Yeasts Associated with Various Amazonian Native Fruits

CARLOS VEGAS¹, AMPARO I. ZAVALETA¹, PAMELA E. CANALES* & BRAULIO ESTEVE-ZARZOSO²

¹Laboratory of Molecular Biology, Faculty of Pharmacy and Biochemistry, Universidad Nacional Mayor de San Marcos, Lima, Peru
²Departament de Bioquímica i Biotecnologia, Facultat d’Enologia, Universitat Rovira i Virgili, Tarragona, Spain

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 native 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, Martinizyyma, 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.

Keywords: 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. Vakdertiová 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.
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 (Garcinia marophylla), cidra (Citrus medica), cocona (Solanium sessiliflorum), pomarrosa (Syzygium jambos), tajeríba (Spondias dulcis), ubos (Spondias mombin), umari (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/EcoRI 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’-TCCG-TAGGTTAAGCTATCG-3’) and ITS4 (5’-TCTCG-CGCTTATTGATATGC-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’-GCAATATCAATAACGGGGAGAAGG-3’) and NL-4 (5’-GGTCCGTGTTTACAAGCAGG-3’) (Kurtzman and Robnett 1998). PCR products were sent to Macrogen (Rockville, USA) for sequencing. Electropherograms for both primers were evaluated using Sequencer 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
Yeast species from Amazonian fruits were estimated 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 (β-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

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

- not evaluated; Not determined – the restriction profile could not be matched to any previously published data.  

---

Table I

Molecular methods for the identification of yeasts isolated from Amazonian native fruits.
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. The identification consensus of all strains was achieved 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/ umgurahui | P. sericea/ umari | S. sessiliflorum/ cocona | S. dulcis/ taperiba | S. mombin/ ubos | S. jambos/ pomarrosa | Total (%) |
| Candida akabanensis           | I                   | P11            |                   |                          |                     |                     |                        |                  |                          |                    |              |                          | 4.9        |
| Candida carpophila            | II                  | P07, P12       | 1                 |                          | 1                    | 1                  |                        |                  |                          |                    |              |                          | 3.7        |
| Candida intermedia            | III                 | P04            |                   |                          |                      | 1                  |                        |                  |                          |                    |              |                          | 1.2        |
| Candida jarnoonii             | IV                  | P05            |                   |                          |                      |                    |                        |                  |                          |                    |              |                          | 1.2        |
| Candida pseudohaemulonii      | V                   | P19            |                   |                          |                      |                    |                        |                  |                          |                    |              |                          | 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 pseudoguillermondi | X              | P30            | 3                 |                          |                      |                    |                        |                  |                          |                    |              |                          | 3.7        |
| Hanseniaspora thailandica     | IX                  | P16, P17, P26, P28, P29 |                   |                          |                      |                    |                        | 4                 | 4                       | 1                  |              |                          | 11.1       |
| Hanseniaspora uvarum          | IX                  | P25            |                   |                          |                      |                    |                        |                  |                          |                    |              |                          | 1.2        |
| Kodamaea ohmeri               | XI                  | P21            | 1                 |                          |                      |                    |                        |                  |                          |                    |              |                          | 1.2        |
| Martinozyma asiatica          | XII                 | P32            |                   |                          |                      |                    |                        |                  |                          |                    |              |                          | 2.5        |
| Meyerozyma caribbica          | II                  | P23            | 2                 |                          |                      |                    |                        |                  |                          |                    |              |                          | 2.5        |
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.

### Table III

| Species                  | Strain profile | Fermentation of carbohydrates | Temperature (°C) | Osm 50% | Acid prod | Tol | Tri |
|-------------------------|----------------|-------------------------------|------------------|---------|-----------|-----|-----|
| *C. akabasensis*        | P11            | + + - - -                    | ✓ ✓✓ ✓ ✓       | -       | + + -     |     |     |
| *C. carpophila*         | P07            | + + - - -                    | ✓ ✓✓ ✓ ✓       | -       | + + -     |     |     |
| *C. intermedia*         | P04            | + + - - +                    | ✓ ✓✓ ✓ ✓ ✓     | -       | + + -     |     |     |
| *C. jaromii*            | P05            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *C. pseudohaemulonii*   | P19            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *C. quercitrusa*        | P01            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *C. tropicalis*         | P06            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *D. hansenii*           | P08            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *D. nepalensis*         | P20            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. opuntiae*           | P13            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. pseudoguillermondii*| P30            | + - - - +                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. thailandica*        | P16            | + - - - +                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. uvarum*             | P17            | + - - - +                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *K. ohmeri*             | P21            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *M. asiatica*           | P32            | - - + - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *M. caribbica*          | P23            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *M. pseudohaemulonii*   | P19            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *M. quercitrusa*        | P01            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *C. tropicalis*         | P06            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *D. hansenii*           | P08            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *D. nepalensis*         | P20            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. opuntiae*           | P13            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. pseudoguillermondii*| P30            | + - - - +                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. thailandica*        | P16            | + - - - +                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *H. uvarum*             | P17            | + - - - +                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *K. ohmeri*             | P21            | + + - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *M. asiatica*           | P32            | - - + - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |
| *M. caribbica*          | P23            | - - - - -                    | ✓ ✓✓ ✓ ✓ ✓     | +       | + + -     |     |     |

*Glu – glucose, Suc – sucrose, Mal – maltose, Sta – starch, Cel – cellobiose

| Temperature (°C) | Osm 50% | Acid prod | Tol | Tri |
|------------------|---------|-----------|-----|-----|
| 4                | ✓ ✓✓ ✓ ✓| ✓ ✓✓ ✓ ✓  | +   |     |
| 15               | ✓ ✓✓ ✓ ✓| ✓ ✓✓ ✓ ✓  | +   |     |
| 30               | ✓ ✓✓ ✓ ✓| ✓ ✓✓ ✓ ✓  | +   |     |
| 37               | ✓ ✓✓ ✓ ✓| ✓ ✓✓ ✓ ✓  | +   |     |

*Osm – osmotic pressure media (50% glucose)

*Tol – tolerance to 1% acetic acid

*Tri – hydrolysis of tributyrin
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 to 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. pseudoguilhermondii 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-Vázquez 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 Garcinia 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 umari 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 hydrolyses 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.

**ORCID**

Pamela E. Canales https://orcid.org/0000-0001-6019-8846

**Acknowledgments**

This work was supported by INNOVATE-PERU, agreement Nº 230-FINCYT-IA-2013, and CIENCIATIVITA, agreement Nº 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.

**Literature**

Al-Sweih N, Khan ZU, Ahmad S, Devarajian L, Khan S, Joseph L, Chandy R. *Kodamaea ohmeri* as an emerging pathogen: a case report and review of the literature. Med Mycol. 2011;49(7):766–770. https://doi.org/10.3109/13693786.2011.572300

Andrews JH, Harris RF. The ecology and biogeography of microorganisms on plant surfaces. Annu Rev Phytopathol. 2000; 38(1):145–180. https://doi.org/10.1146/annurev.phyto.38.1.145

Bhadra B, Begum Z, Shivaji S. *Pichia gargaricae* sp. nov., isolated from a rotten mangosteen fruit (*Garcinia mangostana* L., *Clusia*). Int J Syst Evol Microbiol. 2008; 58(Pt 11):2665–2669. https://doi.org/10.1099/ijs.0.65770-0

Capozzi V, Garofalo C, Chirriati MA, Grieco F, Spanelo G. Microbial terroir and food innovation: the case of yeast biodiversity in wine. Microbiol Res. 2015;181:75–83. https://doi.org/10.1016/j.micres.2015.10.005

Ceugniet A, Drider D, Jacques P, Couchenoy F. Yeast diversity in a traditional French cheese “Tomme dorches” reveals infrequent and frequent species with associated benefits. Food Microbiol. 2015;52:177–184. https://doi.org/10.1016/j.fm.2015.08.001

Chand-Goyal T, Spotts RA. Enumeration of bacterial and yeast colonists of apple fruits and identification of epiphytic yeasts on pear fruits in the Pacific Northwest United States. Microbiol Res. 1996;151(4):427–432. https://doi.org/10.1016/S0923-2508(96)80013-9

Cowan ST, Steel KJ. Cowan and Steel's Manual for the Identification of Medical Bacteria (second ed.). London (UK): Cambridge University Press; 1974.

Da Silva EG, Borges MdF, Medina C, Piccoli RH, Schwam RF. Pectinolytic enzymes secreted by yeasts from tropical fruits. FEMS Yeast Res. 2005; 5(5):859–865. https://doi.org/10.1016/j.femsyr.2005.02.006

Dellaccassa E, Trenchs O, Farina L, Debernardis F, Perez G, Boido E, Carrau F. Pineapple (*Ananas comosus* L. *Merr.*) wine production in Angola: characterisation of volatile aroma compounds and yeast native flora. Int J Food Microbiol. 2017; 24:161–167. https://doi.org/10.1016/j.ijfoodmicro.2016.10.014

El Sheikh AF, Condour A, Metayer I, Nguyen DD, Loiseau G, Montet D. Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: preliminary application to *Physalis* fruits from Egypt. Yeast. 2009;26(10):567–573. https://doi.org/10.1002/yea.1707

Esteve-Zarzoso B, Bellcoh C, Urruburu F, Querol A. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int. J Syst Bacteriol. 1999; 49(Pt 1):329–337. https://doi.org/10.1099/00207713-49-1-329

Ezekool TO, Gupta AK, Mienie C, Popoola TO, Bezuidenhout CC. PCR-denaturing gradient gel electrophoresis analysis of microbial community in *soy-daddawa*, a Nigerian fermented soybean (*Glycine max* (L.) *Merr.*) condiment. Int J Food Microbiol. 2016;220:58–62. https://doi.org/10.1016/j.ijfoodmicro.2016.01.003

Fernández Maura Y, Balzarini T, Clapé Borges P, Eavrad P, De Vuyst I, Daniel HM. The environmental and intrinsic yeast diversity of Cuban cocoa bean heap fermentations. Int J Food Microbiol. 2016;233:34–43. https://doi.org/10.1016/j.ijfoodmicro.2016.06.012

Fernández-Ruiz M, Guinea J, Puig-Asensio M, Zaragoza Ò, Almirante B, Cuencsa-Extella M, Aguado JM; CANDIPOP Project, GEIH-GEMICOMED (SEIMC) and REIPI. Fungemia due to rare opportunistic yeasts: data from a population-based surveillance in Spain. Med Mycol. 2017;55(2):125–136. https://doi.org/10.1093/mmy/myw055

Filho MC, Bertelli MBD, Valle JS, Paccola-Meirelles LD, Linde GA, Barcellos FG, Colauto NB. Genetic diversity and pectinolytic activity of epiphytic yeasts from grape carposphere. Genet Mol Res. 2017;16(2):gmr16029698. https://doi.org/10.4238/gmr16029698

Fleet GH. Yeast interactions and wine flavour. Int J Food Microbiol. 2003;86(1–2):11–22. https://doi.org/10.1016/S0168-1605(03)00245-9

Fonseca Á, Inácio J. Phyloplane yeasts. In: Péter G, Rosa C, editors. Biodiversity and ecophysiology of yeasts. Berlin Heidelberg (Germany): Springer-Verlag; 2006. p. 263–301.
Gori K, Ryssel M, Arneborg N, Jespersen L. Isolation and identification of the microbiota of Danish farmhouse and industrially produced surface-ripened cheeses. Microb Ecol. 2013;65(3):602–615. https://doi.org/10.1007/s00248-012-0138-3

Grondin E, Sing ASC, Caro Y, Raherimandimbiny M, Randrianiernana AL, James S, Nueno-Palop C, François JM, Petit T. A comparative study on the potential of epiphytic yeasts isolated from tropical fruits to produce flavoring compounds. Int J Food Microbiol. 2015;203:101–108. https://doi.org/10.1016/j.ijfoodmicro.2015.02.032

Guillamón JM, Sábaté J, Barrio E, Cano J, Querol A. Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. Arch Microbiol. 1998;169(5):387–392. https://doi.org/10.1007/s002030050587

Irkin R, Dogan S, Dergirmencioglu N, Diken ME, Guldas M. Phenolic content, antioxidant activities and stimulatory roles of citrus fruits on some lactic acid bacteria. Arch Biol Sci. 2015;67(4):1313–1321. https://doi.org/10.2298/ABSI40909108I

Janisiewicz WJ, Kurtzman CP, Buyer JS. Yeasts associated with nectarines and their potential for biological control of brown rot. Yeast. 2010;27(7):389–398. https://doi.org/10.1002/yea.1763

Jindamakorat S, Ninomiya S, Lintong S, Yonngmanitchai W, Kawasaki H. Three new species of bipolar budding yeasts of the genus *Hanseniaspora* and its anamorph *Kloechera* isolated in Thailand. FEMS Yeast Res. 2009;9(8):1327–1337. https://doi.org/10.1111/j.1567-1364.2009.00568.x

Koricha AD, Han DY, Bacha K, Bai FY. Occurrence and molecular identification of wild yeasts from Jimma Zone, South West Ethiopia. Microorganisms. 2019;7(12):633. https://doi.org/10.3390/microorganisms7120633

Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874. https://doi.org/10.1093/molbev/msw054

Kurtzman CP, Fell JW, Boekhout T, Vincent R. Methods for Isolation, Phenotypic Characterization and Maintenance of Yeasts. In: Kurtzman CP, Fell JW, Boekhout T, editors. The Yeasts: a Taxonomic Study. Amsterdam (The Netherlands): Elsevier B.V.; 2011b. p. 87–110.

Kurtzman CP, Fell JW, Boekhout T. The Yeasts, a Taxonomic Study. Amsterdam (The Netherlands): Elsevier B.V.; 2011a. p. 2354.

Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek. 1998;73(4):331–371. https://doi.org/10.1023/a:1001761008817

Kurtzman CP. Description of *Martiniozyma* gen. nov. and transfer of seven *Candida* species to *Saturnispora* as new combinations. Antonie Van Leeuwenhoek. 2015;108(4):803–809. https://doi.org/10.1007/s11746-015-0536-x

Lane MM, Burke N, Karreman R, Wolfe KH, O’Byrne CP, Morrissey JP. Physiological and metabolic diversity in the yeast *Klyveromyces marxianus*. Antonie Van Leeuwenhoek. 2011;100(4):507–519. https://doi.org/10.1007/s10482-011-9606-x

Larkin MA, Blackshields G, Brown NP, Chenna R, McGinnigle PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23(21):2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Las Heras-Vazquez FJ, Mingorange-Cazorla I, Clemente-Jimenez JM, Rodriguez-Vico F. Identification of yeast species from orange fruit and juice by RFLP and sequence analysis of the 5.8 S rRNA gene and the two internal transcribed spacers. FEMS Yeast Res. 2003;3(1):3–9.

Limtong S, Kaeiwwichian R, Am-In S, Nakase T, Lee CF, Yongmanitchai W. *Candida asiatica* sp. nov., an anamorphic ascomycetous yeast species isolated from natural samples from Thailand, Taiwan, and Japan. Antonie Van Leeuwenhoek. 2010;98(4):475–481. https://doi.org/10.1007/s10482-010-9463-z

Limtong S, Kaeiwwichian R, Yongmanitchai W, Kawasahi H. Diversity of culturable yeasts in phylloplane of sugarcane in Thailand and their capability to produce indole-3-acetic acid. World J Microbiol Biotechnol. 2014;30(6):1785–1796. https://doi.org/10.1007/s11274-014-1602-7

Molnáravá J, Vadkertirová R, Stratilová E. Extracellular enzymatic activities and physiological profiles of yeasts colonizing fruit trees. J Basic Microbiol. 2013;53:1–11.

Montúfar R, Laffargue A, Pintaud J-C, Hamon S, Avallone D, Dussert S. *Oenocarpus bataua* Mart. (Areciaceae): rediscovering a source of high oleic vegetable oil from Amazonia. J Am Oil Chem Soc. 2010;87(2):167–172. https://doi.org/10.1111/j.1746-0099-2010-5821.x

Morais PB, Martins MB, Klaczko LB, Mendonça-Hagler LC, Hagler AN. Yeast succession in the Amazon fruit *Parahancornia anna* as resource partitioning among *Drosophila* spp. Appl Environ Microbiol. 1995;61(12):4251–4257.

Oh BJ, Shin JH, Kim MN, Sung H, Lee K, Joo MY, Shin MG, Suh SP, Ryang DW. Biofilm formation and genotyping of *Candida haemulonii*, *Candida pseudohaemulonii*, and a proposed new species (*Candida auris*) isolates from Korea. Med Mycol. 2011;49(1):98–102. https://doi.org/10.3109/13693786.2010.493563

Padilla B, García-Fernández D, González B, Izidoro I, Esteve-Zarzoso B, Beltran G, et al. Yeast biodiversity from DOQ uninoculated unfermented fermentations. Front Microbiol. 2016;7:930. https://doi.org/10.3389/fmicb.2016.00990

Prista C, Michan C, Miranda IM, Ramos J. The halotolerant *Debaryomyces Hansenii*, the Cinderella of non-conventional yeasts. Yeast. 2016;33(10):523–533. https://doi.org/10.1002/yea.3177

Querol A, Barrio E, Huerta T, Ramon D. Molecular monitoring of wine fermentations conducted by active dry yeast strains. Appl Environ Microbiol. 1992;58(9):2948–2953. https://doi.org/10.1128/AEM.58.9.2948-2953.1992

Quirist LA, De Filippo C, Strati F, Stefanini I, Sordi M, Andlid T, Felis GE, Mattarelli P, Cavalieri D. Isolation, identification and characterization of yeasts from fermented goat milk of the Yaghnob Valley in Tajikistan. Front Microbiol. 2016;7:1690. https://doi.org/10.3389/fmicb.2016.01690

Raveendran S, Parameswaran B, Ummalaya SB, Abraham A, Mathew AK, Madhavan A, Rebello S, Pandey A. Applications of microbial enzymes in food industry. Food Technol Biotechnol. 2018;56(1):16–30. https://doi.org/10.17113/fib.56.18.5491

Romelle FD, Rani A, Manohar RS. Chemical composition of some selected fruit peels. J Environ Microbiol. 2016;4(4):12–21. https://doi.org/10.3389/fmicb.2016.00916

Ruiz-Moyano S, Martín A, Villalobos MC, Calle A, Serradilla MJ, Córdoba MG, Hernández A. Yeasts isolated from figs (*Ficus carica* L.) as biocontrol agents of postharvest fruit diseases. Food Microbiol. 2016;57:45–53. https://doi.org/10.1016/j.fm.2016.01.003

Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sanchez-Porco C, Martin S, Mellado E, Ventosa A. Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. J Appl Microbiol. 2003;94(2):295–300. https://doi.org/10.1046/j.1365-2672.2003.01834.x

Sierra G. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie Van Leeuwenhoek. 1957;23(1):15–22. https://doi.org/10.1007/bf02545855
Sipiczki M. Dimorphic cycle in *Candida citri* sp. nov., a novel yeast species isolated from rotting fruit in Borneo. FEMS Yeast Res. 2011;11(2):202–208. https://doi.org/10.1111/j.1567-1364.2010.00708.x

Starmer WT, Lachance M-A. Yeast Ecology. In: Kurtzman CP, Fell JW, Boekhout T, editors. The Yeasts, a Taxonomic Study. Amsterdam (The Netherlands): Elsevier B.V.; 2011. p. 65–83.

Sugita T, Takashima M, Poonwan N, Mekha N. *Candida pseudohaemulonii* sp. nov., an amphotericin B-and azole-resistant yeast species, isolated from the blood of a patient from Thailand. Microbiol Immunol. 2006;50(6):469–473. https://doi.org/10.1111/j.1348-0421.2006.tb03816.x

Teather RM, Wood PJ. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol. 1982;43(4):777–780.

Tournas VH, Katsoudas E. Mould and yeast flora in fresh berries, grapes and citrus fruits. Int J Food Microbiol. 2005;105(1):11–17. https://doi.org/10.1016/j.ijfoodmicro.2005.05.002

Trindade RC, Resende MA, Silva CM, Rosa CA. Yeasts associated with fresh and frozen pulps of Brazilian tropical fruits. Syst Appl Microbiol. 2002;25(2):294–300. https://doi.org/10.1078/0723-2020-00089

Vadkertiová R, Molnárová J, Vránová D, Sláviková E. Yeasts and yeast-like organisms associated with fruits and blossoms of different fruit trees. Can J Microbiol. 2012;58(12):1344–1352. https://doi.org/10.1139/cjm-2012-0468

Versalovic J, Koeth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. 1991;19(24):6823–6831. https://doi.org/10.1093/nar/19.24.6823

Villachica H. [Fruit trees and promising vegetables of the Amazonia] (in Spanish). Lima: Tratado de Cooperacion Amazonica, Secretaria Pro-Tempore; 1996. p. 337.

Visintin S, Alessandria V, Valente A, Dolci P, Cocolin L. Molecular identification and physiological characterization of yeasts, lactic acid bacteria and acetic acid bacteria isolated from heap and box cocoa bean fermentations in West Africa. Int J Food Microbiol. 2016;216:69–78. https://doi.org/10.1016/j.ijfoodmicro.2015.09.004

White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: A guide to methods and applications. Cambridge (USA): Academic Press; 1990. p. 315–322.