International Journal of Microbiological Research 4 (2): 119-131, 2013
ISSN 2079-2093
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DOI: 10.5829/idosi.ijmr.2013.4.2.7377

The Molecular Phylogenetic Diversity of Bacteria and Fungi Associated with the Cerrado Soil from Different Regions of Minas Gerais, Brazil

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Abstract: The microbial community structures associated with the soils from three regions of the Brazilian Cerrado of Minas Gerais, Brazil were evaluated using polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) of bacterial 16S rRNA (V3 and V6-V8 hypervariable regions) and fungal 18S rRNA and Internal Transcribed Spacer (ITS) regions. Soil chemical analysis was also evaluated and correlated with microbial community structures. The 16S rRNA sequences were assigned to the genera *Bacillus, Klebsiella, Enterobacter, Pantoea, Escherichia and Leuconostoc.* The DGGE profiles with Eukarya specific primers amplified bands related to the yeasts *Zygosaccharomyces, Candida* and *Lachancea* as well as to the filamentous fungus *Cladosporium*. The microbial fingerprints of the soils in the analyzed areas were similar during the rainy and dry season. The pH of the soil samples from the Passos, Luminárias and Arcos regions ranged from 4.7 to 5.5, 5.0 to 5.4 and 4.1 to 5.0, respectively. The chemical properties were no significant differences in the concentrations of phosphorus, magnesium and organic matter in the soils among the studied areas. Samples from the Arcos area show large amounts of aluminum in the rainy season and hydrogen+aluminum in the rainy and dry season. These results show that the microbial fingerprint was not affected by the high levels of aluminum in the soil. The results are important for understanding how the microbial communities in the Cerrado area are described in bacterial and fungal profiles.

Key words: Brazilian Savanna · PCR-DGGE · Microdiversity · Soil

INTRODUCTION

The Brazilian Cerrado biome contains savanna-like vegetation and occupies approximately 24% of the Brazilian territory. This area is characterized by high temperatures (22-27°C), rainfall (800-1.600 mm) and solar radiation (475-500 Cal cm^{-2} day^{-1}). Soils in this biome are highly weathered, which makes the soils acidic, poor in nutrients and rich in iron and aluminum oxides. The Brazilian Cerrado soil is atypical in that it is fire susceptible, has high aluminum levels and has experienced anthropogenic action. However, due to the excellent physical advances in agriculture within the last 30 years, the Cerrado has becoming a leading area for grain production in Brazil [1]. This biome is being threatened by increasing agricultural expansion and it is unclear how this conversion in pastoral areas impacts the microbial communities [2].

Some studies have investigated the structure of the soil microbial community. For instance, Castro *et al*. [3] showed a remarkable difference in soil fungal communities among a riverbank forest area, a pasture and a soybean plantation in Cerrado soils using ribosomal intergenic spacer analysis. Peixoto *et al*. [4] described a pioneering study using the universal rpoB gene (RNA polymerase b-subunit gene), as an alternative to the 16S rRNA gene, to evaluate total bacterial soil communities of Cerrado soils. In a later study, the use of this approach to compare tillage and no-tillage (NT) systems in Cerrado soils was shown to be efficient in detecting changes in the soil bacterial community structure (BCS) caused by alterations in soil conditions [2]. In soil the main activities of the organisms are decomposing organic matter, humus production, nutrient cycling and energy (including the fixation of atmospheric nitrogen), production of complex

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compounds that contribute to soil aggregation, decomposition of xenobiotics and biological control pests and diseases [5].

The government of Minas Gerais is involved in preserving this ecosystem; in particular, the Biota Project includes a characterization of the bacterial and fungal microbial communities. Microorganisms are a critical component of the ecosystems, as they mediate 80-90% of the processes that occur in the soil [6] and are key players in the carbon and nitrogen biogeochemical cycles. The functions and diversity of bacterial and fungal communities could be a more efficient and dynamic indicator of soil quality than those based on physical and chemical properties. However, little is known about the factors that drive diversity not only due to the complexity of communities but also because not all microorganisms can be cultured under laboratory settings [6]. Molecular techniques have often been applied to microbial ecology studies to explore the microbial diversity and analyze the structure of these communities [3, 6-8]. Culture-independent, molecular approaches have proven to be powerful tools towards providing a more complete inventory of the microbial diversity in environmental samples [8] and polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) has been applied successfully to assess the microbiota in soil [7]. Assessing the diversity in the Cerrado soil is an important aspect in the quest to maintain soil biodiversity [8]. This study is important in order to elucidate the different types of microorganisms and their importance in Cerrado soil.

The objectives of this study were first, to perform a molecular survey based on the sequencing of different rDNA regions of the bacterial and fungal communities associated with the Cerrado soil and second, to compare the bacterial and fungal communities of the Cerrado soil with the physicochemical characteristics of these soils.

MATERIALS AND METHODS

Site Descriptions and Soil Sampling: Soil samples were collected from three sites in Minas Gerais, Brazil. Each sampling point was geo-referenced and a general description of each site is given in Figure 1 and Table 1.

Thirty composited samples were collected in protected areas during January (high rainfall, 1000-2100 mm) and August (low rainfall, 20-200 mm) of 2010. Each sample consisted of 12 subsamples, collected at a depth of 0-20 cm within a circle of 3-6 m in diameter from the center (Fig. 1c). Before collecting the subsamples at each sampling point, all the equipment was flamed to prevent cross-contamination from earlier points. The soil samples were placed in sterile Nasco® plastic bags and stored at 4°C until further use.

DNA Extractions and PCR-DGGE Analyses: Each sample (approximately 0.25 g soil, wet weight) was transferred into a plastic tube and the DNA was extracted using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The genomic DNA was used as template for PCR amplification of target bacterial or fungal ribosomal regions and for denaturing gradient gel electrophoresis (DGGE) analyses. Two primers sets were used to analyze each microbial community. The information about the primers and the conditions for the PCR and PCR-DGGE analyses are shown in Table 2.

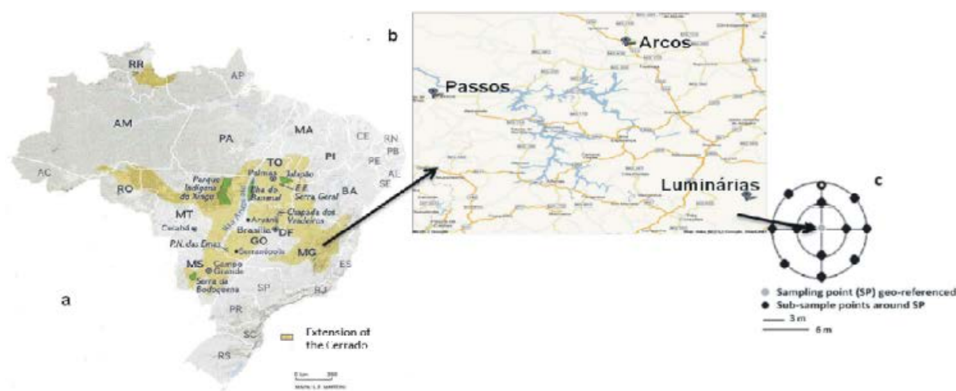

Fig. 1: A. Location of Cerrado soil in Minas Gerais, Brazil. B. Cities where samples were collected. C. Distribution of sampling point. Sampling point scheme: one composed soil sample (12 sub-samples) was collected around each sampling point.

Table 1: Location of the Brazilian Cerrado soil collection sites

	Site name	Location
PA	Point 1	20°49'57.7"S; 046°30'29.3"W
	Point 2	20°49'56.8"S; 046°30'30.1"W
	Point 3	20°49'48.0"S; 046°30'54.9"W
	Point 4	20°49'47.1"S; 046°30'54.5"W
	Point 5	20°49'47.8"S; 046°30'51.5"W
LU	Point 6	21°37'51.0"S; 044°58'22.7"W
	Point 7	21°37'50.6"S; 044°58'22.7"W
	Point 8	21°37'51.5"S; 044°59'11.0"W
	Point 9	21°37'55.3"S; 044°59'29.3"W
	Point 10	21°37'54.6"S; 044°59'54.0"W
AC	Point 11	20°16'27.7"S; 045°29'14.6"W
	Point 12	20°14'47.9"S; 045°25'35.9"W
	Point 13	20°14'51"S; 045°31'40.8"W
	Point 14	20°14'48.6"S; 045°31'33.4"W
	Point 15	20°14'58.0"S; 045°31'54.0"W

*Abbreviations: PA - Passos; LU - Luminárias; AC - Arcos.

Table 2: DGGE-PCR primers used to detect fungal and bacterial communities in the soils of the Brazilian Cerrado

Primer	Sequence (5' - 3')	Community	Target	PCR conditions	DGGE conditions	References
968fGC	AAC GCG AAG AAC CTT ACGC clamp connected to the 5' end of 968f	Bacterial	V6-V8 region of the 16S rRNA gene	Condition 1	16 h at 70 V at 60°C.	b
1401r ITS1fGC	CGG TGT GTA CAA GAC CC TCC GTA GGT GAA CCT GCG GGC clamp connected to the 5' end of ITS1gc	Fungal	ITS region of the rDNA	Condition 1	16 h at 70 V at 60°C.	a
ITS4r 338fGC	TCCTCCGCTTATTGATATGC GCA CGG GGG GAC TCC TAC GGG AGG CAG CAGGC clamp connected to the 5' end of 338fgc	Bacterial	V3 region of the 16S rRNA gene	Condition 1	6 h at 70 V at 60°C.	b
518r NS3fGC	ATT ACC GCG GCT GCT GG GCA AGT CTG GTG CCA GCA GCCGC clamp connected to the 5' end of NS3gc	Fungal	18S region of the rDNA	Condition 2	16 h at 70 V at 60°C.	b
YM951r	TTG GCA AAT GCT TTC GC					

* GC clamp - CGC CCG CCG CGC GCG GCG GGC GGG GCG GG, f - forward primer; r - reverse primer aWhite et al. (1990); bMagalhães et al. (2010) Condition 1 - Denatured for 5 min at 95°C. 30 cycles: denaturing at 92°C for 60 s, annealing at 55°C for 60 s and extension at 72°C for 60 s; final extension for 10 min at 72°C. Condition 2 - 35 cycles instead of 30.

Sequencing of DGGE Bands and Multivariate Analysis: Selected bands from the PCR-DGGE gels were excised with a sterile blade and the DNA was separated using the kit QIAEX II (Qiagen, Chatsworth, CA, USA) according to the manufacturer's protocol. The DNA recovered from each DGGE band was reamplified using the same primers to amplify the target bacterial and fungal regions (Table 2), except they did not include the GC clamp. The PCR amplicons were sequenced by Macrogen Inc. (Seoul, South Korea). GenBank (http://www. ncbi. nlm. nih. gov/BLAST/) was used to determine the closest known relatives of the partial ribosomal DNA sequences obtained. The compositional similarity between the microbial communities at each different site in the Brazilian Cerrado was based on the presence or absence of amplicons detected by DGGE. The gels were normalized to internal reference bands and analyzed using the BioNumerics software (version 1.5, Applied Maths, Kortrijk, Belgium) program to determine the diversity of the amplicons. Similarities between profiles were calculated using the Jaccard coefficient and an average linkage (unpaired group method with arithmetic means; UPGMA) dendrogram was separately derived for the prokaryotic and eukaryotic PCR-DGGE profiles.

Physicochemical Analysis of the Brazilian Cerrado Soil: The physical and chemical characteristics of the soil were analyzed using approximately 200 g of each sample. The soils were analyzed according to the method described by Embrapa [9] and measurements were taken for the concentration of potassium (K), phosphorus (P), aluminum (Al), magnesium (Mg), organic matter (OM), pH,

hydrogen+aluminum (H+Al), calculation of exchangeable bases (SB) and soil texture. The SAS System 9.1 (SAS Institute Inc. Cary, NC, USA) was used for statistical analysis with the general linear model (GLM) procedure. Correlations between the microbiota in the Brazilian Cerrado soils and the physicochemical soil variables were subjected to statistical analysis (principal component analysis, or PCA) using the Unscrambler® 9.7 software (CAMO, Oslo, Norway).

RESULTS

The Evaluation of Different Primers to Assess Bacterial, Yeast and Fungal Communities in the Brazilian Cerrado Soil and Microbial Identification: In this study, four of the mostly used primers for PCR-DGGE were selected to profile the microbial communities in the Cerrado soil of Minas Gerais, Brazil. Two primer sets targeted different regions of bacterial 16S rDNA, namely 968fGC and 1401r (V6-V8 region), 338fGC and 518r (V3 region) and two primer pairs ITS1 and ITS2, NS3 and YM951 targeted the fungal ITS (internal transcribed spacer) and 18S rDNA regions, respectively. All of the analyzed primer pairs gave satisfactory amplification of the samples.

This study is important in order to elucidate the different types of microorganisms and their importance in Cerrado soil. Also check the difference of primers to evaluate a community. The bacterial and fungal DGGE profiles of the three Cerrado soils are shown in Figure 2. These profiles showed a few strong dominating bands and a great number of faint bands. In general, the bands corresponding to the yeast Zygosaccharomyces sp. and *Lachancea meyersii* and to the bacteria *Enterobacter cowanii* were found in all soil samples (Fig. 2, bands a, c and n, respectively). This result could indicate that these yeast and bacteria are the dominant species in the Brazilian Cerrado soil samples from Minas Gerais.

Amplification with the fungal (Figs. 2a and 2b) primer pair ITS1fGC and ITS4r provides a diversity of bands, indicating the presence of the unculturable *zygomycete*, *Cladosporium* sp. and the uncultured soil fungus, *Cladosporium oxysporium*, which were not amplified by the primers NS3fGC and YM951r. The primer pairs were able to differentiate filamentous fungi from yeast. In relation to bacteria ecology, the primers 338fGC and 518r, which amplified the V3 region, were able to amplify a greater variety of bands that indicated the presence of *Pantoea agglomerans*, *Leuconostoc mesenteroides*, *Enterobacter* sp. and *Escherichia coli,* which were not amplified by the primers 968fGC and 1401r (Figs. 2c and 2d). This can be justified by the PCR conditions that may have improved the performance of this primer.

Table 3 and Figure 2 show the molecular diversity of bacteria and fungi found in the different Cerrado regions of Minas Gerais, Brazil. The bands in the acrylamide gels were excised and re-amplified and the eluted DNA fragments were sequenced with the primers NS3f and YM951r, ITS1f and ITS4r, 968f and 1401r, 338f and 518r (Fig 2). The sequences obtained had =98% identity with sequences available in GenBank.

In the eukaryotic community analysis, band a was identified as the yeast *Zygosaccharomyces* sp. , band b was identified as Candida sp. and band c as *Lachancea meyersii*. Bands d, e and f were identified as an unculturable fungi, ascomycota and zygomycete, respectively. Band g was identified as *Cladosporium sp*. band h was identified as an unculturable soil fungus and band i was identified as *Cladosporium oxysporium*. It was not possible to identify band j; the band was excised from the gel, but could not be assigned to any fungal species (Figs. 2a and 2b). In the prokaryotic community analysis, bands k to x (Figs. 2c and 2d) were identified as different bacterial species. Band k was identified as an unculturable soil bacterium. Band l was identified as *Bacillus macerans* and band m as *Klebsiella pneumoniae*. Band n was identified as *Enterobacter cowanii* and band p was identified as unculturable bacterium. Bands q and s could not be assigned to any bacterial species. Band t was identified as *Pantoea agglomerans* and band u as *Escherichia coli*. Band v was identified as *Enterobacter* sp. and band x was identified as *Leuconostoc mesenteroides*. Bands o and r were identified as *Bacillus subtilis*.

Among the yeasts, *Lachancea meyersii* was found in all samples (Passos, Luminárias and Arcos city) taken from the rainy and dry season using the primers ITS1fGC and ITS4r. Using the primers 968f and 1401r, it was possible to observe little variation between the profiles. The microorganism *Enterobacter cowanii* was observed in all samples taken from both the rainy and dry season. Compared to the primers 968f and 1401r, it was possible to verify a larger number of bands in the profiles. In the Passos and Luminárias samples, there was little variation between the rainy and dry season, as exhibited by few differences in the bands. In the Arcos region, no differences were observed in the profiles from samples taken during the rainy season and dry season.

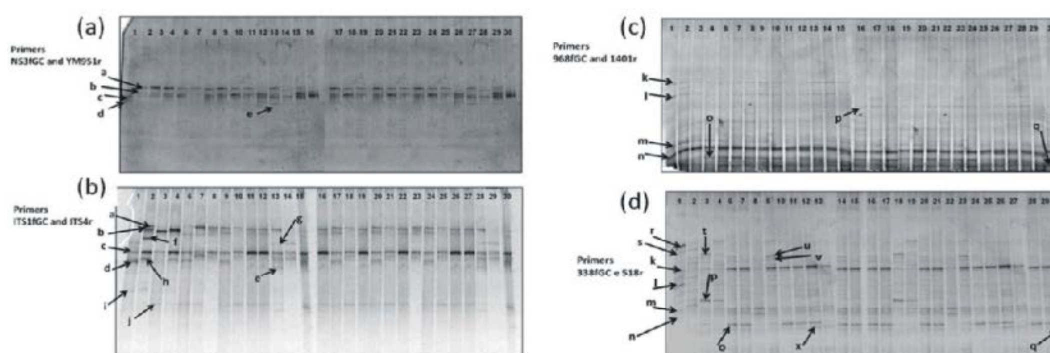

Fig. 2: DGGE profiles of the fungal 18S (A) and ITS (B) rDNA fragments amplified from soil samples. Band a - *Zygosaccharomyces* sp. AF017728.1 (98%). Band b - *Candida* sp. GI190714325 (99%). Band c - *Lachancea meyersii* AY645661.1(99%). Band d - Uncultured fungus AB534344 (99%). Band e - Uncultured ascomycota GQ404775 (99%). Band f - Uncultured zygomycete AY969178 (99%). Band g - *Cladosporium* sp. FJ950740 (98%). Band h - Uncultured soil fungus DQ421005.1 (99%). Band i - *Cladosporium oxysporium* AJ300332.1 (100%). Band j - not identified. Abbreviations: Region of Passos 1-4 (Rainy season) and 16-20 (dry season); Luminárias 6-10 (rainy season) and 21-25 (dry season); Arcos 11-15 (rainy season) and 26 -30 (dry season). DGGE profiles of bacterial 16S rDNA V6-V8 regions (b) and V3 region (c) amplified from soil samples. Band k - Uncultured soil bacterium GU598579.1 (99%). Band l - *Bacillus macerans* AB281478 (100%). Band m - *Klebsiella pneumoniae* CP000964 (99%). Bands n - *Enterobacter cowanii* FJ357832 (99%). Band o and r - *Bacillus subtilis* EU000054 (99%). Bands p - uncultured bacterium EF014703.1 (99%). Band q and s - not identified. Band t - *Pantoea agglomerans* DQ122371 (99%). Band u - *Escherichia coli* EU026432 (100%). Band v - *Enterobacter* sp. EU304794 (98%). Band x - *Leuconostoc mesenteroides* AB02741 (99%). Abbreviations: Region of Passos 1-4 (rainy season) and 16-20 (dry season); Luminárias 6-10 (rainy season) and 21-25 (dry season); Arcos 11-15 (rainy season) and 26 -30 (dry season).

Table 3: Bacteria and fungi community from different Cerrado regions of Minas Gerais, Brazil

Cerrado	Season	Bacteria	Yeast	Filamentous fungi
PA	Rainy	Bacillus macerans, Bacillus subtilis, Enterobacter cowanii, Klebsiella pneumoniae, Pantoea agglomerans	Candida sp.Lachancea meyersii Zygosaccharomyces sp.	Cladosporium oxysporum
	Dry	Bacillus subtilis, Enterobacter cowanii, Enterobacter sp., Escherichia coli, Klebsiella pneumoniae, Leuconostoc mesenteroides, Pantoea agglomerans	Candida sp.Lachancea meyersii Zygosaccharomyces sp.	
LU	Rainy	Bacillus macerans, Bacillus subtilis, Enterobacter cowanii, Enterobacter sp., Escherichia coli, Klebsiella pneumoniae, Leuconostoc mesenteroides,	Candida sp.Lachancea meyersii Zygosaccharomyces sp.	
	Dry	Bacillus subtilis, Enterobacter cowanii, Enterobacter sp., Escherichia coli, Klebsiella pneumonia, Leuconostoc mesenteroides	Candida sp.Lachancea meyersii Zygosaccharomyces sp.	
AC	Rainy	Bacillus macerans, Bacillus subtilis, Enterobacter cowanii, Enterobacter sp., Escherichia coli, Klebsiella pneumoniae, Leuconostoc mesenteroides	Candida sp.Lachancea meyersii Zygosaccharomyces sp.	Cladosporium sp.
	Dry	Bacillus subtilis, Enterobacter cowanii, Klebsiella pneumoniae, Leuconostoc mesenteroides	Candida sp.Lachancea meyersii Zygosaccharomyces sp.	Cladosporium sp.

* Abbreviations: PA - Passos; LU - Luminárias; AC - Arcos.

Among the profiles amplified by the primers NS3fGC and YM951r, the four regions sampled in Passos showed the same profile of bands in both the rainy and dry season. In the Luminárias region, the same profile was found, with the exception of sample 7 in which the dry season presented more bands than in the rainy season (sample 22). The profiles from the Arcos soils were different between the seasons. It was possible to observe a greater diversity in the profiles of the samples obtained during the rainy season (samples 11-15) compared to the samples taken during the dry season (samples 26-30) (Fig. 2).

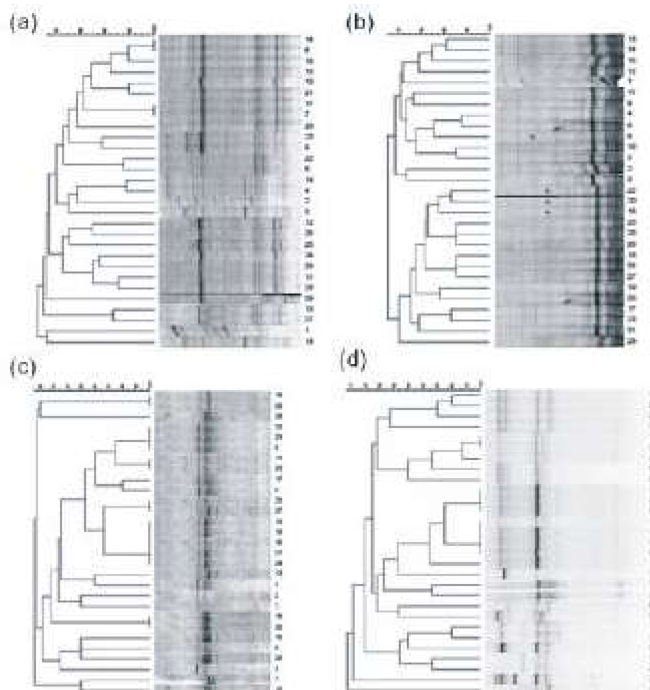

Fig. 3: Cluster analysis of bacteria. A. Cluster analysis of the V6-V8 regions of the 16S rDNA amplicons present in samples of the Brazilian Cerrado soil. B. Cluster analysis of the V3 region of the 16S rDNA amplicons present in sample Cerrado soil. Abbreviations: Region of Passos 1-4 (rainy season) and 16-20 (dry season); Luminárias 6-10 (rainy season) and 21-25 (dry season); Arcos 11-15 (rainy season) and 26 -30 (dry season). Cluster analysis of fungi: C. Cluster analysis region of the 18S rDNA amplicons present in the samples of the Cerrado soil. D. Cluster analysis of the ITS rDNA amplicons present in samples of the Brazilian Cerrado soil. Abbreviations: Region of Passos 1-4 (rainy season) and 16-20 (dry season); Luminárias 6-10 (rainy season) and 21-25 (dry season); Arcos 11-15 (rainy season) and 26 -30 (dry season).

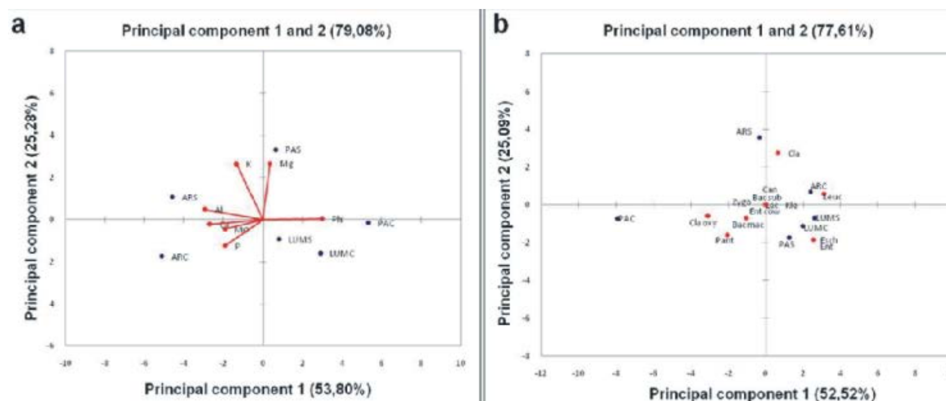

Fig. 4: Principal component analysis (PCA) of microbial and chemical characteristics of the Brazilian Cerrado soil of Minas Gerais. A. PCA of chemical characteristics. B. PCA of microbial characteristics. Abbreviations: K = Potassium; P = Phosphorus; Al = Aluminum, Ca = Calcium, Mg = Magnesium; PAC = Passos (rainy season); PAS = Passos (dry season); ARC = Arcos (rainy season); ARS = Arcos (dry season); LUMC = Luminárias (rainy season); LUMS = Luminárias (dry season); Zygo = *Zygosaccharomyces* sp.; Can = *Candida* sp.; Lac = *Lachancea meyersii*, Cla = *Cladosporium* sp.; Cla oxy = *Cladosporium oxysporum;* Bac mac = *Bacillus macerans;* Kle = *Klebsiella pneumoniae;* Ent cow = *Enterobacter cowanii;* Bac sub = *Bacillus subtilis;* Pant = *Pantoea agglomerans;* Esch = *Escherichia coli;* Ent = *Enterobacter* sp.; Leuc = *Leuconostoc mesenteroides.*

Table 4: Chemical and physical characteristics of the Brazilian Cerrado soil samples

Sample and Season			pH	Pmg/dm^3	Kmg/dm^3	Mgmg/dm^3	Almg/dm^3	H+AlCmol/dm^3	MOdag/Kg	SBmg/dm^3	Texture
PA	Rainy	Point 1	5.3±0.1a	1.5±0.1a	25±1a	0.1±0.0a	0.6±0.1a	3.6±0.1a	1.4±0.1a	0.3±0.1a	Sandy loam
		Point 2	5.4±0.1a	1.5±0.1a	56±2a	0.1±0.0a	0.6±0.1a	4.5±0.1a	2.0±0.1a	0.4±0.1a	Medium loam
		Point 3	5.5±0.1a	1.2±0.1a	33±1a	0.2±0.0a	0.4±0.1a	2.6±0.1a	1.1±0.1a	0.3±0.1a	Medium loam
		Point 4	5.5±0.1a	1.0±0.1a	70±1b	0.1±0.0a	0.5±0.1a	3.6±0.1a	1.5±0.1a	0.5±0.1a	Medium loam
		Point 5	5.4±0.1a	0.7±0.1a	9±1b	0.1±0.0a	0.1±0.1a	1.7±0.1a	0.4±0.1b	0.2±0.1a	Medium loam
LU	Rainy	Point 6	5.4±0.1a	1.2±0.1a	28±1a	0.2±0.0a	0.5±0.1a	7.9±0.1a	3.4±0.1a	0.3±0.1a	Clay loam
		Point 7	5.0±0.1a	1.5±0.1a	20±1a	0.1±0.0a	0.5±0.1a	7.9±0.1a	2.6±0.1a	0.3±0.1a	Clay loam
		Point 8	5.1±0.1a	1.2±0.1a	11±1b	0.2±0.0a	0.3±0.1a	2.6±0.1a	1.1±0.1a	0.2±0.1a	Sandy loam
		Point 9	5.2±0.1a	2.0±0.1a	20±1a	0.1±0.0a	0.9±0.2a	7.0±0.1a	2.4±0.1a	0.3±0.1a	Medium loam
		Point 10	5.1±0.1a	1.5±0.1a	34±1a	0.1±0.0a	0.8±0.1a	8.8±0.3b	2.7±0.1a	0.3±0.1a	Clay loam
AC	Rainy	Point 11	5.0±0.1a	1.2±0.1a	48±1a	0.1±0.0a	0.6±0.1a	4.0±0.1a	1.6±0.1a	0.7±0.1a	Clay loam
		Point 12	4.6±0.1a	0.7±0.1a	39±1a	0.1±0.0a	1.0±0.1a	6.3±0.1a	2.0±0.1a	0.3±0.1a	Clay loam
		Point 13	4.1±0.1a	1.8±0.1a	27±1a	0.3±0.0a	2.1±0.1b	15.3±1b	3.4±0.1a	0.3±0.1a	Clay loam
		Point 14	4.1±0.1a	1.8±0.1a	33±1a	0.1±0.0a	2.4±0.1b	17.1±2b	4.0±0.1a	0.3±0.1a	Clay loam
		Point 15	5.0±0.1a	1.8±0.1a	69±2b	0.1±0.0a	1.8±0.1b	12.3±1b	2.7±0.1a	0.4±0.1a	Clay loam
PA	Dry	Point 1	4.7±0.1a	1.7±0.1a	113.8±1b	0.1±0.0a	0.2±0.1a	13.7±0.1b	1.9±0.1a	0.5±0.1a	Sandy loam
		Point 2	5.1±0.1a	1.7±0.1a	88.9±1b	0.1±0.0a	0.4±0.1a	5.6±0.1a	2.4±0.1a	0.7±0.1a	Medium loam
		Point 3	5.1±0.1a	1.4±0.1a	137.28±1b	0.1±0.0a	0.4±0.1a	4.5±0.1a	2.2±0.1a	0.8±0.1a	Medium loam
		Point 4	5.1±0.1a	1.7±0.1a	117±1b	0.1±0.0a	0.5±0.1a	5.0±0.1a	1. 9±0.1a	0.9±0.1a	Medium loam
		Point 5	5.2±0.1a	1.4±0.1a	54±1b	0.1±0.0a	0.2±0.1a	4.5±0.1a	1.7±0.1a	0.4±0.1a	Medium loam
LU	Dry	Point 6	5.1±0.1a	2.5±0.1a	37.4±1a	0.1±0.0a	0.6±0.1a	6.3±0.1a	2.2±0.1a	0.1±0.1a	Clay loam
		Point 7	5.1±0.1a	0.9±0.1a	37.4±1a	0.1±0.0a	1.5±0.1b	7.0±0.1a	2.8±0.1a	0.2±0.1a	Clay loam
		Point 8	5.2±0.1a	0.9±0.1a	39±1a	0.1±0.0a	0.7±0.1a	7.8±0.1a	2.8±0.1a	0.2±0.1a	Sandy loam
		Point 9	5±0.1a	1.2±0.1a	46±1a	0.1±0.0a	1.5±0.1b	10.9±0.1b	3.0±0.1a	0.3±0.1a	Medium loam
		Point 10	5±0.1a	1.2±0.1a	67±1b	0.1±0.0a	1.4±0.1b	9.88±0.1b	3.1±0.1a	0.3±0.1a	Clay loam
AC	Dry	Point 11	4.7±0.1a	2.0±0.1a	149.7±1b	0.1±0.0a	0,4±0.1a	8.7±0.1a	2.4±0.1a	1.3±0.1a	Clay loam
		Point 12	4.8±0.1a	1.4±0.1a	48.3±1a	0.6±0.0a	0.1±0.1a	7.0±0.1a	1.8±0.1a	0.2±0.1a	Clay loam
		Point 13	4.3±0.1a	1.4±0.1a	54.6±1a	0.1±0.0a	0.1±0.1a	15.3±1b	2.8±0.1a	0.3±0.1a	Clay loam
		Point 14	4.2±0.1a	1.7±0.1a	39.0±1a	0.1±0.0a	0.1±0.1a	17.1±1b	3.0±0.1a	0.2±0.1a	Clay loam
		Point 15	4.8±0.1a	1.2±0.1a	84.2±2b	0.1±0.0a	0.1±0.1a	10.9±1b	1.9±0.1a	0.4±0.1a	Clay loam

* Data are average values of duplicate±standard deviation. Different letters indicate significant differences ($p < 0.05$). Soil classification in sandy loam (content clay <15), medium loam (content clay between 15 and 35) and clay loam (content clay = 35). Abbreviations: PA - Passos; LU - Luminárias; AC - Arcos. K - Potassium; P - Phosphorus; Al - Aluminum; Ca - Calcium; Mg - Magnesium; H+Al - Hydrogen + Aluminum; OM - Organic Matter; SB - (exchangeable bases) the sum of Ca, Mg, Na and K.

A cluster analysis of the DGGE gels according to Jaccard correlation was performed to compare the microbial communities and dendrograms were constructed using UPGMA methods (Fig. 3). A DGGE profile analysis of the samples of the Cerrado soil from Minas Gerais showed two distinct groups of eukaryotic (Figs. 3c end 3d) and prokaryotic (Figs. 3a and 3b) communities. For the bacterial community, which used primers 338f and 518r (Fig. 3a), the first group includes samples from Passos 1 and 18, but in both seasons. The remaining samples were grouped into the second group. The samples from Arcos, Passos and Luminárias regions (6-14 and 7-17) showed 100% of similarity. The primers 968f and 1401r (Fig. 3b) could distinguish the groups in the samples collected during the rainy and dry season. For the fungal community, which used the primers NS3fGC and YM951r (Fig. 3c), it was possible to verify three groups: group 1 with 1 and 12 samples from the Passos and Arcos regions, respectively, with similarity of 48% or more; group 2 formed by the Arcos samples (14 and 28 with similarity of 100%); and group 3 formed by Arcos samples (14, 26 and 28 with similarity between 20%) and by the Passos, Luminárias and Arcos samples (9, 11, 20, 22, 23), with similarity of 70% or more. The samples amplified with the primers ITS1fGC and ITS4r (Fig. 3d) were all grouped together, except for sample 1. Therefore, compared to the bacterial species, there was low variation in the fungal species between the dry and rainy seasons.

Physicochemical Properties of the Brazilian Cerrado Soil Samples: In order to analyze the physicochemical differences of the Cerrado soils in dry and rainy seasons and its influence on the microorganisms fingerprint was made to analyze the soil chemistry and biochemistry. The chemical and biochemical properties of the Cerrado soil samples taken during the rainy and dry season from the Passos, Luminárias and Arcos regions are shown in Table 4. The pH of the soils ranged from 4.7 to 5.5, 5.0 to 5.4 and 4.1 to 5.0 in the Passos, Luminárias and Arcos regions, respectively.

There were no significant differences in the concentrations of phosphorus, magnesium and acidity among the three studied areas. The Arcos region had large amounts of aluminum during the rainy season and large amounts of hydrogen+aluminum during the rainy and dry seasons. In contrast, in the rainy season Arcos also showed large amounts of organic matter that unseen in the dry season in the same region, but it was not

possible to observe interference of these large amounts aluminum in community fingerprints. The amount of organic matter also differed in Luminárias, demonstrating much in the dry season in most samples than in the rainy season. According to the soil texture, it was possible to observe differences in the sampling sites of the Passos and Luminárias regions. In general, the physical and chemical characteristics for all the soils analyzed were similar.

Multivariate Analysis of the Chemical and Microbial Characteristics of the Brazilian Cerrado Soils: A multivariate analysis using frequency values for the chemical and microbial characteristics of the Brazilian Cerrado soils was performed (Figs. 4a and 4b). Compared to the Luminárias and Passos regions, the samples from the Arcos region was different due to the high concentrations of phosphorus during the rainy season and high concentrations of aluminum and potassium during the dry season. In relation to the microbial characterization, the yeasts *Zygosaccharomyces sp*. *Candida* sp. and *L. meyersii* and the bacteria *B. subtilis*, *E. cowanii* and *K. pneumoniae* were found in all of the soil samples, demonstrating that these species are abundant in the preserved Cerrado soils. The fungus *Cladosporium* sp. was found in the Arcos samples taken during both the rainy and dry seasons. The soils from the Luminárias (rainy and dry season) and Passos (dry season) regions were characterized by the presence of *E. coli*, *Enterobacter* sp. and *L. mesenteroides* (Fig. 4b).

DISCUSSION

The Cerrado biome has two distinct seasons: the dry season (from May to September) and the rainy season (from November to April). An important factor relevant to this study is that the soil samples were collected at the peak of the seasons, causing a greater distinction between the samples because the water was either limited or abundant [3]. The samples were collected in January (high rainfall, 1000-2100 mm) and August (low rainfall, 20-200 mm) of 2010. The important caveat is the heterogeneity of microorganisms distribution in soil, where their growth usually is observed in patches rather than a homogeneous distribution [10-12]. We have tried to minimize this possible bias simply by collecting samples from different spots in each area studied and mixing them to obtain a composite sample.

In this study, the PCR-DGGE technique was used and all of the analyzed primer pairs gave satisfactory amplification of the samples (Fig. 2). The microbial community profiles can be highly dependent on the PCR primers used [11, 13, 14]. The fungal communities assessed by molecular techniques appeared to be more diverse when fungal DNA was amplified with the primer pair ITS1r/ITS4f than with the primer pair NS3f/YM951r. Ascomycete genera with a wide range of morphological and physiological differences possess almost identical 18S rRNA sequences [15]. This was probably the main reason for lower diversity of fungi in the soil of the Cerrado revealed by primer pair NS3f/YM951r. On the contrary, the high variability of the ribosomal ITS region may be more suitable for a high level of dis crimination [16-18]. In your studies, the two primer pairs targeting V3 and V6-V8 regions of the 16S rDNA of bacteria, but at each sampling date higher diversity was revealed by the primer pair 338GC/518 (for V3 region). It has been shown that targeting different rDNA regions may lead to different results in terms of microbial composition, e.g. co-migration of DNA from different species in the same band and the formation of multiple bands due to amplification of genes from a single genome [19], thus providing incorrect information about the dominance and diversity of certain ribotypes in the community. In fact, a few researchers have noticed that PCR ampli?cation of 16S rDNA is highly biased. Results described by Cocolin *et al*. [20] showed that only the 41f-130r primer set gave PCR products that allowed differentiation by DGGE in their study of bacterial community in Italian sausages. Hansen *et al*. [21] concluded that the PCR bias was dependent on the position of the primer sites and suggested that community diversity analysis based on PCR ampli?cation of 16S rDNA should involve in at least two different primer sets. Li *et al*. [22] investigated the presence of additional bands in the region V3 that the region V8 in soils planted with rice. In our studies the V3 region showed more bands than the V6-V8 region. Coincidentally, the results from this study are partly in accordance with the study by Yu and Morrison [23] on microbial diversity of rumen and gastrointestinal microbiomes and Li *et al*. [22] in soils of rice, their results revealed that the DGGE profiles of the V3 region were best and its produced better-DGGE profiles than other multiple V-region amplicons. Probably just metagenomic studies are able to enter into an appropriate depth of a given community within the environment.

Although many studies have clearly demonstrated the broad applicability of PCR-DGGE to discriminate among target bacteria/fungal [6, 11, 19, 24], the selection

of an appropriate primer set in accordance with the study objectives is critical because the target region of rDNA, the length of the target sequence and the sequence of each primer can affect the results [25]. Various methods to assess soil communities have been developed and have great potential to contribute to a better understanding of the ecological role of microbiota in soil habitats [3, 6- 8]. A variety of primers specific for the 18S and ITS regions of fungi have been designed [13]. The ITS1 [17] and ITS4 [26] primers appear to be specific for ascomycota, basidiomycota and zygomycota fungi [27, 28]. Yeasts belonging to the *Candida* and *Zygosaccharomyces* genera and also the filamentous fungi *Cladosporium sp*. were found in the soil sample [29]. These species were also found in our work, suggesting that they might be ubiquitous to the Brazilian soil. Recently, Bresolin *et al*. [6] observed microbiota present in soil using DNA analysis by PCR-DGGE. The microbial species isolated were related to the uncultivable soil fungus and bacteria found in this study.

Among the genera and species of yeast and fungi found in this study, *L. meyersii* is the first species in the genus of *Lachancea* collected from soil. According to Fell *et al*. [30], the other species of *Lachancea* have been isolated from plants, plant products or plant-associated insects and mangroves. The specific ecological niche of *L. meyersii* has not been determined and the habitat may be soil or possibly a niche associated with plants. Our finding is the first to report that this genus is present in the Brazilian Cerrado soil.

In this study, we could not obtain high quality sequences for some bands, such as a, g and v, for homology identification (similarity of 98% - *Zygosaccharomyces sp. Cladosporium sp*. and *Enterobacter sp*.). These bands may represent new species, but more studies are needed.

Zygosaccharomyces is a yeast genus often associated with food spoilage. The general characteristics of *Zygosaccharomyces* yeasts are the ability to ferment of sugars, osmotolerance, resistance to preservatives, including sulfite, sorbic acid and ethanol, the formation of heat-resistant ascospores and fructophily (preference for fructose). *Zygosaccharomyces* includes some of the most osmotolerant organisms known to resist concentrations of food preservatives vastly in excess of those normally or legally encountered. Foods particularly at risk are acidic, pH 2.5-5.0 and contain high concentrations of fermentable sugars [31]. The characteristics of the resistant microorganisms to different environments may be related to their presence in soils.

The *Cladosporium* genus is a black mold, but not the "toxic black mold." *Cladosporium* is one of the largest genera of hypomycetes. These species are among the most common fungi isolated from almost any environment in the world. Many species are plant pathogens, while others are regularly encountered as contaminants and spoilage agents in food or industrial products, as many are frequently associated with asthmatic complaints and endophytic fungi [32]. *Cladosporium* commonly grows on insulation in alternating current systems lacking sufficient ventilation and sometimes on walls and wallpaper in rooms where insulation is lacking. *Cladosporium* is also found on foundation walls, on basement and crawlspace ceiling joists and subflooring [33]. However, more recent data suggest that they are present in most soil samples [34]. This fact may be related to the buried condition of the soils, leading to reduced rates of oxygen and moisture. The *Candida* genus is commonly found in soils. Several species of *Candida* have been reported in forest soil from Taiwan, including the new species *C. jianshihensis*, *C. yuanshanicus, C. dajiaensis* and *C. sanyiensis* [35]. Shin *et al*. [36] also found the *Candida* genus in Korean soils.

Our results show the presence of DNA some bacterial species in the Brazilian Cerrado soil (Figs. 2c and 2d; Table 3). *B. subtilis* were identified as bands at different positions (bands o and r, Figs. 2c and 2d). These multiple banding patterns might be due to sequence microheterogeneity from multiple copies of the 16S rDNA gene found in this strain [37]. *Bacillus* sp. is commonly found in soil samples [4, 38, 39, 40].

Klebsiella sp. Enterobacter sp. P. agglomerans and *E. coli* were found in the soil samples collected for this study. This species include in γ-Proteobacteria group, the Proteobacteria group are one of the largest groups within prokaryotes. Genome reductions have been associated with an intracellular life style [41]. Quirino *et al*. [41] found in the soil of the Cerrado stricto sense of 1.4% to 4.3% of β-Proteobacteria, γ-Proteobacteria, δ-Proteobacteria, Fibrobacteres, Bacteriodetes and Gemmatomonadetes. *L. mesenteroides* was not reported in previous studies in Cerrado soil; however, it was found in the samples in this study. The presence of this species in the soil could be associated with plants and fruits present in the vegetation of the Cerrado region.

In this study, several bands were identified that were related to unculturable soil microorganisms (bands e, f, g, h, k and p of the Fig. 2). This result is particularly relevant considering that microbial communities of the Brazilian Cerrado soils have been poorly investigated to date. Several studies have shown diversity in the soil microbiota in the Brazilian Cerrado by analyzing the 16S and 18S rDNA region [3, 39, 41-44]. Therefore, this study shows the importance of using two different primers to amplify the 16S and 18S rDNA of bacterial and fungal communities in the Brazilian Cerrado soil. The use of multiple prime sets can increase the detection of the total microbial community. Castro *et al*. [3] found groups of communities that were "uncultured" by identifying the prominence of these native Cerrado soils in Goiás, Brazil. A similar result was observed by Jizheng *et al*. [43] in the Australian Cerrado soil, a margin of the forest soil amended with soybean planting and pastures.

Microorganisms are the key drivers of biogeochemical processes in the soil. Thus, it is important to evaluate the physicochemical properties of the soil and how these properties could be related to microbial profiles in different soils [1]. These changes in the soil affect the native microbial populations. Seasonal variations in the pH of the soil can lead to changes in the distribution patterns of the microbial species because bacteria prefer neutral to alkaline conditions whereas yeasts and filamentous fungi prefer the acidic conditions [6]. This work was possible to verify that despite the Cerrado soils are acids, it didn’t prevent the detection of bacteria in DGGE fingerprints. The pHs of the Cerrado soils were similar; therefore, there was not great variation of the microbial population. Regarding soil moisture, according Santos *et al*. [45] hydric stress reduces microbial populations in forest soils. However, the rains apparently did not affect the profile of the microbial community fingerprint. The higher soil moisture during the rainy season can be correlated with an increase in the amount of organic matter in the soil. The greater amount of rain it increases the layer of vegetable biomass into the soil and it thus higher amount of organic matter and organisms present in this environment [46].

The Arcos region had large amounts of aluminum during the rainy season and large amount hydrogen+aluminum during the rainy and dry season. High quantities of soluble aluminum in the soil cause toxicity in plants, as aluminum competes with other elements, such as essential nutrients, for the same chemical sites and promotes soil impoverishment [46]. However, in this study, the microbiota is not affected by high aluminum levels in the soil due to its similarity with the other sampled sites. The three analyzed areas showed no significant differences in organic matter values, which may be due to the similarity in the vegetation and riverbank forest of the three areas [3].

Altogether, the results obtained using different pairs of primers show a diverse DGGE profile, suggesting the presence of a robust, dominant microbial consortium. These results could be explained by the dif?culty in obtaining high quality DNA suitable for PCR directly from the soil samples. The initial template DNA and template competition may affect the detection of rare microorganisms in the microbial population [47]. Despite the limitations inherent to molecular biology techniques (e.g. DNA extraction method, amplification) and the limited size of sequencing data, our results are relevant for understanding the microbial community in the Cerrado areas and will be used to evaluate the degree of impact in the Cerrado soils. This study shows that the physicochemical and microbial characteristics of the soil are similar in the three areas and suggests that knowledge of the microorganisms and chemical properties of the soil are an important first step in characterizing a soil type.

Therefore, the results presented in this study high light the importance of the Cerrado biome and the consequences of the expansion of human activity on microbial community richness. While the flora and fauna are often the focus of studies and preservation efforts, the microbial community is neglected. Our study shows that a new view of the importance of Cerrado that takes into consideration the microbial communities should be developed. In addition, it is clear that many questions regarding the microbial communities of Cerrado remain to be addressed in future studies.

In summary, this research was the first study to characterize the bacterial and fungal community present in Brazilian Cerrado soil of Minas Gerais. The application of PCR-DGGE techniques that do not rely on cultivation for bacterial and yeast community analysis confirms that the microbial ecosystems of Cerrado soil support a wide diversity of organisms, which may be responsible for some characteristics of these soils.

ACKNOWLEDGEMENTS

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenaçγo de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundaçγo de Amparo à pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

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