE rhizospheric and bulk soil sampled in the forest, and in the agriculture managed soil, being subsequently cultivated with caupi bean. Additionally, the *phz*F gene coding for a key enzyme in the phenazine biosynthesis was detected and quantified. Gene polymophism (Terminal Restriction Fragment Length Polymorphism, T-RFLP) analysis revealed differences in the bacterial community structure between colonized rhizospheric and bulk soil, but there were no differences concerning the 16S rRNA gene copy number. Besides, the *phz*F gene copy number was higher in the rhizospheric than in the bulk soil, without any difference between forest and agricultural soils. This work confirms that the type of soil and the interaction between plants and microorganisms are key factors that shape the structure and diversity of bacterial communities and represent a biotechnological potential, with the possibility of finding natural compounds for use in biological control.

Key words: Amazonian Dark Earth, rhizosphere, quantitative polymerase chain reaction (PCR), terminal restriction fragment length polymorphism (T-RFLP), microbial diversity.

INTRODUCTION

Soil bacterial communities carry genes with yet unknown functions that could possibly be important concerning biotechnological applications. Plant commensal bacteria play an important role contributing to their protection from harmful organisms as also to their physiology (Berlec,

2012). To date, molecular techniques make possible to identify and characterize these microorganisms, particularly functional bacterial communities associated to plants. In this way, the Amazonian Dark Earth, also known as Amazonian Black Soil or "Terra Preta" (ADE),

represents a kind of soil that is colonized by bacterial communities with potential biotechnological contribution (Lehmann et al., 2003; Lima et al., 2002). These soils are characterized by a dark coloration due to the high charcoal concentration, originated from traditional burnings, utilized by the Amazonian ancient populations. It is found in diverse sites in the amazon biome, exhibiting elevated pH values, fertility and microbiological diversity (Navarrete et al., 2010). Due to these characteristics, the ADE soils behave as reservoir of specific bacterial genes participating in certain functions, e.g. in the nitrogen and carbon cycle, coding for proteins responsible for degradation processes and secondary compounds synthesis (Germano et al., 2012; Brossi et al., 2014; Nakamura et al., 2014; Lima et al., 2014). It is feasible that many genes could be expressed under the interaction between microorganisms and plants as their products are important sources of bioactive metabolites. Besides, bacterial organisms pose relevant role in the production of compounds utilized in the pharmaceutical, food and farming industry. Certainly, the detection of secondary compounds in the environment produced by prokaryotic cells has been increasing in face of the improvement of molecular tools that make possible their application in biocontrol of plant pathogens in commercial crops.

The phenazine, produced by bacterial organisms commonly found in plants rhizosphere, is a pigmented nitrogenated heterocyclic compound that exhibits an ample inhibitory activity against eukaryotic and prokaryotic cells (Mavrodi et al., 2010; Lovic et al., 1993). Microorganisms' competition and survival are the main factors involved in the phenazine synthesis, mainly in the root, in their natural habitat as their producers are allowed to carry out redox reactions, e.g. Nicotinamide Adenine Dinucleotide (NAD) oxidation and Reactive Oxygen Species (ROS) generation (Guttenberger et al., 2018).

The phenazine-1-carboxylic acid compound, produced by the *Pseudomonas fluorescens* strain 2-79, was initially reported by Mavrodi et al. (2010), in significant concentrations of 27 to 43 ng g^{-1} in rhizospheric soils, suggesting that this antibiotic exerts great activity in the root. The phenazine synthesis is carried out by an *Operon* composed of seven biosynthetic genes (*phz* ABCDEFG) in *Pseudomonas* species (Fitzpatrick, 2009). Through root exudates, plant adapts to the soil microbiota community shaping the surrounding rhizophere in a dynamic and vital biochemical relationship (Jacoby et al., 2017).

Despite scientific advances in soil microbiology, the majority of soil microorganisms are still unknown.

Previously to molecular genetics methodology aiming to identify bacterial organisms, phenotypic identification was currently used, based on colony morphology, color and bacterium individual morphology and staining properties, and until now culture and staining techniques are coupled to genomic studies. The fact that every living unicellular or multicellular organism share common properties displaying unique profile coded in the DNA, the most conserved sequences that show specific variations among different organisms were selected and employed to distinguish and group bioorganisms in separate taxonomic units. Therefore, the most promising genes were those coding for ribosomal RNA, 5S, 16S and 23S rRNA and also interspace sequences of these genes. Nowadays, the DNA sequence coding for the 16S rRNA subunit is the most commonly employed for prokaryote phylogenetic studies (Palys et al., 1977; Pace, 1977; Suárez Moya, 2017; Larkin and Martiny, 2017; Fierer, 2017).

The frequency of bacteria and gene coding for the phenazine compound present in rhizospheric ADE soil was quantified and described, utilizing specific probes that determined conserved regions of the 16S rRNA bacterial gene and the *phz*F gene. Besides, it was possible to quantify the studied genes and to determine the molecular profile of bacterial communities in these soils. It is important here to inform about the role of functional bacterial community in rhizospheric and bulk ADE soils cultivated with the caupi bean [*Vigna unguiculata* (L.) Wald].

MATERIALS AND METHODS

This research work was conducted in the Laboratory of Phytopathology of the Instituto Nacional de Pesquisas da Amazônia-INPA, Manaus-AM and in the Laboratory of Cellular and Molecular Biology of the Centro de Energia Nuclear na Agricultura, Universidade de São Paulo (CENA)-USP. The organization of the experimental procedures is described in the flowchart (Figure 1).

Soil sampling

Samples of the ADE soil were collected in the year 2013, in the Hatahara site located in the Amazonas state, in the Iranduba county (Figure 2), in two distinct areas, the forest area (03°16'494''S 60°12'340''O) and the agricultural area (03°16'516''S 60°12'275''O). Sampling procedure was carried out in five points, starting in a central location in the North, South, East and West positions. Collection points were separated from each other by 15 m, and two samples were collected from each point at a depth of 20 cm in the soil.

The chemical analysis of soil attributes under the different studied environments was previously realized in the experimental

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SOIL SAMPLE COLLECTION

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CHEMICAL ANALYSIS

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VEGETATION HOUSE EXPERIMENT

↓

COLLECTION OF RHIZOSPHERIC AND BULK SOILS

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DNA EXTRATION

AMPLIFICATION OF 16S rRNA AND phzF GENES BY QUANTITATIVE PCR   T-RFLP ANALYSIS OF A BACTERIAL COMMUNITY IN SOIL SAMPLES
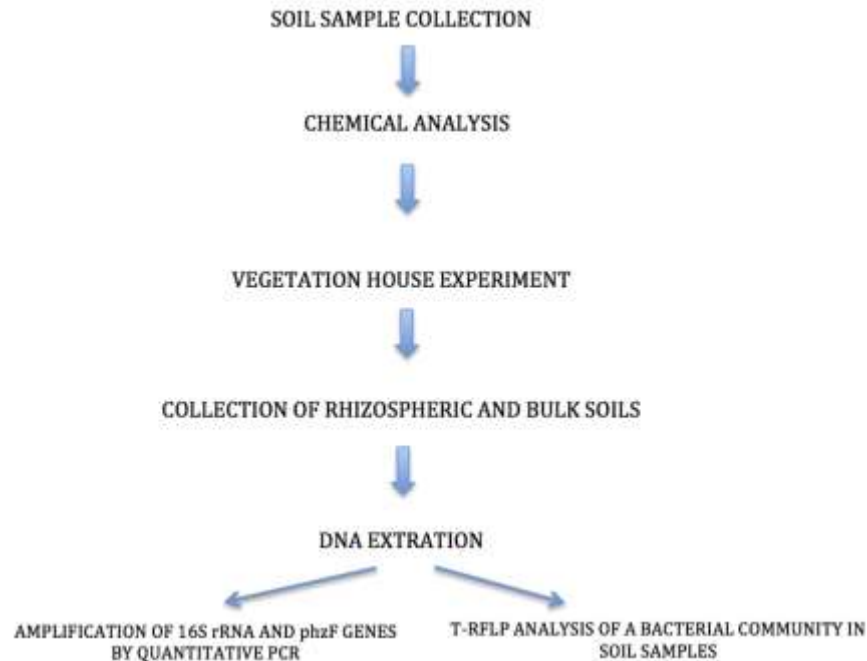
Figure 1. Flowchart of experimental procedures carried out in this research work.

Figure 2. Geographical location of Hatahara site, Iranduba county in the Amazonas state where the soil was collected.

set up of the mesocosm. From each area, forest and agricultural, 400 g of soil samples were collected. The analyses were carried out in triplicate, in the laboratory of the Department of Soil Science in the Escola Superior de Agricultura Luiz de Queiroz-ESALQ, Piracicaba-SP. For phosphorus (P) and Aluminum (Al) dosage, the extraction was done by calorimetry utilizing an ion exchange resin and potassium chloride, respectively. Acidity potential (H+Al) was determined with the Shoemaker Mac lean e Pratt buffer; organic matter (OM) and the sum of exchangeable bases (EB) were determined by the Walkley-Black method; Calcium (Ca), Manganese (Mg) and Iron (Fe) ions contents were determined by atomic absorption spectrometry, extracted with an ionic exchange resin (Raij et al., 2001).

The experimental design was totally random, with a factorial scheme of 2×2×2, being two soil samples, agricultural and forest; two systems, with and without plants; two genes, 16S rRNA and *PhzF*, with 15 repetitions. Collected samples at points of each area (forest and agricultural) were homogenized, yielding a composed sample representing each area, distributed in 10 pots of 3.6 kg capacity. After asepsis, by total immersion in 70% alcohol for 10 min, washing in *milli*Q water and dried in towel paper, 5 seeds of the caupi bean [*V. unguiculata* (L.) Wald] (Crioula-INPA variety) were sown in 5 pots containing soil sample of each area. After the germination period, 2 plants were kept until the end of the experiment. In order to control the experiments, 5 pots were kept exclusively with the collected soil, without seeds, under the same conditions of pots with germinated plants. The experimental design was completely randomized and carried out in the vegetation house

located at the Instituto Nacional de Pesquisas da Amazônia-INPA, Manaus-AM.

At 45 days post seeding, in the flowering period, rhizospheric soils were collected from each pot with 2 caupi bean plants, from both forest and agricultural area, and also from pots with just soil without plants, treated in the same conditions as earlier stated. The soil collection was done utilizing a sterilized firm bristle brush. From each pot with plants, 3 samples of rhizospheric soil were collected, as also from pots without plants but with soil only (bulk soil), without the influence of beans root. The soil samples were kept at -20°C until DNA extraction.

Metagenomics analysis

Genomic DNA was extracted from rhizospheric and bulk soil in triplicate, utilizing the Power lyzer DNA Extraction^{TM} Kit (MoBio, Carlsbad, CA). In microtubes containing glass beads, 0.25 g of each sample was added and gently homogenized. After cell lysis, total DNA was extracted according to the manufacturer's instructions, ascertaining the DNA quality by measuring the sample in the spectrophotometer, Nanodrop 1000 (Thermo Scientific, Waltham, EUA).

The bacterial 16S rRNA gene segment was PCR amplified utilizing the primers U968F (5' AAC GCG AAC AAG CTT AC 3') and R1387 (5'CGG TGT GTA CAA GGC CCG GGA ACG 3') (Heuer et al., 1997), yielding an amplicon of 400 bp. The primers pair Ps_up1 (5'ATCTTCACCCCGGTCAACG3') and Ps_low1 (5'CCRTAGGCCGGTGAGAAC3') were applied for PCR synthesis of the *phz*F gene, generating a segment of approximately 427 bp (Mavrodi et al., 2010). The quantitative PCR reactions were run in a total volume of 10 µL containing 5 µL of the SYBR Green Rox (qPCR Kit, Fermentas, Brazil), 1.0 µL of each primer (5 pmol), 2 µL of *milli*Q water and 50 ng of the purified product. PCR conditions were optimized to each primer.

Also, for the 16S rRNA gene amplification, the following conditions were optimized: 94°C for 10 min, 40 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 40 s. At the end of the reaction, a melting curve was included in the following conditions: 95°C for 15 s, 56°C for 1 min with temperature of 95°C for 15 seconds, and collection at 0.7°C. For the *phz*F gene, the optimized conditions were: 94°C for 4 min, 94°C for 1 min, 62°C for 30 s, 72°C for 45 s, and the melting curve was obtained in the following conditions: 95°C for 15 s, 62°C for 1 min, 95°C for 15 s. The reactions were carried out in the StepOnePlus$^{™}$ Real Time PCR System equipment (Applied Biosystems).

Specific and cloned fragments of the 16S rRNA and *phz*F genes were utilized to build the standard curve. The amplicon positive control was purified utilizing the GFX^{TM} PCR DNA and Gel Band Purification kits (GE Healthcare), following the manufacturer's instruction, and quantified in the spectrophotometer model ND-2000 (Nanodrop Technologies, Walthan, EUA), calculating the gene copy number per gram of soil. The standard curves were generated applying 10 serial dilutions, varying from 10^2 to 10^7 gene copy per µL. The cycle threshold (Ct) values were utilized as normalizers, identifying the cumulative points of amplicons in each sample by the generated fluorescence in each cycle. Aiming to verify if there was difference between genes copy number found in soils of each area assayed, analysis of variance was conducted at significant level of p<0.05 using the Software Graphpad Prism version 5. The analysis data, when necessary, were transformed to log^{10} and analyzed according to the relative species abundance.

Terminal restriction fragment length polymorphism (T-RFLP) analysis of a bacterial community in soil samples

T-RFLP analysis was carried out to determine the bacterial community structure of agricultural and forest soils. The bacterial 16S rRNA gene was PCR amplified utilizing DNA samples obtained from rhizospheric and bulk ADE soils. The PCR was set up employing the primers: 27F AGAGTTTGATCMTGGCTCA and 1492R TACGGYTACCTTGTTACGACTT (Edwards et al., 1989; Woese et al., 1990). In order to subsequently detect the amplicons by the T-RFLP technique, the primer 27F was labeled with the 6-carboxyfluorescein (6-FAM) in its 5' terminal.

The amplified fragments were purified utilizing the GFX^{TM} PCR DNA kit and the Gel Band Purification kit (GE Healthcare), following the fabricant's instructions. The purified amplicons were submitted to reactions with the *Msp*I restriction enzyme (Invitrogen, EUA), and the digested products were sodium acetate/EDTA and absolute ethanol precipitated. To carry out the analysis of the Terminal Restriction Fragments (T-RFs), the precipitated digested product was resuspended in highly deionized formamide (Hi-Di formamide) and GeneScan 500 ROX Size Standard (Applied Biosystems).

The T-RFLP data was analyzed in a file generated by the sequencer's Data Collection Software, in the Peak Scanner software v 1.0 (Applied Biosystems) to determine the length of restriction fragments termini by comparison to pattern of fragments termini size (Trotha et al., 2002). The data were exported to an Excel electronic worksheet (Microsoft) and converted to a matrix for further multivariate analysis. The evaluation of the bacterial community structure obtained by T-RFs profiles was done by analysis in a multidimensional scale (multidimensional scaling, MDS) by stress levels of 0.06 and 0.01. The test was calculated based on the Bray-Curtis similarity coefficient, utilizing the Primer 6 software (Playmouth Marine Laboratory, Primer E, United Kingdom) (Clarke, 1993). Subsequently, a similarity analysis was run to determine statistical differences among the samples analyzed. Biological data were run in the Assistat Software version 7.7 beta (Silva, 1996) and submitted to variance analysis, and the means compared by the Duncan test at 5% probability. The redundancy and multivariate analysis were carried out to evaluate the correlation of bacterial communities' structural composition with chemical attributes of the agricultural and forest soil. The Canoco Software, version 4.5 (Biometris, Wageningen, Netherlands) was utilized.

RESULTS

Soil chemical analysis

There were significant statistical differences when comparing the chemical analysis data of collected samples in the forest and agricultural area (Table 1). Sulphur (S) and potassium (K) concentrations presented 64.2 and 33.6% differences in the forest and agricultural areas, respectively. Concerning Magnesium (Mg) and Calcium (Ca) contents, the differences were 31 and 0.7% respectively. There were no statistical significant differences in the parameters of Phosphorus (P) and Iron (Fe) content, acidity potential (H+Al), bases sum and ionic exchange capacity.

Bacterial and phzF gene diversity in rhizospheric and bulk soils

The gene copy number of the bacterial 16S rRNA in the forest soil determined by qPCR was 3.85×10^9 copies/g of soil in rhizospheric soils and 3.94×10^9 copies/g of soil in

Table 1. Chemical analysis of the Amazonian Dark Soil (ADE) in the forest and agricultural areas.

Area	Chemical parameters[1]										
	pH	P	S (**)	K (**)	Ca (*)	Mg (*)	H+Al	SB	CTC	Fe	M.O
Forest	5.5±011[a]	372±42.9[a]	4.3±0.57[b]	0.73±0.057[b]	158±16.7[a]	6.6±1.1[b]	32±1.7[a]	165.6±19.7[a]	197.5±19.7[a]	101±63.2[a]	67±3.6[a]
Agricultural	5.3±0.1[a]	409±61.77[a]	12±0.57[a]	1.1±0.057[a]	157±12.6b	9.6±0.57[a]	40±6.1[a]	168.1±6.7[a]	209.2±6.7[a]	79.3±4.5[a]	73.6±6.6[a]
CV%	1.99%	13.20%	6.90%	6.08%	9.39%	11.18%	12.36%	9.45%	7.20%	49.7%	7.60%
Differences forest vs. agricultural	3.7%	9.1%	64.2%	33.6%	0.7%	31%	20%	1.4%	5.5%	21.4%	8.9%

Calcium (Ca), Aluminum (Al), Magnesium (Mg); Acidity Potential (H+Al), Bases Sum (SB) and Cationic Exchange Capacity (CTC) are represented in mmolc dm^{-3}; organic matter (MO) in g dm^{-3}; Phospohorum (P), Iron (Fe) and Sulphur (S) in mg dm^{-3}. [1]Means with standard deviation. **Means followed by different letters differ statistically between them by the F test ($p<0.01$). *Means followed by different letters differ statistically between them by the F test ($p<0.05$).

bulk soils, while in the agricultural soils, there were $3.51×10^8$ copies/g of soil in rhizospheric and $2.79×10^8$ copies/g of soil in bulk soils. Despite numeric differences, there were no significant differences by the Duncan test at 5% probability concerning the gene copy number of the bacterial 16S rRNA between rhizospheric and bulk soils in the same environment (Figure 3a). Nevertheless, when the gene copy number was compared between different environments, the quantity was higher in the forest, for both rhizospheric and bulk soils (without statistical differences between them), that figure out a great bacterial diversity than in the agricultural soil. It was also possible to observe that, in the agricultural soil, the gene copy number was higher in the rhizospheric soil than in the bulk soil (without statistical differences).

The molecular analysis of the *phz*F gene allowed us to observe the gene frequency in the studied environment. When the *phz*F gene copy number was compared in rhizospheric soils obtained from the forest and agricultural area, a higher number of copies, $4.82×10^8$ copies/g of soil, in the agricultural area could be observed, as the number of copies in the forest area was $3.11×10^8$ copies/g of soil. In the bulk soil samples, there was no statistical difference by the Duncan test at 5% probability for the same parameter analyzed.

The results showed that soil samples collected in the forest area, after cultivation with caupi bean, had 1.26 times more the *phz*F gene copy number than the bulk soil ($2.46×10^8$ copies/g of soil), with no significant statistical difference. In agricultural soil samples, the *phz*F gene copy number was also higher in rhizospheric environment, $4.82×10^8$ copies/g of soil, than in bulk soil, $2.75×10^8$ copies/g of soil (Figure 3b).

Comparison of bacterial communities in rhizospheric and bulk soils

The T-RFLP analysis was performed to access the bacterial community structure colonizing the rhizospheric soil cultivated with caupi bean, and to compare to the bulk soil, collected in the forest and agricultural area, based on separated T-RFs for the bacterial 16S rRNA marker among ADE soils.

The results of T-RFLP analysis showed a clear distinction in the bacterial community structure between the soil collected in the forest and agricultural areas, as also, soil samples that were influenced by the caupi bean roots and control, and bulk soil. The data exhibited joining at 55% similarity, mainly high among biological replicates (Figure 4).

The results presented global R values above 0.83 to rhizospheric and bulk soil samples, in the agricultural environment, and 0.704 in the forest environment for rhizospheric and bulk soil samples, being statistically significant at $p<0.05$ level by the ANOSIM test. The analysis of ordered MDS clearly showed difference of bacterial communities between soil samples. This structural difference could be related to different characteristics of each soil, and mainly to the influence of caupi bean roots.

It was also possible to observe, from the profiles generated by the T-RFPL analysis, the differences in the bacterial community structures in rhizospheric soil cultivated with caupi bean and bulk soils, likewise among the areas where the soils were collected (forest and agricultural), which make us to infer that the environment influenced in this composition. In the soil of forest environment, it was observed that the caupi bean rhizosphere was more homogeneous than in the bulk soil (Figure 5), and it was also more gathering samples were noted.

Furthermore, the RDA analysis results of T-RFLP profiles showed that these bacterial communities also differed in the structure, meaning that there was integration of the replies
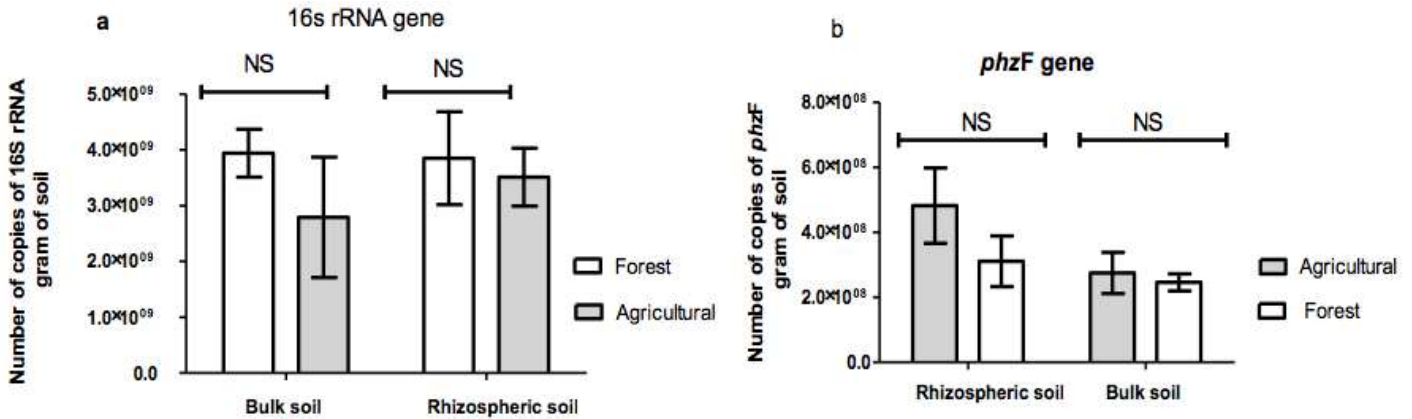
Figure 3. Mean of gene copy number of the bacterial 16S rRNA: (a) and number of phzF gene copy (b) determined by the quantitative PCR. Samples refer to ADE rhizospheric soil cultivated with caupi bean in two environment (forest and agricultural).
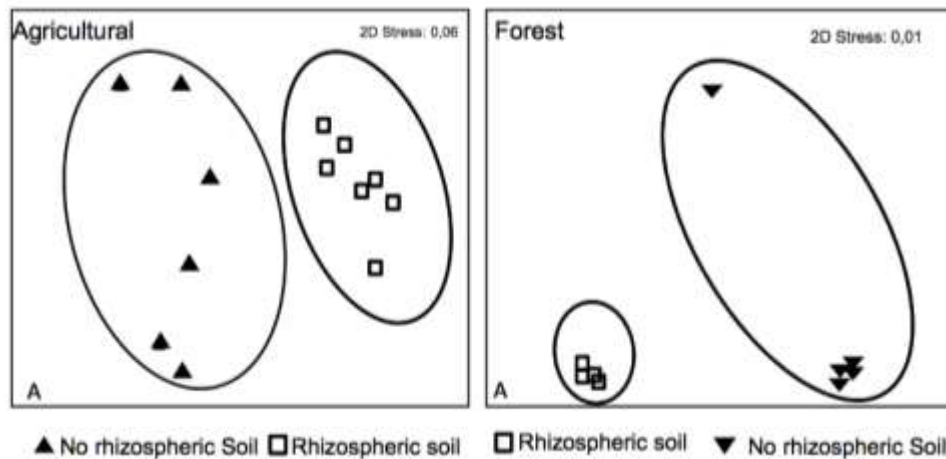

Figure 4. Multidimensional scale anelusis (MDS) based on T-RFs seperated by the 16S rRNA among areas and soil samples.

relating to both types of soils (rhizospheric and bulk soils) of each area. Anyway, there were no elements associated to the data variability, as those were responsible for 40% in the agricultural area and 44% in the forest as shown in Figure 6. The results also showed a distinct correlation of the chemical parameters with the bacterial communities among samples of rhizospheric and bulk soils.

DISCUSSION

Concerning the soil chemical analysis, it is important to reinforce that the ADE composition of stable pyrogenic organic matter allied to elevated contents of Phosphorus, Calcium, Magnesium and Carbon, which constitute a unique micro ecosystem, make it highly fertile (Glasser,

2007; Madari et al., 2006; Falcão et al., 2010; Lehmann et al., 2003). The pH of the forest soil was slightly elevated as compared to the soil in agricultural area, possibly explained by the burning of forest vegetation carried out by ancient communities as a practice to prepare the soil for new cultivation, which promotes the permutation exchange of cations from the ashes to the soil, causing pH acidification as described by Cenciani et al. (2009).

The molecular analysis of bacterial and phzF gene diversity in rhizospheric and bulk soils shows a great potential as reservoirs of new antibiotics. Distinct bacterial species colonizing soil communities, and also associated with plants roots produce natural antibiotics as a way to suppress pathogens attack. Gouda et al. (2018) emphasize the importance of plant growth promoting rhizobacteria as the best option for the plant
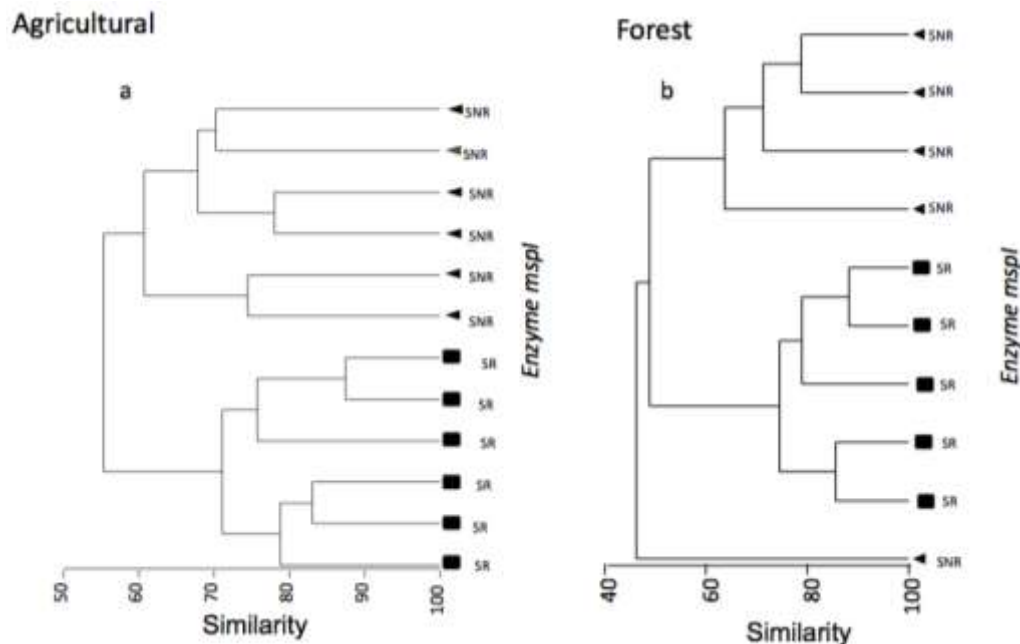
Figure 5. Similarity join between rhizopheric (SR) and bulk soil (SNR) collected in the forest (a) and agricultural environment (b). Obtained samples were grouped with algorithm, through the *Mspl* enzyme segmentation performed by CLUSTER analysis in Primer 6 software (Plymouth Marine Laboratory, PrimerE, United Kingdom).
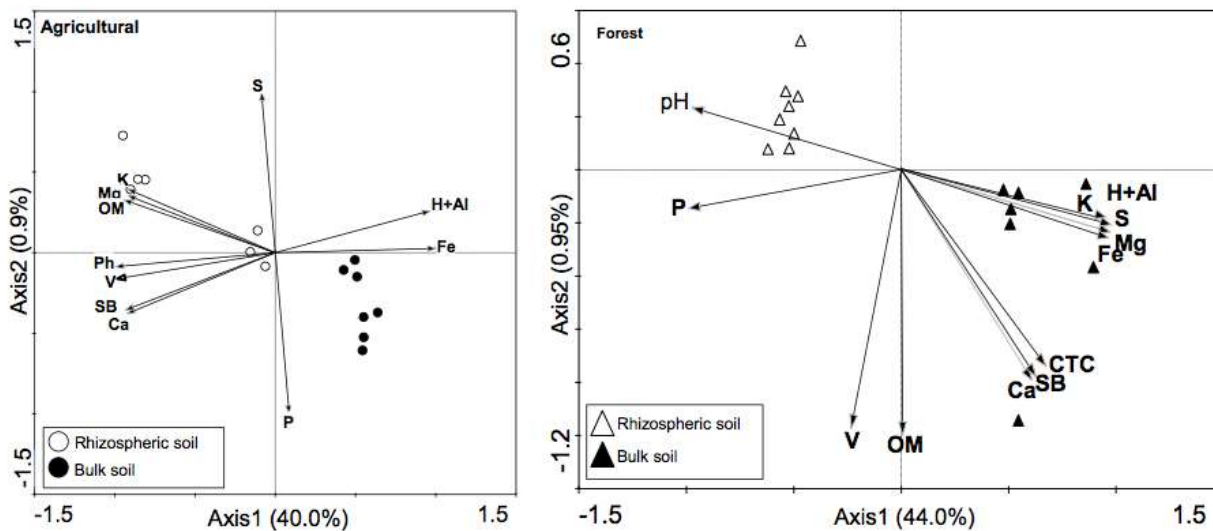

Figure 6. Redundancy analysis (RDA) of bacterial communities determined by the T-RFLP technique of chemical characteristics in the agricultural and forest soil.

and soil health instead of chemical fertilizers and pesticides. Rashid and Chung (2017) discuss plant hormone signaling regulation and biosynthesis by rhizobacteria and rhizofungi likewise the jasmonic acid, ethylene and salicylic acid pathways which trigger protection mechanisms against pathogens and insects.

The quantitative PCR analysis aimed at obtaining the total gene copy number of the bacterial 16S rRNA and the *phz*F gene in rhizospheric and bulk soil samples was precise concerning the gene copy quantification, allowing us to compare the frequency among soil samples in both forest and agricultural environments. The results suggest

that chemical, physical and biologic environmental conditions of the caupi bean rhizosphere could favor the incorporation of specific groups of bacterial organisms as mentioned elsewhere (Hinsinger et al., 2009). Undetected statistical difference concerning the elevated abundance of bacterial organisms in the rhizospheric and bulk soils seems to be related to the physicochemical stability and plant diversity in the ADE environment. The biological, chemical and physical conditions of the rhizosphere environment stimulate the plants to release organic ions to the environment, favoring the colonization or not of specific groups of prokaryotic organisms. Also, in the rhizosphere environment, the temperature and nutritional stress could directly affect its composition around the roots (Raiesi et al., 2015; Compant et al., 2005; Moreira and Siqueira, 2006). These findings focus on studies towards these groups of diversified bacterial organisms colonizing the plant rhizosphere microenvironment, considering the possibility of isolating and characterizing bacterial species through methods dependent or not on culture, manly to produce secondary metabolites as antibiotics.

The data obtained here suggest that the caupi bean rhizosphere microenvironment aggregates specific groups of bacterial organisms that harbor and/or express the *phz*F gene. After studying the evolution and dispersion of the *phz*F gene in rhizospheric soil samples, it corroborated the diversity of phenazine-producing bacterial organisms as previously discussed by Mavrodi et al. (2010), furthermore, implying that most of them are plant associated microorganisms and also that the *Pseudomonas* genus keeps mechanisms of gene conservation, while the *Burkholderia* and *Pectobacterium* genus operate mechanisms of horizontal gene transfer. The intra-genotypic diversity in a group of microorganisms that share the same antagonistic characteristics provides a largely unexplored resource to improve the biological control of plant pathogens (Raaijmakers et al., 2001; Weller et al., 2002).

The *phz*F gene diversity in the natural environment permits specific comprehension and regulation of these processes by some groups of bacteria that are relevant for the production of natural biocompounds *in vitro*. The results of this study add improvements to isolate and characterize phenazine-bacterial producers in soil systems, likewise in the plants rhizosphere. Besides, some groups of prokaryotes, colonizing the plant rhizosphere, produce phenazine when stimulated by phytopathogens attack.

It was observed that the caupi beans roots considerably influenced the bacterial community structure. It is also considered that the general characteristics of each area, forest and agricultural, could have shaped the bacterial community profile. Studies carried out by Lima et al. (2014), with ADE rhizospheric soil samples and controls, demonstrated in ordered MDS analysis, taking in account T-RFLP data that the rhizosphere of 2 leguminous species shaped the bacterial community structure which summed to soil properties.

The interaction among plants and bacteria is coordinated by intrinsic metabolic processes in both organisms, in a mutual recognition processes (Dini-Andreote and van Elsas, 2013). Furthermore, bacteria respond distinctly to the compounds secreted by plants roots (Melo and Azevedo, 2008), dictated by the soils' properties, which strongly influence the bacterial community structure (Jesus et al., 2009).

The characterization of bacterial communities in rhizospheric environments could contribute to future studies related to the soil quality and usefulness of these microorganisms in the environment as nitrogen biological fixation, bioremed* ation, production of enzymes involved in plastic degradation, disease control in plants and pathogen suppression in the soil (Bettiol et al., 2005). Similar studies with soils from the Amazon region showed that bacterial communities were influenced by the land use and chemical characteristics of the vegetation metabolites (Jesus et al., 2009; Navarrete et al., 2010; Lima et al., 2014).

Studies of the functional bacterial communities in the ADE indicated that the land use interfered on the amount of bacterial organisms and, the soil high fertility is associated to the microbial diversity detected when compared with other Amazonian soils (Brossi et al., 2014). In the present results, there was clustering of these bacterial communities, even though without significant differences. The historic events in the studied areas suggest a positive influence in the direct maintenance of the bacterial communities structure.

Conclusion

The 16S rRNA and *phz*F genes were detected in the caupi bean rhizospheric soil applying the quantitative PCR assay, demonstrating that the gene copy number of *phz*F was elevated in rhizospheric soils in the forest and agricultural environment. The bacterial 16S rRNA copy gene number was elevated in bulk soil collected in the forest environment, emphasizing the bacterial diversity in relation to the agricultural environment. The bacterial community profile showed divergence according to plant type and roots influence.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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