Polish Journal of Microbiology
2020, Vol. 69, No 3, 251–261
https://doi.org/10.33073/pjm-2020-027

ORIGINAL PAPER

Yeasts Associated with Various Amazonian Native Fruits

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Submitted 14 March 2020, revised 27 May 2020, accepted 28 May 2020

Abstract

Yeasts, commonly present on the surface of fruits, are of industrial interest for the production of enzymes, flavorings, and bioactive compounds, and have many other scientific uses. The Amazonian rainforest may be a good source of new species or strains of yeasts, but their presence on Amazonian fruits is unknown. The aim of this study was to identify and characterize yeasts isolated from Amazonian native fruits using molecular and phenotypic methods. In total, 81 yeast isolates were obtained from 10 fruits species. Rep-PCR showed 29 strain profiles. Using a combination of restriction-fragment length polymorphism (RFLP) of the 5.8S-ITS region and D1/D2 sequencing of the 26S rRNA gene, 16 species were identified belonging to genera *Candida*, *Debaryomyces*, *Hanseniaspora*, *Kodamaea*, *Martiniozyma*, and *Meyerozyma*. The most dominant species were *Candida tropicalis*, *Debaryomyces hansenii*, *Hanseniaspora opuntiae*, and *Hanseniaspora thailandica*. *H. opuntiae* and *H. thailandica* showed the highest number of the strain profiles. Phenotypic profiles were variable between species, and even among strains. Screening for hydrolases showed lipolytic activity in only one isolate, while proteolytic, cellulolytic and amylolytic capabilities were not detected. Yeast presence among fruits varied, with cidra (*Citrus medica*) and ungurahui (*Oenocarpus bataua*) having the highest number of species associated. This investigation broadens the understanding and possible biotechnological uses of yeast strains obtained from Amazonian native fruits.

Key words: yeast diversity, fruit, Amazonia, PCR-RFLP, 5.8S-ITS

Introduction

Fruits constitute excellent habitats for yeasts, mainly due to their low pH, availability of nutrients, and active fruit-associated vectors. These traits are variable across the type and maturity of the fruit. Changes in the community in response to varying availability of nutrients, production of mycotoxins, and the arrival of new yeast species are evident (Tournas and Katsoudas 2005; Starmer and Lachance 2011).

The majority of research has focused on the diversity of yeasts on grapes and wine-related samples due to their application in the winemaking process (Guillamón et al. 1998; Filho et al. 2017), although some expansions have been made beyond this zone of interest. Koricha et al. (2019) identified yeasts from lemon, mango, and guava fruits, with *Candida albicans*, *Debaryomyces hansenii*, *Kodamaea ohmeri*, *Rhodotorula mucilaginosa*, among others, found to be present. Vadkertiová et al. (2012) studied the diversity of yeasts and yeast-like microbes associated with fruits and blossoms of apple, plum, and pear orchards in Slovakia. Trindade et al. (2002) investigated yeasts inhabiting the fresh and frozen pulps of Brazilian tropical fruits. Notably, some fruits have been described as sources of new yeasts (Bhadra et al. 2008; Sipiczki 2011).

Yeast diversity on the wide variety of Amazonian native fruits (ANF) has not been widely investigated, with reports focusing mainly on other tropical fruits like passion fruit (*Passiflora edulis*), mangaba (*Hancornia speciosa*), umbu (*Spondias tuberosa*), and acerola (*Malpighia glabra*) (Trindade et al. 2002; Da Silva et al. 2005; Grondin et al. 2015). The Amazonian rainforest's environmental characteristics suggest the possibility of finding diverse yeast communities, including new species or strains with new characteristics of biotechnological interest (Morais et al. 1995; Da Silva et al. 2005). Yeasts represent a promising source for obtaining microbial enzymes (Trindade et al. 2002; Da Silva et al. 2005; Raveendran et al. 2018), flavorings (Grondin et al.

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2015), and can be used as biocontrol agents for postharvest fruit diseases (Janisiewicz et al. 2010; Ruiz-Moyano et al. 2016). Yeasts or their metabolites isolated from the Amazonian rainforest may display unique characteristics due to the particularities of their habitat. Accordingly, this study aimed to identify and characterize yeasts isolated from Amazonian native fruits using molecular and phenotypic methods to glimpse also their potential biotechnological features.

Experimental

Materials and Methods

Fruit samples. One hundred specimens from ten different species (ten of each species) of Amazonian native fruits (ANF) were obtained from a small rustic market in the city of Iquitos (Amazonian region of Peru), which is supplied with fruits from different localities of the region, in July 2015. At that time, temperature was on average 25°C with the least amount of rains of the year. Fruits were all ripe with no apparent spoilage. Fruits were transported in refrigerated and sterile bags to Lima for laboratory analysis. The following ANF were employed in this study: aguaje (*Mauritia flexuosa*), camu camu (*Myrciaria dubia*), charichuelo (*Garciniama crophylla*), cidra (*Citrus medica*), cocona (*Solanum sessiliflorum*), pomarrosa (*Syzygium jambos*), taperiba (*Spondias dulcis*), ubos (*Spondias mombin*), umarí (*Poraqueiba sericea*), and ungurahui (*Oenocarpus bataua*).

Yeast isolation. For surface sampling, the same species of ANF were pooled and washed under aseptic conditions with sterile water which was used for further preparation of serial decimal dilutions in liquid YPD medium (1% yeast extract, 2% peptone, 2% glucose w/v), supplemented with chloramphenicol (100 mg/l; AppliChem GmbH, Germany). Aliquots of several dilutions were spread onto YPD plates and incubated at 30°C for 24 h. Ten colonies from each fruit were selected based on different colony morphologies (form, size, color, margin, and elevation) for further purification. Yeast colonies were identified and characterized genotypically and phenotypically.

DNA extraction, rep-PCR, and RFLP-PCR of the 5.8S-ITS region. DNA extraction was performed as per Querol et al. (1992) with a slight modification in the use of lyticase (3.3 U · µ/l; Sigma, USA) instead of zymolase. For discrimination at the strain level, PCR of the repetitive extragenic palindromic sequences (rep-PCR) (Versalovic et al. 1991) was performed using a primer $(GTG)_5$ (5'-GTG GTG GTG GTG GTG-3') as described by Gori et al. (2013). Amplification products were separated by electrophoresis on 0.8% agarose gel using the 100 bp Plus DNA (Thermo Scientific, USA) and Lambda DNA/*EcoR* I + *Hind* III Marker (Thermo Scientific, USA) ladders. One representative of each strain pattern obtained was chosen for the RFLP-PCR analysis of the 5.8S-ITS region.

PCRs were carried out using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) in order to amplify the 5.8S rRNA gene and 2 internal transcribed spacers (ITS1 and ITS2), according to the methodology described by Esteve-Zarzoso et al. (1999). PCR products were digested by the restriction enzymes *Hinf* I, *Cfo* I, and *Hae* III (Thermo Scientific, USA) following the manufacturer's instructions. PCR products and their restriction fragments were separated by electrophoresis on 1 and 2% agarose gels, respectively. Gels were stained with ethidium bromide, and DNA fragments were visualized under UV. Sizes were estimated by comparison against a DNA ladder (100 bp Plus; Thermo Fisher Scientific, USA). Preliminary identification of restriction profiles was determined by comparison with those previously reported (Guillamón et al. 1998; Esteve-Zarzoso et al. 1999).

Sequencing and phylogenetic analysis. The strains were subjected to sequencing. D1/D2 domains of the 26S rRNA gene were amplified using primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG-3') (Kurtzman and Robnett 1998). PCR products were sent to Macrogen (Rockville, USA) for sequencing. Electropherograms for both primers were evaluated using Sequencher version 4.1.4 (Gene Codes, USA) and contigs for each sample were assembled. Sequences are deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) under the accession codes MF979591-MF979618 and MF979620.

To construct our dataset, similar sequences were searched using the BlastN algorithm against the GenBank database. Identities were matched at 99–100% similarity. In addition, we searched for similar sequences against the Mycobank database (http://www.mycobank.org) using its pairwise sequence alignment tool (MolecularID) for confirming results. Both of these results provided preliminary information on the identification of each sample. Following this, preliminary information was used to search for the corresponding type strain sequences described by Kurtzman et al. (2011a). If accession codes for any type strain were not present, they were searched in GenBank (Fig. 1). For phylogenetic tree construction, we aligned multiple sequences using ClustalX 2.1 (Larkin et al. 2007). Long flanks were removed to obtain a similar alignment size for all sequences. MEGA7 (Kumar et al. 2016) was used to estimate conserved and variable positions and a genetic distance matrix (K2P). This matrix was used

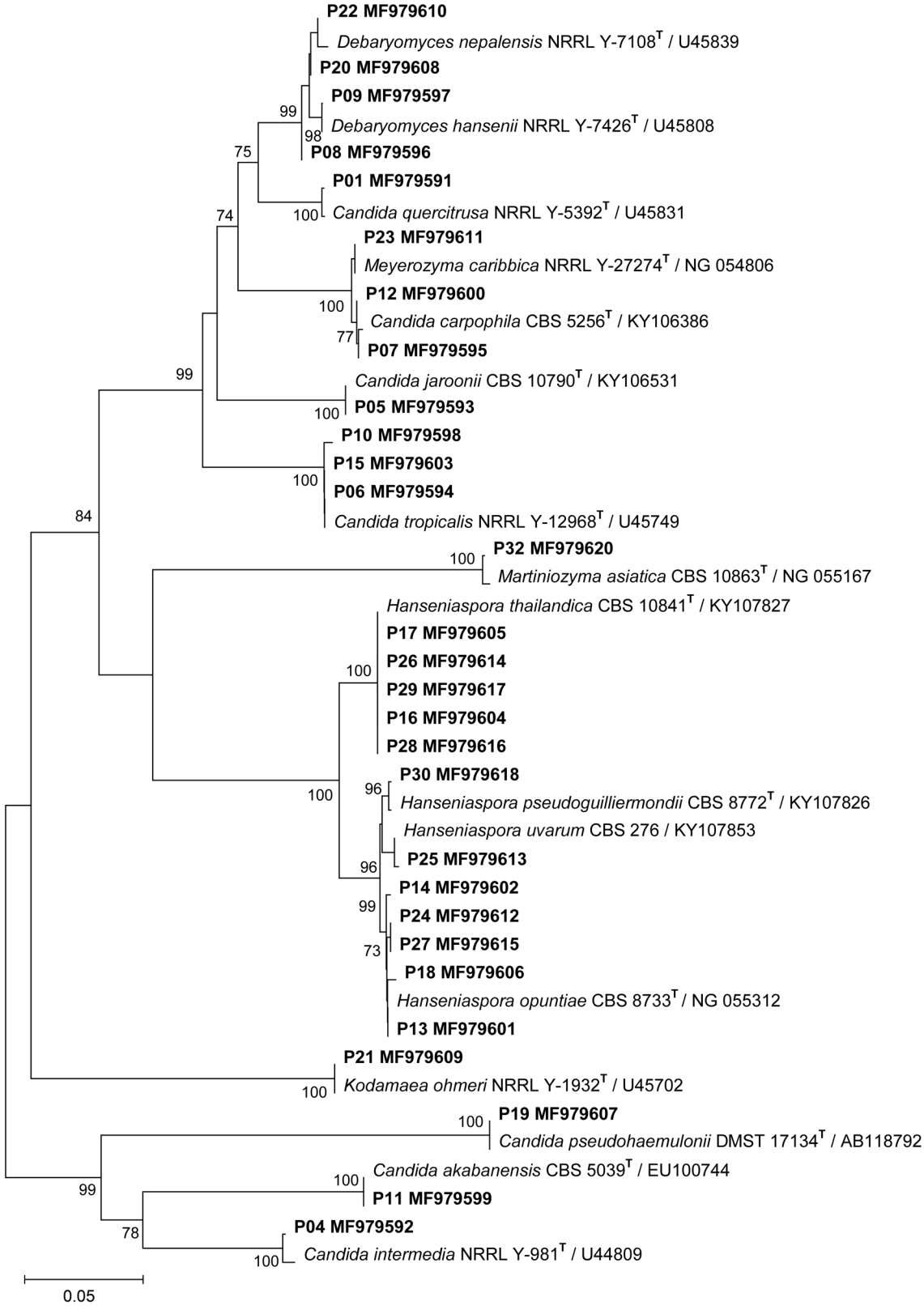

Fig. 1. Neighbor-joining tree of strains obtained from ANF (codes and accession numbers are highlighted in bold) and the corresponding type strains. Bootstrap values in nodes that received >70 of support are shown. Culture collection codes of type strains[T] and their accession numbers are shown.

to estimate a tree using the neighbor-joining method (Saitou and Nei 1987). Branch support was estimated using 1,000 bootstrap replicates.

Phenotypic characterization. Physiological tests were performed as described by Kurtzman et al. (2011b) with slight modifications, including fermentation of

sugars (D-glucose, sucrose, maltose, lactose, starch, and cellobiose), growth at high concentrations of glucose (50 and 60%), acidity production on YPD medium supplemented with 2% $CaCO_3$, and tolerance of 1% of acetic acid in a liquid medium. Growth at 4, 15, 30, and 37°C was evaluated in YPD broth. Production of extracellular hydrolases was tested on YPD plates supplemented with the specific substrates and incubated at 30°C for 48 h. Esterase activity was determined by the formation of precipitate around the growth using 1% Tween 80 as substrate (Sierra 1957). Degradation of tributyrin for lipase production was evaluated through the formation of zones of clearing around colonies. The cellulolytic activity was investigated using carboxymethyl cellulose as a substrate following the methodology of Teather and Wood (1982) which uses Congo red as an indicator. Production of proteases was evaluated using 1% skim milk as substrate. Casein hydrolysis was evident by zones of clearing around colonies. The amylolytic activity was tested using starch (2 g/l) as substrate after flooding plates with a solution of Lugol's iodine (Cowan and Steel 1974). Zones of clearing around the growth revealed the production of amylases (Sánchez-Porro et al. 2003).

Results

A total of 81 yeast isolates were obtained from one hundred specimens of 10 different fruit species from the Peruvian Amazonia. Typing at the strain level by rep-PCR discriminated 29 strain profiles (Table I). Our

Table I
Molecular methods for the identification of yeasts isolated from Amazonian native fruits.

Strain profile	Method of identification		Identification consensus
	Restriction profile	D1/D2 26S ribosomal RNA sequencing	
P01	Not determined	*C. quercitrusa*	*Candida quercitrusa*
P04	Not determined	*C. intermedia*	*Candida intermedia*
P05	Not determined	*C. jaroonii*	*Candida jaroonii*
P06	*C. tropicalis*	*C. tropicalis*	*Candida tropicalis*
P07	Not determined	*C. carpophila*	*Candida carpophila*
P08	*D. hansenii*	*D. hansenii*	*Debaryomyces hansenii*
P09	*D. hansenii*	*D. hansenii*	*Debaryomyces hansenii*
P10	–	*C. tropicalis*	*Candida tropicalis*
P11	Not determined	*C. akabanensis*	*Candida akabanensis*
P12	Not determined	*C. carpophila*	*Candida carpophila*
P13	*H. guilliermondii/H. uvarum*	*H. opuntiae*	*Hanseniaspora opuntiae*
P14	*H. guilliermondii/H. uvarum*	*H. opuntiae*	*Hanseniaspora opuntiae*
P15	*C. tropicalis*	*C. tropicalis*	*Candida tropicalis*
P16	*H. guilliermondii/H. uvarum*	*H. thailandica*	*Hanseniaspora thailandica*
P17	*H. guilliermondii/H. uvarum*	*H. thailandica*	*Hanseniaspora thailandica*
P18	*H. guilliermondii/H. uvarum*	*H. opuntiae*	*Hanseniaspora opuntiae*
P19	Not determined	*C. pseudohaemulonii*	*Candida pseudohaemulonii*
P20	*D. hansenii*	*D. nepalensis*	*Debaryomyces nepalensis*
P21	*C. incommunis*	*K. ohmeri*	*Kodamaea ohmeri*
P22	*D. hansenii*	*D. nepalensis*	*Debaryomyces nepalensis*
P23	Not determined	*Meyerozyma caribbica*	*Meyerozyma caribbica*
P24	*H. guilliermondii/H. uvarum*	*H. opuntiae*	*Hanseniaspora opuntiae*
P25	*H. guilliermondii/H. uvarum*	*H. uvarum*	*Hanseniaspora uvarum*
P26	–	*H. thailandica*	*Hanseniaspora thailandica*
P27	*H. guilliermondii/H. uvarum*	*H. opuntiae*	*Hanseniaspora opuntiae*
P28	*H. guilliermondii/H. uvarum*	*H. thailandica*	*Hanseniaspora thailandica*
P29	*H. guilliermondii/H. uvarum*	*H. thailandica*	*Hanseniaspora thailandica*
P30	*H. guilliermondii/H. uvarum*	*H. pseudoguillermondii*	*Hanseniaspora pseudoguillermondii*
P32	*C. sake*	*Martiniozyma asiatica*	*Martiniozyma asiatica*

– not evaluated; Not determinated – the restriction profile could not been matched to any previously published data

naming system for the strain profiles employed the letter P, followed by a 2-digit number. One representative of each strain profile was chosen for further RFLP analysis of the 5.8S-ITS region. Using this methodology, we were able to distinguish 12 restriction profiles (Table II).

Phylogenetic analysis of the D1/D2 domains of the 26S rRNA gene was used to identify all 29 representative strains, revealing 16 species belonging to 6 genera. Our dataset comprised 45 sequences from the 26S rRNA partial gene (Fig. 1). The final alignment resulted in 578 aligned positions and 288 variable sites. All isolates were identified as ascomycetous and non-*Saccharomyces* species. *Hanseniaspora* (40.7%) was the most common genus, followed by *Candida* (35.6%), and *Debaryomyces* (17.3%). *H. opuntiae* (24.7%) was found to be the most prevalent species among all the isolates, followed by *C. tropicalis* with 16.0%. *D. hansenii* and *H. thailandica* were also present at 11.1% of strains obtained (Table II). The highest number of strain profiles was observed in both *H. opuntiae* and *H. thailandica* (five strain profiles each), followed by *C. tropicalis* (three strain profiles). *C. carpophila*, *D. hansenii*, and *D. nepalensis* exhibited two strain profiles; others exhibited one (Table II).

Analysis of yeast presence on Amazonian fruits showed that *H. opuntiae* was found most frequently across the ANF of our study, with a presence on five of the studied fruits, while *C. tropicalis* was the second most common, with presence on four of the studied fruits. Yeasts species tended to cluster together with multiple species present on each fruit. The highest number of species of yeasts was found associated with cidra (five species) and ungurahui (four species) fruits, while the lowest was found in camu camu and charichuelo fruits, with only one species being associated with each. Pomarrosa, taperiba, and ubos fruits shared a similar yeast profile, with each harboring *H. opuntiae* and *H. thailandica*. Cidra and taperiba showed the highest number of strain profiles (data not shown). Cidra exhibited six strain profiles corresponding to five yeast species; taperiba showed six strain profiles, from three yeast species (Table II).

The biochemical profiles of selected yeasts (one representative per each strain profile in most cases) are presented in Table III. Some phenotypic traits were investigated as hydrolytic capabilities for potential biotechnological applications. Isolates showed a diverse range of phenotypic characteristics, with differentiation evident even between strains belonging to the same species. Fermentation of lactose and growth at 60% glucose was negative in all isolates tested. Hydrolytic capabilities were rarely detected, and lipolytic activity was determined in only one isolate (P11 strain profile). Degradation of Tween 80, carboxymethyl cellulose, casein, gelatin, and starch was not evident in any strain.

Discussion

Fruits possess essential traits that make them suitable habitats for yeasts. In this study, we isolated yeasts from 10 ripe ANF of the region of Loreto, Peruvian Amazonia, and belonging to the genera *Citrus*, *Garciniama*, *Mauritia*, *Myrciaria*, *Oenocarpus*, *Poraqueiba, Solanum*, *Spondias*, and *Syzygium*. Repetitive sequence-based PCR (rep-PCR) yielded 29 strain profiles of yeasts from these fruits. Although this method was initially developed for fingerprinting bacterial genomes (Versalovic et al. 1991), it has also been applied in describing fungal diversity in various samples (Ceugniez et al. 2015; Filho et al. 2017).

For preliminary visualization and identification of the microbial community, we conducted an RFLP analysis of the amplified 5.8S rRNA gene with the two flanking internal transcribed spacers ITS1 and ITS2 (Esteve-Zarzoso et al. 1999), yielding 12 restriction profiles. The majority of RFLP restriction profiles could not be matched with previous reports (Guillamón et al. 1998; Esteve-Zarzoso et al. 1999). As far as we know, restricted profiles belonging to our strains P01, P04, P05, P07, P11, P12, P19, and P23 had no match to any previously published strains. Meanwhile, P13, P14, P16, P17, P18, P24, P25, P27, P28, P29, and P30 were similar to *H. opuntiae*, *H. pseudoguilliermondii*, *H. thailandica* or *H. uvarum*. This is probably because the methodology in question includes only a limited number of strains currently isolated from other types of fruits and environments. Nonetheless, this approach provided important information about the profiles and, in some cases, the species. We found that several yeast species exhibited the same restriction profile of the ITS region. For example, *C. carpophila* and *Meyerozyma caribbica* (restriction profile II, Table II). However, all the species could be differentiated using rRNA gene sequencing (Jindamorakot et al. 2009).

When various typing methods are used together, higher-profile diversity can be observed than when single methods are used (Padilla et al. 2016). Thus, for the purpose of supporting and determining results, we also carried out the sequencing of the D1/D2 domains of the 26S rRNA gene (Kurtzman and Robnett 1998). The identification consensus of all strains was achieved by analyzing the information gathered from these combined techniques. Individual identities were ascribed to each of 81 isolates grouped in 29 strain profiles (Table I).

The distribution of species and strains varied across the ANF in this study. Communities were dominated by the genus *Hanseniaspora*, followed by *Candida* and *Debaryomyces*. More than one yeast species was present on all fruits except camu camu and charichuelo (Table II). In the conditions of this study, we believe that the nature of the fruit peels (chemical composition,

Table II
Source and incidence of yeast species and strains isolated from ANF.

Species	Restriction profile	Strain profile	Number of isolates from each fruit										
			C. medica/ cidra	*G. crophylla/ charichuelo*	*M. flexuosa/ aguaje*	*M. dubia/ camu camu*	*O. bataua/ ungurahui*	*P. sericea /umarí*	*S. sessiliflorum/ cocona*	*S. dulcis/ taperiba*	*S. mombin/ ubos*	*S. jambos/ pomarrosa*	Total (%)
Candida akabanensis	I	P11			4								4.9
Candida carpophila	II	P07, P12	1		1		1						3.7
Candida intermedia	III	P04					1						1.2
Candida jaroonii	IV	P05					1						1.2
Candida pseudohaemulonii	V	P19	1										1.2
Candida quercitrusa	VI	P01				6							7.4
Candida tropicalis	VII	P06, P10, P15			4		6		1	2			16.0
Debaryomyces hansenii	VIII	P08, P09		9									11.1
Debaryomyces nepalensis	VIII	P20, P22	5										6.2
Hanseniaspora opuntiae	IX	P13, P14, P18, P24, P27						1	7	4	4	4	24.7
Hanseniaspora pseudoguillermondii	X	P30						3					3.7
Hanseniaspora thailandica	IX	P16, P17, P26, P28, P29								4	4	1	11.1
Hanseniaspora uvarum	IX	P25										1	1.2
Kodamaea ohmeri	XI	P21	1										1.2
Martiniozyma asiatica	XII	P32						2					2.5
Meyerozyma caribbica	II	P23	2										2.5

Table III
Biochemical tests performed on yeasts isolated from ANF.

Species	Strain profile	Fermentation of carbohydrates[a]					Temperature (°C)[b]				Osm[c] 50%	Acid prod[d]	Tol[e]	Tri[f]
		Glu	Suc	Mal	Sta	Cel	4	15	30	37				
C. akabanensis	P11	+	+	+	–	–	✓	✓✓✓	✓✓✓	–	–	+	–	+
C. carpophila	P07	+	+	–	–	–	–	✓✓✓	✓✓✓	–	+	+	–	–
	P12	+	+	–	–	–	–	✓✓	✓✓✓	–	+	–	–	–
C. intermedia	P04	+	+	+	–	+	✓	✓✓✓	✓✓✓	–	–	+	–	–
C. jaroonii	P05	–	–	–	–	–	–	✓✓	✓✓	✓	+	+	–	–
C. pseudohaemulonii	P19	+	+	–	–	–	–	✓✓✓	✓✓✓	✓✓✓	+	–	–	–
C. quercitrusa	P01	–	–	–	–	–	–	✓✓	✓✓	✓✓	+	–	–	–
C. tropicalis	P06	–	–	–	–	–	–	✓	✓✓	✓✓	+	–	–	–
	P10	+	+	+	–	–	✓	✓✓✓	✓✓✓	✓✓✓	+	+	+	–
	P15	+	+	+	–	–	✓	✓✓✓	✓✓✓	✓✓✓	–	+	–	–
D. hansenii	P08	–	–	–	–	–	–	✓✓✓	✓✓✓	✓	+	–	+	–
	P09	–	–	–	–	–	–	✓	✓✓	–	+	–	–	–
D. nepalensis	P20	+	+	–	–	–	–	✓✓	✓✓	✓✓	+	–	–	–
	P22	–	–	–	–	–	–	✓	✓✓	–	+	–	–	–
H. opuntiae	P13	+	+	–	–	–	–	✓✓✓	✓✓✓	–	+	+	+	–
	P14	+	+	–	–	–	–	✓✓	✓✓✓	✓	+	+	–	–
	P18	+	+	–	–	+	–	✓✓✓	✓✓✓	–	–	+	–	–
	P24	–	–	–	–	–	–	✓✓	✓✓✓	–	+	–	+	–
	P27	+	–	–	–	–	–	✓✓	✓✓✓	–	+	+	–	–
H. pseudoguillermondii	P30	+	–	–	–	+	✓	✓✓✓	✓✓✓	✓	–	+	–	–
H. thailandica	P16	+	–	–	–	+	–	✓✓✓	✓✓✓	–	–	+	+	–
	P17	+	–	–	–	–	–	✓✓	✓✓✓	✓✓	–	+	–	–
	P26	–	–	–	–	–	–	✓✓✓	✓✓✓	–	+	–	–	–
	P28	+	–	–	–	–	–	✓✓✓	✓✓✓	✓✓	–	+	–	–
	P29	+	–	–	–	–	–	✓✓	✓✓✓	–	–	+	–	–
H. uvarum	P25	–	–	–	–	–	–	✓✓	✓	✓	+	–	–	–
K. ohmeri	P21	+	+	–	–	–	–	✓	✓✓	–	+	–	–	–
M. asiatica	P32	–	–	+	+	–	–	✓✓	✓✓✓	–	–	–	+	–
M. caribbica	P23	–	–	–	–	–	–	✓✓	✓✓	–	+	–	–	–

[a] Glu – glucose, Suc – sucrose, Mal – maltose, Sta – starch, Cel – cellobiose
[b] Growth at different temperatures in liquid media where ✓: 0.02–0.5, ✓✓: 0.5–1, ✓✓✓ : >1 (OD_{600})
[c] Growth in high osmotic pressure media (50% glucose)
[d] Acid prod – acid production
[e] Tol – tolerance to 1% acetic acid
[f] Tri – hydrolysis of tributyrin

thickness, aspect) may be one of the principal reasons for the yeast profiles observed on the ANF, but further research into the nature of these fruits is needed.

The characteristics of fruits strongly influence the diversity of yeasts and other microbes found in their adherent communities. The peels of fruits can contain various proportions of carbohydrates, crude fibers, lipids, crude proteins, minerals, and anti-nutrients. Adherent microbes must develop ways to access such materials (Villachica 1996; Romelle et al. 2016). Additionally, some fruit skins are thinner than others or have indentations that make them more prone to yeast colonization (Tournas and Katsoudas 2005). Interestingly, the highest number of yeast species was found associated with cidra fruit, a citrus species with low pH, and ungurahui, which is considered one of the most useful plants for indigenous people in Amazonia. Also, cidra harbors strains belonging to four genera (the highest number of genera among our ANF), possibly because low pH is a favorable condition for yeast growth. The range of pH of citrus fruits tends to be between 2.3 and 3.6 (Irkin et al. 2015). Ungurahui is employed for medicinal and cosmetic purposes, and to prepare a milk-like alcoholic beverage called chicha (Montúfar et al. 2010). Ungurahui was found colonized by members of the *Candida* genus, which may explain why the fruit is used to produce fermented alcoholic beverages, as frequently different species of *Candida* are present

in fruits used for alcoholic fermentation (Fleet 2003; Capozzi et al. 2015).

Conditions such as climate, geography, and other factors also interact to determine yeast diversity on the fruit surfaces (Andrews and Harris 2000; Fonseca and Inácio 2006; El Sheikha et al. 2009). Similarly, the stage of fruit maturity also plays an important role in determining the composition of yeast communities (Morais et al. 1995), though in our study, all fruit samples were mature. Hence, we showed the composition of yeast communities at that stage. Thus, nutrient changes and physicochemical characteristics exert an effect on the diversity of yeasts.

The same species identified in this study have been reported in other investigations using samples as diverse as non-Amazonian fruits, other plant surfaces, grape-associated products or even clinical samples (Kurtzman et al. 2011a). The genera *Hanseniaspora* and *Candida* have been typically associated with grape juice in the first stages of alcoholic fermentation during winemaking and have been identified as the main genera in some yeast diversity studies on fruits (Trindade et al. 2002; Vadkertiová et al. 2012; Grondin et al. 2015). *H. opuntiae* have been mainly found in the microbiota of cocoa bean fermentations (Fernández Maura et al. 2016). *H. opuntiae* have also been identified in the pineapple vinification process in Angola (Dellacassa et al. 2017). *H. opuntiae* can be referred as a ubiquitous yeast in nature. This fact is corroborated in our study, where *H. opuntiae* was found among half of the fruits tested and exhibited the high number of strains.

H. thailandica was first reported by Jindamorakot et al. (2009) in samples of insect frass, crabapple mangrove (*Sonneratia caseolaris*) flowers, lichen, and rotted *Psidium guajava* fruit from different locations in Thailand. In our study, both *H. opuntiae* and *H. thailandica* showed various strain profiles and tended to be present in consortium with other species of the *Hanseniaspora* genus (Table II). In contrast to the other representatives of the *Hanseniaspora* genus in our study, *H. pseudoguilliermondii* and *H. uvarum* showed low prevalence.

Trindade et al. (2002) isolated yeasts from fresh and frozen pulps of the Brazilian tropical fruits pitanga (*Eugenia uniflora*), mangaba (*Hancornia speciosa*), umbu (*Spondias tuberosa*), and acerola (*Malpighia glaba*). The authors found 405 different strains belonging to 42 ascomycetous and 28 basidiomycetous species, including various species of *Candida*, *Hanseniaspora*, *Kluyveromyces*, *Rhodotorula*, and *Saccharomyces*, among others. *Candida* showed the highest species richness, as was also the case in our investigation. However, we observed only ascomycetous yeasts, and one reason for this could be the temperature of 30°C we used for the isolation of yeasts. Surprisingly, none of the isolates identified by Trindade et al. (2002) were coincident with our results. This could be due to the nature of the fruits.

The *Candida* genus is widely found in yeast diversity studies on fruits. *Candida tropicalis* has been described in various ecological niches (Las Heras-Vazquez et al. 2003; Limtong et al. 2014). *C. pseudohaemulonii* is ordinarily found in clinical samples at hospitals (Sugita et al. 2006; Oh et al. 2011). However, we found that *C. pseudohaemulonii* is also associated with citrus fruit in consortium with other yeast genera representing novel information.

D. hansenii was found on pear fruit surfaces by Chand-Goyal and Spotts (1996) from diverse areas in the Pacific Northwest United States. Interestingly, *D. hansenii* has been described as harboring particular features for biotechnological applications (Prista et al. 2016). In our investigation, *D. hansenii* appeared to prefer *Garciniama crophylla* tree fruit as a habitat and was the only yeast species found on this fruit.

K. ohmeri has been mainly reported as a rare human pathogen (Al-Sweih et al. 2011; Fernández-Ruiz et al. 2017). However, it has also been described as being associated with food (Ezeokoli et al. 2016). In our work, we found *K. ohmeri* associated with cidra. The genus *Martiniozyma* has recently been described (Kurtzman 2015), and *C. asiatica* is now recognized as *Martiniozyma asiatica*. *M. asiatica* has been previously detected in natural samples from various Asian countries (Limtong et al. 2010). In our study, *M. asiatica* tended to cohabit with other yeast species associated with umarí fruit.

In order to analyze phenotypic characteristics of the isolates, and possibly find useful traits for biotechnological purposes (Da Silva et al. 2005; Molnárová et al. 2013), some phenotypic tests were carried out. Variation in phenotypic traits of the species compared to previous reports (Kurtzman et al. 2011a) may be due to diverse factors, including the dynamic environmental conditions of Amazonia, which may influence the physiological features. Certain environmental conditions may switch specific genes on or off, causing the broad strain variation. In addition, the patterns we observed could also be ascribed to the effects of fruit species. These factors have been shown to contribute to species variation (Lane et al. 2011; Qvirist et al. 2016).

In terms of hydrolytic capabilities, lipase production was only detected in *C. akabanensis*, which was isolated exclusively from aguaje, a fruit with high fatty acid content. It is possible that *C. akabanensis* employs lipase to, in some way, utilize the fatty acids present in the pulp. More generally, however, the rarity of hydrolytic activity detected in our study is not unexpected, since it appears that these yeasts tend to use straightforward sources of carbon such as simple sugars (glucose in most cases, Table III). Ecologically, this is a cost-effective strategy,

considering that the surface of fruits in the Amazonia tends to constitute harsh environmental conditions. It is important to consider that strains can be very heterogeneous both genetically and biochemically (Prista et al. 2016; Visintin et al. 2016), and also this variability can be strongly influenced by the nutritional composition of the samples they are obtained from. Furthermore, as far as we know, there are no reports of the same tests for hydrolases for all the species of this study to compare. Thus, the majority of negative hydrolytic profiles appear in agreement with the information described by Kurtzman et al. (2011a). Nevertheless, we recommend investigating hydrolytic capabilities using basal nutrients different from YPD and non-synthetic or residual substrates because non-natural substrates can result in a different biochemical response of the yeasts. More suitable substrates can be starch of potato, olive oil, or beef suet.

Comparing our results with previous works shows that yeasts are ubiquitous on different fruits, and even in different types of samples. The surface of Amazonian fruits, although a hostile environment, can be an interesting source of yeast strains displaying diverse phenotypic traits. Different yeasts found in the ANF studied seem to be influenced mainly by the nature of the fruits and their environment. ANF may constitute a good source of new species or strains of yeasts with particular characteristics for biotechnological purposes. Further investigation is needed in order to explore the potential industrial applications of these yeasts in food, feed ingredients, biocatalysis, or biocontrol.

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Acknowledgments

This work was supported by INNOVATE-PERU, agreement N° 230-FINCyT-IA-2013, and CIENCIACTIVA, agreement N° 007-FONDECYT-2014.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect this publication's contents and/or claim authorship rights to this publication.

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