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Yeasts diversity in Brazilian Cerrado soils: Study of the enzymatic activities

Fernanda Paula Carvalho[1], Angélica Cristina de Souza[1], Karina Teixeira Magalhães-Guedes[1]*, Disney Ribeiro Dias[2], Cristina Ferreira Silva[1] and Rosane Freitas Schwan[1]

[1]Department of Biology, Federal University of Lavras (UFLA), Campus Universitário, 37.200-000, Lavras, MG, Brazil.
[2]Department of Food Science, Federal University of Lavras (UFLA), Campus Universitário, 37.200-000, Lavras, MG, Brazil.

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A total of 307 yeasts strains were isolated from native Cerrado (Brazilian Savannah) soils collected at Passos, Luminárias and Arcos areas in Minas Gerais State, Brazil. The soils were chemically characterized. Ten yeast genera (*Candida, Cryptococcus, Debaryomyces, Kazachstania, Kodamaea, Lindnera, Pichia, Schwanniomyces, Torulaspora* and *Trichosporon*) and 23 species in both rainy and dry seasons were identified. All genera were abundant during the dry season. The pH values of the soil from the Passos, Luminárias and Arcos areas varied from 4.1 to 5.5. There were no significant differences in the concentrations of phosphorus, magnesium and organic matter in the soils among the studied areas. The Arcos area contained large amounts of aluminum during the rainy season and both hydrogen and aluminum in the rainy and dry seasons. The yeast populations identified seemed to be unaffected by the high levels of aluminum in the soil. The API ZYM® (BioMérieux, France) system was employed to characterize the extracellular enzymatic activity profiles of the yeast isolates. The results of the API ZYM® test showed differences in the extracellular enzyme profiles among the yeast species. Some isolates that belong to the same species showed enzymatic profiles that differed from one another. Our study is the first to describe yeasts isolated from Brazilian Cerrado soils from Minas Gerais State and demonstrates their ability to produce enzymes that may be of potential industrial interest.

Key words: Enzymes, Brazilian soils, yeasts biodiversity, Cerrado.

INTRODUCTION

The Cerrado is the second largest biome in Brazil, covering an area of approximately 2 million km^2 and accounting for 24% of the national territory. This area encompasses land within the Minas Gerais, Goiás, Tocantins, Bahia, Maranhão, Mato Grosso, Mato Grosso do Sul and Piauí states and the Distrito Federal in Central Brazil. The climate of this biome is tropical, characterized by well-defined seasons, including dry winters (April to September) and rainy summers (October to March). The Cerrado soils are deep acidic nutrient-poor and contain high concentrations of aluminum (Lopes and Cox, 1997). The Cerrado vegetation is heterogeneous, ranging from tall dense savanna woodlands (cerradão), through progressively less arboreal formations (cerrado sensu strictu), to nearly treeless grasslands (campo sujo) (Furley, 1999). The variation of vegetation contributes to the extensive biodiversity found within this biome. Indeed, the Cerrado region is considered a biodiversity hotspot and is a priority

*Corresponding author: E-mail: gt-magalhaes@uol.com.br. Tel.: (+55) 35 38291614. Fax: (+55) 35 38291100.

for conservation efforts (Myers et al., 2000; Bustamante et al., 2012). The expansion of agriculture, forest fires and urban growth has led to reductions in the native Cerrado, making it the most threatened biome in South America. It is estimated that by 2030, the Brazilian Cerrado will be eliminated if no actions are taken to preserve this biome (Bresolin et al., 2010).

Some studies have been conducted to show the diversity of the flora and fauna of the Brazilian Cerrado biome (Bustamante et al., 2012; Bitar et al., 2012). Others have demonstrated the presence of several types of bacteria and fungi in the Cerrado soils (Castro et al., 2008; Bresolin et al., 2010; Nascimento et al., 2012), however, the diversity of yeasts in the native cerrado soils of Minas Gerais is yet to be documented.

Yeasts occur in a wide range of soil types from vastly diverse geographical areas ranging from arctic zones to the tropics (Gareth-Jones and Ka-Lai, 2012). Combinations of temperature, humidity, chemical composition, geographic location and other factors influence the diversity of yeasts found in these environments (Vishniac, 2006). Many yeast genera have been isolated from soils, such as *Kluyveromyces*, *Lipomyces*, *Schwanniomyces* and *Schizoblatosporium.* Other genera that have been isolated from soils taken from locations in Brazil and throughout the world include *Debaryomyces*, *Hansenula*, *Candida*, *Brettanomyces*, *Rhodotorula*, *Cryptococcus*, *Trichosporon* and *Torulopsis* (Bresolin et al., 2010).

Yeasts present in the soil contribute to the mineralization process, enhance plant growth, maintain soil structure, contribute to nutrient transformations, control phytopathogens and are useful in bioremediation approaches (Botha, 2011). In addition, yeasts are important sources of biomolecules such as enzymes that are commonly used in industrial applications. Currently, enzymes from microbial sources are used in food processing, detergents, textiles and pharmaceuticals, molecular biology and biofuels (Romo-Sánchez et al., 2010).

In recent years, the interest in production of enzymes has increased due to several potential applications, such as the production of bioenergy and biofuels, and application in textile and paper industries (Soccol et al., 2010). Many studies have been published seeking new microorganisms to produce enzyme with higher specific activity and efficiency. Several strategies are available for improving the production and efficiency of cellulases, including optimization of the fermentation process, genetic difications and mutagenesis. However, at present, the task of finding a good producer of enzymes still arouses the interest of researchers. There is great interest in finding microorganism species that are not yet cataloged as interesting producers of inputs to industry in general, as well as optimizing production processes of these inputs from known microorganisms.

It is important to highlight the need to isolate and characterize yeasts from different habitats to enhance species preservation efforts, in addition to ascertaining the biotechnological potential that these microorganisms may present (Romo-Sánchez et al., 2010). In this study, we assessed the diversity, geographical and seasonal distribution, and biotechnologically relevant enzyme production of yeasts inhabiting the native cerrado within Arcos, Passos and Luminárias municipalities in Minas Gerais, Brazil.

MATERIALS AND METHODS

Sampling sites and sample collection

Thirty composite soil samples of the native cerrado were collected from three areas in Minas Gerais, Brazil: Passos (PA), Arcos (AR) and Luminárias (LU). The samples were taken during the months of January (highest rainfall season, designated “r”) and August (dry season, designated “d”) in the year 2010. Five composite samples were collected (A, B, C, D and E) (Figure 1a and b) by region, comprising 15 georeferenced samples, with coordinates ranging from 20° 14’ to 21° 37’ south and 44° 58’ to 46° 30’ west. Each composite sample consisted of 12 simple sub-samples, collected at a depth of 0 to 20 cm, in two concentric circles with a radius of 3 and 6 m from the center (Figure 1c). The soil samples were extracted with a flamed-sterilized auger, mixed, packed in sterile bags, transported to the laboratory and stored at 4°C for later use (Moreira et al., 2009).

Isolation of yeasts

Yeasts were isolated from yeast extract peptone dextrose media (YEPD) (Merck, SP, Brazil) containing 2% glucose, 2% peptone and 1% yeast extract. Serial dilutions of the suspension were plated on YEPD media acidified to pH 3.5 to prevent yeasts growth. Plates were incubated under aerobic conditions at 28°C for 24 at 72 h. A number of colonies, corresponding to the square root of the number of colony of each morphotype, were purified by repeated streaking on YEPD (pH 3.5) (FDA, 1998). The purified isolates were maintained at -80°C in YEPD broth containing 20% glycerol.

Identification of yeasts

Internal transcribed spacer (ITS) regions were sequenced to identify the yeasts, using the methodology of Ramos et al. (2010). The ITS region was amplified using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) according to Kusari et al. (2012). PCR conditions were: initial denaturation at 94°C for 3 min; 35 cycles at 94°C for 1 min, 52°C for 45 s and 72°C for 1 min; a final extension step at 72°C for 7 min; then held at 4°C. Amplification of the ITS region was confirmed by electrophoresis. The ITS region was sequenced by Macrogen, (Seoul, Korea) and compared with sequences reported in GenBank using the BLAST algorithm.

Diversity indices of species

The species diversity of yeasts isolated from the cerrado areas of Arcos, Passos and Luminárias was evaluated using the PAST software (version 2.15), which calculated the number of taxa (S), the total number of individuals (n), equitability ($J = H'/Hmax$), dominance ($D= \Sigma (n_i/n)^2$), Simpson’s index (1-D) and Shannon’s index ($H = -\Sigma(n_i/n)\ln(n_i/n)$, where n_i is the number of individuals of the taxon I, and n is the total number of OTUs (Hammer et al., 2001).

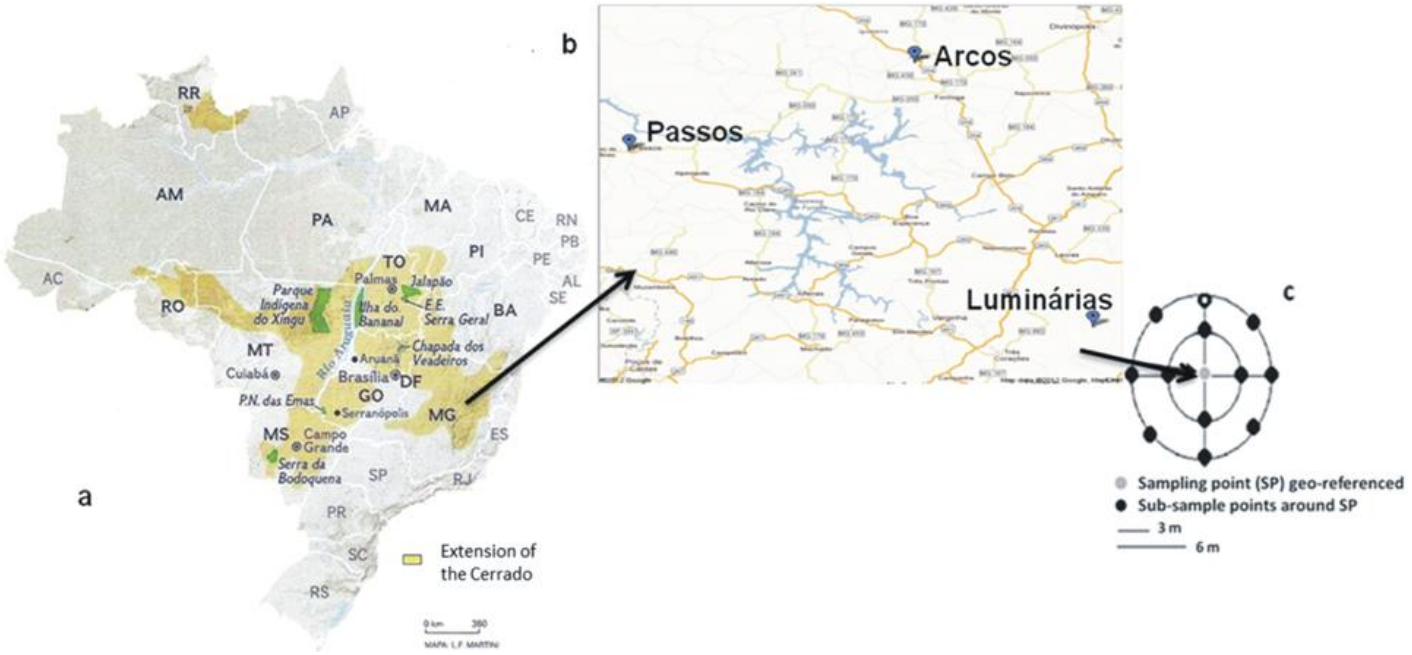

Figure 1. Geographical location of collection areas (b) and sampling strategy (c) in the Brazilian Cerrado (a), in Minas Gerais.

Physicochemical analysis of soils

The soil samples were forwarded to the Soil Fertility and Plant Nutrition Laboratory at the Federal University of Lavras and analyzed according to Empresa Brasileira de Pesquisa Agropecuária (Embrapa, 1997). The concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), exchangeable acidity (H^+Al), organic matter (OM), sand, silt and clay were evaluated. These results were used to calculate other parameters, such as the base sum (SB) Ca, Mg and K, effective cation exchange capacity (t), cation exchange capacity at pH 7.0 (T) and base saturation of CTC at pH 7.0 (V%), aluminum saturation and texture (m%).

Screening for cellulase and xylanase enzymes

Cellulase and xylanase activities were assessed on plates containing CMC agar media (0.2% carboxymethylcellulose, 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% KCl, 0.02% peptone and 1.7% agar) (Kasana et al., 2008) and xylan-agar (0.67% yeast nitrogen base, 1% beech wood xylan, and 1.8% agar) (Merck, SP, Brazil) (Bhadra et al., 2008). The plates were inoculated with 10 µL aliquots of each isolate, standardized to 10^7 cells/mL, and incubated at 28 and 26°C for 48 h and 15 days, respectively. After the incubation period, the plate was flooded with iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 min to identify isolates producing enzymes. The formation of a clear halo around the colonies was considered a positive result, indicating the presence of the given enzyme.

Evaluation of enzymes by the API ZYM® test

The API ZYM® (BioMérieux, France) test was used to evaluate the enzymatic profiles of isolates of different yeasts species. The API ZYM® gallery is composed of 20 cupules that contain enzymatic substrates, with a single cupule serving as a control. This system allows the semi-quantitative assessment of 19 enzymes, including glycosyl hydrolases (α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase), esterases (esterase, esterase lipase and lipase), aminopeptidases (leucine arylamidase, valine arylamidase, and cystine arylamidase), proteases (trypsin, α-chymotrypsin) and phosphatases (alkaline phosphatase, acidic phosphatase and naphthol-AS-BI-phosphohydrolase).

The API-ZYM® test was used according to the manufacturer's instructions (API bioMérieux, Marcy l'Etoile, France). The enzyme activity was graded on a scale from 0 to 5 with the API ZYM® color reaction chart, with "0" indicating a negative reaction and "5" a reaction of maximum intensity. Values of 1-4 indicate intermediate reactions commensurate with the intensity of the reaction.

Analysis

The PAST software (version 2.15) was used for principal component analysis (PCA) and canonical correspondence analysis (CCA) (Hammer et al., 2001). Principal component analysis was performed using a correlation matrix in which the values of abiotic variables, such as soil chemical composition, were used to identify similarities between the samples that were collected at different locations. CCA was employed to correlate the distribution of yeasts with the abiotic soil variables and different regions sampled.

RESULTS AND DISCUSSION

Soils and species composition and diversity analysis

The chemical and biochemical properties of the cerrado soils from the Passos, Luminárias and Arcos areas during the rainy and dry seasons are shown in Table 1. The results for the thirty samples of cerrado soils were

ordered by PCA, in which the principal components accounted for 70.98% of the total variance. The first principal component accounted for 48.59% and the second component accounted for 22.39% of the total variability. The generated scatterplot revealed relationships between the areas studied; grouping the similar soils into four groups (Figure 2). This analysis did not show any discernible pattern in the distribution for soils belonging to the same area or season. Groups III and IV covered a larger number of areas and were influenced mainly by the aluminum saturation index (m%) and pH, respectively. Most soils of the Arcos and Luminárias areas contained high levels of Al, H + Al and m%. These soils had high acidity and aluminum concentration, consistent with the values found in cerrado soils that have been reported by others (Furley, 1999; Lopes and Cox, 1997). Considering the t, V% and SB parameters, which relate to soil fertility, the areas belonging to groups I and II correspond with the most fertile conditions of all the areas assessed.

The total yeasts counts were compared between the rainy and dry seasons. A statistically significant difference ($p < 0.05$) was observed for all the areas (Table 2). The dry season exhibited higher microbial counts (~9 log CFU/g) when compared with the rainy season (~8 log CFU/g).

The analysis of the ITS region sequence amplification products led to the identification of ten yeast genera: *Candida*, *Cryptococcus*, *Debaryomyces*, *Kazachstania*, *Kodamaea*, *Lindnera*, *Pichia*, *Schwanniomyces*, *Torulaspora* and *Trichosporon* (Table 3 and Figure 3). Several yeasts genera have been frequently isolated from various soils from different locations. In Amazonian soils, species belonging to the *Candida*, *Rhodotorula, Wangiella, Trichosporon* and *Pichia* genera were found (Mok et al., 1984). In addition, *Bullera*, *Candida*, *Cryptococcus*, *Saccharomyces*, *Williopsis, Rhodotorula and Sporidiobolus* genera were isolated from fertile soils collected from Costa Rica (Vishniac, 2006). Yeasts belonging to the *Aureobasidium*, *Bulleromyces*, *Candida*, *Cryptococcus*, *Cystofilobasidium*, *Debaryomyces*, *Geotrichum*, *Kluyveromyces*, *Pichia*, *Rhodotorula*, *Sporobolomyces*, *Trichosporon* and *Williopsis* genera were isolated from Amazon soils (Vital et al., 2002). The above mentioned areas contain distinct soil and climate characteristics, and yet, several yeast genera isolated from these soils were also found in the Brazilian Cerrado soils.

We identified twenty-three distinct Operational Taxonomic Units (OTUs); 22 OTUs were present in samples taken during the dry season, and 9 OTUs were present in rainy season samples (Table 4). The different yeast species are members of the *Saccharomycetaceae* (18), *Tremellaceae* (3), *Trichosporonaceae* (1) and *Wickerhanomyceteae* (1) families. Figure 2 show yeast species morphotypes isolated in the Brazilian Cerrado in Minas Gerais.

A total of 307 distinct yeast were isolated. The higher yeast number was in Passos in the dry season (121). The percent of organisms from each genus was as follows: *Candida* (58.6%), *Torulaspora* (13.4%), *Schwanniomyces* (11.4%), *Cryptococcus* (8.5%) and *Lindnera* (6.2%). *Debaryomyces, Kazachstania*, *Kodamaea*, *Pichia* and *Trichosporon* corresponded to 2% of the isolated organisms. Loureiro and colleagues (2005) isolated the genus *Candida* mainly in soil and beach water in the Northeast of Brazil (Pernambuco).

Species of the *Cryptococcus* genera have also been isolated frequently from soil (Vital et al., 2002). Organisms that were isolated with high frequency include *Candida* sp. (40%), *Candida tetrigidarum* (30%) and *Candida tropicalis* (30%). The isolates number of other species ranged from 3.3 to 16.7%, with 18 species that showed a number below 10% (Table 4). Species that were found in the greatest numbers, because they grow and persist within the habitat and originate within the system, are considered autochthonous, whereas species isolated in smaller quantities are considered allochthonous and may originate from outside the system (Botha, 2011).

Yeast species that were common in both seasons (dry and rainy) correspond to 22% of isolates (*Candida glabatra*, *Candida neerlandica, Candida tetrigidarum*, *C. tropicalis* and *Torulaspora globosa*). The yeasts *Candida labiduridarum*, *Candida laurentti* and *Pichia kudriavzevii* were isolated only in the rainy season, while a greater diversity of species was obtained in the dry season (*Candida frijolensis, Candida orthopsilosis, Candida railenensis, Candida sojae, Candida flavescens*, *Candida humicola*, *D. hansenii, Kazachstania exigua*, *Kodamaea ohmeri, Lindnera saturnus*, *Schwanniomyces* sp., *Schwanniomyces polymorphus*, *Schwanniomyces pseudopolymorphus*, *Schwanniomyces vanrijiae*, *Torulaspora malleae* and *Trichosporon loubieri*).

Among the various genera isolated, the relative abundance of the Ascomycota and Basidiomycota phyla was 80 and 20%, respectively. Vital et al. (2002) also found the Ascomycota as most abundant phylum in Amazonian soils. However, other authors detected higher numbers of basidiomycetous yeasts in the soils of Austria and other countries located along the latitudinal gradient ranging from >77°S to > 64°N (Vishniac, 2006).

The species richness, dominance, evenness and the Shannon diversity index and Simpson's index for different regions during both seasons (dry and rainy) were evaluated. The highest species richness was measured in dry season (S). With respect to regional differences, the Passos area demonstrated the highest degree of species richness (fourteen species). This was followed by Arcos and last of all Luminárias, in which species numbers varied from five to eight species (Table 5).

The Passos area showed the highest Shannon and Simpson indexes in the dry season, indicating higher diversity in this area. In all areas, we observed a dominant point. This means there is one dominant taxon

Table 1. Chemical and physical characteristics of the Brazilian Cerrado soil samples.

Sample and Season			pH	P mg/dm^3	K mg/dm^3	Mg mg/dm^3	Al mg/dm^3	H+Al Cmol/dm^3	OM dag/Kg	SB mg/dm^3	Texture
		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
PA	**Rainy**	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
		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
LU	**Rainy**	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
		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
AC	**Rainy**	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
		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	3.9±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
PA	**Dry**	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
		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
LU	**Dry**	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
		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
AC	**Dry**	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 as sandy (clay content <15), medium (clay content between 15 and 35) and clay (clay content ≥ 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.

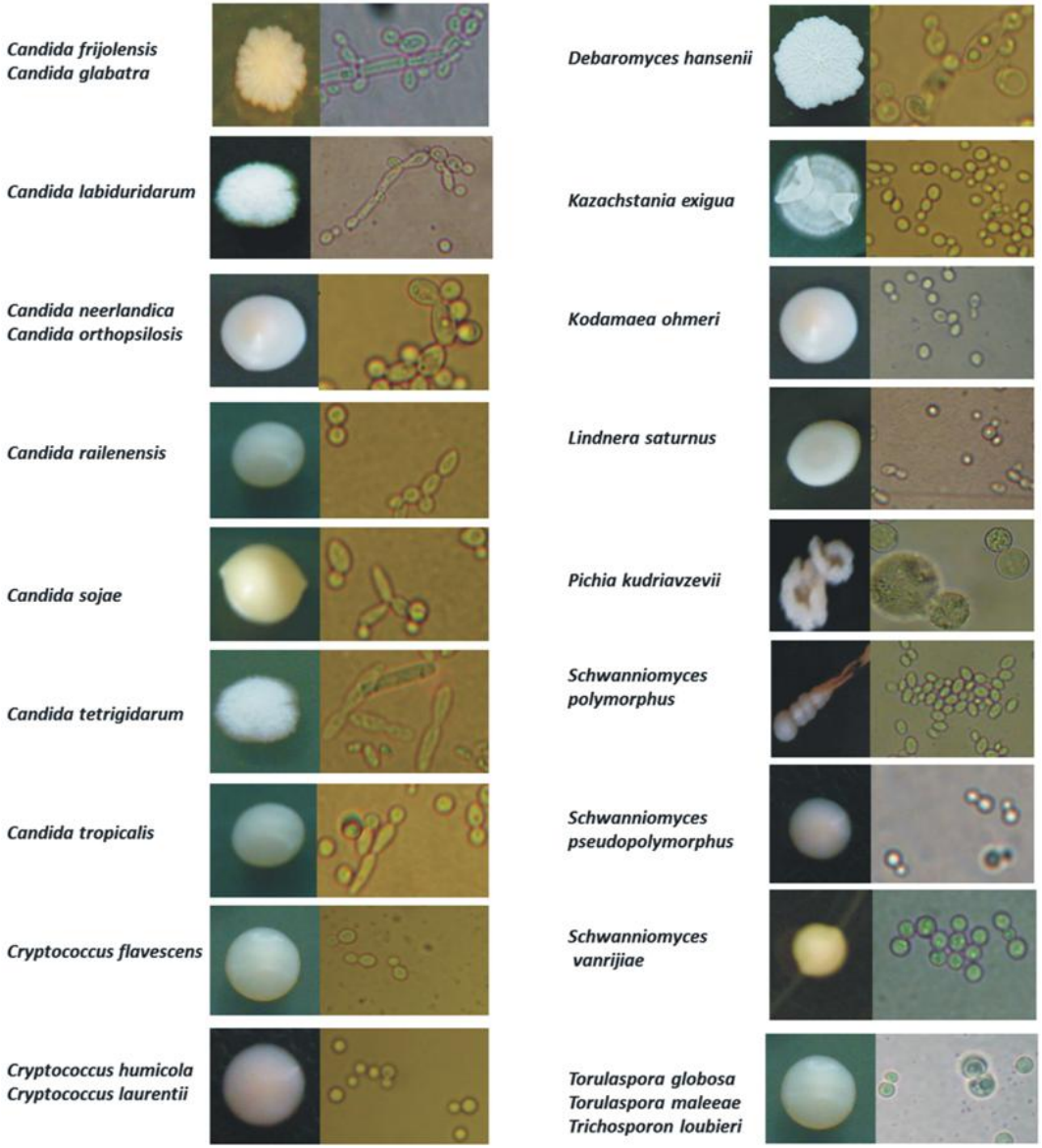

Figure 2. Yeast species morphotypes isolated in the Brazilian Cerrado in Minas Gerais.

Table 2. Isolates count of the population of soil in YEPD medium during the rainy and dry season.

Region	Rainy Season	Dry Season
Arcos	9.1 log CFU/g ±0.2^{c}	9.1 log CFU/g ±0.2^{c}
Luminárias	7.8 log CFU/g ±0.1^{a}	8.9 log CFU/g ±0.2^{c}
Passos	7.8 log CFU/g ±0.1^{a}	8.8 log CFU/g ±0.1^{c}

Data are mean values of duplicate ± standard deviation; different letters indicate significant differences ($p < 0.05$).

within the ecosystem (Table 5).

The values of equitability (J) refer to the distribution pattern of the number of individuals among species. The J values ranged from 0.70 to 0.86, with the lowest value found in Luminárias during the dry season, and the highest values corresponded to the Arcos and Passos regions, also during the dry season (Table 5).

The results of Canonical Correspondence Analysis (CCA) (Figure 4) of the yeast species isolated from the three regions in the rainy season showed that *C. glabatra, P. kudriavzevii, Candida* sp.*, C. tetrigidarum, C. tropicalis* and *T. globosa* are associated with high values of SB, K and Ca. *Candida labiduridarum* and *Candida neerlandica* were associated with OM, P, t, T, Al, H + Al, Mg and m. *Cryptococcus laurentii* was isolated only in Passos soil and is not associated with any specific characteristic of the soil.

In the dry season (Figure 5), the parameters V, Mg and P-rem correspond with the yeasts *C. humicola*, *D. hansenii* and *L. saturnus*. The species *K. ohmeri, S. polymorphus, S. pseudopolymorphus, Schwanniomyces* sp.*,* and *S. vanrijiae* were not associated with any particular chemical characteristic of the soil, but all of them were isolated in soils with high levels of silt. High aluminum concentrations and potential acidity associated with other factors correspond with 13 species of yeasts (*C. frijolensis, C. neerlandica, C. orthopsilosis, C. railenensis, C. sojae, Candida* sp.*, C. tetrigidarum, C. tropicalis, C. flavescens, K. exigua, T. globosa, T. maleeae* and *T. loubieri*). Approximately 90% of *Candida* species were isolated in soils that had high levels of aluminum and potential acidity, suggesting that these species are tolerant to these factors. In addition to the influence of the chemical and physical characteristics of the soil, the growth and survival of a particular yeast species in a given soil sample may not depend solely on the intrinsic abilities of the yeast but rather result from the cumulative interactions within the soil microbial community (Martini, 1992; Botha, 2011).

Enzymatic profile of yeasts

According to the 307 yeasts evaluated, 18 (5.8%) isolates were positive for cellulase and xylanase, and six (1.9%) were positive for xylanase only (*data not shown*). These isolates were identified as *Cryptococcus laurentii, Cryptococcus flavescens, Cryptococcus humicola* and *C. neerlandica*. *C. laurentii* was isolated during the rainy season in the Passos region and produced the enzymes cellulase and xylanase. Other isolates produce xylanase exclusively, including *C. humicola* and *C. neerlandica,* which were isolated from Passos, along with *C. flavescens* isolated from Arcos during the dry season. Parachin et al. (2009) isolated *Cryptococcus flavus* from leaves and flowers of the Brazilian Cerrado, which exhibits amylase, carboxymethyl cellulase and xylanase activities. Cellulases can be used for the improved processing of cellulosic fibers, in stone washing, as detergent additives and as biofuel and waste treatments (Dhillon et al., 2012). Xylanases have many applications in paper production, in fermentation and in food Industries, as well as in waste treatment (Parachin et al., 2009).

The API ZYM® system was employed to characterize the extracellular enzymatic activities of the different genera of yeast isolated from Brazilian Cerrado soil of Minas Gerais State (Table 6). The results demonstrate differences in the extracellular enzymes produced by the different species evaluated. In addition, some isolates belonging to the same species showed different enzymatic profiles.

All isolates showed some esterase activity. The isolates that produced esterase and esterase lipase enzymes with relatively high intensity (4 to 5) were 22 and 6%, respectively. *Candida* sp., *C. neerlandica, C. tetrigidarum, C. humicola, K. exigua, P. kudriavezevii, S. pseudopolymorphus, S. vanrijiae* and *T. loubieri* showed the greatest amount of esterase activity. The highest esterase lipase activity was obtained by the *C. orthopsilosis* and *C. laurentii* isolates.

The proportion of isolates exhibiting high glycosyl hydrolase enzyme activity was 2.0% (α-galactosidase), 6.1% (β-galactosidase), 30.6% (α-glucosidase), 16.3% (β-glucosidase) and 20.4% (N-acetyl-β-glucosamidase). No isolate was able to produce the enzymes β-glucuronidase, α-mannosidase or α-fucosidase. The cerrado soil isolates with the most prolific production of enzymes were *S. vanrijiae* (α-galactosidase), *C. laurentii* (β-galactosidase), *C. labiduridarum, C. neerlandica, C. orthopsilosis, C. sojae, C. tropicalis, S. polymorphus, S. pseudopolymorphus, S. vanrijiae* and *T. globosa* (α-glucosidase), *C. laurentii, L. saturnus, S. polymorphus* and *S. pseudopolymorphus (β-glucosidase), C. frijolensis, C. neerlandica, C. tropicalis, Candida* sp. and *T. globosa* (N-acetyl-β-glucosamidase).

All isolates showed high leucine arylamidase activity, being that this enzyme a good measure of the proteolytic activity of microrganisms. This enzyme catalyzes the cleavage of leucine and other hydrophobic amino acids from the amino terminus of protein or peptide substrates (Flores et al., 2000). The best producers of valine arylamidase were *Candida* sp., *C. orthopsilosis, C. sojae, C. humicola, K. exigua, L. Saturnus* and *T. globosa,* corres-

Table 3. Identification of representative yeasts isolated from Arcos, Passos e Luminárias.

Group	Identified Isolate Number	Isolate Code	Accession Number	Identity (%)	Closest GenBank fit (species name)
I	3	UFLA CES-Y 816	EF658666.1	97	*Candida frijolensis*
		UFLA CES-Y 818	EF658666.1	97	
		UFLA CES-Y 835	EF658666.1	97	
II	7	UFLA CES-Y 557	AY939793.1	100	*Candida glabatra*
		UFLA CES-Y 561	AY939793.1	100	
		UFLA CES-Y 563	AY939793.1	99	
III	1	UFLA CES-Y 590	FJ623629.1	100	*Candida labiduridarum*
IV	13	UFLA CES-Y 580	FJ623628.1	98	*Candida neerlandica*
		UFLA CES-Y 284	EF658663.1	100	
V	11	UFLA CES-Y 597	FN812686.1	99	*Candida orthopsilosis*
		UFLA CES-Y 614	FN812686.2	99	
		UFLA CES-Y 625	FN812686.1	99	
VI	4	UFLA CES-Y 758	HQ438300.1	99	*Candida railenensis*
		UFLA CES-Y 759	FM178302.1	99	
		UFLA CES-Y 760	HQ438303.1	99	
VII	3	UFLA CES-Y 777	FN424104.1	99	*Candida sojae*
		UFLA CES-Y 781	FN424104.1	99	
		UFLA CES-Y 784	FN424104.2	99	
VIII	24	UFLA CES-Y 279	FJ623630.1	98	*Candida tetrigidarum*
		UFLA CES-Y 601	FJ623630.1	98	
		UFLA CES-Y 836	FJ623630.1	98	
IX	18	UFLA CES-Y 598	EF194842.1	100	*Candida tropicalis*
		UFLA CES-Y 621	EF568038.1	99	
		UFLA CES-Y 783	JF916546.1	99	
X	2	UFLA CES-Y 726	JF279290.1	100	*Cryptococcus flavescens*
		UFLA CES-Y 735	JF279290.1	99	
XI	2	UFLA CES-Y 682	AY382335.1	99	*Cryptococcus humicola*
		UFLA CES-Y 683	EF377334.1	99	
XII	18	UFLA CES-Y 521	JN626987.2	100	*Cryptococcus laurentii*
		UFLA CES-Y 546	JN627015.2	100	
		UFLA CES-Y 548	JN627003.2	100	
XIII	1	UFLA CES-Y 678	EF193070.1	99	*Debaromyces hansenii*
XIV	2	UFLA CES-Y 838	AY046170.1	100	*Kazachstania exigua*
		UFLA CES-Y 839	AY046170.1	100	
XV	1	UFLA CES-Y 648	AF219004.1	100	*Kodamaea ohmeri*
XVI	9	UFLA CES-Y 669	EF194844.1	99	*Lindnera saturnus*
		UFLA CES-Y 676	EF194844.1	100	
		UFLA CES-Y 681	EF194844.1	100	
XVII	2	UFLA CES-Y 542	FR819718.1	100	*Pichia kudriavzevii*
		UFLA CES-Y 543	JF920160.1	100	
XVIII	3	UFLA CES-Y 641	AJ586523.1	99	*Schwanniomyces polymorphus*
		UFLA CES-Y 646	AJ586523.1	99	
		UFLA CES-Y 652	AJ586523.1	99	
XIX	5	UFLA CES-Y 635	EF198011.1	99	*Schwanniomyces pseudopolymorphus*
		UFLA CES-Y 638	EF198011.1	99	
		UFLA CES-Y 649	EF198011.1	99	
XX	10	UFLA CES-Y 657	AB054104.1	100	*Schwanniomyces vanrijiae*

Table 3. Contd.

		UFLA CES-Y 661	AB054104.1	100	
		UFLA CES-Y 680	AB054104.1	100	
XXI	15	UFLA CES-Y 64	AY046184.1	99	*Torulaspora globosa*
		UFLA CES-Y 832	FN428932.1	99	
		UFLA CES-Y 846	AY046184.1	99	
XXII	4	UFLA CES-Y 775	AB304160.1	100	*Torulaspora maleeae*
		UFLA CES-Y 782	AB304160.1	100	
		UFLA CES-Y 785	AB304160.1	99	
XXIII	1	UFLA CES-Y 631	HM585351.1	99	*Trichosporon loubieri*

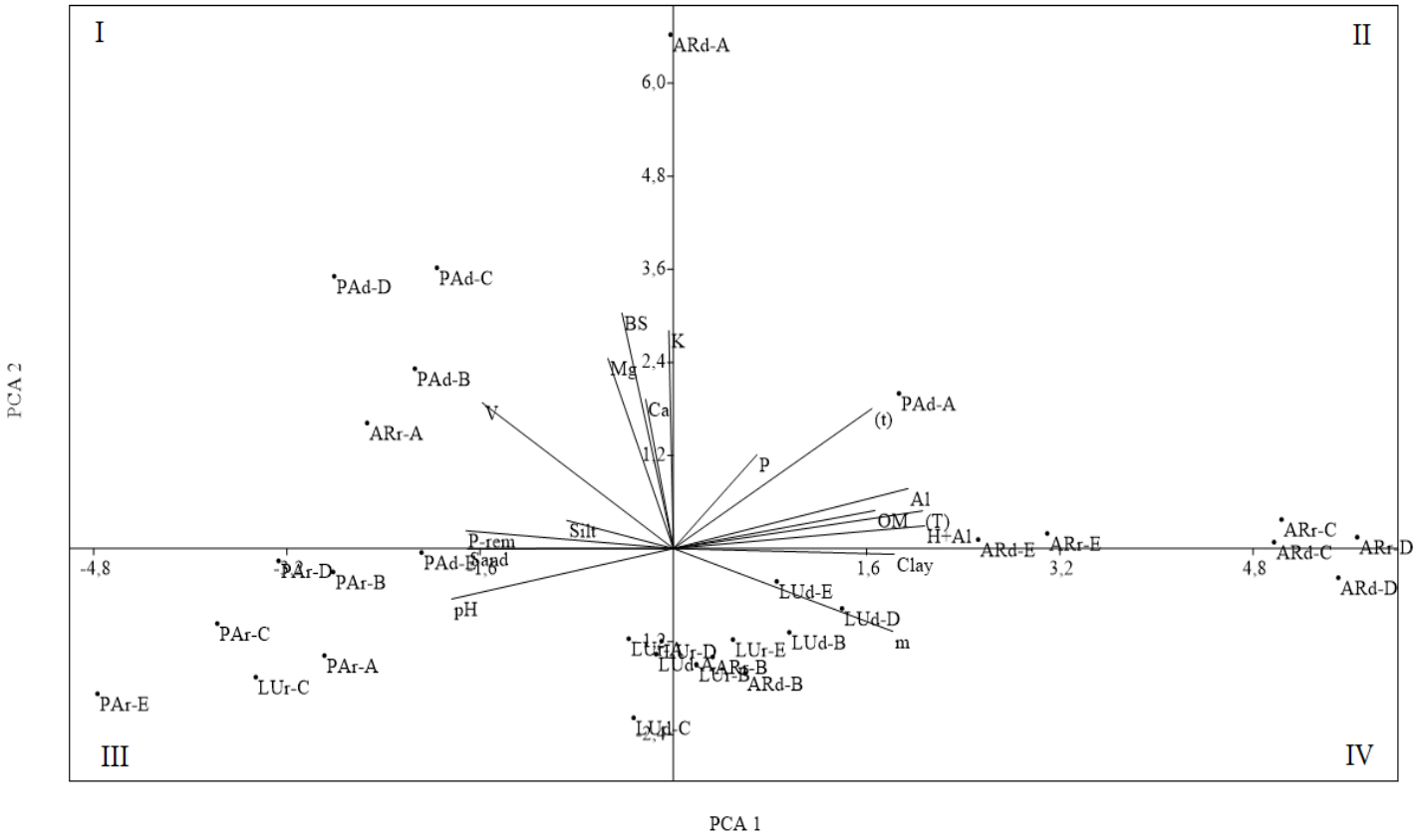

Figure 3. Principal components analysis (PCA) of chemical and physical atributes of 30 soil samples of Brazil cerrado of regions Arcos (AR), Passos (PA) and Luminárias (LU) in five points (A, B, C, D and E) in dry (d) and rainy seasons (r). Abbreviations: P– phosphorus; K – potassium; Ca– calcium; Mg– magnesium; Al– aluminium; H $^{+}$Al- exchangeable acidity; OM- organic matter; SB- sum of Ca, Mg and K; t- effective cation exchange capacity; T- cation exchange capacity at pH 7,0; V%- base saturation of CTC at pH 7.0; m%- aluminium saturation.

ponding to 28.6% of isolates. The proportion of isolates that produced highly active alkaline phosphatase, acidic phosphatase and naphthol-AS-BI-phosphohydrolase (phosphatases), were 2, 53 and 4.1%, respectively. The organisms that demonstrated a great amount of enzyme activity were as follows: *S. vanrijiae* (alkaline phosphatase), *C. glabatra, C. neerlandica, C. orthopsilosis, C. railenensis, C. tetrigidarum, Candida tropicalis, Candida* sp., *Cryptococcus flavescens, Cryptococcus humicola, Cryptococcus laurentii, K. ohmeri, S. polymorphus, S. pseudopolymorphus, S. vanrijiae, T. globosa* and *T. loubieri* (acidic phosphatase), and *C. laurentii and S. vanrijiae* (naphthol-AS-BI-phosphohydrolase).

We did not isolate any producers of lipase, β-

Table 4. Distribuition of yeast species in dry and rainy season, OTUs, richness (S), abundance and isolate number (frequency) (TF) quantification.

OTUs	Arcos			Passos			Luminárias			TA	TF (%)
	Rainy	Dry	A	Rainy	Dry	A	Rainy	Dry	A		
Candida frijolensis			0			0		3	3	3	16.7
Candida glabatra	10		10	1		1	1	1	2	13	66.7
Candida labiduridarum	1		1			0			0	1	16.7
Candida neerlandica	11		11		2	2	14	5	19	32	66.7
Candida orthopsilosis			0		11	11			0	11	16.7
Candida railenensis		4	4			0			0	4	16.7
Candida sojae		3	3			0			0	3	16.7
Candida sp.	5	16	21	12	26	38	5	4	9	68	100
Candida tetrigidarum	3	4	7	4	7	11	5	1	6	24	100
Candida tropicalis	1	2	3		13	13	1	4	5	21	83.3
Cryptococcus flavescens		2	2			0			0	2	16.7
Cryptococcus humicola			0		5	5			0	5	16.7
Cryptococcus laurentii			0	18		18			0	18	16.7
Debaromyces hansenii			0		1	1			0	1	16.7
Kazachstania exigua			0			0		2	2	2	16.7
Kodamaea ohmeri			0		1	1			0	1	16.7
Lindnera saturnus			0		19	19			0	19	16.7
Pichia kudriavzevii			0	2		2			0	2	16.7
Schwanniomyces polymorphus			0		3	3			0	3	16.7
Schwanniomyces pseudopolymorphus			0		5	5			0	5	16.7
Schwanniomyces sp.			0		17	17			0	17	16.7
Schwanniomyces vanrijiae			0		10	10			0	10	16.7
Torulaspora globosa			0			0	6	27	33	33	33.3
Torulaspora maleeae		8	8			0			0	8	16.7
Trichosporon loubieri			0		1	1			0	1	16.7
Total Abundance	31	39	**70**	37	121	**158**	32	47	79	**307**	
Richness (S)	6	7	**10**	4	14	**17**	6	8	**8**	**25**	

A= abundance, as the sum of occurrence in both seasons, TA = total abundance, as the sum of occurrence in all samples, TF = total frequency (isolate number), the sum of occurrence in all samples.

Table 5. Richness and diversity indices of yeasts isolated from the Brazilian Cerrado by sampling area and season.

Diversity indexes	Sampling sites					
	Arcos		Passos		Luminárias	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
Richness (S)	6	7	5	14	6	8
Individuals	31	39	37	121	32	47
Dominance (D)	0.27	0.24	0.36	0.12	0.28	0.36
Simpson (1-D)	0.73	0.76	0.64	0.88	0.72	0.64
Shannon (H)	1.47	1.66	1.21	2.27	1.47	1.45
Equitability (J)	0.82	0.85	0.75	0.86	0.82	0.70

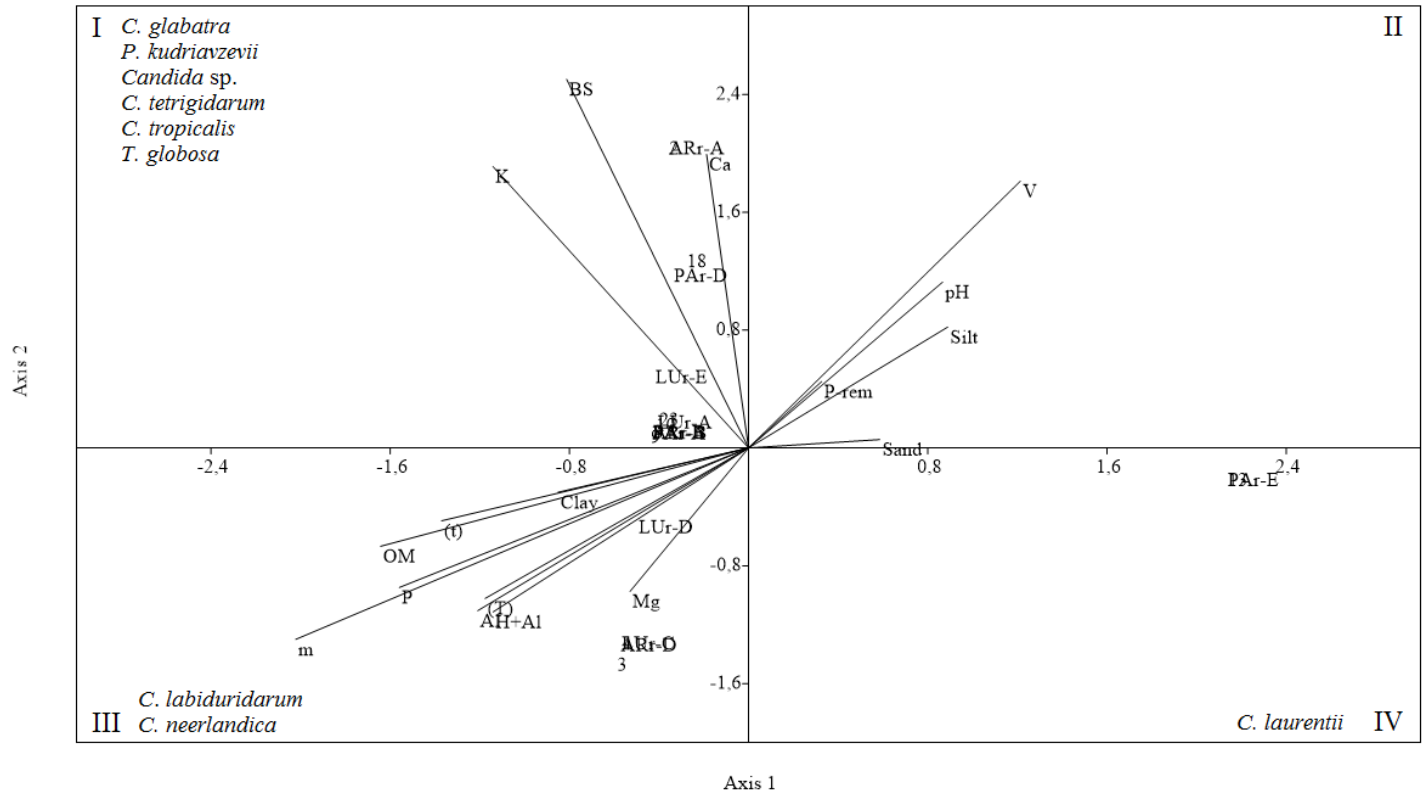

Figure 4. Canonical correspondence analysis (CCA) of soil yeasts species of Arcos (AR), Passos (PA) and Luminárias (LU) collection areas during rainy season (r). 2- *Candida glabatra*; 3- *C. labiduridarum*; 4- *C. neerlandica*; 8- *Candida* sp.; 9- *C. tetrigidarum*; 10- *C. tropicalis*; 13- *Cryptococcus laurentii*; 18- *Pichia kudriavzevii*; 23- *Torulaspora globosa.*

glucuronidase, α-mannosidase, α-fucosidase, trypsin, α-chymotrypsin, or cysteine arylamidase with medium or high intensity. The *S. vanrijiae* yeast produced most enzymes with the greatest activities, including esterase, leucine arylamidase, acidic phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. *S. vanrijiae* was the only producer of alkaline phosphatase and α-galactosidase. Only *S. vanrijiae* (CES UFLA Y-657) and *C. laurentii* (CES UFLA 519) demonstrated significant naphthol-AS-BI-phosphohydrolase production.

The *Cryptococcus* genera, the third most abundantly isolated genus in this work, showed high production of various enzymes, including β-glucosidase and β-galactosidase. The β-glucosidase enzymes associated with other complex carbohydrates may be potentially useful in the food industry, biofuel production and textile

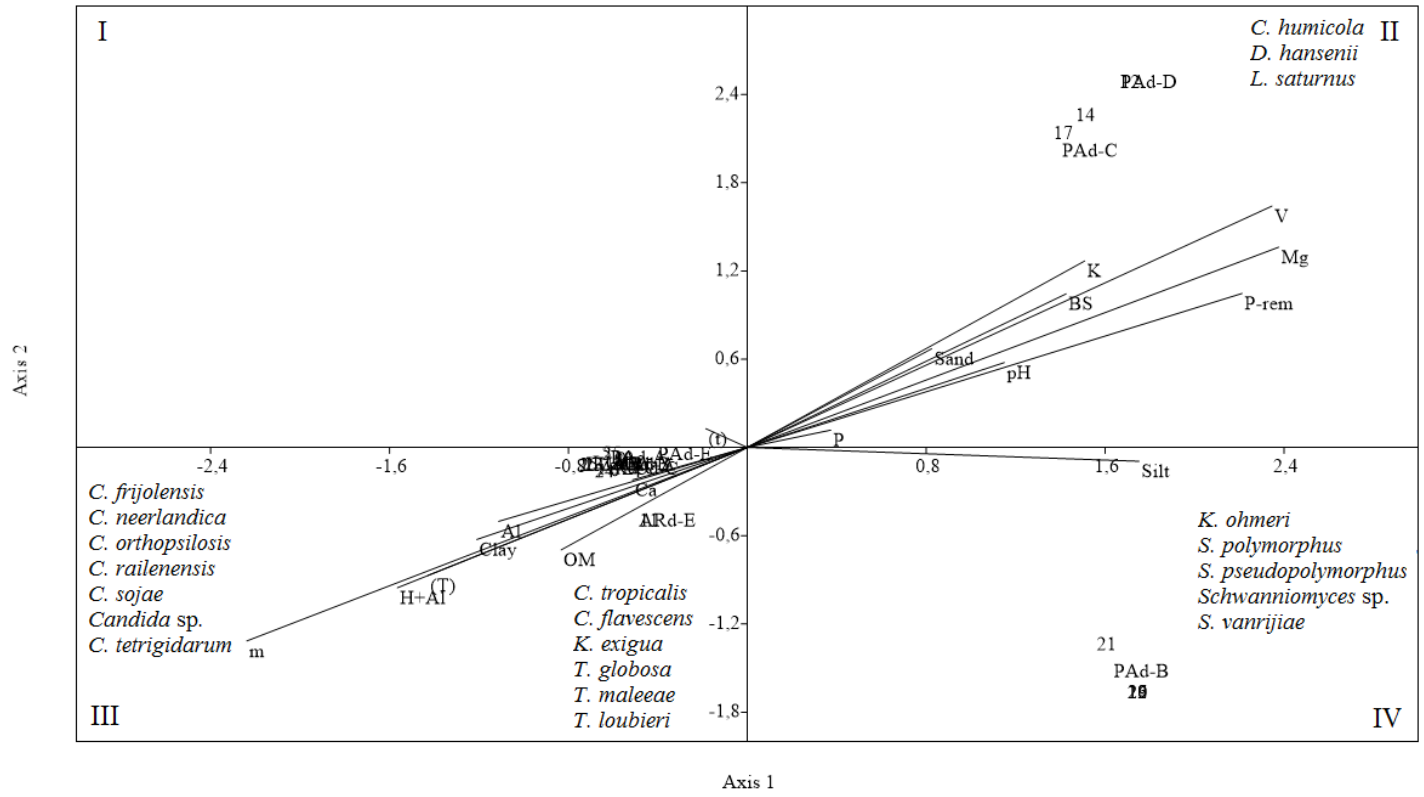

Figure 5. Canonical correspondence analysis (CCA) of soil yeasts species of Arcos (AR), Passos (PA) and Luminárias (LU) collection areas during dry season (d). 1- *Candida frijolensis*; 2- *C. glabatra*; 4- *C. neerlandica*; 5- *C. orthopsilosis*; 6- *C. railenensis*; 7- *C. sojae*; 8- *Candida* sp.; 9- *C. tetrigidarum*; 10- *C. tropicalis*; 11- *C. flavescens*; 12- *Cryptococcus humicola*; 14- *Debaromyces hansenii*; 15- *Kazachistania exígua*; 16- *Kodamaea ohmeri*; 17- *Lindnera saturnus*; 19- *Schwannyomices polymorphus*; 20- *S. pseudopolymorphus*; 21- *Schwanniomyces* sp.; 22- *S. vanrijiae*; 23- *Torulaspora globosa*; 24- *T. maleeae*; 25- *Trichosporon loubieri.*

production, among others (Dhillon et al., 2012; Lavenson et al., 2012). *C. laurentii* UFLA CES 519, UFLA CES 523 and UFLA CES 526 were the only isolates that produced β-galactosidase with high activity. This enzyme is used in the hydrolysis of lactose, and it is commercially produced by *Kluyveromyces lactis* (Romo-Sánchez et al., 2010).

The Cerrado yeasts showed differences in the enzyme profiles for isolates of the same species. Therefore, it may be possible that yeast species isolated from tropical regions vary somewhat from those described in the literature (Dhillon et al., 2012; Lavenson et al., 2012).

Conclusions

To our knowledge, our study is the first that describes the isolation of specific yeast strains from Brazilian Cerrado soil from Minas Gerais States. All genera were abundant during the dry season. This means that the analyzed soil microorganisms prefer low humidity. In addition, we have demonstrated their ability to produce enzymes of Industrial interest. Characterization of this resource may also contribute to the development of a microbial database, providing data on the properties and enzyme characteristics of yeast isolates for potential industrial and technological applications.

Perspectives

This study highlights the ability of Cerrado soil yeasts to produce enzymes that show potential for industrial applications. The yeast isolates that are the best at producing selected enzymes are being tested for scale-up production on different substrates.

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Table 6. Comparison of enzymatic activities of yeasts strains determined by the API ZYM®.

Species	Code	Enzyme																		
		Alkaline phosphatase	Esterase (C 4)	Esterase Lipase (C 8)	Lipase (C 14)	Leucine arylamidase	Valine arylamidase	Cystine arylamidase	Trypsin	α-chymiotrypsin	Acidic phosphatase	Naphtyl-AS-BI-phosphohydrolase	α-galactosidase	β-galactosidase	β-glucuronidase	α-glucosidase	β-glucosidase	N-acetyl-β-glucosaminidase	α-mannosidase	α-fucosidase
Candida frijolensis	UFLA CES-Y 816	1	3	3	0	5	3	2	0	0	3	2	0	0	0	1	0	5	0	0
	UFLA CES-Y 818	1	3	3	0	5	3	2	0	0	3	2	0	0	0	1	0	5	0	0
Candida glabatra	UFLA CES-Y 557	1	2	3	0	5	3	2	0	0	4	2	0	0	0	0	0	0	0	0
	UFLA CES-Y 566	1	2	3	0	5	3	2	0	0	4	2	0	0	0	0	0	0	0	0
	UFLA CES-Y 558	1	2	3	0	5	3	2	0	0	4	2	0	0	0	0	0	0	0	0
Candida labiduridarum	UFLA CES-Y 590	2	3	3	1	5	3	2	0	0	3	1	0	0	0	5	2	0	0	0
Candida neerlandica	UFLA CES-Y 580	1	2	3	0	5	3	2	1	0	2	2	0	0	0	4	1	1	0	1
	UFLA CES-Y 284	2	4	3	1	5	3	1	0	0	4	1	0	1	0	2	0	5	0	0
	UFLA CES-Y 707	1	2	3	0	5	3	2	1	0	2	2	0	0	0	4	1	1	0	1
	UFLA CES-Y 708	1	2	3	0	5	3	2	1	0	2	2	0	0	0	4	1	1	0	1
Candida orthopsilosis	UFLA CES-Y 606	2	2	4	1	5	4	2	0	0	5	1	0	0	0	4	0	0	0	0
	UFLA CES-Y 614	2	2	4	1	5	4	2	0	0	5	1	0	0	0	4	0	0	0	0
Candida railenensis	UFLA CES-Y 758	2	2	3	1	5	3	2	0	0	4	1	0	0	0	2	3	1	0	0
	UFLA CES-Y 759	2	2	3	1	5	3	2	0	0	4	1	0	0	0	2	3	1	0	0
Candida sojae	UFLA CES-Y 781	1	3	3	1	5	4	1	0	0	1	1	0	0	0	5	3	0	0	0
	UFLA CES-Y 784	1	3	3	1	5	4	1	0	0	1	1	0	0	0	5	3	0	0	0
Candida tetrigidarum	UFLA CES-Y 68	1	4	3	1	5	3	2	0	0	4	1	0	0	0	3	1	5	0	1
	UFLA CES-Y 289	1	4	3	1	5	3	2	0	0	4	1	0	0	0	3	1	5	0	1
Candida tropicalis	UFLA CES-Y 783	1	3	2	0	5	3	2	0	0	2	2	0	0	0	5	3	0	1	0
	UFLA CES-Y 621	1	3	3	0	5	3	2	1	0	4	2	0	0	0	4	0	5	0	0
	UFLA CES-Y 593	1	3	3	0	5	3	2	1	0	4	2	0	0	0	4	0	5	0	0
Candida sp.	UFLA CES-Y 278	1	4	3	1	5	3	2	0	0	4	1	0	0	0	3	1	5	0	1
	UFLA CES-Y 761	2	2	3	1	5	3	2	0	0	4	1	0	0	0	2	3	5	0	0
	UFLA CES-Y 778	1	3	3	1	5	4	1	0	0	1	1	0	0	0	0	0	0	0	0
Cryptococcus flavescens	UFLA CES-Y 726	1	3	2	0	5	1	0	0	0	5	1	0	0	0	0	1	0	0	0
Cryptococcus humicola	UFLA CES-Y 682	1	2	2	1	5	4	2	1	0	5	3	0	0	0	1	0	0	0	0

Table 6. Contd.

	UFLA CES-Y 683	1	4	3	1	5	1	1	1	0	3	2	0	0	0	1	1	0	0	0
Cryptococcus laurentii	UFLA CES-Y 519	2	3	4	1	5	3	1	0	0	5	5	1	5	0	2	4	0	0	0
	UFLA CES-Y 523	1	2	3	1	5	3	2	0	0	5	2	0	5	0	1	4	0	0	0
	UFLA CES-Y 526	1	2	3	1	5	3	2	0	0	5	2	0	5	0	1	4	0	0	0
Debaromyces hansenii	UFLA CES-Y 678	2	3	3	1	5	3	1	0	0	2	1	0	0	0	0	0	0	0	0
Kazachstania exigua	UFLA CES-Y 838	1	2	2	1	5	3	1	1	0	2	1	0	0	0	0	0	1	0	1
	UFLA CES-Y 839	1	4	3	1	5	4	3	0	0	2	1	0	0	0	0	0	0	0	0
Kodamaea ohmeri	UFLA CES-Y 648	2	3	3	0	5	2	1	0	0	5	2	0	0	0	2	1	0	0	0
Lindnera saturnus	UFLA CES-Y 676	0	2	2	1	5	4	3	0	0	2	3	0	0	0	1	5	0	0	0
	UFLA CES-Y 681	0	2	2	1	5	4	3	0	0	2	3	0	0	0	1	5	0	0	0
	UFLA CES-Y 667	0	2	2	1	5	4	3	0	0	2	3	0	0	0	1	5	0	0	0
Pichia kudriavzevii	UFLA CES-Y 542	0	4	3	1	5	4	3	0	0	2	2	0	0	0	0	0	0	0	0
	UFLA CES-Y 543	0	4	3	1	5	4	3	0	0	2	2	0	0	0	0	0	0	0	0
Schwanniomyces polymorphus	UFLA CES-Y 652	2	2	3	1	5	3	1	0	0	5	1	0	0	0	5	4	1	0	0
Schwanniomyces pseudopolymorphus	UFLA CES-Y 632	2	4	3	1	5	3	1	0	0	5	3	1	0	0	5	4	1	0	0
Schwanniomyces vanrijiae	UFLA CES-Y 657	4	4	3	1	5	3	2	0	1	5	4	4	0	0	5	0	0	0	0
Torulaspora globosa	UFLA CES-Y 64	2	1	2	1	5	4	2	0	0	5	1	0	0	0	0	0	0	0	0
	UFLA CES-Y 817	2	1	2	1	5	4	2	0	0	5	1	0	0	0	0	0	0	0	0
	UFLA CES-Y 61	1	3	3	1	5	3	2	0	0	3	1	0	0	0	4	0	5	0	0
Torulaspora malleae	UFLA CES-Y 775	1	3	2	1	5	3	2	0	0	1	1	0	0	0	0	0	1	0	0
	UFLA CES-Y 782	1	3	2	1	5	3	2	0	0	1	1	0	0	0	0	0	1	0	0
	UFLA CES-Y 771	1	3	2	1	5	3	2	0	0	1	1	0	0	0	0	0	1	0	0
Trichosporon loubieri	UFLA CES-Y 631	2	4	3	0	5	2	1	0	0	5	2	0	0	0	2	2	2	0	0
No activity (0)		5	0	0	14	0	0	1	41	48	0	0	46	45	49	15	24	27	48	42
Low intensity (1)		28	2	0	35	0	2	12	8	1	6	24	2	1	0	9	9	11	1	7
Medium intensity (2-3)		15	36	46	0	0	33	36	0	0	17	23	0	0	0	10	8	1	0	0
High intensity (4-5)		1	11	3	0	49	14	0	0	0	26	2	1	3	0	15	8	10	0	0

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