YEAST COMMUNITIES STRUCTURE IN FRUITS OF DIFFERENT NATIVE PLANT SPECIES OF BRAZILIAN CERRADO

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Abstract: In this study, we evaluated the occurrence and diversity of endophytic and total (endophytic + epiphytic) yeasts in fruits of seven Cerrado native plant species in two Conservation Units from the Distrito Federal, Brasília, Brazil. Healthy and mature fruits were collected for the isolation of yeasts using MYGP medium. After five days, the colonies were counted and the morphologically distinct yeasts were isolated axenic culture. The molecular identification of the isolates was performed using ITS region (rDNA). A total of 69 specimens of yeasts were isolated. The *Guapira graciliflora* (endophytic) and *Ouratea hexasperma* (total) hosts had higher yeast densities per gram of fruit and together with the *Brosimum gaudichaudii* showed higher species diversity. Yeasts belonging to the Ascomycota Phylum were predominant. The most frequent genera of yeasts were *Candida*, *Aureobasidium* and *Hannaella*. Others genera such as *Hanseniaspora*, *Meyerozyma*, *Filobasidium*, *Cryptococcus*, *Symmetrospora*, *Sirobasidium*, *Meira*, *Rhodosporidiobolus*, *Papiliotrema* and *Erythrobasidium* were identified. This is the first report of the occurrence of endophytic yeasts in fruits of *G. graciliflora* and represents the host with greater density and diversity of species of endophytic yeasts. In addition, expands the knowledge about the occurrence of yeasts associated with the fruits of seven native plant species of the Cerrado.

Keywords: host specificity, endophytes diversity, fruit-associated yeasts.

ESTRUTURA DE COMUNIDADES DE LEVEDURAS EM FRUTOS DE DIFERENTES ESPÉCIES NATIVAS DE PLANTAS DO CERRADO BRASILEIRO

Resumo: Neste estudo, avaliamos a ocorrência e diversidade de leveduras endofíticas e totais (endofíticas + epifíticas) em frutos de sete espécies de plantas nativas do Cerrado em duas Unidades de Conservação do Distrito Federal. Frutos aparentemente sadios foram coletados para o isolamento de leveduras utilizando o meio MYGP. Após cinco dias foi realizada a contagem de colônias e as leveduras morfologicamente distintas foram isoladas em cultura axênica. A identificação molecular dos isolados foi realizada pelo sequenciamento da região ITS (rDNA). Um total de 69 leveduras foram isoladas e a densidade de levedura por grama de fruto foi semelhante entre os hospedeiros. As espécies *Guapira graciliflora* (endofítico), *Ouratea hexasperma* (total) e *Brosimum gaudichaudii* (total) apresentaram maior diversidade de espécies. Leveduras pertencentes ao Filo Ascomycota foram predominantes. Os gêneros de leveduras mais frequentes foram *Candida*, *Aureobasidium* e *Hannaella*. Outros gêneros como *Hanseniaspora*, *Meyerozyma*, *Filobasidium*, *Cryptococcus*, *Symmetrospora*, *Sirobasidium*, *Meira*, *Rhodosporidiobolus*, *Papiliotrema* e *Erythrobasidium* foram identificados. Este estudo é o primeiro relato da ocorrência de leveduras endofíticas em frutos de *G. graciliflora*, que aqui mostrou ser a hospedeira com maior diversidade de espécies de leveduras endofíticas. Adicionalmente, o presente trabalho expande o conhecimento sobre a ocorrência de leveduras associadas aos frutos de sete espécies de plantas nativas do Cerrado brasileiro.

Palavras-chave: especificidade de hospedeiro, diversidade de endofíticos, leveduras em frutos.

INTRODUCTION

The Brazilian Cerrado is considered one of the largest, most diverse and most threatened Savannah in the world, with a high degree of endemism of plants and animals (Cardoso Da Silva & Bates, 2002). The Cerrado region has about twelve thousand species of vascular plants, and 44% of those are endemic (Cardoso da Silva & Bates, 2002). These plants are suitable niches for many microorganisms and yeasts that are commonly found in roots and leaves (Ferreira et al., 2017; Sperandio et al., 2015), underground reserve organs (Isaeva et al., 2010), stem and barks (Bhadra et al., 2008; Ferreira et al., 2017), fruits (Moreira et al., 2015; Sperandio et al., 2015), nectar (Barbosa et al., 2012), and flowers (Rosa et al., 2009).

The occurrence of yeasts has been observed in fruits of native plants of Cerrado, such as *Eugenia lutescens* Cambess. (perinha-do-Cerrado), *Campomanesia xanthocarpa* O.Berg. (gabiroba), *Brosimum guadichaudii* Trécul. (mamacadela) (Moreira et al., 2015), *Mauritia flexuosa* L.f. (buriti), *Bactris inundata* Mart. (tucum), *Attalea maripa* (Aubl.) Mart. (inajá) and *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. (macaúba) (Oliveira, 2015a), *Annona crassiflora* Mart. (araticum), *Hancornia speciosa* Gomes (mangaba), *Caryocar brasiliense* Cambess. (pequi), *Anacardium humile* A.St.-Hil. (cajuzinho-do-cerrado), and others (Oliveira, 2015b); fruits and leafs of *Byrsonima crassifolia* (L.) Kunth. (muricizão) and *Eugenia dysenterica* (Mart.)DC. (cagaita) (Sperandio et al., 2015). In addition, yeasts were isolated from bracts of *Heliconia psittacorum* L.f. (Helicônia-papaguaio) and flowers of *Centropogon cornutu*s (L.) Druce (a-ninga) (Barbosa et al., 2012; Rosa et al., 2009). Many of these yeasts have an important biotechnological role, such as post-harvest biological control (Sperandio et al., 2015), production of enzymes such as cellulase, lipase, proteases (Oliveira, 2015a), and production of secondary compounds that promote plant growth (Moller et al., 2016).

Yeasts are commonly found on surfaces (epiphytic) and internal tissues of plants (endophytic), depending on substrates with high concentrations of readily available compounds such as fruits and other reserve organs (Isaeva et al., 2010). Most of the epiphytic yeasts have morphological and physiological characteristics that allow surviving adverse conditions, with the occurrence in internal tissues a possible adaptation to survive the external factors.

Endophytic yeasts interact physiologically with their host, where it favors the maintenance of plant fitness (Moller et al., 2016). Many endophytic yeasts have already been reported as important agents in biotechnological processes such as production of phytohormones (Moller et al., 2016), antimicrobial substances (Vieira et al., 2012) and biocontrol of pathogens (Sperandio et al., 2015).

Even with the efforts and work of some authors cited above, data on the occurrence and distribution of the total (epiphytic + endophytic) and endophytic yeasts in fruits of native Cerrado plants are still very limited. For this reason, the occurrence and diversity of total and endophytic yeasts were evaluated in fruits of seven native plants collected in the Cerrado of the Federal District, located in the Brazilian Midwest region. The yeast diversity was evaluated by molecular identification. The aims of the present study were: a) to isolate, identify and evaluate the density of the yeast population (total and endophytes); b) to evaluate the diversity and distribution of yeast species in fruits of seven host species of plants in two different Conservation Units of Federal District, observing if the yeast communities share the same host species.

MATERIAL AND METHODS

FRUITS SAMPLING

Healthy and mature fruits of seven native Cerrado plants species were collected in two Conservation Units of the Federal District of Brazil (Tab. 1). Fruits of three different plants of each species host were collected in December of 2015 during the rainy period using gloves and sterilized plastic bags, and taken in refrigerated thermal boxes to the laboratory, where they were processed within two days.

Tab. 1. Species of Cerrado native plants, popular name, isolation method for yeast detection and collection site.

Species	Popular name	Family	Type of isolation	Sampling site
Ouratea hexasperma (A. St. - Hil.) Baill.	Vassoura-de-bruxa	Ochnaceae	Epiphytic	Reserva Ecológica do IBGE
Brosimum gaudichaudii Trécul.	Mamacadela	Moraceae	Epiphytic	Reserva Ecológica do IBGE
Passiflora nitida Kunth	Maracujá-do-Cerrado	Passifloraceae	Epiphytic	Reserva Ecológica do IBGE
Myrcia tomentosa (Aubl.) DC.	Araçacinho	Myrtaceae	Epiphytic	Jardim Botânico de Brasília
Byrsonima coccolobifolia Kunth	Murici	Malpighiaceae	Epiphytic	Jardim Botânico de Brasília
Caryocar brasiliense Cambess.	Pequi	Caryocaraceae	Endophytic	Jardim Botânico de Brasília
Guapira graciliflora (Mart. ex Schmidt)	Maria-mole	Nyctaginaceae	Endophytic	Jardim Botânico de Brasília

ISOLATION OF TOTAL YEASTS (EPIPHYTIC + ENDOPHYTIC)

For the isolation of total yeasts, fruits of three different plants of the hosts *Ouratea hexasperma*, *Brosimum gaudichaudii*, *Passiflora nitida* (fruit peel), *Myrcia tomentosa* and *Byrsonima coccolobifolia* were macerated, homogenized and placed in Erlenmeyer flasks containing 0.8% peptone aqueous solution (w/v) in a 9:1 ratio of peptone solution volume to sample volume and stirred for 20 minutes at 150 rpm. Serial dilution was performed up to 10-3, and 0.1 mL of this solution was inoculated into MYGP culture medium (0.3% malt extract, 0.3% yeast extract, 5% peptone and 1% glucose) (Masoud et al., 2004) and incubated at room temperature. After incubation, the Colony Forming Unit (CFU) number was determined, and then the morphologically distinct yeasts colonies were isolated to obtain pure culture. All yeast isolates were stored in glycerol (25%) in a freezer at -80 °C.

ENDOPHYTIC YEASTS ISOLATION

The fruits of *Guapira graciliflora*, *Caryocar brasiliense* and *Passiflora nitida* had their surfaces disinfected as described by Araújo et al. (2001), which consists of washing with 70% alcohol for one minute, followed by washing with sodium hypochlorite (4%) for four minutes and successive washes with autoclaved distilled water. For *C. brasiliense* and *P. nitida*, the sampling was done by internal scraping of the pulp. Isolation of endophytic yeasts followed the same methodology of total yeast isolation described above.

ISOLATES IDENTIFICATION

DNA extraction was performed from the cell pellet using the chloroform and isoamyl alcohol method, using glass beads for mechanical lysis of the cells and extraction buffer (2% Triton, 1% SDS, 100 mM NaCl, 10 mM Tris pH 8 and 1 mM EDTA pH 8) (Kurtzmann & Fell, 1998).

The identification of the isolates was performed by the amplification of the ITS (Internal Transcribed Spacer) region of the rRNA, using primers ITS 4 (5 'TCCTCCGCTTATTGATATGC 3') and ITS 5 (5 'GGAAGTAAA AGTCGTAACAAGG 3'). The amplifications were performed to a final volume of 25 µl containing 20-30 ng of DNA, 200 µM dNTPs (dATP, dCTP, dGTP, dTTP), 10X Buffer, 1.25U Taq DNA polymerase, 0.2 µl of each initiator and deionized water (Milli-Q) sterilized. The thermocycling program consisted of: initial denaturation at 94 °C for five minutes, followed by 33 cycles of 30 seconds at 94 °C, one minute at 58 °C and two minutes at 72 °C, with a final extension of 72 °C for seven minutes. The amplified fragments were sent for sequencing at ACTGene Molecular

Analyses (Rio Grande do Sul, Brazil) using the ABI 3130xl Sequencer Applied Bio Systems according to Sanger's methodology (Sanger et al. 1997). For species identification, the obtained nucleotide sequences were compared with sequences deposited in the NCBI GenBank (National Center for Biotechnology Information), using the BLASTn algorithm (Altschul et al., 1990).

Diversity and Distribution of Isolates

For analysis of distribution and sharing of isolates among hosts, a matrix of presence (1) and absence (0) of isolates was created for all seven studied hosts. An ellipse represented a host and showed the number of isolates per host and the number of isolates shared between hosts (ellipses intersection region).

The Past program (version 3.13) was used to calculate the diversity index Dominance (D), Shannon (H), Simpson (1-D) (Hammer et al., 2001). The diversity indexes were calculated based on an abundance matrix (isolated versus host), using the abundance data of the yeast species found in each analyzed host plants.

Results

Population density, identification and composition of total yeasts

A total of 37 yeasts were isolated from fruits of five species of host plants, ten of which were isolated from *O. hexasperma*, eight from *B. gaudichaudii*, five from *M. tomentosa*, eight from *P. nitida* and six from *B. coccolobifolia*. All hosts presented density of Colony Forming Units (CFU) per gram of fruit, except for *B. coccolobifolia* that did not have enough colonies for CFU count (Tab. 2).

Tab. 2. Density of yeasts per gram of fruit (CFU g.fruit^{-1}) in seven hosts of Cerrado native plants.

Total (epiphytic + endophytic)			**Endophytic**		
Plant host	Number of isolates	CFU g.fruit^{-1}	Plant host	Number of isolates	CFU g fruit^{-1}
Ouratea hexasperma	10	5,34x10^{-1}	*Guapira graciliflora*	11	1,46x10^{-1}
Brosimum gaudichaudii	8	4,33x10^{-1}	*Caryocar brasiliense*	21	7,1x10^{-2}
Myrcia tomentosa	5	1,89x10^{-1}	*Passiflora nitida*	0	WG
Passiflora nitida	8	4,31x10^{-1}			
Byrsonima coccolobifolia	6	W-CFU			
Total of isolates	37		32		

WG = Without growth; W-CFU = without colony forming units

Of the 37 total yeasts isolated, 20 (54.10%) belong to the Phylum Ascomycota and 17 (45.90%) belong to the Phylum Basidiomycota (Figure 1A). In relation to the Phylum Ascomycota, the genera were: *Aureobasidium*, *Candida* and *Meyerozyma*. The most prevalent yeast species of this Phylum were *Aureobasidium leucospermi*, *Meyerozyma guilliermondii* and *Candida* sp. corresponding to 65% of isolates (Figure 1B).

Among Basidiomycota, the genera founded were: *Filobasidium*, *Hannaella*, *Rhodosporidiobolus*, *Papiliotrema*, *Erythrobasidium*, *Symmetrospora* and *Meira*. The most abundant species were *Hannaella pagnoccae*, *Rhodosporidiobolus ruineniae*, *Filobasidium magnum* and *Filobasidium* sp., representing 41% of the isolates (Figure 1B). Despite the lower abundance of isolated yeasts, this Phylum showed higher species richness.

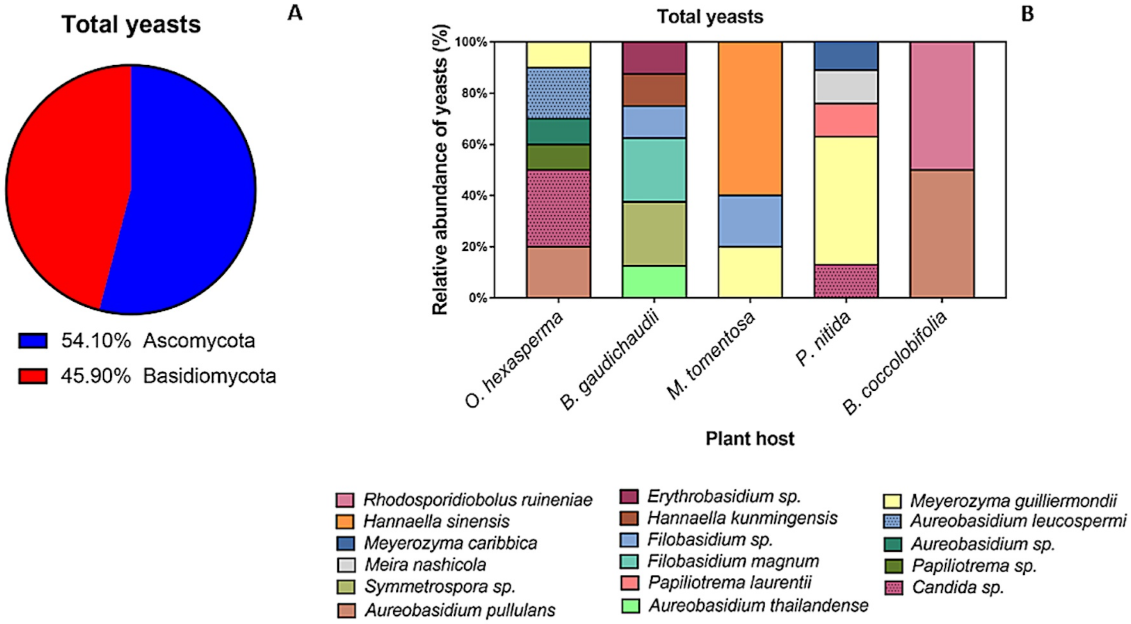

Fig. 1. Composition of the total yeast community in fruits of five host plant species native to the Brazilian Cerrado. A. Percentage of yeasts belonging to the Ascomycota and Basidiomycota phyla. B. Relative abundance of yeast species.

POPULATION DENSITY, IDENTIFICATION AND COMPOSITION OF ENDOPHYTIC YEASTS

Thirty-two endophytic yeasts were isolated from *G. graciliflora* and *C. brasiliense* hosts, 11 of which were isolated from *G. graciliflora* and 21 from *C. brasiliense*. These hosts showed similar cell density per gram of fruit (Tab. 2). There was no growth of endophytic yeasts in *P. nitida* pulp (Maracujá-do-Cerrado), although growth in the total yeast assay was observed using the bark of this fruit (Tab. 2).

Of the 32 endophytic yeasts isolated, 25 (78.80%) belong to the Ascomycota Phylum and seven (21.20%) belong to the Basidiomycota Phylum (Figure 2A). For Ascomycota, the most common genera were *Candida*, *Aureobasidium* and *Hanseniaspora*. The most predominant endophytic yeast species were *Aureoba-*

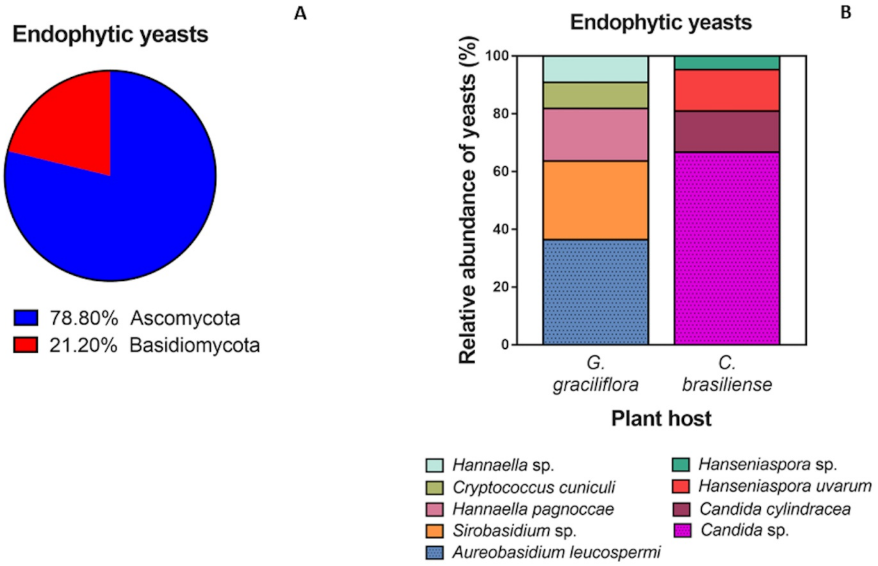

Fig. 2. Composition of the community of endophytic yeasts in *Guapira graciliflora* and *Caryocar brasiliense* fruits. A. Percentage of yeasts of Ascomycota and Basidiomycota phyla. B. Relative abundance of isolated yeast species.

sidium leucospermi, *Hanseniaspora uvarum* and *Candida* sp. corresponding to 84% of the yeasts found in this Phylum (Figure 2B).

The most abundant genera were *Sirobasidium* and *Hannaella* belonging to the Basidiomycota. The most common species were *Sirobasidium* sp. and *Hannaella pagnoccae*, representing 71% of the species of this Phylum (Figure 2B).

DIVERSITY AND DISTRIBUTION OF SPECIES AMONG HOSTS

The diversity of the total and endophytic yeasts was analyzed using the Shannon and Simpson indices. The host *B. gaudichaudii*, *O. hexasperma* (total) and *G. graciliflora* (endophytic) had the highest species richness (Shannon: 1.733, 1.696, and 1.468 respectively) (Tab. 3). In addition, it presented the highest cell densities per gram of fruit (Tab. 2).

The largest number of yeasts shared was isolated in the *O. hexasperma* (Fig. 3), with the genus *Papiliotrema* occurring only in this host. The most abundant species in host *G. graciliflora* was *Aureobasidium leucospermi*, which also occurred in host *O. hexasperma* (Tab. 4). On the other hand, the yeast *Hannaella pagnoccae*, *Sirobasidium* sp. and *C. cuniculi* occurred exclusively in this host. For host *C. brasiliense*, the most common genus isolated was *Candida*,

Tab. 3. Diversity of yeasts isolated from fruits of seven host plants native to the Cerrado.

	Plants hosts						
	Totals (epiphytics + endophytics)					**endophytics**	
	Ouratea hexasperma	*Brosimum gaudichaudii*	*Passiflora nitida*	*Myrcia tomentosa*	*Byrsonima coccolobifolia*	*Caryocar brasiliense*	*Guapira graciliflora*
Richness (S)	6	6	5	3	2	4	5
Numbers of isolates	10	8	8	5	6	21	11
Dominance(D)	0,2	0,1875	0,3125	0,44	0,5	0,4875	0,2562
Simpson (1-D)	0,8	0,8125	0,6875	0,56	0,5	0,5125	0,7438
Shannon (H)	1,696	1,733	1,386	0,9503	0,6931	0,9713	1,468
Equitabilidade (J)	0,9464	0,9671	0,8614	0,865	1	0,7006	0,9122

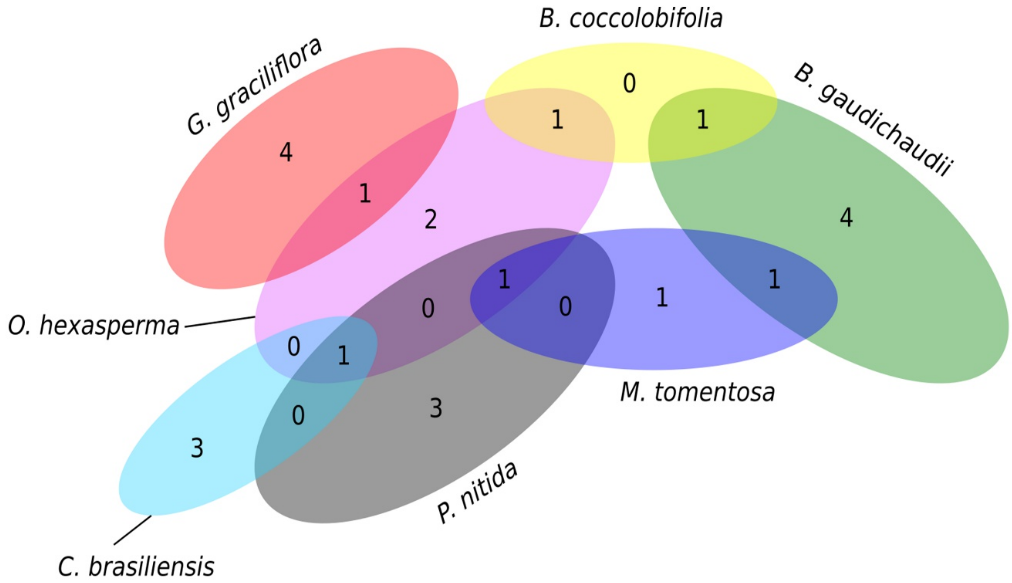

Fig. 3. Distribution of yeast species in fruits of *Ouratea hexasperma, Brosimum gaudichaudii*, *Passiflora nitida*, *Myrcia tomentosa*, *Byrsonima coccolobifolia* (totals) and *Caryocar brasiliense* and *Guapira graciliflora* (endophytics). Each ellipse represents the different species of host plants and the intersections of the ellipses show the yeast species that occurred in more than one host.

being the genus most prevalent in other hosts (Tab. 4). The species *Candida cylindracea* and *H. uvarum* occurred exclusively in this host.

DISCUSSION

All plants studied were found to be yeast-friendly habitats, with the exception of *P. nitida* pulp. Yeast density per gram of fruit was similar in total and endophytic isolation hosts (Tab. 2). Yeasts belonging to the Ascomycota Phylum were more abundant in both total and endophytic isolation, with the genera *Aureobasidium* and *Candida* being the most abundant in total and endophytic isolation, respectively. Additionally, *B. gaudichaudii*, *O. hexasperma* and *G. graciliflora* species had higher species richness, according to the Simpsons and Shannon indices, and

Tab. 4. Identification of yeasts accession number, coverage and sequence identity on GenBank.

Host	Isolation method	Isolate ID	Species or genus	Best blast match [GenBank]	ID (%)
Ouratea hexasperma	Epiphytic	IBGE3.1	*Aureobasidium pullulans*	LC277150	98
	Epiphytic	IBGE3.3	*Candida sp.*	KY104256; KY848362	81
	Epiphytic	IBGE3.4	*Papiliotrema sp.*	NR121465; LC191348	89
	Epiphytic	IBGE3.5	*Aerobasidium sp.*	EF197817	93
	Epiphytic	IBGE3.6	*Aureobasidium leucospermi*	JN712488	98
	Epiphytic	IBGE3.8	*Aureobasidium pullulans*	LC277150	98
	Epiphytic	IBGE3.9	*Candida sp.*	EF197951; KP132412	80
	Epiphytic	IBGE3.10	*Aureobasidium leucospermi*	JN712488	98
	Epiphytic	IBGE3.11	*Candida sp.*	KY104256; KY848364	81
	Epiphytic	IBGE3.12	*Meyerozyma guilliermondii*	JF508433	99
Brosimum gaudichaudii	Epiphytic	IBGE7.2	*Aureobasidium thailandense*	NR147337	94
	Epiphytic	IBGE7.4	*Symmetrospora sp.*	KY105573	94
	Epiphytic	IBGE7.5	*Filobasidium magnum*	KY103435	99
	Epiphytic	IBGE7.7	*Filobasidium magnum*	KY103435	99
	Epiphytic	IBGE7.8	*Filobasidium sp.*	KY103450; KT323965	85
	Epiphytic	IBGE7.9	*Hannaella kunmingensis*	JN181161	96
	Epiphytic	IBGE7.10	*Erythrobasidium sp.*	KY103393	97
	Epiphytic	IBGE7.11	*Symmetrospora sp.*	KY105520; KY105570	98
Passiflora nitida	Epiphytic	IBGE8.1	*Meyerozyma guilliermondii*	JF508433	99
	Epiphytic	IBGE8.2	*Papiliotrema laurentii*	LT594997	90
	Epiphytic	IBGE8.3	*Meyerozyma guilliermondii*	JF508433	99
	Epiphytic	IBGE8.4	*Meira nashicola*	JX682675	96
	Epiphytic	IBGE8.5	*Meyerozyma caribbica*	KP132412	99
	Epiphytic	IBGE8.6	*Meyerozyma guilliermondii*	JF508433	99
	Epiphytic	IBGE8.7	*Meyerozyma guilliermondii*	JF508433	99
	Epiphytic	IBGE8.8	*Candida sp.*	KY102187; EF194842	93
Myrcia tomentosa	Epiphytic	JB5.1	*Hannaella sinensis*	KX781278	99
	Epiphytic	JB5.2	*Hannaella sinensis*	KX781278	99
	Epiphytic	JB5.3	*Hannaella sinensis*	KX781278	99
	Epiphytic	JB5.4	*Meyerozyma guilliermondii*	JF508433	99
	Epiphytic	JB5.5	*Filabasidium sp.*	KY103450	81

continuation...

Byrsonima coccolobifolia	Epiphytic	JB9.3	*Aureobasidium pullulans*	EU715320	98
	Epiphytic	JB9.5	*Aureobasidium pullulans*	LC277150	98
	Epiphytic	JB9.6	*Rhodosporidiobolus ruineniae*	KY104710	98
	Epiphytic	JB9.7	*Rhodosporidiobolus ruineniae*	KY104710	98
	Epiphytic	JB9.8	*Rhodosporidiobolus ruineniae*	KY104710	98
	Epiphytic	JB9.9	*Aureobasidium pullulans*	LC277150	98
Caryocar brasiliense	Endophytic	PQ1	*Candida sp.*	KY101945	90
	Endophytic	PQ2	*Candida cylindracea*	KY102093	88
	Endophytic	PQ3	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ6	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ7	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ8	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ9	*Hanseniaspora uvarum*	KY103567	94
	Endophytic	PQ10	*Candida sp.*	KY101945; KY102093	90
	Endophytic	PQ11	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ13	*Hanseniaspora sp.*	KY103529; KF747754	96
	Endophytic	PQ14	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ15	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ16	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ17	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ18	*Candida cylindracea*	KY102039	99
	Endophytic	PQ19	*Hanseniaspora uvarum*	KY103567	94
	Endophytic	PQ20	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ21	*Candida cylindracea*	KY102039	99
	Endophytic	PQ22	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ23	*Candida sp.*	KY102355; KC349937	99
	Endophytic	PQ24	*Hanseniaspora uvarum*	KY103567	94
Guapira graciliflora	Endophytic	JB2.1	*Aureobasidium leucospermi*	JN712488	98
	Endophytic	JB2.3	*Hannaella pagnoccae*	KY103507	96
	Endophytic	JB2.4	*Hannaella sp.*	KY103515; KY103498	97
	Endophytic	JB2.6	*Sirobasidium sp.*	AF444330; AF042430	92
	Endophytic	JB2.7	*Sirobasidium sp.*	AF444330; AF042430	92
	Endophytic	JB2.8	*Sirobasidium sp.*	AF444330; AF042430	92
	Endophytic	JB2.9	*Aureobasidium leucospermi*	JN712488	98
	Endophytic	JB2.10	*Aureobasidium leucospermi*	JN712488	98
	Endophytic	JB2.11	*Hannaella pagnoccae*	KY103507	96
	Endophytic	JB2.13	*Cryptococcus cuniculi*	KM079158	86
	Endophytic	JB2.14	*Aureobasidium leucospermi*	JN712488	98

O. hexasperma presented higher occurrence of isolated yeasts in other hosts, while other species occurred in specific hosts.

The density and occurrence of yeasts can be influenced by biotic and abiotic factors such as seasonality, ontogenic stage, morphology, physiology of the fruits, ecology (xerophyte, mesophyte) of the host plant, type of substrate, maturation degree of the fruit and by the interactions between the species, such as in the production of 'killer's toxins' and competition for substrate(Glushakova & Chernov 2007, 2010; Morais et al. 1995). For example, in fruits of *Malus domestica* (Borkh.) Borkh, and *Pyrus communis* L., the density of endophytic yeasts was higher during the period when the fruit was fully ripe (Glushakova & Kachalkin, 2017a). However, the diversity of yeasts does not always reflect a higher density of cells per gram of fruit (Isaeva et al., 2010).

In our study, fruits were collected ripe and the diversity index (Tab. 3) may have been influenced mainly by the physiology of the host, by the type of substrate and interactions among the species of yeasts, considering that fruits were collected in the neighboring areas, submitted to the same environmental conditions. Glushakova & Kachalkin (2017b) found a similar result, showing the diversity yeasts was higher in mature fruits, mainly due to the increase in the concentration of free sugars.

Specific conditions in certain tissues of some hosts may be a disadvantage for colonization by yeasts. In the host *P. nitida*, for example, no yeast growth in the isolated fruit pulps. Antifungal compounds produced by *Passiflora* have been reported to control the growth of yeasts of the genus *Candida albicans* (Galvão et al., 2010), corroborating the existence of antifungal activity in the *Passiflora* spp. pulp that can prevent the colonization of yeasts in their fruits. However, it is possible that antifungal activity is restricted to the pulp, since we detected growth of yeasts on the bark of *P. nitida*. Studies focusing on the *Passiflora* spp. microbiome deserve a lot of attention, since the information so far points to a great potential of these microorganisms in biotechnological applications.

The high occurrence of Ascomycetous yeasts in fruits was also observed in other studies with the Cerrado species, such as *Eugenia lutescens*, *Campomanesia xanthocarpa*, and *Brosimum guadichaudii*, in which the occurrence of Ascomycetous yeasts was higher than Basidiomycetous yeasts with the predominance of the genus *Aureobasidium* (Moreira et al., 2015; Trindade et al., 2002).

Although a single yeast species may occur in internal and external tissues, there are properties that differentiate the yeast communities in these two environments (Isaeva et al., 2010). Ascomycetous yeasts, for example, occur in fruit pulps (Glushakova & Chernov 2010; Isaeva et al., 2010). Basidiomycetous yeasts are more common on fruit surfaces, where the exposition to environmental conditions are higher than internal tissues. These yeasts synthetize complex compounds such as pigments (carotenoids and melanin) and polysaccharide capsules, which allows them to remain in this environment (Glushakova & Kachalkin, 2017a). However, there are cases in which Ascomycetous yeasts are more adapted to low humidity and desiccation than Basidiomycetous yeasts (Fernández et al., 2012).

Many of the yeasts species found in this work such as *H. uvarum*, *M. guilliermondii*, *M. caribbica*, *Filobasidium magnum*, *Rhodotorula* sp., *Cryptococcus* sp., *Candida* sp., among others, have already been reported as endophytic yeasts (Glushakova & Kachalkin, 2017a; Isaeva et al., 2010; Camatti-Sartori et al., 2005). Endophytic yeasts isolated from trees show the importance of these microorganisms in the production of phytohormones, antagonistic activity against phytopathogenic microorganisms, and the production of 'killer toxins', emphasizing important ecological functions for the fitness of the plant (Moller et al., 2016). From the 35 species cited in Moller et al. (2016), *M. caribbica*, *M. guilliermondii* and *Papiliotrema laurentii* (total yeasts) were identified in fruits used in this study.

The genera of yeasts found in this work are commonly found in fruits from several environments, mainly *Candida*, *Hanseniaspora*, *Aureobasidium*, and *Rhodotorula* (Isaeva et al., 2010; Liu et al., 2017; Morais et al., 1995; Trindade et al., 2002; Vadkertiová et al., 2012). On the other hand, the occurrence of yeasts in fruits of native plants of the Cerrado predominated for the genera *Candida* and *Aureobasidium* isolated on the hosts *Annona crassiflora*, *C. brasiliense*, *E. dysenterica*, *Psidium cattleyanum*, *Butia capitata*, *E. lutescens*, *C. xanthocarpa*, *B. guadichaudii*, *Byrsonima crassifolia* and *Syagurs oleracea* (Moreira et al., 2015; Oliveira, 2015b; Sperandio et al., 2015; Vale et al., 2015). The distribution of these yeasts in several hosts demonstrates the ability to colonize different types of fruits, which explains the fact that these genera are the most common in fruits. Many yeast species isolated from fruits, such as *A. pullulans* (Ippolito et al., 2000; Lima et al., 1997; Sperandio et al., 2015), *H. uvarum* (Liu et al., 2017) and *Candida* sp. (Lima et al., 1997; Liu et al., 2017) are used in the biological control of pathogenic post-harvest fungi in fruits.

For the host *B. gaudichaudii*, other genera such as *Symmetrospora*, *Filobasidium*, *Hannaella*, and *Erythrobasidium* were found in this work, different from those encountered by Moreira et al. (2015). In *C. brasiliense* only the species *H. uvarum* was isolated in a previous study (Oliveir, 2015b). Other genera of yeasts such as *Meyerozyma*, *Debaryomyces*, *Lodderomyces* and *Rhynchogastrema* were reported for the host *C. brasiliense*, all with enzymatic potential in the production of cellulase, protease, amylase and pectinase (Oliveira, 2015b).

Some yeasts occupy different types of environments and may be specific to a particular host. This distribution (broad or more specific) is related to the type of substrate available as energy source and also to the vectors responsible for the dispersion of yeasts and other factors (i.e. Abranches et al., 2000; Glushakova & Kachalkin, 2017b; Grondin et al., 2015; Morais et al., 1995). The *M. guilliermondii* species was also isolated endophytically (Moller et al., 2016), but commonly occurs in an epiphytic manner in fruits (Vale et al., 2015).

This is the first comprehensive set of data on asymptomatic yeasts associated with fruits of the species *O. hexasperma*, *B. coccolobifolia*, and *M. tomentosa*, with possibly two new species of yeasts belonging to the genus *Filobasidium* sp. (JB5.5 e IBGE 7.8). This also represents the first report of isolation of endophytic yeasts from the host *G. graciliflora*. The JB5.5 and IBGE7.8 isolates had a good quality sequence, but the percentage of identity (97% and 98%, respectively) and coverage (95% and 97%, respectively) were very low (Kurtzman & Fell, 2006), and these characteristics classify these isolates as probable new species.

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