Dominant yeasts associated to mango (*Mangifera indica*) and rose apple (*Syzygium malaccense*) fruit pulps investigated by culture-based methods

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Abstract: The biotechnological potential of yeasts associated to different habitats in Colombia has been poorly studied, especially the yeasts associated with different plant structures. Fruit pulps are interesting substrates mainly for the growth of yeast species, that can positively affect the productivity and quality of some bioeconomic species. Therefore, the objective of this study was to identify the dominant yeast species associated with mango and rose apple fruit pulps in Cali, Colombia. A total of 90 isolates were obtained, which were grouped considering their colony morphology. The D1/D2 domain of the large ribosomal RNA gene (LSU rRNA gene) or internal transcribed spacer (ITS) 1, ribosomal gene 5.8S and ITS 2 (ITS) regions of one to several representative isolates from each group was sequenced and compared with type strains for identification. The species *Hanseniaspora thailandica, H. opuntiae* and *Clavispora lusitaniae* were reported as shared by both fruits, specific for rose apple (*H. uvarum, Pichia terricola, Rhodosporidiobolus ruineniae* and *Candida albicans*), or for Mango (*Meyerozyma caribbica, M. guilliermondii, C. natalensis, Aureobasidium pullulans, Pichia* sp*., Saturnispora diversa* and *C. jaroonii*). Two morphotypes were not identified at the taxonomic level of species and were reported as candidates for new species, belonging to the genera *Wickerhamomyces* and *Pichia*.

Key words: *Mangifera indica, Syzygium malaccense,* molecular identification, yeasts, Colombia.

INTRODUCTION

Yeasts have been reported in almost all natural and artificial ecosystems. The conditions that prevail in each substrate determine the ecology, metabolic activity, growth and survival of different yeast species (Deak 2009). Fruits, composed mainly of water and carbohydrates, provide an ideal environment for the growth and maintenance of ascomycetous yeasts. Mango (*Mangifera indica*) and the rose apple (or "Pomarroso" in Colombia, *Syzygium malaccense*) are highly nutritional fruits that contain carbohydrates, proteins, fats, minerals and vitamins, specifically vitamin A (beta carotene),

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B1, B2, and vitamin C (ascorbic acid) (at: http://www.traditionaltree.org/). As the fruit matures, vitamin C concentrations decrease, and glucose, fructose and sucrose levels increase (reported here as ºBrix), which can be used by fermentative yeasts. In this sense, the fermentative capacity, enzymatic activity and other physiological properties of yeasts are of interest and have been extensively explored in other countries (Pampulha and Loureiro 1989, Palnitkar and Lachke 1990, Mejía et al. 2009, Thierfelder et al. 2011). However, in Colombia the composition or biotechnological potential of these yeasts is less documented (Ulloa et al. 2009).

Colombia has been widely recognized as a center of mega-biodiversity at the level of both plant and animal species, a situation that is determined thanks to its privileged variety of ecological niches. This condition allows intuiting the presence of an equally mega-diverse profile in the population of microorganisms in our territory (Velez 2009). Few reports in the literature analysed the diversity of yeasts associated with mango or rose apple. In the case of mango, Gaviria and Osorio (2012) identified the yeasts associated with mango inflorescences in the same region (Cali, Colombia), finding 13 different species. These results suggest the possibility that some of these species may possibly be shared in the pulp of the fruit. Similarly, Jager et al. (2001) evaluated the yeasts of the mango phylloplane, finding species of the genera *Aureobasidium, Cryptococcus*, and *Sporobolomyces*. On the other hand, Trujillo and Echeverry (2015) evaluated the yeasts associated with the rose apple in the Department of Huila (Colombia), identifying 6 groups at the genus level and 4 unidentified morphotypes. The main drawback they had was the identification using biochemical methods, which did not allow the identification to the species level, and therefore did not have comparable results. In this way, evaluating yeasts associated with different plant structures is of great interest, not only to understand their

ecological role, perpetuity in plants or even possible co-evolution, but also to find strains that may be of biotechnological interest (Ulloa et al. 2009).

The identification of yeasts should be carried out using molecular methods such as the sequence analysis of ribosomal regions, such as the D1/D2 domain of the LSU rRNA gene and ITS region, and different thresholds would be compared with type strain sequences for identification at the species level (Kurtzman et al. 2015). In this sense, Vu et al. (2016) analysed the sequences of both regions of the entire yeast collection belonging to the Westerdijk Fungal Biodiversity Institute and determined an identity threshold of 98.41% for the ITS region (98.31% for Ascomycetes and 98.61% for Basidiomycetes) and an identity threshold of 99.51% for the D1/D2 domain of the LSU rRNA gene (99.41% for Ascomycetes and 99.51 for Basidiomycetes) as optimal cut-off criteria to reach the species level. Thus, the objective of this work was to identify the dominant community of yeasts associated with mango and rose apple fruit pulps in Santiago de Cali, Colombia, using sequence-based analysis.

MATERIALS AND METHODS

SAMPLING AND YEAST ISOLATION

Yeasts were obtained between 2010 and 2011 from the pulps of mango and rose apple (or "pomarroso") fruits in Santiago de Cali, Colombia. This city is framed within a climate of tropical valley, with precipitation up to 1200 mm, average temperature of 26ºC, minimum of 19ºC and maximum of 34ºC. 50 mangoes were picked from a fruit distribution center (origin of mangoes: Valle del Cauca, Colombia) and 50 rose apples were randomly collected at Universidad del Valle. Each fruit was collected in sterile bags and immediately transported under refrigeration to the laboratory of molecular biology of microorganism at Universidad del Valle. The individual fruits were classified following Bartrina

(2006) and sorted according to their coloration resulting in 10 healthy orange-yellow mango fruits (n = 10) and 15 magenta-crimson rose apples (n = 15) being analysed.

To clean the exterior of the fruits and avoid contaminating the pulp, the fruits were sterilized by washing them for one minute in three consecutive solutions. First, a wash of one minute with hypochlorite (1.5% v/v), then one minute with 70% ethanol (v/v) and finally one minute with sterile distilled water. The skin was removed, and the pulp homogenized. The Brix degrees were measured in the macerated fruit product using a manual refractometer (RHB Westover 32ATC, USA). Twenty-five grams of pulp extract were mixed in 50 mL malt extract medium (20 g/L of malt extract, 20 g/L of glucose and 1 g/L of peptone) and incubated at 28ºC for 48h on a rotary shaker at 150 rpm.

After the enrichment step, serial dilutions were made in peptone water and the 10^{-1} to 10^{-3} dilutions for mango, and the 10^{-4} to 10^{-6} for rose apple were plated in yeast peptone dextrose agar (YPDA) medium (10 g/L yeast extract, 20 g/L mycological peptone, 20 g/L glucose and 20 g/L agar; ascomycete yeasts) and malt extract agar (MEA) (20 g/L malt extract, 20 g/L glucose, 1 g/L peptone and 15 g/L agar; basidiomycete yeasts), both supplemented with 25 mg/L of ampicillin and chloramphenicol to inhibit bacterial growth. The plates were incubated at room temperature for 48 hours and five to ten representative colonies of each plate were subsequently isolated and purified through several repetitions of streaking on YPDA or MEA culture medium. Finally, the isolates were preserved using a mixture of YPD medium (70%) and glycerol (30%) and stored at -20°C until further analysis.

MORPHOLOGICAL CHARACTERIZATION AND DNA SEQUENCING

All isolates were characterized for colony and cell morphology using a light microscope (Olympus

CH30, USA). The following criteria were considered: shape, elevation, margin of the colony, cell shape and type of asexual reproduction. This data was used to group the isolates by morphotypes.

DNA extraction was performed according to Osorio-Cadavid et al. (2009). The concentration and purity of the DNA were determined by spectrophotometry at 260 nm and 260 nm/280 nm ratio using a Nanodrop 2000 (Thermo Scientific ® v1.0 USA) and by agarose gels (1% w / v), stained with ethidium bromide 1.5% (w / v) and excited at 240 nm.

The sequencing of the D1/D2 domain of the LSU rRNA gene or ITS region of representative isolates from each morphological group was carried out according to Kurtzman et al. (2015). For the amplification of the the D1/D2 domain of the LSU rRNA gene, primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') were used. For the ITS region, primers ITS5 (5´-GGAAGTAAAAGTCGTAACAAGG-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) were used. The reactions were performed in a thermal cycler (Multigene-Labnet, USA) under the following conditions: initial denaturation at 94ºC for 5 min, 30 cycles of denaturation at 94ºC for 1 min, pairing at 55ºC for 30 seconds, and extension at 72°C for 1 min, with a final extension of 10 min at 72°C (Osorio-Cadavid et al. 2008).

The PCR products were purified and sequenced by Macrogen (USA) and Corpogen (Colombia) under standardized conditions. Subsequently, the sequences were manually edited, assembled and compared to the sequence of the type strain reported in GenBank using the BLAST algorithm. Sequences with identity equal to or greater than 99% were identified at the taxonomic level of species. Strains LUM023, LUM031 (the D1/D2 domain of the LSU rRNA gene) and LUM055 (ITS) were identified using phylogenetic analysis. First, we determined the best substitution model for

each alignment (Clustal W). Second, we used the Maximum Likelihood method with all sites, and a bootstrap test of 1000 replicates. All phylogenetic trees were constructed with the software MEGA X ®.

This article does not contain any studies with human participants performed by any of the authors.

RESULTS

A total of 90 representative isolates were obtained, out of which 48 were isolated from rose apple and 42 from mango. The rose apples were classified in three groups according to their Brix degrees (or ºBrix): less than 8 ºBrix (three samples), between 8.1 and 10 ºBrix (eight samples), between 10.1 and 12 ºBrix (five samples). In total, 15 rose apples were selected from the 50 fruits randomly sampled. For mango, four intervals were determined: less than or equal to 12 ºBrix (one sample), 15 to 15.9 ºBrix (three samples), 16 to 16.9 ºBrix (two samples), and 20 ºBrix or higher (four samples). In total, 10 mango fruits were selected and analysed from the 50 sampled fruits. Table I shows the number of isolates obtained from each range of dissolved sugars per fruit.

YEAST IDENTIFICATION

Groupings were made considering both the colony and cell morphologies of the isolates to facilitate their identification. The isolates obtained from rose apples were divided into 8 groups (morphotypes) and the ones from mango fruits into 13 morphotypes.

One to five representative isolates from each morphotype (or single isolates if it was the case), were identified by comparing the sequence of the the D1/D2 domain of the LSU rRNA gene or ITS region with the type strain sequences deposited in the GenBank database. For rose apples, eight different species were identified within the genera *Hanseniaspora, Pichia, Rhodosporidiobolus,*

TABLE I
Sample distribution by Brix degrees ranges and number of isolates for each selected sample and range.

Range	Number of samples	Number of isolates
Rose Apple		
< 8 ºBrix	2	15
8.1-10 ºBrix	8	25
10.1-12 ºBrix	5	8
Total	15	48
Mango		
≤12 ºBrix	1	10
15-15.9 ºBrix	3	23
16-16.9 ºBrix	2	7
> 20 ºBrix	4	2
Total	10	42

Rhodotorula, Candida and *Clavispora* (Table II, Figure 1). For mango, 11 species were identified, belonging to the genera *Hanseniaspora, Candida, Clavispora, Meyerozyma, Aureobasidium* and *Pichia* (Table II, Figure 1). The sequences of the representative isolates for each morphotype were deposited in the GenBank database (Table II). Groups M6 and M7 could not be identified at the taxonomic level of species by sequence comparison and are therefore reported as candidates for a new species within the genera *Wickerhamomyces* (Figure S1 - Supplementary Material) and *Pichia* (Figure S2), according to the phylogenetic analysis.

According to Figure 1, both fruits shared three yeast species: *Hanseniaspora thailandica, H. opuntiae* and *Clavispora lusitaniae*. The most dominant species in rose apple and mango were *H. thailandica* and *Wickerhamomyces* sp., respectively. Other species, such as *Candida natalensis, Aureobasidium melanogenum, Pichia* sp., *Saturnispora diversa, Hanseniaspora opuntiae* and *H. thailandica* only had one isolate each in mango. In rose apple, the species *Rhodosporidiobolus ruineniae* had one isolate, followed by *A. thailandense, Clavispora lusitaniae* and *H. opuntiae* with two isolates per species.
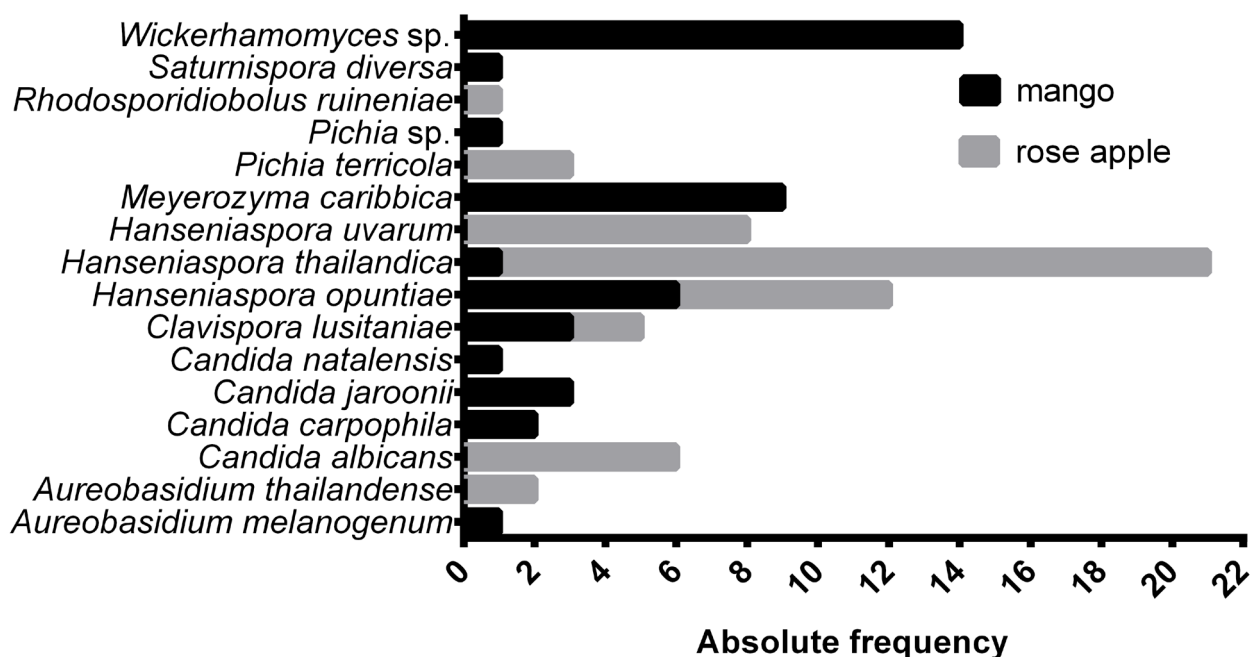
Figure 1 - Absolute frequency of yeast species obtained from mango and rose apple pulps. Each value corresponded to the sum of the strains isolated for each species.

The distribution of species and isolates by ranges of ºBrix was analysed (Figure 2). Isolates of the species *Wickerhamomyces* sp. were found in all ranges of ºBrix for mango. In rose apple, *H. uvarum* was isolated in most ranges, except for values equal to or greater than 20 ºBrix. In the range from 15 to 15.9 ºBrix for mango, 18 isolates from five different species were obtained, which suggests that it is the range of dissolved sugars with the highest yield in terms of richness of ascomycete yeasts (Figure 2). For rose apple, most species, except *Clavispora lusitaniae*, were found in the range of 8.1 to 10 ºBrix, followed by the rank equal to or less than 8 ºBrix, which presented half of the identified species for this fruit.

DISCUSSION

ANALYSIS OF SAMPLING AND GROUPING

In this work two culture media namely YPDA and MEA were used, with the intention of promoting the growth of dominant ascomycetes and basidiomycetes yeasts from mango and rose apple,

respectively. It should be noted that the sampling and development of the research was culture-dependent, so the analyses of different species found are biased by the type of methodology used. According to Baxter and Van der Linde (1999), the MEA is the most suitable medium for the growth of most basidiomycetes. On the other hand, culture media such as GYP or YPD are widely used in methods for obtaining ascomycete yeasts, such as *Saccharomyces cerevisiae* (Ausubel 2002).

The grouping of isolates and the subsequent identification of group representatives is a common practice in laboratories with limited economic resources. According to Vásquez et al. (2016), several characteristics are used for this purpose, depending on the aim of the project, and one or more techniques can be selected at the same time. Among them, the most used is morphological characterization and genetic profiling (for example Microsatellite-Primed-PCR Fingerprinting). Considering the high biodiversity present in Colombia and the little research done on the

TABLE II
Morphotypes and identified yeast species using molecular identification.

Morphotype[1]	Isolates	Number of isolates	Species	Identity with type strain	LSU GenBank accession	ITS GenBank accession
M1	LUM035*, LUM043, LUM045, LUM048, LUM050, LUM051	6	*Hanseniaspora opuntiae*	99%	MH892857	
M2	LUM001*, LUM003*, LUM007, LUM008, LUM011, LUM012,	6	*Meyerozyma caribbica*	99%	MH892850, MH892851	
M4	LUM009*, LUM054, LUM053	3	*Meyerozyma caribbica*	100%	MH892853	
M3	LUM005, LUM006*	2	*Candida carpophila*	100%	MH892852	
M5	LUM024, LUM025*, LUM026*	3	*Clavispora lusitaniae*	99%		MK367571, MK367572
M6	LUM020, LUM022, LUM023*, LUM030, LUM033, LUM034, LUM036, LUM040, LUM046, LUM049, LUM031*, LUM032, LUM037, LUM039	14	*Wickerhamomyces* sp.	92%	JQ682648, JQ682649	
M7	LUM055*	1	*Pichia* sp.	94%		MK256280
M8	LUM042*, LUM044, LUM047	3	*Candida jaroonii*	99%	MH892858	
M10	LUM014*	1	*Candida natalensis*	99%	MH892854	
M11	LUM015*	1	*Aureobasidium melanogenum*	100%	MH892855	
M12	LUM029*	1	*Saturnispora diversa*	99%	MH892856	
M13	LUM052*	1	*Hanseniaspora thailandica*	99%	MH892859	
P1	LUP060*, LUP061, LUP062, LUP063, LUP064, LUP065	6	*Candida albicans*	99%	MH892875	
P2	LUP059*, LUP066	2	*Aureobasidium thailandense*	99%	MH892874	

TABLE II (continuation)

Morphotype[1]	Isolates	Number of isolates	Species	Identity with type strain	LSU GenBank accession	ITS GenBank accession
P3	LUP033*, LUP035 LUP007, LUP015, LUP025, LUP008	6	*Hanseniaspora opuntiae*	99%	MH892866	
P4	LUP020*, LUP030*, LUP036*, LUP058*, LUP070*, LUP068, LUP076, LUP067, LUP071, LUP072, LUP073, LUP074, LUP075, LUP037, LUP034, LUP054, LUP051, LUP052, LUP049, LUP047	20	*Hanseniaspora thailandica*	99%	MH892864, MH892865, MH892867, MH892873, MH892876	
P5	LUP004*, LUP006*, LUP039*, LUP040, LUP041, LUP014, LUP024, LUP021	8	*Hanseniaspora uvarum*	99%	MH892860, MH892861, MH892869, MH892870	
P6	LUP018*, LUP023	2	*Clavispora lusitaniae*	100%	MH892862	
P7	LUP046*, LUP038*, LUP019*	3	*Pichia terricola*	99-100%	MH892872, MH892868, MH892863	
P8	LUP045*	1	*Rhodosporidiobolus ruineniae*	99%	MH892871	

[1]The initial letter of each isolate indicates isolation source: M (mango), P ("pomarroso" or rose apple).
* Sequenced isolates.

yeast-like communities associated with different environments, this work contributes to the knowledge of the dominant yeast species present in fruits, microhabitats with different concentration of dissolved sugars, that can provide a new source of fermentative native isolates, identified through a polyphasic approach.

The type of substrate will select the types of yeast that can grow on it by determining the nutritional sources available and is considered one of the most important ecological factors. In general, yeasts are associated with almost all types of environments, including soil, freshwater, seawater, plants and animals (Deak 2009). According to the results obtained, the culture medium MEA achieved the isolation of both basidiomycetes (with the genus *Rhodosporidiobolus*) and ascomycetes (*H. uvarum, P. terricola* and *S. diversa*), which agrees with Yurkov et al. (2011). When both Phyla were compared, the results showed greater richness and abundance of ascomycete yeasts than basidiomycetes. This result agrees with the literature, since it was expected that more species of ascomycetes would be found in fruits, while basidiomycetes predominate in oligotrophic environments, such as plant structures different from flowers and fruits (Trindade et al. 2002, Carrillo 2003).

According to the previous postulate, the physico-chemical composition of both mango
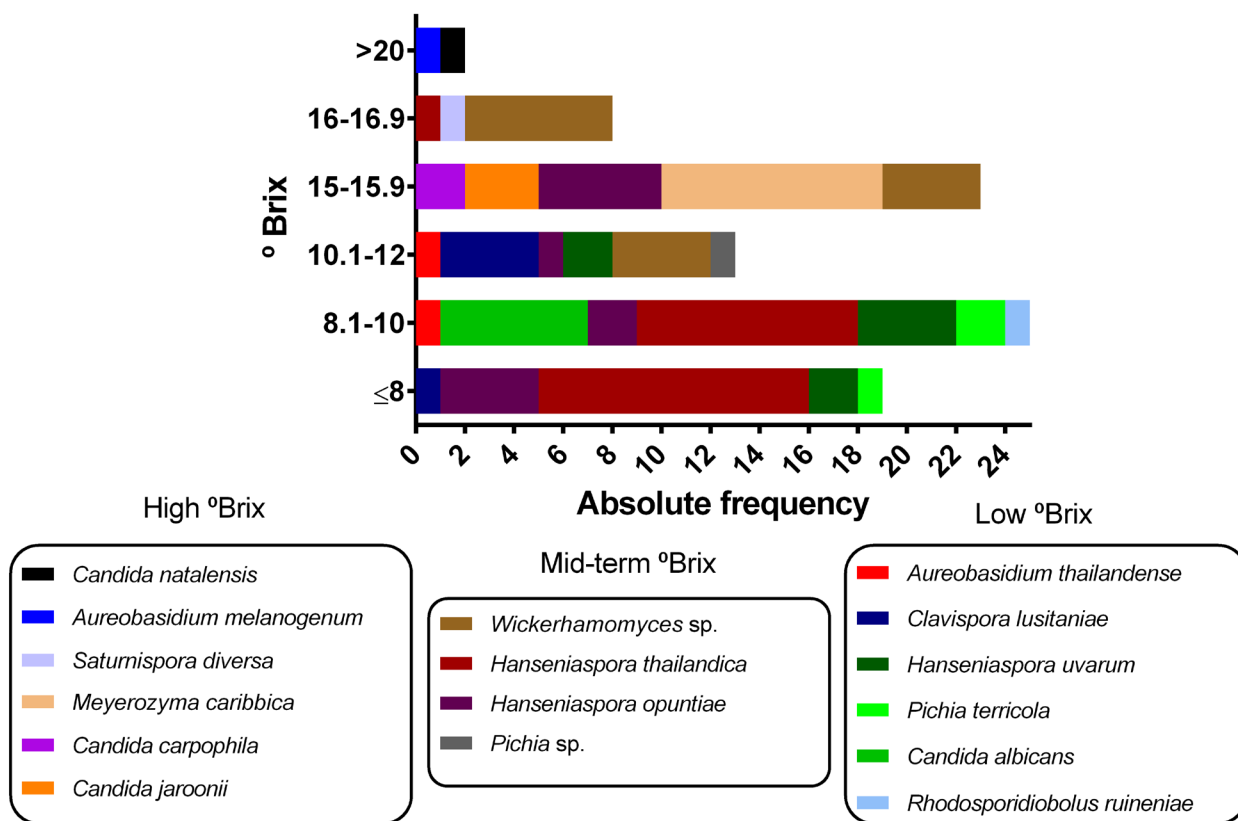
Figure 2 - Absolute frequency of yeast species found in mango and rose apple, classified according to ºBrix for each sample.

(*Mangifera indica*) and rose apple (*Syzygium malaccense*) provides all sources of carbon, nitrogen, minerals, vitamins and growth factors to allow the development of ascomycete yeasts (Deak 2009). This work evaluated and classified the sampled fruits according to the concentration of dissolved solids (ºBrix) and found several ranges varying between 8 and 12 ºBrix for rose apple and 12-20 ºBrix for mango (Figure 2). The results agree with the concentrations of sugars obtained by Somda et al. (2011) and Lu et al. (2018). The species *H. uvarum* and *Wickerhamomyces* sp. were present in a wider range of ºBrix. Nevertheless, *H. uvarum* presented isolates in the lower range (between 8 and 12 ºBrix and specific for rose apple), suggesting that these strains tolerate a lower concentration of sugars. On the other hand, *Wickerhamomyces* sp. presented isolates in the range between 10 and 17 ºBrix, being specific for mango and associated with

higher concentration of sugar content (relatively). The wide range of strains suggests that these species may be able to grow over a broad spectrum, perhaps ensuring their survival for long periods of time. The species *Pichia terricola, Candida albicans* and *Rhodosporidiobolus ruineniae* were isolated only at concentrations below 10 ºBrix, suggesting a possible adaptation to fruits with lower ºBrix.

ANALYSIS OF THE YEAST COMMUNITY

Eleven different species were isolated from mango, two of them correspond to undescribed species. The hypothesis of finding new species of ascomycete yeasts associated with *Mangifera indica* has already been reported. Santos et al. (2015) reported a new species associated with mango called *Ogataea mangiferae*, which was isolated from the phylloplane of this species in Belo Horizonte, Brazil. It should be noted that the

species reported by Santos et al. (2015) and the two species found in this study are not conspecific (identity in sequence less than 85%). On the other hand, nine species (*A. pullulans, C. jaroonii, Cl. lusitaniae, H. thailandica, H. opuntiae, K. marxianus M. caribbica, M. guilliermondii* and *S. diversa*) have been reported in previous studies as associated with fruits worldwide (Kurtzman et al. 2011, Buenrostro-Figueroa et al. 2018, Ting et al. 2018) or at the local level (Lopez-Arboleda et al. 2010, Mambuscay et al. 2013). Several papers reported the genus *Candida* in mango (Suresh et al. 1982, Feoli et al. 1997, Poubol and Izumi 2005). However, this is the first report of *C. natalensis* in fruits. *S. cerevisiae* was not detected in our results despite the enrichment method. The enrichment would probably increase its proliferation. The fact this species was not present suggests that possibly this species is not related with this substrate, as reported in other works (Suresh et al. 1982, Buenrostro-Figueroa et al. 2018).

The different species found in the rose apple pulp are consistent with the results obtained by Trujillo and Echeverry (2015). However, the results of this study identified yeasts up to the taxonomic level of species (using molecular tools), while Trujillo and Echeverry (2015) only managed to identify up to genera based on dichotomous keys. For example, they reported the genera *Hanseniaspora, Candida, Aureobasidium* (as a dimorphic fungus), *Rhodotorula, Meyerozyma* (reported as *Pichia*) and *Clavispora*. This is the first report of *C. albicans* associated to the pulp of rose apple. Since it is a human commensal yeast, and it may become pathogenic under different environmental variables, we considered the possibility of human contamination or non-aseptic conditions, but this notion was disregarded due to the fact that it did not appear associated with the mango pulp, and *C. albicans* was already isolated from environmental substrates at Universidad del Valle (Silva-Bedoya et al. 2014). Finally, the work

of Trujillo and Echeverry (2015) reported yeasts associated with the pulp of rose apple in the city of Neiva (Huila, Colombia), which is latitudinally close to the city of Cali (Valle del Cauca, Colombia), but separated by the Central Mountain range of the Andes, which could involve a geographical barrier for the appearance of unique species for each geographical location.

The genus *Aureobasidium* comprises fungi with yeast-like characteristics and is widely distributed in the world (Gostinčar et al. 2014), having soils, phylloplane, bark and other plant structures, rocks, monuments and limestone as growth substrates (Urzi et al. 1999). It has also been recognized as a pollutant in paper mills, optical lenses and hypersaline habitats or coastal waters (Nagahama 2006, Li et al. 2007). It has several biotechnological applications, mainly in the generation of bioproducts for biocontrol in fruits, grains and vegetables (Di Francesco et al. 2015), as well as to produce enzymes such as amylases, lipases, alkaline proteases, hydroxylases, β-fructofuranosidases, maltosyltransferases, among others (Chi et al. 2009, Wongwatanapaiboon et al. 2016, Turk and Gostinčar 2018). This genus was already reported by Feoli et al. (1997) in their search for microorganisms with pectinolytic activity in mango, together with other species of the genus *Candida*. Feoli et al. (1997) used Saboraud and Nutrient agar to isolate the yeasts, suggesting that the culture media and the enrichment step affected the yeast community.

The genus *Meyerozyma* was described by Kurtzman and Suzuki (2010) as part of the taxonomic reorganization within the Pichiaceae clade. The species *M. guilliermondii* (Formerly *Pichia guilliermondii*) is associated with flowers, fruits and foodstuffs and it is opportunistic in humans and animals (Kurtzman et al. 2011, Corte et al. 2015).

The genus *Hanseniaspora* presented three species, which could correspond to the three

morphotypes found by Trujillo and Echeverry (2015). This genus is widely distributed and frequently isolated from soil, insects and fruit musts or initial stages of fruit fermentation, even at the regional and local level (Lopez-Arboleda et al. 2010, Kurtzman et al. 2011, Gaviria and Osorio 2012, Ramirez-Castrillon et al. 2017). This genus was one of the most abundant in rose apple, fact that may be related with the concentration of dissolved solutes, which is lower when compared to mango. However, the presence of species of this genus is associated with decomposition of fruits or deterioration of fermentations (Morais et al. 1995), or positively with the release of enzymes of organoleptic interest such as glycosidases or xylosidases (Manzanares et al. 1999, Rodriguez et al. 2007). *H. opuntiae* and *H. thailandica* were isolated from both fruits (Figure 1). According to Ting et al. (2018), the *Hanseniaspora* genus is found in several fruit pulps and/or processed fruits, among them fig (Ruiz-Moyano et al. 2016), grapes (Nisiotou and Nychas 2007), pear (Ting et al. 2018), cactus (Cadez et al. 2003, Ganter et al. 2017), kiwi (Niu et al. 2015), pineapple (Lopez-Arboleda et al. 2010, Dellacassa et al. 2017) suggesting its ubiquitous characteristic in this type of substrates. Luan et al. (2018) suggested co-fermentations of *H. opuntiae* together with *S. cerevisiae* in order to obtain organoleptically differentiated wines. In addition, Ruiz-Moyano et al. (2016) suggested *H. opuntiae* as a biocontrol agent for food contaminating moulds.

In the rose apple, *H. thailandica* was the most abundant species, in contrast to mango that only presented an isolate. This species has been reported in different substrates, such as phylloplane (Limtong and Nasanit 2017), lakes (Silva-Bedoya et al. 2014), lichen, flowers and decaying fruits of guava (Jindamorakot et al. 2009). Groenewald et al. (2017) reported this species as one of the most abundant in crop collections associated with fruits. The presence of this species in the lakes of

the Universidad del Valle, as well as in the fruits of plants associated with these lakes, suggest a possible co-location of these species due to the fall of the fruits to the lakes and their subsequent survival in aquatic environments (Silva-Bedoya et al. 2014). Subsequent studies should evaluate if this hypothesis can be established.

R. ruineniae has few reports in South America. This species has only been reported by Guamán-Burneo and Carvajal-Barriga (2009) in forest environments of Ecuador, while Maciel et al. (2013) isolated from coconut water and reconstituted juices in Belo Horizonte, Brazil. It is possible that several taxonomic changes in this species make the traceability of their isolation difficult. However, its report in few works suggests its low frequency or low sampling effort in this type of substrates.

Our results showed the presence of *Cl. lusitaniae* associated with mango and rose apple pulps. The species *Cl. lusitaniae* has been reported previously associated with mango phylloplane (Rao et al. 2008, Limtong and Koowadjanakul 2012) and other plant species, such as cactus (Starmer et al. 1990), fig (*Ficus carica*), guava (*Psidium guajava*) or banana (*Musa paradisiaca*) (Limtong and Koowadjanakul 2012). It has also been reported as the most abundant species associated with acetic rot at the base of the agave leaves used to produce Tequila (Kurtzman et al. 2011). Finally, Trujillo and Echeverry (2015) also found it associated with rose apple in Neiva (Huila, Colombia).

In conclusion, the community of yeasts associated with the fruit pulp of mango and rose apple in the city of Cali (Valle del Cauca, Colombia) reflects a high richness and abundance of species, that varies according to the Brix degrees present in the fruit. The species *H. opuntiae, H. thailandica* and *Cl. lusitaniae* were shared for both types of fruit and the genus *Hanseniaspora* was the most abundant for the rose apple. Two possible new species have been reported belonging to genera *Wickerhamomyces* and *Pichia*, associated

with mango. Several species have been reported with biotechnological interest, either to produce enzymes or organoleptic compounds, or for the transformation of raw material into bioproducts. Finally, some yeast species are usually associated with decaying organic matter, so this type of yeast could colonize fruits for further degradation.

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AUTHOR CONTRIBUTIONS

MRC, wrote the paper, analysed data; LMU, performed research; LMSB, analysed the data; EOC, conceived and designed the study.

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SUPPLEMENTARY MATERIAL

Figure S1 - Phylogeny of yeast species in the *Wickerhamomyces* clade inferred from the D1/D2 domain of the LSU rRNA gene region. The tree backbone was constructed using Maximum Likelihood method. The best substitution model was Tamura-Nei 93 (G+I). Bootstrap percentages over 70% from 1000 bootstrap replicates are shown. Bar = 0.2 substitutions per nucleotide position.

Figure S2 - Phylogeny of yeast species in the *Pichia* clade inferred from the ITS region. The tree backbone was constructed using Maximum Likelihood method. The best substitution model was Kimura-2-Parameter (G+I). Bootstrap percentages over 70% from 1000 bootstrap replicates are shown. Bar = 2 substitutions per nucleotide position.