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ORIGINAL ARTICLE

Yeasts isolated from tropical fruit ice creams: diversity, antifungal susceptibility and adherence to buccal epithelial cells

Leveduras isoladas de sorvetes de frutas tropicais: diversidade, susceptibilidade a antifúngicos e adesão a células epiteliais bucais

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Abstract

Fruit-based ice creams are products widely consumed in tropical countries and, because of their composition, can be a good source for microbial growth, including opportunistic pathogens. The aims of this study were to characterize the yeast populations present in Brazilian fruit-based ice creams, and to investigate the antifungal susceptibility to amphotericin B, fluconazole and itraconazole, and the ability of the isolates that were able to grow at 37 ºC to adhere to buccal epithelial cells (BEC). Two hundred and sixty-seven yeast isolates obtained from the ice cream samples were identified as belonging to 29 species, with counts that ranged from 1.5 to 5.2 log CFU/mL. The predominant species were *Candida intermedia, Torulaspora delbrueckii, C. parapsilosis, Clavispora lusitaniae, Saccharomyces cerevisiae* and *Pichia kudriavzevii.* At least 16 yeast species isolated in this study have been reported as opportunistic pathogens. Forty-one yeast isolates showed resistance or dose-dependent susceptibility to at least one of the antifungal drugs tested. One isolate of *C. parapsilosis* was resistant to all the antifungals tested and showed ability to adhere to BEC. The percentage of adhesion to BEC was high mainly for isolates of *P. kudriavzevii, Meyerozyma guilliermondii, C. parapsilosis, S. cerevisiae* and *Debaromyces hansenii*. The data suggest that the presence of these opportunistic yeasts as contaminants in ice creams may represent a potential risk to the final consumer, especially to immunocompromised individuals who may consume these products.

Keywords: Yeast occurrence; Ice creams samples; Antifungal activity; Adhesion; Pathogens; Opportunistic microorganisms.

Resumo

Os sorvetes à base de frutas são produtos amplamente consumidos em países tropicais e, devido à sua composição, podem ser uma boa fonte de crescimento microbiano, incluindo patógenos oportunistas. Os objetivos deste estudo foram caracterizar as populações de leveduras presentes nos sorvetes brasileiros à base de frutas e investigar sua susceptibilidade aos antifúngicos anfotericina B, fluconazol e itraconazol, e a capacidade dos isolados, que puderam crescer a 37 °C, de aderir em células epiteliais bucais. Duzentos e sessenta e sete isolados de levedura obtidos das amostras de sorvete foram identificados como pertencentes a 29 espécies, com contagens variando de 1,5 a 5,2 log UFC/mL. As espécies predominantes foram *Candida intermedia*, *Torulaspora delbrueckii*, *C. parapsilosis*, *Clavispora lusitaniae*, *Saccharomyces cerevisiae* e *Pichia kudriavzevii*. Pelo menos 16 espécies de leveduras isoladas neste estudo foram relatadas como agentes patogênicos oportunistas. Quarenta e um isolados de leveduras apresentaram resistência ou susceptibilidade dose-dependente a pelo menos um dos antifúngicos testados. Um isolado de *C. parapsilosis* foi resistente a todos os antifúngicos testados e mostrou capacidade de aderir a células epiteliais bucais. A porcentagem de adesão foi alta principalmente para isolados de *P. kudriavzevii*, *Meyerozyma guilliermondii*, *C. parapsilosis*, *S. cerevisiae* e *Debaromyces hansenii*. Os dados sugerem que a presença dessas leveduras oportunistas como contaminantes nos sorvetes pode representar um risco para o consumidor final, especialmente para indivíduos imunocomprometidos que podem consumir esses produtos.

Palavras-chave: Ocorrência de leveduras; Amostras de sorvetes; Atividade antifúngica; Adesão; Patógenos; Microrganismos oportunistas.

1 Introduction

Ice cream is a major product of the dairy industry and is widely consumed in tropical countries due to the high temperatures in these regions (Farias et al., 2006; Arbuckle, 2013; Lima et al., 2016). It is prepared by freezing a mixture of pasteurized milk products (milk, condensed milk, milk powder and cream), sugar, emulsifiers, stabilisers, flavouring and colouring agents, which are combined in a desirable proportion (Ahmed et al., 2009; Lee et al., 2009; Ambily & Beena, 2012). Ice creams, usually made from dairy products, are often combined with fruits. In Brazil, tropical fruits that are commonly used in the preparation of fruit-based ice cream include mango, pineapple, passion fruit, strawberry and guava. Fruit-based ice creams are especially susceptible to yeast contamination since the unprocessed fruit pulps used in the ice cream preparation frequently have high counts of these microorganisms (Trindade et al., 2002). The high content of nutrients like lactose, sugar and proteins, and the final pH, make them an excellent growth medium for yeasts, even though stored at low temperatures (Lee et al., 2009; Ambily & Beena, 2012). These products may represent a specialised ecological environment for the selective occurrence and growth of certain yeast species, such as *Debaryomyces hansenii (=Candida famata*), *Naganishia albida (=Cryptococcus albidus), Saitozyma flava (=Cryptococcus flavus), Rhodosporidium glutinis (=Rhodotorula glutinis)* and *Rhodotorula mucilaginosa,* which have been isolated from ice creams (Fleet & Mian, 1987).

The presence of pathogens may cause serious disease outbreaks in humans who consume contaminated ice cream (Jadhav & Raut, 2014; Lima et al., 2016). The consumption of contaminated ice creams has led to fungal outbreaks in Asia, Europe and North America (Chugh, 1996; Djuretic et al., 1997; Jadhav & Raut, 2014). In Brazil, the relatively simple manufacturing process, combined with a lack of obligation to register the product, has increased the number of factories characterised by deficient sanitary-hygienic practices (Lima et al., 2016). Moreover, the use of unprocessed fruit and the absence of thermal treatment contribute to the presence of pathogens in the product (Jadhav & Raut, 2014; Lima et al., 2016).

The consumption of contaminated foods can be a risk to vulnerable age groups, such as children and elderly people, and also to immunosuppressed patients. Hence, it is necessary to maintain a high microbiological safety standard in these products (Kanbakan et al., 2004). In addition, the presence of high counts of yeast species that are not included in the microorganisms specified by the Brazilian sanitary

legislation, may represent a risk to consumers, since many of these species have been reported as spoilage microorganisms or opportunistic pathogens (Kurtzman et al., 2011; Maciel et al., 2013). Low pH values (3.5-5.5) and high sugar levels are factors that can promote the development of yeasts in ice creams (Lima et al., 2016). The yeast species found in food are almost entirely non-pathogenic (Tribst et al., 2009; Sundh & Melin, 2011; Maciel et al., 2013). However, opportunistic pathogenic species including *Candida parapsilosis* and *Pichia kudriavzevii (*formerly *C. krusei*) have been isolated from food and beverages (Stratford, 2006; Sundh & Melin, 2011; Maciel et al., 2013). Environmental strains of opportunistic yeast species can be resistant to antifungal drugs and could be a risk to consumers (Brilhante et al., 2013; Maciel et al., 2013). The adhesion of the pathogenic yeasts to host cells or abiotic surfaces is considered an important virulence factor and is essential for colonisation and survival in the host (Johann et al., 2007; Höfs et al., 2016).

The aim of this study was thus to characterise the yeast communities present in tropical fruit-based ice creams obtained from the Brazilian market. In addition, the antifungal resistance of the species able to grow at 37 °C was investigated, which could be considered a prerequisite phenotype for invasive mycoses (Perfect, 2006), and the ability of these yeasts to adhere to buccal epithelial cells (BEC) was also tested.

2 Material and methods

2.1 Sample collection and yeast isolation

Fifty-one fruit-based ice cream samples were obtained from seven commercial establishments with industrial production and seven with artisanal production. The fruit-based ice cream samples obtained included 12 pineapple (*Ananas comosus*), 11 açaí (the Amazon fruit *Euterpe oleracea*), five guava (*Psidium guajava*), nine mango (*Mangifera indica*) and 14 passion fruit (*Passiflora edulis*) ice creams. The samples were obtained in the municipality of Belo Horizonte, Minas Gerais state, Brazil, from July 2010 to January 2011. They were placed in disposable plastic cups that were provided by the establishments and then immediately transferred to sterile flasks, transported to the laboratory in isothermal packaging and examined within 24 hours.

Aliquots (10 mL) of each sample were diluted in 90 mL sterile 0.1% buffered peptone water before inoculation. To determine the yeast population counts, triplicate aliquots (0.1 mL) of appropriate decimal dilutions were spread on yeast extract–malt extract agar (YMA; 1% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2% agar) supplemented with 10 mg% chloramphenicol. The plates were incubated at 25 °C for 3 to 10 days. After growth, the different yeast morphotypes were enumerated and representative colonies of each morphotype were purified by repeated streaking on YMA plates. The purified samples were maintained at −80 °C prior to identification.

2.2 Yeast identification

The yeasts were characterised morphologically and physiologically by standard methods (Kurtzman et al., 2011). Growth at 37 °C was assessed on glucose-yeast nitrogen base agar and the results recorded after 4 days (Kurtzman et al., 2011). Isolates with identical morphological and physiological characteristics were grouped together and subjected to PCR fingerprinting using the primer EI1 (5'-CTGGCTTGGTGTATGT-3') (Lopes et al., 1998). Yeast strains with identical PCR fingerprint patterns were grouped together and putatively considered to belong to the same species (Lopes et al., 1998; Canelhas et al., 2011). At least 50% of the yeast isolates of each molecular group were identified by sequencing of the internal transcribed spacer (ITS)-5.8S region and the D1/D2 variable domains of the large subunit of the rRNA gene, as described previously (Lachance et al., 1999). The amplified DNA was concentrated, cleaned, and sequenced in an ABI 3130 Genetic Analyzer automated sequencing system using BigDye v3.1 and the POP7 polymer. The sequences obtained were compared with those

in the GenBank database using the Basic Local Alignment Search Tool – BLAST (National Center for Biotechnology Information, 2018; Altschul et al., 1997).

2.3 Antifungal drug susceptibility

Yeast susceptibility to amphotericin B (Sigma, USA), itraconazole (Jansen Pharmaceutical, Belgium) and fluconazole (Pfizer Pharmaceutical, USA) was determined for the isolates able to grow at 37 °C. The determinations were made using the broth microdilution method in accordance with the Clinical Laboratory Standards Institute protocol M27-A3 (Clinical and Laboratory Standards Institute, 2008). Yeast suspensions were prepared by the spectrophotometric method, with a final inoculum of $(1.5 \pm 1.0) \times 10^3$ cells/ml. A 100 µL aliquot of yeast suspension was added to each well of the microdilution trays. The final concentrations of fluconazole ranged from 0.125 to 64 µg/mL and the final concentrations of itraconazole and amphotericin B ranged from 0.031 to 16 µg/mL. The plates were incubated at 35 °C and the minimum inhibitory concentration (MIC) endpoints were read visually after 24 and 48 h. Drug and yeast-free controls were included in all the experiments.

After incubation, the MICs of amphotericin B were read as the lowest concentration at which no cell growth was visualised. The MICs of the other drugs were read as the lowest concentration at which a prominent decrease (approximately 80%) in turbidity relative to that in the control well was observed. The reference values used for the susceptibility tests in vitro (µg/mL) were those published by Clinical and Laboratory Standards Institute (2008).

2.4 Yeast adhesion to BEC

Yeast cells were suspended at 10^6 cells/mL in a 1 mL volume for each yeast isolate and 4 mL of Sabouraud dextrose broth subsequently added. These samples were incubated at 37 °C for 1 hour with agitation at 120 rpm, and then centrifuged for 10 minutes at 3,000 × g, washed twice with PBS and finally suspended in 3 mL of PBS.

The method described by Kimura & Pearsall (1978) and Ellepola & Samaranayake (1998a, b), and modified by Johann et al. (2007), was used in the preparation of buccal epithelial cells (BECs) for the adherence assays. To this end, BECs from healthy adults were collected in the morning with the aid of sterile swabs and dispensed in 10 mL of PBS. The BEC suspensions were washed four times with PBS and collected by centrifugation at 3,000 × g for 10 minutes before being resuspended in PBS at a final concentration of 10^5cells/mL. Once prepared, 0.5 mL of BEC suspension and 0.5 mL of yeast suspension were mixed gently and incubated in a rotary shaker at 37 °C for 1h. At the end of this period, each solution containing BEC was filtered through polycarbonate filters (pore size 12 µm), washed with 100 mL of PBS to remove non-adhered yeasts and the filters placed on glass slides. The preparations were air-dried, fixed with heat, stained with crystal violet and the yeasts adhered to the buccal cells counted under a light microscope (×40). For each slide, 50 BEC were evaluated and the number of associated yeast cells counted. Clumped, folded or overlapping BECs were excluded.

3 Results and discussion

Two hundred and sixty-seven yeast isolates belonging to 29 species were isolated from the fruit-based ice creams. The yeast populations ranged from 1.5 to 5.2 log colony forming units (CFU)/mL. Table 1 details the occurrence of yeasts in the ice creams analysed in the present study.

Table 1. Yeast species counts (CFU/mL) and the number of positive samples isolated from ice creams made with fruit juices or fruit pulps.

Yeast species	Ice cream (n=51)
Aureobasidium pullulans	1.5 - 1.8 (8)
Candida akabanensis (*Metschnikowia* clade)	3.1 (1)
C. etchellsii (*Starmerella* clade)	5.2 (1)
C. intermedia (*Metschnikowia* clade)	1.5 - 5.2 (28)
C. orthopsilosis (*C. albicans/Lodderomyces* clade)	3.22 (1)
C. pararugosa (*Wickerhamiella* clade)	3-4 (9)
C. parapsilosis (*C. albicans/Lodderomyces* clade)	1.82 - 4.63 (17)
C. quercitrusa (*Kurtzmaniella* clade)	1.8 (1)
C. sake (*C. albicans/Lodderomyces* clade)	4.1 (1)
Clavispora lusitaniae	3.2 - 4.6 (15)
Papiliotrema flavescens	3.3 - 4.4 (2)
Pa. laurentii	1.5 (1)
Debaryomyces hansenii	1.5 - 4 (4)
Meira argovae	1.5 (1)
Meyerozyma guilliermondii	3.7 - 4.1 (2)
Pichia kudriavzevii	1.5 - 3.6 (10)
P. manshurica	1.5 - 1.9 (2)
P. membranifaciens	1.5 (1)
P. norvegensis	4.0 (1)
P. occidentalis	3.2 (1)
Rhodotorula mucilaginosa	1.5 - 3.5 (3)
Saccharomyces cerevisiae	1.5 - 4.9 (14)
Torulaspora delbrueckii	1.5 - 4.8 (26)
Trichosporon faecal	1.5 (1)
Trichosporon ovoides	3.1 - 4.4 (4)
Trichosporon sp.	1.5 (2)
Wickerhamomyces anomalus	1.8 (1)
Zygoascus hellenicus	3.5 (1)
Zygosaccharomyces bailii	3.2 - 3.8 (2)

n: number of samples; ( ) number of samples in which the species was isolated.

The prevalent yeast species isolated from the ice creams were *Candida intermedia, Torulaspora delbrueckii, C. parapsilosis, Clavispora lusitaniae, Saccharomyces cerevisiae* and *Pichia kudriavzevii.* Most of these can be considered as spoilage agents and have been isolated from beverages and foods (Trindade et al., 2002; Tribst et al., 2009; Kurtzman et al., 2011; Maciel et al., 2013). Thirteen yeast species occurred only in one ice cream sample and could be considered as accidental contaminants of these products. These species were *C. akabanensis, C. etchellsii, C. orthopsilosis, C. quercitrusa, C. sake, Papiliotrema laurentii, Meira argovae, P. membranifaciens, P. norvegensis, P. occidentalis, Trichosporon faecale*, *Wickerhamomyces anomalus* and *Zygoascus hellenicus*. Some of these yeasts can be found associated with flowers, fruits, plant surfaces and insects (Kurtzman et al., 2011). Yeast contamination of ice creams can be caused by inappropriate storage and handling conditions or associated with the fruit pulps used in the preparation of these foods.

At least 16 yeast species isolated in this study have been reported as opportunistic pathogens (Miceli et al., 2011; Maciel et al., 2013; Taj-Aldeen et al., 2014). *Candida intermedia, C. orthopsilosis, C. pararugosa, C. quercitrusa, C. sake, P. kudriavzevii, Cl. lusitaniae,* and *W. anomalus* have been associated with life-threatening infections in immunocompromised hosts (Kurtzman et al., 2011; Miceli et al., 2011; Maciel et al., 2013; Taj-Aldeen et al., 2014; Oliveira et al., 2014; Westblade et al., 2015; Fernández-Ruiz et al., 2017) and hence their detection in the ice cream samples could indicate a risk to consumers in Brazil. *C. parapsilosis* is the third most common *Candida* species in Brazil which causes invasive fungal infections (Colombo et al., 2006). *S. cerevisiae* and *Pa. laurentii* (former *Cr. laurentii*), which were both found in ice creams in this

study, may act as opportunistic agents and cause infections in their hosts (Anoop et al., 2015; Thomson et al., 2017; Martínez et al., 2017).

Considering that many of the yeasts found in the present study are species with several reports of systemic disease, mainly in immunocompromised patients, it was of interest to examine the yeast isolates for indications that they may really pose a threat to human health. For this reason, 68 yeast isolates obtained from ice cream samples that were able to grow at 37 °C, were tested to determine their susceptibility to the antifungal agents fluconazole, itraconazole and amphotericin B (Table 2). The capacity of the yeast isolates that were resistant or displayed a dose-dependent susceptibility to the antifungal agents to adhere to BEC was also tested, since adherence is an essential step in fungal pathogenesis and is an important virulence factor of pathogenic yeasts (Table 3) (Johann et al., 2007; Costa et al., 2012).

Table 2. Minimum inhibitory concentrations (µg/mL) of fluconazole, itraconazole and amphotericin B against fruit ice cream yeast isolates able to grow at 37 °C.

Yeast species	Ice cream made with fruit juices or pulps (n=79)[a]	Fluconazole (µg/mL)	Itraconazole (µg/mL)	Amphotericin B (µg/mL)
Candida etchellsii	1	0.5 (1-0-0)[b]	0.031 (1-0-0)	0.031 (1-0-0)
C. intermedia	10	0.25 - 4 (10-0-0)	0.031 - 0.125 (10-0-0)	0.031 - 0.5 (10-0-0)
C. parapsilosis	13	0.25 - ≥ 64 (11-0-2)*	0.031 - 16 (11-1-1)*	0.25 - 16 (10-0-3)*
C. pararugosa	5	0.125 - 8 (5-0-0)	0.031 - 0.062 (5-0-0)	0.031 - 2 (4-0-1)*
C. sake	1	0.25 (0-0-1)*	0.031 (1-0-0)	0.031 (1-0-0)
Clavispora lusitaniae	10	0.25 - 1 (10-0-0)	0.031 - 0.125 (9-0-1)*	0.125 - 8 (9-0-1)*
Debaryomyces hansenii	3	1 - 2 (3-0-0)	0.031 - 0.062 (3-0-0)	1 - 2 (1-0-2)*
Meyerozyma guilliermondii	2	1 - 4 (2-0-0)	0.5 (0-0-2)*	0.5 - 2 (1-0-1)*
Pichia kudriavzevii	3	32 - 64 (0-2-1)*	0.25 - 0.5 (0-3-0)*	2 - 16 (0-0-3)*
P. manshurica	1	64 (0-0-1)*	0.062 (1-0-0)	16 (0-0-1)*
P. norvegensis	1	32 (0-1-0)*	0.125 (1-0-0)	1 (1-0-0)
P. occidentalis	1	0.125 (1-0-0)	0.5 (0-1-0)*	0.031 (1-0-0)
Saccharomyces cerevisiae	11	0.5 - 16 (10-1-0)*	0.031 - 4 (5-0-6)*	0.031 - 1 (9-0-2)*
Torulaspora delbrueckii	14	2 - 64 (13-0-1)*	0.031 - 0.125 (14-0-0)	0.031 - 0.5 (14-0-0)
Trichosporon ovoides	1	0.125 (1-0-0)	0.031 (1-0-0)	0.062 (1-0-0)
Trichosporon sp.	1	2 (1-0-0)	0.125 (1-0-0)	1 (1-0-0)
Wickerhamomyces anomalus	1	64 (0-0-1)*	1 (0-0-1)*	0.25 (1-0-0)

[a]The number of yeast isolates tested. [b]The first number in parenthesis represents the number of susceptible isolates; the second number represents the number of dose-dependent susceptible isolates; the third number represents the number of resistant isolates. *Species that showed resistance or dose-dependent susceptible isolates are shown with asterisks.

Table 3. Adhesion of the yeast isolates from ice creams to buccal epithelial cells.

Yeast species*	Number of adherent yeast cells	Percentage of adhesion (%)	Resistant to	Dose-dependent susceptibility to
Candida parapsilosis (3)	7-10	22.5-32.2	Amphotericin B, Fluconazole, Itraconazole	Itraconazole
C. sake (1)	2	6.4	Fluconazole	
C. pararugosa (1)	2	6.4	Amphotericin B	
Clavispora lusitaniae (1)	6	19.3	Amphotericin B, Itraconazole	
Debaryomyces hansenii (1)	8	25.8	Amphotericin B	
Meyerozyma guilliermondii (1)	11	35.4	Amphotericin B, Itraconazole	
Pichia kudriavzevii (2)	2-14	6.4-45.1	Amphotericin B, Fluconazole	Fluconazole, Itraconazole
P. manshurica (1)	1	3.2	Fluconazole, Amphotericin B	
Saccharomyces cerevisiae (5)	1-9	3.2-29.0	Itraconazole, Amphotericin B	Fluconazole
Wickerhamomyces anomalus (1)	5	16.1	Fluconazole, Itraconazole	

*Number in parenthesis represents the number of yeast isolates tested.

Nearly all the yeasts obtained from the fruit ice creams were susceptible to fluconazole, except isolates of *C. sake*, *C. parapsilosis, P. kudriavzevii, P. manshurica, P. norvegensis, T. delbrueckii* and *W. anomalus*,

which were resistant to this antifungal agent and presented MIC values that ranged from 0.25 to 64 µg/mL (Table 2). One isolate of *P. kudriavzevii*, resistant to fluconazole, presented the highest percentage of adhesion to BEC (45.1%). This isolate was also resistant to amphotericin B (Table 3).

Resistance to itraconazole was observed in 11 isolates belonging to the species *C. parapsilosis, Cl. lusitaniae, M. guilliermondii, S. cerevisiae* and *W. anomalus*, with MIC values ranging from 0.031 to 16 µg/mL (Table 2). One isolate of *C. parapsilosis* and two isolates of *P. kudriavzevii* were susceptible to itraconazole in a dose-dependent manner, and showed adhesion rates of 32.2% and 45.1%, respectively, to BEC (Table 3).

Fourteen isolates displayed resistance to amphotericin B, identified as *C. parapsilosis* (n=3), *C. pararugosa* (n=1), *Cl. lusitaniae* (n=1), *D. hansenii* (n=2), *M. guilliermondii* (n=1), *P. kudriavzevii* (n=3), *P. manshurica* (n=1) and *S. cerevisiae* (n=2), with MIC values ranging from 0.125 to 16 µg/mL. None of the isolates obtained from ice creams showed dose-dependent susceptibility to amphotericin B.

In summary, 41 yeast isolates from ice creams exhibited resistance or dose-dependent susceptibility to at least one of the antifungal drugs tested (Table 2). One isolate of *C. parapsilosis* (SL.17.7) was resistant to all three anti-fungal agents and was also able to adhere to BEC (adherence rate of 25.5%) (Table 3). *C. parapsilosis* is an opportunistic commensal responsible for various mycoses (Miceli et al., 2011). The finding of antifungal-resistant strains of *C. parapsilosis* is particularly interesting, because it is the third most frequent agent of candidemia in Brazil (Colombo et al., 2006). Trofa et al. (2008) and Pinhati et al. (2016) also reported that *C. parapsilosis* is an emerging human pathogen that has dramatically increased in significance and prevalence over the past three decades, becoming one of the leading causes of invasive candidiasis worldwide. Individuals at highest risk for severe yeast infections include neonates and patients in intensive care units, as well as patients with cancer and neutropenia (Trofa et al., 2008; Pinhati et al., 2016; Villalobos et al., 2016). Although it is difficult to evaluate the risk associated with the consumption of contaminated food, the presence of many strains with varying susceptibility to the three commonly used antifungal drugs suggests a potential threat to consumers such as immunocompromised individuals, who consume these products.

Only a few classes of antifungal drugs are available, so the emergence of resistance to single drug classes and to multidrug resistance greatly hampers patient management. The mechanisms of antifungal drug resistance are mostly shared by both resistant strains displaying inherently reduced susceptibility and by those acquiring resistance during therapy. The molecular mechanisms that cause drug resistance are naturally occurring in less susceptible species and are acquired in strains of susceptible organisms. These mechanisms include altered drug affinity and target abundance, reduced intracellular drug levels mediated by efflux transporters, and permeability barriers associated with biofilms. Genetic factors regulating these mechanisms, as well as cellular factors important for stress adaptation, are also important to better understand the emergence of antifungal drug resistance (Cowen et al., 2014).

The adherence to BEC, which was highest for *P. kudriavzevii* isolates, followed by *M. guilliermondii, C. parapsilosis, S. cerevisiae* and *D. hansenii*, is an important factor that can be evaluated. Successful colonisation and infection by a microorganism often depends on the initial capacity to adhere to host tissues (Trofa et al., 2008). The production of enzymes and the ability to adhere to epithelial and endothelial cells have been studied thoroughly as virulence factors in opportunistic *Candida* species and in other groups of yeasts. The adhesion of micro-organisms to host mucosal surfaces is a prerequisite for colonization and infection (Lyon & Resende, 2006). The presence of these yeasts as contaminants in ice creams may represent a risk to consumers.

Finally, the results suggest that fruit-based ice creams may represent a potential risk to consumers in Brazil, especially to vulnerable age groups and immunosuppressed patients. It is difficult to evaluate the risk associated with the consumption of contaminated food. Yeast contaminants that can affect the ice cream quality may also represent a risk to humans, and may have originated from poorly sanitized equipment,

contaminated water and other raw materials, combined with the absence of an appropriate thermal treatment. These microorganisms may act as opportunistic pathogens and cause serious infections in humans (Kurtzman et al., 2011; Miceli et al., 2011; Maciel et al., 2013; Taj-Aldeen et al., 2014; Oliveira et al., 2014; Westblade et al., 2015; Fernández-Ruiz et al., 2017). In Brazil, the lack of sanitary inspection and obligation to register this kind of product has increased the number of factories with deficient sanitary-hygienic practices.

4. Conclusion

The results led to the characterization of the yeast communities present in tropical fruit-based ice creams obtained on the Brazilian market. In addition, yeast species that may be considered pathogenic microorganisms were isolated. The presence of pathogenic yeasts resistant to the three most commonly used antifungal drugs represents a potential threat to the consumer. These findings also suggest that sanitary rules and hygienic practices have not been properly adopted during the manufacturing, transport and storage stages of the products analysed. Thus this work provides an alert to the potential risk posed by these frozen products to public health.

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