Biota Neotropica 21(4): e20201184, 2021
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

Yeasts in native fruits from Brazilian neotropical savannah: occurrence, diversity and enzymatic potential

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VALE, H.M.M., REIS, J.B.A., OLIVEIRA, M., MOREIRA, G.A.M., BOMFIM, C.A. **Yeasts in native fruits from Brazilian neotropical savannah: occurrence, diversity and enzymatic potential.** Biota Neotropica 21(4): e20201184. https://doi.org/10.1590/1676-0611-BN-2020-1184

Abstract: Cerrado is the second largest phytogeographic domain in Brazil, with a huge ethnobotany variety, including fruit species that stand out for their economic, industrial, biotechnological and medicinal potential. The objective of this study was to characterize the diversity of culturable yeasts and their potential for the production of hydrolytic enzymes in fruits of 13 species of native plants of the Cerrado in Brazil. Sequencing the 26S rRNA gene identified the isolates. The enzymatic potential was evaluated using specific substrates for the enzymes amylases, cellulases, proteases, and pectinases. Nine of the 13 fruit species analyzed showed yeast growth, totaling 82 isolates, identified in 26 species. The phylum Ascomycota predominated over Basidiomycota. The fruits of *Butia capitata* presented the highest species richness. *Candida* and *Meyerozyma* were the most frequent genera. About 57% of the isolates were able to produce at least one of the enzymes analyzed. The species *Papiliotrema flavescens, Hanseniaspora meyeri, Meyerozyma guilliermondii*, and *Rhodotorula mucilaginosa* produced all the enzymes tested. The results were found to expand the knowledge about the yeast communities present in fruits of the Cerrado native plants, evidencing the presence of species shared among the plants, and their potential for biotechnological use in the future.
Keywords: *hydrolytic enzymes;* Candida*; Cerrado domain;* Meyerozyma*; fruit pulp.*

Leveduras em frutos nativos do Cerrado: ocorrência, diversidade e potencial enzimático

Resumo: O Cerrado é o segundo maior domínio fitogeográfico do Brasil, com grande variedade etnobotânica, incluindo espécies frutíferas que se destacam por seu potencial econômico, industrial, biotecnológico e medicinal. O objetivo deste trabalho foi caracterizar a diversidade de leveduras cultiváveis e seu potencial para a produção de enzimas hidrolíticas em frutos de 13 espécies de plantas nativas do Cerrado brasileiro. O sequenciamento do gene 26S rRNA identificou os isolados. O potencial enzimático foi avaliado utilizando substratos específicos para as enzimas amilases, celulases, proteases e pectinases. Nove das 13 espécies de frutos analisadas apresentaram crescimento de levedura, totalizando 82 isolados, identificados em 26 espécies. O filo Ascomycota predominou sobre Basidiomycota. Os frutos de *Butia capitata* apresentaram a maior riqueza de espécies. *Candida* e *Meyerozyma* foram os gêneros mais frequentes. Cerca de 57% dos isolados foram capazes de produzir pelo menos uma das enzimas analisadas. As espécies *Papiliotrema flavescens, Hanseniaspora meyeri, Meyerozyma guilliermondii* e *Rhodotorula mucilaginosa* produziram todas as enzimas testadas. Os resultados encontrados ampliam o conhecimento sobre as comunidades de leveduras presentes nos frutos das plantas nativas do Cerrado, evidenciando a presença de espécies compartilhadas entre as plantas, e seu potencial para uso biotecnológico no futuro.
Palavras-chave: *Enzimas hidrolíticas;* Candida*; Domínio Cerrado;* Meyerozyma*; Polpa de frutas.*

Introduction

The Cerrado is the second largest phytogeographic domain in Brazil occupying about 2 million km^2 with phytophysiomic formations of tropical fields, savannah, and seasonal forest (Klink, 2005; Buzatti et al. 2018). This biome is considered a biodiversity hotspot characterized by having a high number of endemic vascular plant species (Souza et al. 2016; Buzatti et al. 2018). It is estimated that there are more than 4.400 plants native to this biome and, among this huge ethnobotanical variety, fruit species stand out for their economic, industrial, biotechnological, and medicinal value (Wantzen et al. 2012; Machado et al. 2014; Costas et al. 2018).

Among the native fruit species of the Brazilian Cerrado, some of the most studied and used for economic and biotechnological purposes are the *Anacardium humile* A.St.-Hil (Cerrado cashew) (Silva et al. 2013; Araújo et al. 2018), *Caryocar brasiliense* Cambess (pequi) (Paz et al. 2014) and *Mauritia flexuosa* Lf (buriti) (Castro et al. 2014; Garcia et al. 2015; Pratulea et al. 2019). The fruits of these plant species, besides being rich in nutritional values, are known to present molecules with anti-inflammatory, antioxidant, antimicrobial, thickening, and aromatic properties (Silva et al. 2013; Costas et al. 2018). In addition, to be known for their nutraceutical characteristics, the Cerrado fruits have great economic importance for small farmers and extractivists in the Northwest region of the state of Minas Gerais - Brazil, like the cooperative "Copabase" (www.copabase.org), whose main objective is the commercialization of family and artisanal production products, such as fruit pulps, sweets, cakes, and other food products from the Cerrado fruits (Souza et al. 2018).

Several microbial communities have the ability to colonize the interior of plant organs, known as endophytic microorganisms. The term endophytic concerns to all microorganisms that inhabit the interior of organs, tissues and in the inter-and intracellular space of plant cells in a mutualistic way, and may play crucial roles for the maintenance of plant health or producing plant growth regulators (Liu et al. 2019) and alkaloids that act in the protection of plant tissues against herbivores (Felber et al. 2016). Among these microorganisms, the presence of yeasts in the Cerrado native fruits has been attracting attention because they are important sources of new biotechnological resources (Moreira et al. 2015; Sperandio, et al. 2015; Vale, et al. 2015).

Yeasts represent a portion of the natural microbiota of the plant phyllosphere (leaves, stems, shoots, flowers, and fruits) (Vadkertiová et al. 2012; Ling et al. 2019; Piombo et al. 2020) and more recently has been reported in fruits of the Cerrado native plants such as *Eugenia lutescens* Cambess, *Campomanesia xanthocarpa* (O. Berg) and *Brosimum gaudichaudii* Trécul (Moreira et al. 2015); *Byrsonima crassifolia* Steud and *Eugenia dysenterica* DC (Sperandio et al. 2015) and also in fruits of seven more native species of the Cerrado: *Ouratea hexasperma* Baill, *B. gaudichaudii* Trécul, *Passiflora nitida* Kunth, *Myrcia tomentosa* DC, *Byrsonima coccolobifolia* Kunt, *Guapira graciliflora* (Mart. Ex Schmidt) and *C. brasiliense* Cambess (Coelho et al. 2020). Some of these species have the potential for biological control of post-harvest pathogens, such as *Penicillium digitatum in vitro* and *in vivo* tests (Sperandio et al. 2015), emphasizing the importance of knowledge about yeast diversity in the Cerrado native fruits and its biotechnological application.

Yeasts have a high biochemical and physiological versatility, which make them important sources of biomolecule prospecting, similar to enzymes that are commonly used in industrial applications (Carvalho et al. 2013). Currently, interest in the production of enzymes from microbial sources has increased due to the wide application potential, ranging from the production of bioenergy and biofuels to its application in food, textile and papermaking industries (Romo-Sánchez et al. 2010). However, it is still necessary to search for native yeast species not listed as good producers of active biomolecules. It is important to highlight the need to isolate and characterize yeasts from different habitats, aiming at describing the still unknown diversity, and to verify the biotechnological potential that these microorganisms may present (Romo-Sánchez et al. 2010; Carvalho et al. 2013).

Understanding the importance of the Cerrado bioeconomy, its native plants, and the scarcity of research on yeast diversity in native fruits of this Biome, this study aimed to: a) describe the occurrence, density, and diversity of culturable yeasts in fruits of the Cerrado native plants; and b) evaluate the potential of isolated strains for hydrolytic enzyme production.

Materials and Methods

1. Area of study and sampling of fruits

Samples were collected in the Northwest region of the state of Minas Gerais, in the municipality of Arinos, Brazil (Table 1). Fruits of 13 Cerrado native species were collected, as follows: *Anacardium humile* Mart., *Annona crassiflora* Mart., *Butia capitata* Mart., *Caryocar brasiliense* Cambess, *Eugenia dysenterica* DC., *Hancornia speciosa* Gomes, *Hymenaea stigonocarpa* Mart. Ex Hayne, *Mauritia flexuosa* Lf., *Passiflora cincinnata* Mast., *Psidium cattleyanum* Sabine, *Solanum lycocarpum* A.St-Hil, *Syagrus oleracea* Becc and *Talisia esculenta* Radlk (Figure S1). Samples of healthy fruits, ripe, without perforations and smashes, were collected, stored at 4ºC, and processed in less than 48 hours after collection.

2. Isolation and molecular identification

For the isolation of total yeasts (epiphytic + endophytic), samples of fruits from 13 species of plants were collected, being three different trees for each plant species and three different samples for each tree of the same plant species. Fruits were superficially washed with distilled running water to remove dust and dried naturally at room temperature. Aliquots of 10 g composed of pulp and peel of each fruit sample, in triplicate, macerated in 100 ml of peptone water (0.1%), and then homogenized under agitation at 150 rpm for 30 minutes, and diluted to 10^{-3}. Aliquots of 100µL were seeded in YM agar (0.3% yeast extract; 0.3% malt extract; 0.5% peptone; 10% glucose; 2% agar; 100mg. ml^{-1} chloramphenicol) (Kurtzman & Fell, 1998). The plates were incubated at 28 ºC for 5-7 days. After growth, colony-forming units (CFU) were counted, and isolation in pure cultures was performed from each colony's morphological characteristics. The pure cultures were cryopreserved using YM broth with 25% glycerol in a freezer at -80 ºC.

DNA was extracted according to modified Kurtzman & Fell protocol (1998). The isolates were grown in YM broth for 48 hours at 28 ºC under the agitation of 150 rpm. Cell lysis was performed using 0.1 g of glass beads and 200 µL of extraction buffer and agitated for 1 minute. For protein precipitation, 200 µL of phenol, chloroform, and isoamyl alcohol (25:24:1) was used, and subsequently, the DNA was precipitated using isopropyl alcohol (1 hour at -20ºC). The DNA was resuspended in 30µL of Milli-Q water and stored at -20 ºC.

The DNA amplification was performed according to the Kurtzman & Robnett protocol (1998) using domain D1/D2 of the major ribosomal

Table 1. Cerrado native species host plants collected in the municipality of Arinos, Minas Gerais, Brazil.

Botanical Family	Host species	Popular name	Geographic Coordinates	Date of collection
Anacardiaceae	*Anacardium humile* Mart.	Caju-do-Cerrado	15° 55' 01" S e 46° 6' 20" W	08/08/2014
Annonaceae	*Annona crassiflora* Mart.	Araticum	15° 55' 01" S e 46° 6' 20" W	03/14/2014
Apocynaceae	*Hancornia speciosa* Gomes	Mangaba	15° 55' 01" S e 46° 6' 20" W	03/14/2014
Arecaceae	*Butia capitata* Mart.	Coquinho-azedo	15° 51' 20" S e 45° 43' 52" W	03/16/2014
Arecaceae	*Mauritia flexuosa* Lf.	Buriti	15° 51' 20" S e 45° 43' 52" W	08/16/2014
Arecaceae	*Syagrus oleracea* Becc	Coco-guariroba	16° 1' 12" S e 45° 58' 39" W	03/30/2014
Caryocaraceae	*Caryocar brasiliense* Cambess	Pequi	15° 55' 01" S e 46° 6' 20" W	03/15/2014
Fabaceae	*Hymenaea stigonocarpa* Mart. Ex Hayne	Jatobá-do-Cerrado	15° 51' 20" S e 45° 43' 52" W	08/14/2014
Myrtaceae	*Eugenia dysenterica* DC.	Cagaita	15° 55' 01" S e 46° 6' 20" W	10/12/2014
Myrtaceae	*Psidium cattleyanum* Sabine	Araçá	15° 55' 01" S e 46° 6' 20" W	03/28/2014
Passifloraceae	*Passiflora cincinnata* Mast.	Maracujá-do-Cerrado	16° 1' 12" S e 45° 58' 39" W	05/19/2014
Sapindaceae	*Talisia esculenta* Radlk	Pitomba	15° 51' 20" S e 45° 43' 52" W	03/16/2014
Solanaceae	*Solanum lycocarpum* A.St-Hil	Lobeira	15° 51' 20" S e 45° 43' 52" W	06/09/2014

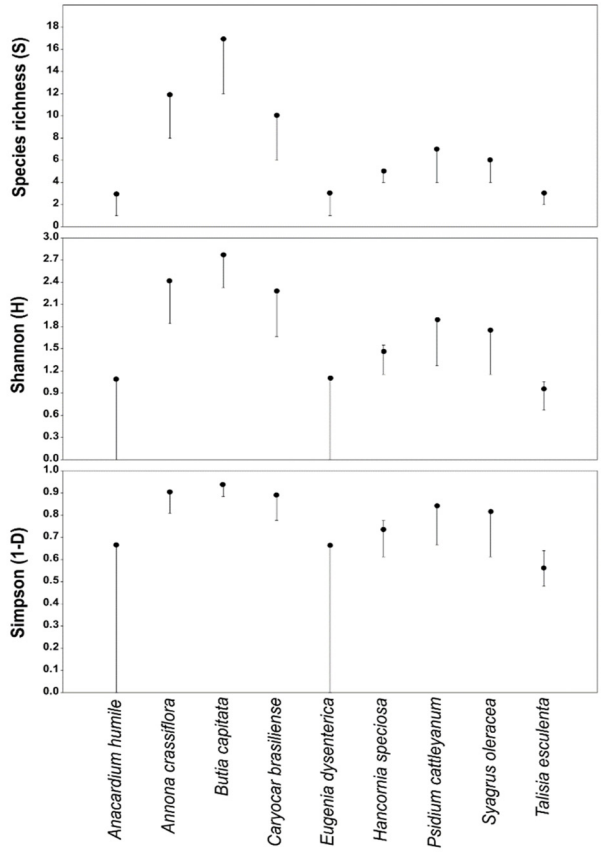

Figure 1. Diversity indexes of yeast communities present in fruits of the Cerrado native plants from the Northwest of Minas Gerais, Arinos, Brazil.

rDNA subunit (LSU) 26S, with the pair of primers: NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG–3') and NL4 (5'-GGT CCG TGT TTC AAG ACG G -3') (O'donnel et al. 1993). The PCR reaction was performed with a final volume of 25µl, containing 20 pmol of each primer,1.5 mM $MgCl_2$, and 0.2 mM dNTPs. The thermocycling program consisted of initial denaturation at 94ºC for 3 minutes, followed by 33 denaturation cycles from 94 ºC to 1 minute, annealing at 56 ºC for 30 seconds, and extension at 72 ºC for 1 minute, and a final extension at 72 ºC for 6 minutes.

The amplicons were purified with the enzyme *ExoSAP-IT*® and sent for sequencing at the Catholic University of Brasília (UCB) using the ABI 3130xl Applied Biosystems sequencer, using the Sanger method (Sanger et al. 1997). The formation of their peaks ascertained the quality of the sequences. These sequences were prepared by removing initial and final noises with the Bio Edit Sequence Alignment Editor version 7.5. After that, they were submitted to the Database of the National Center for Biotechnology Information (NCBI (http://www.ncbi.nlm.nih.gov/), using the program *BLASTn*. The parameters of choice were lower e-value, greater coverage value, and greater identity. The alignment and phylogenetic analyses were made using the *MEGA 6 software*® using the Maximum-likelihood Estimation Method Maxim Verisiillability method (Tamura et al. 2013).

The diversity indices (Species richness S, Simpson 1-D, Shannon H) were calculated based on an abundance matrix (isolates *versus* host plant) using the *Past program* (version 3.13).

3. Enzymatic production

The enzymatic isolates characterization was performed as described in Souza (2008) and Landell (2009) with some modifications. The isolates were cultured in YM broth at 28 °C until reaching the cell density of 10^8 cells/mL. The evaluations were carried out using the "cup plate" methodology, where 100 µl of the yeast solution were inoculated in a perforation (cup) performed with a Pasteur pipette in triplicate at three equidistant points with a diameter of 6 mm. Culture media containing a specific substrate for each enzyme were used. Amylase production was evaluated in Agar-starch (Agar, 18 $g.L^{-1}$; starch, 10 $g.L^{-1}$), was cellulases were evaluated in Agar-CMC (Agar, 18 $g.L^{-1}$, carboxymethylcellulose, 10 $g.L^{-1}$). The Agar-pectin (Agar, 18 $g.L^{-1}$, pectin, 10 $g.L^{-1}$) was used to detect pectinases and Agar-gelatin-milk (Agar, 18 $g.L^{-1}$, 10% gelatin solution, skim milk 10%) for proteases. After a 24-hour incubation period, plates were washed with 0.1 N iodine solution for amylase analysis, Congo red 0.1% for cellulase analysis and 5 N hydrochloric acid for pectinase analysis. No revealing substance was required for protease tests. The formation of degradation halos detected the enzymes and production was expressed by forming the halos (mm).

Results

1. Yeast density and diversity

A total of 82 yeast isolates were recovered from fruits from nine of the 13 hostesses analyzed in this study. Yeast growth was not detected

in the fruits of four plant species, including *S. lycocarpum* (Lobeira), *H. stigonocarpa* (Jatobá-do-Cerrado), *M. flexuosa* (Buriti) and *P. cincinnata* (Maracujá-do-Cerrado). The host *B. capitata* (Coquinho-azedo) presented the highest number of isolates (21), while the fruits of *S. oleracea* (Coco-guariroba) presented the highest population density of yeasts (7.2 x 10^4 CFU .g^{-1} fruit). The fruits of *T. esculenta* (Pitomba), *C. brasiliense* (Pequi) and *E. dysenterica* (Cagaita) presented population densities lower than 1.0 x 10 CFU g^{-1}. The hostesses *A. humile* (Cajuzinho-do-Cerrado) and *E. dysenterica* (Cagaita) presented the smallest number of isolates (three isolates) (Table 2).

The highest richness of yeast species (S) was found in the fruits *of B. capitata,* followed by *A. crassiflora* and *C. brasilienses*. The fruits with the lowest species richness were *A. humile, E. dysenterica,* and *T. esculenta.* The diversity of yeasts in the fruits of plant species, represented by the Shannon (H) and Simpson (1-D) indexes, varied widely from plant to plant (Figure 1). The Shannon and Simpson indexes of *B. capitata* were 2.8 and 1.0, respectively, thus having the highest species diversity values compared to the other analyzed species. *A. crassiflora* occupied the second place with Shannon index of 2.4 and Simpson of 0.9, followed by *C. brasiliense* with Shannon of 2.3 and Simpson of 0.89. *T. esculenta* presented the lowest values in the Shannon and Simpson indexes.

Among the isolates, 26 yeast species were identified in fruit samples from nine plant species where there was colony growth (Table 3). The phylum Ascomycota was predominant, representing 80% of the isolates distributed in 10 genus: *Meyerozyma, Candida, Debaryomyces, Wickerhamomyces, Hanseniaspora, Pichia, Kurtzmaniella, Yarrowia, Eremothecium*, and *Lodderomyces*. Phylum Basidiomycota corresponds to 20% of the isolates, divided into four genus: *Rhynchogastrema, Papiliotrema, Pseudozyma*, and *Rhodotorula.*

The species composition of the culturable yeast communities varied among the different fruits (Figure 2). The species *Pichia terricola* showed higher relative abundance in the fruits of *H. speciosa.* In the fruits *B. capitata* and *P. cattleyanum*, the species *Papiliotrema flavescens* and P. *terricola* showed higher relative abundance.

The genera *Candida* and *Meyerozyma* were the most frequent, present in 89% of the fruits analyzed here. *M. caribbica* was the species with the highest occurrence, present in five different fruits, and the following plant species: *B. capitata* (Coquinho-azedo), *A. crassiflora* (Araticum), *H. speciosa* (Mangaba), *E. dysenterica* (Cagaita) and *A. humile* (Caju-Cerrado). The species *M. guilliermondii, Debaryomyces nepalensis, Hanseniaspora meyeri* and *Pichia terricola* were isolated in 44.4% of the fruits analyzed here. The species *D. fabryi* was less frequent here, exclusively in *B. capitata* fruits.

2. Enzymatic production

Of the 82 isolates, 43 (52,43%) isolates produced at least one of the enzymes sought in this study, and 12 isolates produced amylases, 21 isolates produced cellulases, 25 isolates produced proteases, and 18 isolates produced pectinase. The isolates with higher enzymatic production were recovered from the fruits of *B. capitata, A. crassiflora* and *S. oleracea* (Table 4). Of the 82 isolates, only 39 (47,56%) did not show the care of the enzymes investigated here, being they of the following species *Candida easanensis, Debaryomyces fabryi, D. nepalensis, Hanseniaspora meyeri, Meyerozyma guilliermondii, Pichia terricola, Pseudozyma aphidis, Wickerhamomyces anomalus* and *W. rabaulensis.*

Among the enzymes evaluated, the most produced were proteases being present in 52.08% of the isolates producing the enzymes researched; followed by cellulases, found in 41.66%; pectinases, found in 27.08% of the isolates; and finally, amylases found in 25% of the isolates.

Species of the genus *Candida* are among the best enzymatic producers, with four different species, *C. suratensis, C. oleophila, C. natalensis* and *C. intermedia*; followed by the genus *Hanseniaspora*, with the species *H. opuntiae*, *H. meyeri*, and *H. uvarum*; and finally, the genus *Debaryomyces* and *Meyerozyma*, both with two species. The others genera presented only one species capable of producing one or more of the enzymes tested in this study. Four yeast species produced the four enzymes sought, as *P. flavescens, H. meyeri, M. guilliermondii* and *Rhodotorula mucilaginosa* (Table 4). All these yeast species mentioned were isolated in more than three host species, evidencing that these species may be frequent in several Cerrado fruits.

Table 2. Yeast density per gram of fruit (CFU/g. fruit $^{-1)}$ and number of isolates in fruits of Cerrado native host plants, collected in the municipality of Arinos, Minas Gerais, Brazil.

Botanical Family	Host species	Popular name	Yeast density (CFU/g. $fruit^{-1}$)	Number of yeast isolates
Anacardiaceae	*Anacardium humile* Mart.	Cajuzinho-do-Cerrado	2.2 X 10^3	3
Annonaceae	*Annona crassiflora* Mart.	Araticum	4.4 X 10^2	15
Apocynaceae	*Hancornia speciosa* Gomes	Mangaba	5.8 X10^2	7
Arecaceae	*Butia capitata* Mart.	Coquinho-azedo	5.8 X 10^3	22
Arecaceae	*Mauritia flexuosa* Lf.	Buriti	N/C*	0
Arecaceae	*Syagrus oleracea* Becc	Coco-guariroba	7.2 X 10^4	7
Caryocaraceae	*Caryocar brasiliense* Cambess	Pequi	< 1.0 X 10	11
Fabaceae	*Hymenaea stigonocarpa* Mart. Ex Hayne	Jatobá-do-Cerrado	N/C*	0
Myrtaceae	*Psidium cattleyanum* Sabine	Araçá	2.4 X 10^2	9
Myrtaceae	*Eugenia dysenterica* DC.	Cagaita	< 1.0 X 10	3
Passifloraceae	*Passiflora cincinnata* Mast.	Maracujá-do-Cerrado	N/C	0
Sapindaceae	*Talisia esculenta* Radlk.	Pitomba	< 1.0 X 10	5
Solanaceae	*Solanum lycocarpum* A.St-Hil	Lobeira	N/C*	0
Total:				**82**

*N/C- There was no growth of yeast colonies.

Table 3. Inventory of yeast species in fruits of native plant species of the Northwest Cerrado, Arinos, Minas Gerais, Brazil: host species, isolate ID, yeast species, and GenBank match.

BOTANIC FAMILY	HOST SPECIES	ISOLATED ID	SPECIES	GENBANK MATCH	ID (%)
Anacardiaceae	*Anacardium humile*	CAJ3	*Debaryomyces nepalensis*	JX068681	100
		CAJ2	*Meyerozyma caribbica*	KP797883.1	99
		CAJ4	*Rhynchogastrema complexa*	GU321090.2	99
Annonaceae	*Annona crassiflora*	ARA5	*Candida easanensis*	AY634571.1	99
		ARA26	*Debaryomyces fabryi*	KP263777.1	99
		ARA12	*Debaryomyces fabryi*	KP263777.1	99
		ARA21	*Debaryomyces nepalensis*	JX068681	100
		ARA11	*Hanseniaspora meyeri*	FM200038	99
		ARA16	*Hanseniaspora meyeri*	FM200038	99
		ARA28	*Hanseniaspora opuntiae*	EU386744.1	99
		ARA13	*Lodderomyces sp.*	KF830177.1	95
		ARA22	*Meyerozyma caribbica*	KP797883.1	99
		ARA6	*Meyerozyma guilliermondii*	KM885980	99
		ARA18	*Meyerozyma guilliermondii*	KM885980	99
		ARA3	*Pichia sp.*	AB126678.1	99
		ARA25	*Pseudozyma aphidis*	AB617892.1	98
		ARA23	*Rhodotorula mucilaginosa*	KP760069.1	100
		ARA31	*Rhodotorula mucilaginosa*	KP760069.1	100
		ARA9	*Wickerhamomyces anomalus*	KM978209.1	99
Apocynaceae	*Hancornia speciosa*	MAN12	*Hanseniaspora meyeri*	FM200038	99
		MAN2	*Meyerozyma caribbica*	KP797883.1	99
		MA11	*Pichia terricola*	KJ506735.1	100
		MAN9	*Pichia terricola*	KJ506735.1	100
		MAN1	*Pichia terricola*	KJ506735.1	100
		MAN10	*Rhynchogastrema complexa*	GU321090.2	99
		MAN3	*Wickerhamomyces rabaulensis*	AB858464	100
Arecaceae	*Butia capitata*	COQ2	*Candida easanensis*	AY634571.1	99
		COQ37	*Candida suratensis*	AB500863.1	100
		COQ24	*Debaryomyces nepalensis*	JX068681	100
		COQ18	*Debaryomyces nepalensis*	JX068681	100
		COQ64	*Debaryomyces fabryi*	KP263777.1	99
		COQ11	*Eremothecium sinecaudum*	U43391.1	99
		COQ13	*Hanseniaspora uvarum*	KM589490	100
		COQ55	*Kurtzmaniella natalensis*	KJ794716.1	100
		COQ29	*Lodderomyces sp.*	KF830177.1	95
		COQ3	*Lodderomyces sp.*	KF830177.1	95
		COQ66	*Meyerozyma guilliermondii*	KM885980	99
		COQ35	*Meyerozyma caribbica*	KP797883.1	99
		COQ41	*Papiliotrema flavescens*	LK023746.1	100
		COQ42	*Papiliotrema flavescens*	LK023746.1	100
		COQ40	*Pichia myanmarensis*	AB126678.1	99
		COQ61	*Pichia myanmarensis*	AB126678.1	99
		COQ46	*Pichia terricola*	KJ506735.1	100
		COQ63	*Pichia terricola*	KJ506735.1	100
		COQ39	*Rhynchogastrema complexa*	GU321090.2	99
		COQ59	*Rhodotorula diobovata*	KC442275.1	99
		COQ52	*Yarrowia lipolytica*	KF830179.1	100
Arecaceae	*Syagrus oleracea*	GUA16	*Candida easanensis*	AY634571.1	99
		GUA8	*Hanseniaspora meyeri*	FM200038	99
		GUA5	*Hanseniaspora opuntiae*	EU386744.1	99
		GUA2	*Meyerozyma guilliermondii*	KM885980	99
		GUA3	*Papiliotrema flavescens*	LK023746.1	100
		GUA18	*Rhodotorula mucilaginosa*	KP760069.1	100
		GUA10	*Rhodotorula mucilaginosa*	KP760069.1	100

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Caryocaraceae	*Caryocar brasiliense*	PEQ16	*Debaryomyces nepalensis*	JX06868.1	100
		PEQ2	*Eremothecium sinecaudum*	U43391.1	99
		PEQ12	*Hanseniaspora occidentalis*	JX103176.1	100
		PEQ9	*Hanseniaspora opuntiae*	EU386744.1	99
		PEQ14	*Hanseniaspora uvarum*	KM589490	100
		PEQ18	*Lodderomyces sp.*	KF830177.1	95
		PEQ3	*Meyerozyma guilliermondii*	KM885980	99
		PEQ7	*Meyerozyma guilliermondii*	KM885980	99
		PEQ1	*Pichia terricola*	KJ506735.1	100
		PEQ8	*Rhynchogastrema complexa*	GU321090.2	99
		PEQ5	*Yarrowia lipolytica*	KF830179.1	100
Myrtaceae	*Eugenia dysenterica*	CAG2	*Candida suratensis*	AB500863.1	100
		CAG1	*Meyerozyma caribbica*	KP797883.1	99
		CAG4	*Rhynchogastrema complexa*	GU321090.2	99
Myrtaceae	*Psidium cattleyanum*	ARAA12	*Candida natalensis*	KJ794716.1	100
		ARAA16	*Hanseniaspora meyeri*	FM200038	99
		ARAA2	*Papiliotrema flavescens*	LK023746.1	100
		ARAA3	*Papiliotrema flavescens*	LK023746.1	100
		ARAA15	*Pichia myanmarensis*	AB126678.1	99
		ARAA4	*Pichia terricola*	KJ506735.1	100
		ARAA5	*Pichia terricola*	KJ506735.1	100
		ARAA17	*Rhodotorula diobovata*	KC442275.1	99
		ARAA9	*Yarrowia lipolytica*	KF830179.1	100
Sapindaceae	*Talisia esculenta*	PIT5	*Candida intermedia*	KF830176.1	99
		PIT1	*Eremothecium sinecaudum*	U43391.1	99
		PIT3	*Lodderomyces sp.*	KF830177.1	95
		PIT2	*Lodderomyces sp.*	KF830177.1	95
		PIT4	*Lodderomyces sp.*	KF830177.1	95

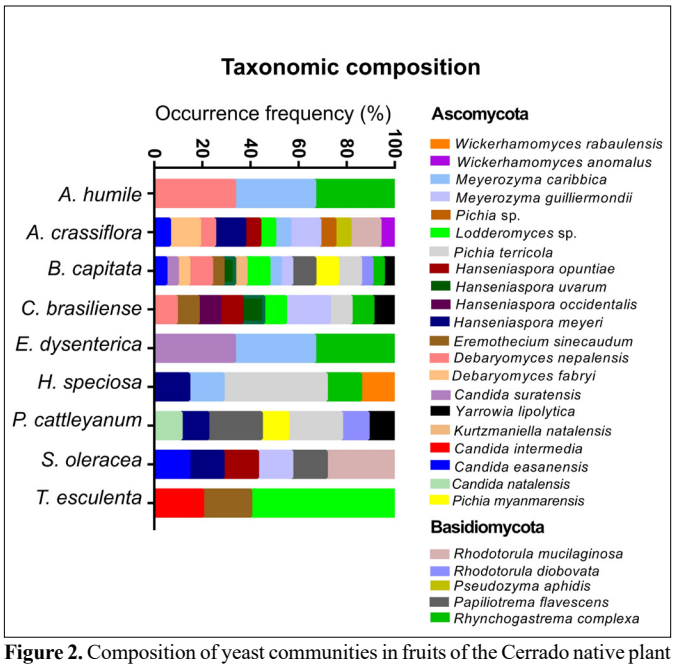

Figure 2. Composition of yeast communities in fruits of the Cerrado native plant species from the Northwest Minas Gerais, Arinos, Brazil.

Discussion

1. Yeasts in Cerrado fruits

The culturable yeast communities diverged among the fruits of the Cerrado native plant species evaluated in this study, regarding cell density and species diversity. The first variable factor among the fruits studied was the density of yeast cells per gram of fruit. The density of microbial cells in the plant may be influenced by abiotic and biotic factors (Hoffman et al. 2008; U'ren et al. 2012), such as seasonality, fruit physiology, host plant ecology, substrate type, fruit morphology, interactions between species, production of 'killer toxins' and competition for substrate (Carvalho et al. 2012; Kusari, et al. 2012; Glushakova & Chernov, 2010). The degree of fruit ripeness can also influence the density, since the more mature the fruit is, the greater the population density of microorganisms that colonize them (Glushakova & Kachalkin 2017). Considering that we use only fully ripe fruits, we hypothesized that the variation in population density among these studied fruits is more related to the differences in the characteristics intrinsic to each fruit species. Variations in nutritional composition (type and quality of available nutrients) and other physical and chemical characteristics have already been reported for the ripe fruits that we studied here (Silva et al. 2008; Rocha 2011; Fujita 2012).

A high population density of yeasts does not always reflect a greater richness of species (Isaeva et al. 2010). We observed these differences in fruits the *A. humile*, which presented three yeast species and 2.2 x 10^3 CFU g^{-1}, and fruits of *C. brasiliense*, which presented 11 yeast species and < 1.0 x 10 CFU g^{-1}. The diversity of yeast communities is also influenced by biotic and abiotic factors (Hoffman et al. 2008; U'ren et al. 2012; Glushakova & Chernov, 2010; Jindamorakot, 2004). Another factor that could interfere in diversity is the mutualistic relationship between microorganisms and plants. The characteristics of the type of colonized tissue can act by selecting the species that would colonize there (Ren et al. 2016; Dhayanithy et al. 2019; Ling et al. 2020). Therefore, we believe that the nutritional characteristics of fruits (quality and availability of nutrients) may be the main factor influencing the richness and abundance of culturable fruit yeast population.

Table 4. Results of amylase, cellulase, protease, and pectinase production tests by yeast isolates of native fruits from the Northwest Cerrado of Minas Gerais, Arinos, Brazil.

SPECIES	PHYLUM	ISOLATE ID	AMYLASE	CELLULASE	PROTEASE	PECTINASE
Candida intermedia	Ascomycota	PIT5	-	-	+	-
Candida suratensis	Ascomycota	COQ37	-	-	+	+
Candida suratensis	Ascomycota	CAG2	-	-	++	-
Debaryomyces fabryi	Ascomycota	ARA26	-	-	+	+
Debaryomyces fabryi	Ascomycota	ARA12	-	-	-	+
Debaryomyces nepalensis	Ascomycota	ARA21	+	-	-	+
Debaryomyces nepalensis	Ascomycota	COQ24	+	+	+	-
Debaryomyces nepalensis	Ascomycota	COQ18	-	-	++	-
Eremothecium sinecaudum	Ascomycota	COQ11	++	++	++	-
Hanseniaspora meyeri	Ascomycota	ARA16	-	+	-	+
Hanseniaspora meyeri	Ascomycota	ARAA16	+	+	-	+
Hanseniaspora opuntiae	Ascomycota	GUA5	++	+	-	-
Hanseniaspora opuntiae	Ascomycota	ARA28	+	+	-	-
Hanseniaspora uvarum	Ascomycota	COQ13	-	-	+	-
Kurtzmaniella natalensis	Ascomycota	COQ55	-	+	-	-
Lodderomyces sp.	Ascomycota	ARA13	-	+	-	-
Lodderomyces sp.	Ascomycota	PIT4	++	-	-	-
Meyerozyma caribbica	Ascomycota	ARA22	-	+	-	-
Meyerozyma caribbica	Ascomycota	CAG1	-	+	+	-
Meyerozyma guilliermondii	Ascomycota	GUA2	-	+	+	+
Meyerozyma guilliermondii	Ascomycota	ARA6	-	+	-	-
Meyerozyma guilliermondii	Ascomycota	COQ66	-	-	-	++
Pichia myanmarensis	Ascomycota	COQ40	-	-	+	-
Pichia terricola	Ascomycota	COQ63	-	+	+	-
Pichia terricola	Ascomycota	MA11	-	-	++	++
Pichia terricola	Ascomycota	MAN9	-	+	+	+
Pichia terricola	Ascomycota	MAN1	-	+	+	+
Pichia sp.	Ascomycota	ARA3	-	+	-	-
Pichia sp.	Ascomycota	ARAA15	+	-	-	-
Yarrowia lipolytica	Ascomycota	PEQ5	++	-	-	-
Yarrowia lipolytica	Ascomycota	ARAA9	-	-	+	+
Papiliotrema flavescens	Basidiomycota	ARAA2	+	-	-	-
Papiliotrema flavescens	Basidiomycota	ARAA3	++	+	-	-
Papiliotrema flavescens	Basidiomycota	GUA3	-	-	+	+
Rhynchogastrema complexa	Basidiomycota	CAJ4	-	-	+	-
Rhynchogastrema complexa	Basidiomycota	CAG4	-	-	++	+
Rhynchogastrema complexa	Basidiomycota	MAN10	-	+	-	-
Rhodotorula diobovata	Basidiomycota	ARAA17	-	-	+	+
Rhodotorula diobovata	Basidiomycota	COQ59	-	-	+	+
Rhodotorula mucilaginosa	Basidiomycota	GUA18	-	++	+	+
Rhodotorula mucilaginosa	Basidiomycota	GUA10	++	+	++	++
Rhodotorula mucilaginosa	Basidiomycota	ARA31	-	-	+	-
Rhodotorula mucilaginosa	Basidiomycota	ARA23	-	-	+	-

(-) absence, (+) halo up to 19 mm and (++) halo ≥ 20 mm.

A total of 82 isolates of yeasts were recovered from the fruits, with a predominance of Ascomycota yeasts. Our results are in agreement with the literature because fruits have a higher predominance of the phylum Ascomycota because it is a habitat rich in simple carbohydrates (Trindade et al. 2008; Negri et al. 2019; Ren et al. 2019) easily assimilated as a carbon source by yeasts (Carvalho et al. 2006). Also, it is already recognized that ascomycetous yeasts tend to predominate within plant tissues (Sperangio et al. 2015), while basidiomycetes are reported in the phylloplane, as they have adaptations to survive this environment, such as the ability to metabolize more carbohydrate sources complexes from the plant cell wall and produce pigments used for protection against ultraviolet rays (Li et al. 2020; Coelho et al. 2020).

Twenty-four yeast species were found, many of them present in more than one fruit species (Table 2). The genus *Candida* (Ascomycota) was the most frequent with 11 isolates. A higher frequency of the genus *Candida* has been observed in fruits of the Cerrado (Coelho et al. 2020). Studies to identify fungi in the Cerrado native fruits using a methodology similar to that used in this study have already been carried out, and many of the yeast species described here have not been reported in these fruits or other fruits (Sperandio et al. 2015; Coelho et al. 2020). Our results corroborate the findings of Sperandio (2015), who described the fruit and foliar yeast communities of *Byrsonima crassifolia* (Murici) and *E. dysenterica* (Cagaita), identifying the species *M. guilliermondii* and *R. mucilaginosa* in common among the those fruits studied. Meanwhile,

for *E. dysenterica* we have also identified the species *C. suratensis* and *Rhynchogastrema complexa*, showing new reports of yeast species colonizing these fruits.

In four plant species analyzed in this study, yeast growth was not detected in fruits. The absence of endophytic colonization yeasts in the fruits of *Passiflora cincinnata* can be explained by the presence of antifungal proteins in the fruits' pulp of the genus *Passiflora* (Jagessar et al. 2017; He et al. 2020). The antifungal activity of this genus seems to be restricted to pulp and seeds since Coelho et al. (2020) reported the presence of 4 yeast genera colonizing the fruit surface of *P. nitida* and also failed to obtain endophytic isolates. Silva (2017) reported the occurrence of yeasts colonizing the phylloplane of *P. incarnata*. The reasons for the absence of growth in the species *Solanum lycocarpum, Hymenaea stigonocarpo*, and *Mauritia flexuosa* do not seem to be linked to the substrate offered yeasts, since the fruits of *S. lycocarpum* present a large amount of carbohydrate and those of *M. flexuosa* large amount of lipid (Negri et al. 2016). Many studies have demonstrated the antimicrobial potential of *H. stigonocarpa* (Barbosa et al. 2015; Dimech et al. 2013; Martines et al. 2015), and *M. flexuosa* (Lima et al. 2006; Batista et al. 2012). These reports may explain the absence of yeasts in these fruits and present information that justifies investigating the phytomedical potential of these plants' products.

The absence of yeasts in fruits of these four plant species may suggest and/or reinforce the hypothesis that they are important sources of biological resources with potential for the production of molecules with antimicrobial action, mainly antifungal. The biotechnological potential of these Cerrado native plants aiming at the development of products for the control of pathogenic fungi deserves to be investigated in future studies. The fruit extract of *S. lycocarpum* (Lobeira) has already shown efficacy against harmful organisms such as the pathogen *Leishmania infantum* (Clementino et al. 2018) and the parasites *Haemonchus contortus* (Oliveira, 2013) and *cytostostomines* (Cyathostominae) (Souza, 2011). In contrast, the leaf extract of *M. flexuosa* demonstrated anti bactericidal activity against the pathogen *Pseudomonas aeruginosa* (Koolen et al. 2013).

2. Production of enzymes by yeasts from the Cerrado native fruits

Of the 82 yeast isolates recovered in this study, 48 (60% of the total) produced one or more of the enzymes studied (cellulase, protease, amylase, and pectinase). A wide variety of molecules and enzymes synthesized by different fungi species have been described (Li et al. 2016). In general, it can be said that fungi colonize environments with low nutrient availability, and as a result, they can present a wide variety of enzyme profiles, such as pectinases, amylases, cellulases, lipases, and protease (Mendes, 2010).

The production of hydrolytic enzymes has been reported as a common trait in plant-associated yeasts. Results similar to those obtained in this study were observed in yeast community (endophytic and epiphytes) isolated from bromeliad, where 40% to 60% presented amylolytic, cellulolytic, and proteolytic potential, stand out as producers of the exoenzymes species of *Candida, Debaryomyces, Metschnikowia, Pichia, Zygosaccharomyces, Cryptococcus, Fellomyces, Kockovaella, Rhodotorula, Sporobolomyces, Tremella, Aureobasidium, Itersonilia* and *Tilletiopsis* (Landell et al. 2006). Among the yeast species isolated in this study, yeasts from the genera *Candida*, *Debaryomyces*, *Hanseniaspora*, *Kloeckera*, *Lodderomyces*, *Pichia*, and *Rhodotorula*, are known for the diversity of synthesized enzymes and are used for the production of enzymes of industrial interest and in fermentative processes (Pretorius, 2000; Buzzini et al. 2002; Coutinho et al. 2013; Kot et al. 2016).

Among the yeast species evaluated, five produced the four enzymes. Few studies report the variety of enzymes produced by the yeasts *P. flavescens, Hanseniaspora meyeri, M. guilliermondii*, and *R. mucilaginosa;* however, these species of fungi are found in the soil, in rocks, in tree trunks, and because they colonize the most diverse environments, they are capable of synthesizing a wide variety of enzymes (Wirth, 2011; Andrade et al. 2012). Despite the need for more time for growth, the genus *Hanseniaspora* can produce proteases and glycolytic enzymes in larger quantities (Fleet, 2008; Comitini et al. 2011). Strains of species *M. guilliermondii* have been considered a great candidate for the biotechnological production of enzymes (Atzmüller et al. 2020).

The predominance of enzymatic groups varied among yeast species. Proteolytic enzymes were found in 25 isolates, thus representing the predominant enzymatic group. Several factors interfere in the enzymatic production of proteases, such as temperature, pH, the concentration of the substrate used, and the metabolism linked to the cell division process (Neves, 2006; Molnárová et al. 2014). Cellulolytic enzymes were found in 21 isolates, and it is known that cellulase production is among wild yeasts (Buzzini et al. 2002; Mendes et al. 2012). The production of pectinases occupied the third place in the number of isolated producers, with 18 isolates, and the degree of maturation of the fruits may have interfered in this result since pectin is a polysaccharide that is part of the cell wall that is depolymerizing with the ripening of the fruits (Trindade 2002; Paiva, 2009). Amylases were the least produced enzymes, which may be related to low starch use as a carbon source (Alberto et al. 2016). Another justification for the low production of amylases among the isolates in this study is that, according to Onofre (2015), these enzymes are produced mainly by saprophytic basidiomycetes filamentous fungi.

The low hydrolytic activities of fungi isolated from tropical regions can be observed due to the difficulties of visualization of these enzymes' production in a solid medium since fungi require a longer period to develop (Orlandelli et al. 2015; Dantas et al. 2017). Although some authors suggest a longer incubation time for evaluating hydrolytic activity in fungi, Marta et al. (2015) obtained positive results for fungal amylase production in a short incubation time (five days), similar to that used in this study. Another important point to be raised about the low enzyme production is that the diffusion of the enzyme and, consequently, the diameter of the hydrolysis halo are influenced by the molecular mass that the enzyme has, which can hinder or even prevent its diffusion in agar (Alberto et al. 2016). Thus, the enzymatic activity index can be considered low or non-existent, even if there is large enzymatic production by the microorganism.

To our knowledge, this study contributes to numerous unpublished findings. There were no reports yet of yeast associated with fruits such as, *A. crassiflora* (Araticum), *S. oleracea* (Coco-guariroba), *B. capitata* (Coquinho-azedo), *H. speciosa* (Mangaba), *T. esculenta* (Pitomba), *S. lycocarpum* (Lobeira), *H. stigonocarpa* (Jatobá-do-Cerrado), and *P. cincinnata* (Maracujá-do-Cerrado), the results of this study is unprecedented both in the analysis of their occurrence colonizing plant organs of the Cerrado biome, the identification of yeasts and their enzymatic potential.

Supplementary Material

The following online material is available for this article:

Figure S1 - *Fruits harvested for analysis: Passiflora cincinnata* (A), *Eugenia dysenterica* (B), *Mauritia flexuosa* (C), *Hymenaea stigonocarpa* (D), *Solanum lycocarpum* (E), *Caryocar brasiliense* (F), *Annona crassiflora* (G), *Psidium cattleyanum* (H), *Butia capitata* (I), *Syagrus oleracea* (J), *Hancornia speciosa* (K), *Talisia esculenta* (L) e *Anacardium humile* (M).

Acknowledgments

We acknowledge the COPABASE employees who helped us collect the fruit samples. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Author Contributions

Helson Mario Martins do Vale: substantial contribution to the conception and design of the study; contribution to data analysis and interpretation; contribution to the preparation of the manuscript; contribution to critical review, adding intellectual content.

Jefferson Brendon Almeida dos Reis: contribution to data analysis and interpretation; contribution to the preparation of the manuscript; contribution to critical review, adding intellectual content.

Marcos de Oliveira: substantial contribution to the conception and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical review, adding intellectual content.

Geisianny Augusta Monteiro Moreira: contribution to data analysis and interpretation; contribution to the preparation of the manuscript; contribution to critical review, adding intellectual content.

Catharine Abreu Bomfim: contribution to data analysis and interpretation; contribution to the preparation of the manuscript; contribution to critical review, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Received: 18/01/2021
Revised: 09/07/2021
Accepted: 21/07/2021
Published online: 27/08/2021