Diversity and antifungal susceptibility of yeasts isolated by multiple-tube fermentation from three freshwater lakes in Brazil

Luciana R. Brandão, Adriana O Medeiros, Mariana C. Duarte, Anne C. Barbosa and Carlos A. Rosa

ABSTRACT

The diversity and antifungal resistance of yeasts able to grow at 37°C and the occurrence of bacterial indicators of water quality were studied in three lakes in Southeastern Brazil. The densities of yeasts, *Escherichia coli, Enterococcus* spp. and *Pseudomonas aeruginosa* were determined by the multiple-tube fermentation technique, and counts of heterotrophic bacteria were determined using the pour plate method. The yeasts were identified using physiological and molecular techniques and their resistance to amphotericin B, itraconazole and fluconazole was tested. Yeast occurrence was significantly correlated only with the density of fecal coliforms. *Candida krusei*, *C. guilliermondii* and *C. tropicalis*, the most frequently isolated yeast species, are associated with fecal contamination of water by warm-blooded animals. Yeast isolates were most resistant to amphotericin B (21.7%), followed by itraconazole (20%) and then fluconazole (2.8%). In addition to tests for the fecal coliform group, the density of yeasts grown at 37°C could be used as a complementary microbial indicator that aquatic environments contain organic matter of human origin. The incidence of yeast species resistant to three antifungal drugs shows that these microorganisms could pose a health risk to the people who use these lakes for recreation.

Key words | antifungal, freshwater, multiple-tube fermentation technique, water quality, yeasts

Luciana R. Brandão
Mariana C. Duarte
Anne C. Barbosa
Carlos A. Rosa (corresponding author)
Departamento de Microbiologia,
ICB, Universidade Federal de Minas Gerais,
C. P. 486,
Belo Horizonte MG 31270-901,
Brazil
Tel.: 55 31 34092751
E-mail: *carlrosa@icb.ufmg.br*

Adriana O Medeiros
Departamento de Botânica,
IB, Universidade Federal da Bahia,
Salvador BA 40170-115,
Brazil

INTRODUCTION

Yeast communities in aquatic environments are highly diverse, and many species may behave as opportunistic pathogens (Hagler & Ahearn 1987). Yeasts such as *Candida tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii* and *C. glabrata*, often isolated from water environments, have been frequently reported as etiological agents of candidiasis (Pfaller *et al.* 2003). Studies on freshwater yeasts have been focused mostly on the application of these microorganisms as organic pollution indicators (Hagler 2006; Nagahama 2006). Some studies have shown a correlation between the occurrence of yeasts in aquatic environments and the presence of fecal pollution indicators (Arvanitidou *et al.* 2002, 2005; de Almeida 2005; Hagler 2006; Medeiros *et al.* 2008). Yeasts could be used as indicators of sewage contamination and recreational water quality as a complement to the coliform counts currently used as indicators of recent fecal pollution (Hagler 2006). Selective methods have been suggested for the isolation of human-associated yeasts, based mainly on incubation at 37°C (Buck 1975; Buck *et al.* 1977; Hagler *et al.* 1986; Hagler 2006). However, few studies have attempted to correlate the occurrence of yeast species able to grow at 37°C in freshwater environments with fecal contamination. Yeast populations respond quickly to organic contamination, and some species could be used as indicators of nutrient enrichment in aquatic environments.

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Many opportunistic yeast species are resistant to a great variety of antifungal drugs. Medeiros *et al.* (2008) reported the occurrence of several yeast species from aquatic environments that showed resistance to antifungal drugs. Yeasts resistant to these drugs could indicate a health risk for the people who use these waters for recreation.

The primary aim of this study was to evaluate the microbiological water quality of three freshwater lakes in Southeastern Brazil in relation to the population levels of yeasts grown at 37°C, as well as *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa* and heterotrophic bacteria. Additionally, we aimed to correlate the yeast counts with the numbers of indicator bacteria of water quality, and to investigate the resistance of yeast species to three antifungal drugs.

MATERIAL AND METHODS

Study area

The samples were collected in three lakes in the paleo-karstic region of the Lagoa Santa plateau in the state of Minas Gerais in Southeastern Brazil (Figure 1). This region is one of the most important carbonate karstic landscapes of Brazil and has great archeological, ecological and tourist potential (Berbet-Born 2000). Lagoa Santa is a paleo-karstic urban lake and is located in center of Lagoa Santa city (19°38′S, 43°53′W). This lake suffers from poor sanitation and eutrophication due to anthropogenic activities, with drastic alterations of the original biota previously recorded (Barbosa *et al.* 1993). The lake of Sumidouro is located in an environmental protection area (APA Karst Lagoa

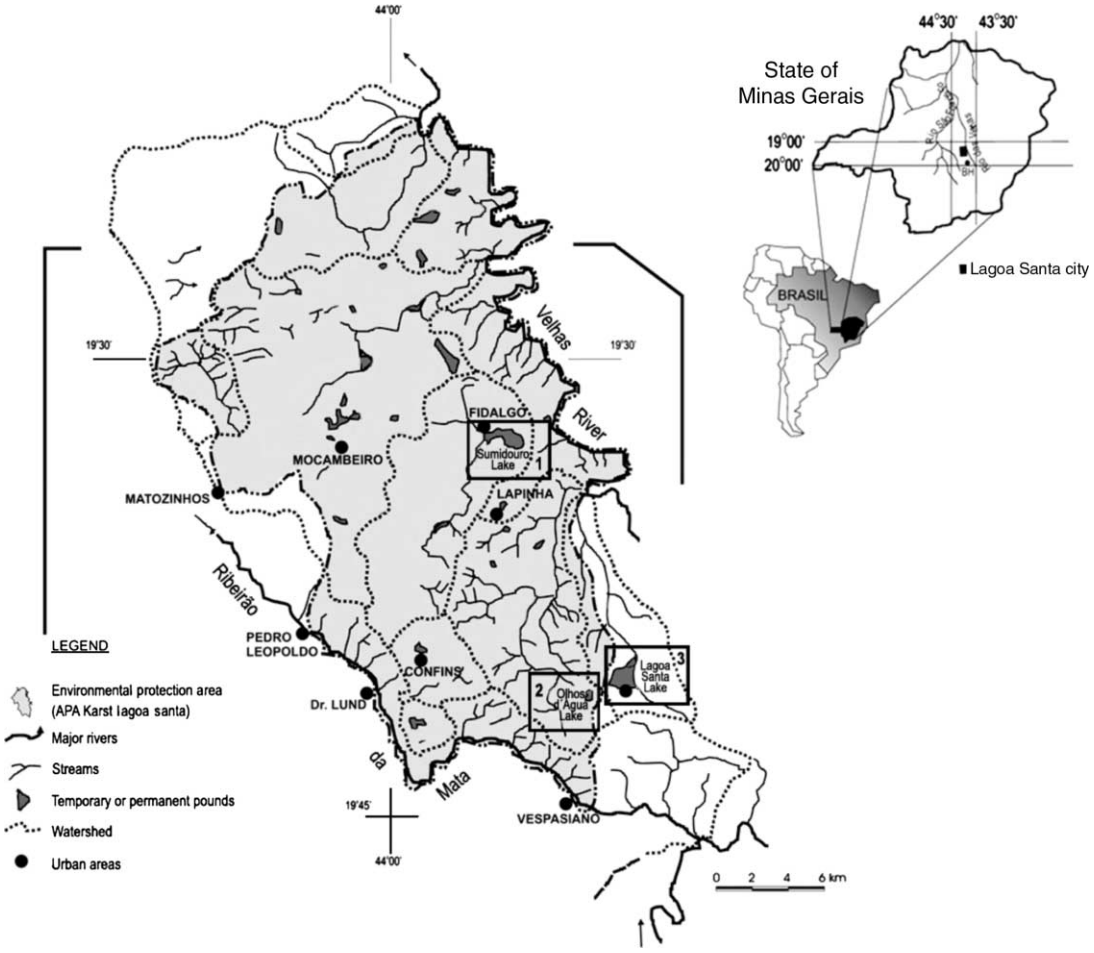

Figure 1 | Map showing the locations of the three lakes studied in Southeastern Brazil. (1) Sumidouro Lake. (2) Olhos D'água Lake. (3) Lagoa Santa Lake (Berbet-Born 2000).

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Santa; 19°32′S, 43°47′W). However, anthropogenic activities such as the construction of houses, tourism, recreational activities and animal farming have affected the environmental quality of this lake during the last 10 years. The Olhos D'Água Lake is considered to be a paleo-karstic lake and is situated in a peri-urban area within the APA of Karst Lagoa Santa. This lake also suffers from different anthropogenic impacts, e.g., growing urbanization and increasing siltation. On the Lagoa Santa plateau, the dry season occurs from May to September and the rainy season from October to April (Madeira & Fernandes 1999).

Sample collection

Using sterile bottles, subsurface water samples from three different points in the littoral zone of each lake were collected monthly, from September 2005 to August 2006. The samples were stored on ice and transported to the laboratory within 8 hours for processing.

Bacteria of sanitary interest

The densities of *E. coli, Enterococcus* spp. and *P. aeruginosa* were determined by the standard multiple-tube technique (Eaton *et al.* 2005). *Escherichia coli* was enumerated using lauryl tryptose broth (Difco, USA) for the presumptive phase, and EC MUG broth (Difco) incubated at 44.5 ± 0.2°C for the confirmatory phase. For the enumeration of *P. aeruginosa*, asparagine and acetamide broths were used. Chromocult Enterococci broth (Merck, Germany) was used for enumeration of *Enterococcus* spp. The results were expressed as the most probable number per 100 ml of water samples (MPN/100 ml). The counts of heterotrophic bacteria were performed using the pour plate method in NWRI agar (peptone 0.3%, soluble casein 0.05%, K_2HPO_4 0.02%, $MgSO_4$ 0.005%, $FeCl_3$ 0.0001% and agar 1.5%) after 48 h of incubation at 37°C (Eaton *et al.* 2005).

Yeast isolation and identification

Yeast quantification was done by the multiple-tube fermentation technique described for members of the coliform group (Eaton *et al.* 2005) with modifications. Volumes of 10, 1 and 0.1 ml of water samples were inoculated in three series of five tubes each of Sabouraud broth (glucose 2%, peptone 1%, yeast extract 0.5% and chloramphenicol 0.02%) that contained small inverted tubes (Durham tubes) to collect any gas that may be produced. The 10-ml volumes of water were inoculated in five tubes with double the concentration of Sabouraud broth, whereas the sample volumes of 1 and 0.1 ml of water were inoculated in two sets of five tubes of regular Sabouraud broth. The tubes were incubated at 37°C for 5 days. After this time, the tubes with microbial growth and/or gas were considered putatively positive for yeast presence, and a loopful of each was streaked on plates with Sabouraud agar containing 0.02% chloramphenicol. The plates were incubated at 37°C for 5 days and the MPN of yeasts able to grow at 37°C was determined from plates on which yeast growth was confirmed. Each yeast morphotype was isolated, purified and maintained at − 80°C for later identification.

Yeasts were characterized by standard methods (Yarrow 1998). The identifications followed the taxonomic keys of Kurtzman & Fell (1998). The PCR fingerprint technique using the primer EI1 (5′-CTGGCTTGGTGTATGT-3′) (de Barros Lopes *et al.* 1998) was used for the preliminary identification of the yeasts. Authentic or type strains of the following yeast species were used as standards for fingerprinting profile comparison: *C. albicans* UFMG 9003, *C. dubliniensis* CBS 7987, *C. glabrata* NCYC 388, *C. guilliermondii* UFMG L-207, *C. krusei* ATCC 20298, *C. lusitaniae* CBS 6936, *Candida parapsilosis* UFMG M2 and *C. tropicalis* ATCC 750.

The identities of yeast strains representing each different PCR fingerprint were confirmed by sequencing the D1/D2 variable domains of the large subunit rDNA. Genomic DNA was prepared from yeast cultures after 2 days of incubation on yeast extract-malt extract agar (yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 1% and agar 2%) using the methodology described by de Barros Lopes *et al.* (1998). The D1/D2 variable domains of the large-subunit rDNA were amplified by PCR using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) according to Lachance *et al.* (1999). The amplified DNA was concentrated, cleaned (Wizard Plus SV Minipreps DNA Purification System; Promega, USA) and sequenced in a MegaBACE™

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1000 automated sequencing system (Amersham Biosciences, USA). The sequence was analyzed with the program DNAMAN, version 4.1 (Lynnon BioSoft, Vaudreuil, QC, Canada). Existing sequences for other yeasts were retrieved from GenBank for comparison.

Antifungal drug susceptibility

Yeast susceptibility to amphotericin B (Sigma, USA), itraconazole (Jansen Pharmaceutical, Belgium) and fluconazole (Pfizer Pharmaceutical, USA) was tested in accordance with the protocol of the Clinical Laboratory Standards Institute (CLSI M27-A_2 2002). The yeast suspension was prepared by the spectrophotometric method, with a final inoculum of $1.5 \pm 1.0 \times 10^3$ cells ml^{-1}. A 100-μl aliquot of yeast suspension was subsequently added to each well of the microdilution trays. The final concentrations of amphotericin B and fluconazole ranged from 0.125 to 64 μg ml^{-1}, while itraconazole ranged from 0.031 to 16 μg ml^{-1}. The trays were incubated at 35°C, and the minimum inhibitory concentration (MIC) endpoints were read after 24–48 hours. Drug- and yeast-free controls were included in all experiments. After incubation, the MICs of amphotericin B were read at the lowest concentration for which no cell growth was visualized. The MICs of the other drugs were read at the lowest concentration for which a prominent decrease (*ca*. 80%) in turbidity relative to the growth control well was observed. The reference values used for susceptibility tests *in vitro* (μg ml^{-1}) were those published by CLSI (2002).

Statistical analysis

Correlations between yeast counts and bacteria of sanitary interest (*E. coli, P. aeruginosa* and *Enterococcus* spp.) were examined by linear regression using Spearman's correlation coefficients (Zar 1996) with the Statistica 6.0 program.

RESULTS AND DISCUSSION

Microbiological indicators

Escherichia coli and *Enterococcus* spp. counts ranged from < 1.8 to 920 MPN/100 ml, and < 1.8 to 1,600 MPN/100 ml in Olhos D'Água Lake, respectively (Table 1). In Lagoa Santa Lake, the counts ranged from < 1.8 to 540 MPN/100 ml for *E. coli*, and < 1.8 to > 1,600 MPN/100 ml for *Enterococcus* spp. These results could be related to the urban location of these lakes, which receive organic matter from different sources. In contrast, Lake Sumidouro, which is located in a protected area, had *E. coli* counts ranging from < 1.8 to 170 MPN/100 ml, and *Enterococcus* spp. from < 1.8 to 240 MPN/100 ml. The MPN of *P. aeruginosa* and total counts of heterotrophic bacteria were highest in all lakes during the rainy season (Table 1). Medeiros *et al.* (2008) analyzed the sanitary conditions of two tropical lakes located in an Atlantic Rain Forest area in Southeastern Brazil, and the MPNs of *E. coli* and *Enterococcus* spp. were lower than those found in the present study and *P. aeruginosa* was not detected. *P. aeruginosa* and enterococci have been isolated from recreational waters and their presence may suggest health risks through body contact, ingestion, or inhalation for people using these waters; consequently these microorganisms have been suggested as indicators of water quality (Eaton *et al.* 2005). Yeasts were isolated only from four samples from Sumidouro Lake. In Lagoa Santa Lake, the MPNs of yeasts ranged from < 1.8 MPN/100 ml to 920 MPN/100 ml (Table 1). The highest yeast counts were found in Olhos D'Água Lake, mainly in the rainy season. The highest counts of bacteria of sanitary interest and yeasts were found in the urban lakes in the rainy season, suggesting the introduction of allochthonous organic matter by run-off and human wastes to these aquatic environments.

Correlation between yeasts and indicator microorganisms of water quality

The yeast counts detected after incubation at 37°C using the multiple-tube fermentation technique showed a positive correlation only with the counts of *E. coli* ($p < 0.05$). Previously, de Almeida (2005) found a positive correlation between the counts of yeasts detected after incubation at 37°C and the occurrence of *E. coli* in the Tagus estuary in Portugal. These authors also found that *C. parapsilosis* was one of the most frequent yeast species, with high counts during autumn, when fecal pollution was abundant.

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Table 1 | Most probable number (MPN/100 ml) of *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa* and yeasts, and total aerobic heterotrophs (cfu/ ml) in three freshwater lakes in Southeastern Brazil

Microorganisms	Lakes	Points	Sept/05	Oct/05	Nov/05	Dec/05	Jan/06	Feb/06	Mar/06	Apr/06	May/06	Jun/06	Jul/06	Aug/06
Escherichia coli	Sumidouro	1	11	4.5	13	17	7.8	4	7.8	23	4.5	48	4	1.8
		2	4	1.8	170	21	2	7.8	4	49	17	2	4.5	4.5
		3	79	<1.8	170	17	<1.8	22	7.8	17	<1.8	11	11	34
	Lagoa Santa	1	33	7.8	41	13	1.8	<1.8	10	17	2	2	3.6	<1.8
		2	<1.8	9.2	24	11	11	10	350	27	14	4.5	31	46
		3	21	<1.8	47	4.5	48	33	350	48	8.2	140	540	13
	Olhos D'água	1	70	3.7	240	8.2	23	8.2	140	13	17	13	240	4.5
		2	920	4.5	240	79	11	1.8	24	33	6.1	25	240	170
		3	220	<1.8	24	34	6.8	11	4	94	6.1	39	13	30
Enterococcus spp.	Sumidouro	1	33	2	6.1	21	3.7	<1.8	<1.8	1.8	10	25	4.5	6.8
		2	110	6.8	5.6	1.8	3.7	5.5	5.5	1.8	4	6.8	14	<1.8
		3	23	<1.8	48	<1.8	<1.8	14	47	34	11	11	7.8	240
	Lagoa Santa	1	79	9.3	1.8	3.7	1.8	150	6.8	<1.8	3.7	27	8.3	25
		2	25	1.8	240	5.6	3.7	4	<1.8	3.6	3.6	130	12	170
		3	>1,600	<1.8	10	8.2	21	1.8	<1.8	10	3.7	>1,600	15	79
	Olhos D'água	1	79	8.2	14	<1.8	1.8	>1,600	1.8	8.2	5.5	4	4.5	7.8
		2	25	1.8	8.3	5.6	5.5	>1,600	3.7	540	2	4	5.6	14
		3	>1,600	6	40	5.6	<1.8	150	3.7	47	9.3	6.8	<1.8	9.2
Pseudomonas aeruginosa	Sumidouro	1	<1.8	<1.8	<1.8	2	2	17	<1.8	<1.8	<1.8	1.8	<1.8	<1.8
		2	<1.8	14	33	21	2	<1.8	540	<1.8	4.5	<1.8	<1.8	6.8
		3	<1.8	14	12	2	49	47	3.6	17	<1.8	<1.8	<1.8	8.2
	Lagoa Santa	1	26	70	17	2	26	27	6.1	<1.8	<1.8	<1.8	<1.8	10
		2	280	79	<1.8	14	4.5	10	<1.8	<1.8	<1.8	<1.8	3.7	14
		3	4.5	<1.8	40	<1.8	2	120	5.5	8.1	1.8	<1.8	<1.8	17
	Olhos D'água	1	49	3.7	12	<1.8	<1.8	<1.8	3.6	<1.8	<1.8	<1.8	1.8	2
		2	24	<1.8	8.3	6.1	<1.8	3.7	<1.8	<1.8	<1.8	<1.8	<1.8	1.8
		3	21	1.8	40	5.6	<1.8	24	3.7	3.7	<1.8	<1.8	<1.8	<1.8
Yeasts	Sumidouro	1	2	<1.8	4	2	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
		2	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
		3	<1.8	<1.8	<1.8	<1.8	<1.8	1.8	2	<1.8	<1.8	<1.8	<1.8	<1.8
	Lagoa Santa	1	1.8	1.8	<1.8	<1.8	<1.8	21	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
		2	<1.8	14	84	9.2	<1.8	48	6.8	<1.8	<1.8	1.8	<1.8	<1.8
		3	1.8	7.8	1.8	6.8	2	920	6.8	48	<1.8	24	<1.8	<1.8

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Table 1 | (continued)

Microorganisms	Lakes	Points	Sept/05	Oct/05	Nov/05	Dec/05	Jan/06	Feb/06	Mar/06	Apr/06	May/06	Jun/06	Jul/06	Aug/06
	Olhos D'água	1	5.5	17	1.8	2	9.3	27	6.8	350	33	1.8	1.8	25
		2	1.8	<1.8	13	2	<1.8	25	3.7	31	27	4.5	4.5	11
		3	1.8	<1.8	47	1.8	2	15	4	4	11	3.7	3.7	<1.8
Heterotrophic bacteria	Sumidouro	1	ND	1,000	1,400	22,050	>30,000	8,500	2,400	3,550	1,400	3,000	16,150	5,050
		2	ND	8,200	>30,000	3,000	>30,000	6,050	>30,000	>30,000	400	3,000	1,500	1,950
		3	ND	26,250	13,100	27,650	>30,000	>30,000	>30,000	>30,000	2,250	3,000	6,200	4,750
	Lagoa Santa	1	ND	3,600	2,700	15,450	20,000	3,200	>30,000	2,550	700	2,295	2,650	2,900
		2	ND	4,550	4,050	3,750	>30,000	5,150	>30,000	19,800	900	3,000	3,000	11,150
		3	ND	2,850	10,600	8,150	>30,000	4,950	>30,000	>30,000	ND	3,000	1,380	7,900
	Olhos D'água	1	ND	24,950	2,550	>30,000	>30,000	8,350	>30,000	26,550	2,400	3,000	2,825	16,400
		2	ND	25,500	11,200	28,750	22,333	13,500	28,800	26,250	1,700	3,000	3,850	17,300
		3	ND	3,750	2,700	28,250	>30,000	1,910	28,800	>30,000	550	3,000	3,000	15,850

Most species isolated in our study were opportunistic fungal pathogens, and most of them are also associated with fecal contamination. The positive correlation between yeast and *E. coli* counts in our study suggests that the multiple-tube fermentation technique to determine the yeast counts could be used as an indicator of sewage contamination of recreational waters and as a complement to fecal coliform counts. Yeasts survive longer in water and are more resistant to stress conditions than *E. coli*. Sunlight, predation by protozoa and bacteriophage lysis are the biggest threats to the survival of *E. coli* in aquatic environments (Chandran & Hatha 2005). Yeasts may be more resistant to these abiotic and biotic factors than *E. coli* in freshwater, and both could be used to indicate the environmental quality of aquatic ecosystems.

Yeast identification

A total of 206 yeast isolates were obtained and identified as belonging to nine genera and 33 species (Table 2). An incubation temperature of 37°C was selective for opportunistic yeast pathogens, which were obtained in high numbers in our work. *Candida krusei, C. guilliermondii, K. apis* and *C. tropicalis* were the yeast species most frequently isolated. These species are often reported in aquatic environments with high levels of organic matter from industrial and domestic waste (Hagler 2006; Nagahama 2006; Vogel *et al.* 2007; Medeiros *et al.* 2008). Except for *K. apis*, these yeasts have traditionally been associated with the intestinal tract of warm-blooded animals and it was suggested that they could be used as fecal pollution indicators (de Almeida 2005; Hagler 2006). Other yeast species were isolated in low counts, mainly during the rainy season, and are of terrestrial origin; these species probably reflect inputs from sources such as soil and plant debris.

The isolation of certain yeast species from these lakes is noteworthy. Two isolates of *Trichosporon coremiiforme* were obtained from Olhos D'Água Lake. This species has been associated with summer-type hypersensitivity pneumonitis, subcutaneous abscesses and urinary tract infections (Sugita *et al.* 2004; Rodriguez-Tudela *et al.* 2005). The most likely origins of this yeast in the water samples are waste effluents discharged into the lake. *Saccharomyces cerevisiae* was found in the urban lakes Olhos D'Água and Lagoa

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Table 2 | Yeast species isolated from three paleo-karstic lakes of the Lagoa Santa region in Brazil

Species	Number of isolates	Rainy season Lake			Dry season Lake		
		Sumidouro	Lagoa Santa	Olhos D'Água	Sumidouro	Lagoa Santa	Olhos D'Água
Candida atlantica	1		1				
Candida butyri	1		1				
Candida famata	1					1	
Candida glabrata	7	2		2			3
Candida guilliermondii	28		7	13		3	5
Candida incospicua	1					1	
Candida krusei	76	3	31	31		1	10
Candida parapsilosis	5	3					2
Candida rugosa	4			3		1	
Candida sonorensis	3					3	
Candida sp. UFMG 117	1			1			
Candida stelimallicola	1					1	
Candida tropicalis	18	1	5	6	1	5	
Candida xylopsoci	2	1		1			
Candida zeylanoides	1	1					
Dipodascus ingens	1			1			
Hanseniaspora meyeri	1			1			
Kloeckera apis	21		13	8			
Kluyveromyces marxianus	2						2
Pichia burtonii	1			1			
Pichia caribbica	2		1	1			
Pichia fabianii	1	1					
Pichia fermentans	1			1			
Pichia galeiformis	1		1				
Pichia guilliermondii	5		1			2	2
Pichia japonica	1		1				
Pichia manshurica	4		2	1		1	
Pichia kluyvery	1						1
Pichia rabaulensis	1			1			
Pichia rhodanensis	3			2			1
Saccharomyces cerevisiae	7		4	1		1	1
Torulaspora globosa	1		1				
Trichosporon coremiiforme	2			1			1
Total	206	12	69	76	1	20	28

Santa. Since *S. cerevisiae* strains are not common in aquatic habitats (Slaviková & Vadkertiová 1997*a*,*b*; Hagler 2006), its occurrence in these lakes could indicate contamination of the water with bakery effluents.

Antifungal drug susceptibility

Of the 175 yeast isolates, 66 were resistant to at least one antifungal drug tested (Table 3). The numbers of yeasts resistant to the antifungal drugs were relatively high, mainly

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Table 3 | Values of MIC_{80} for three antifungal drugs against yeasts isolated from three lakes in Southeastern Brazil

Species	Number of isolates	Lake	Fluconazole	Itraconazole	Amphotericin B
Candida atlantica	1	Lagoa Santa	**64**	0.5	0.25
Candida butyri	1	Lagoa Santa	0.125	0.125	0.031
Candida famata	1	Lagoa Santa	32*	0.25	0.5
Candida glabrata	5	Olhos D'Água	1–32*	0.25–**16**	0.062–**2**
Candida glabrata	2	Sumidouro	32*	**16**	**2–8**
Candida guilliermondii	8	Lagoa Santa	0.5– > **64**	0.031–**1**	0.125–**2**
Candida guilliermondii	19	Olhos d'água	0.25–4	0.125–**16**	0.125–**2**
Candida incospicua	1	Lagoa Santa	1	0.062	**4**
Candida krusei	31	Lagoa Santa	1– > **64**	0.25–**16**	0.031–**8**
Candida krusei	38	Olhos D'Água	0.062–**64**	0.031–**1**	0.125–**4**
Candida krusei	1	Sumidouro	32*	0.5	1
Candida parapsilosis	3	Lagoa Santa	0.5–8*	0.125–0.25	0.125–1
Candida parapsilosis	2	Olhos D'Água	0.5–1	0.25	0.25–0.5
Candida rugosa	1	Lagoa Santa	1	0. 062	1
Candida rugosa	3	Olhos D'Água	1–8*	0.5	1–**2**
Candida sonorensis	3	Lagoa Santa	8*–16*	0.25–**1**	0.5–1
Candida sp. UFMG 117	1	Olhos D'Água	0.25	0.0625	0.5
Candida stellimalicola	1	Lagoa Santa	16	0.5	**4**
Candida tropicalis	8	Lagoa Santa	0.25–**64**	0.062–**1**	0.031–**2**
Candida tropicalis	4	Olhos D'Água	1–32*	0.062–**16**	0.5–**2**
Candida tropicalis	2	Sumidouro	0.5–1	0.031–0.062	0.125–0.5
Candida xylopsoci	1	Olhos D'Água	16*	0.5	0.25
Candida xylopsoci	1	Sumidouro	16*	0.5	**2**
Kloeckera apis	3	Lagoa Santa	2–32*	0.5–**1**	0.031–0.5
Kloeckera apis	5	Olhos D'Água	2–32*	0.25–**16**	0.25–**2**
Pichia burtonii	1	Olhos D'Água	4	0.031	0.031
Pichia caribbica	1	Lagoa Santa	1	0.25	0.125
Pichia fabianii	1	Sumidouro	4	0.5	0.5
Pichia fermentans	1	Olhos D'Água	32*	0.5	0.5
Pichia galeiformis	1	Lagoa Santa	32*	0.25	0.25
Pichia guilliermondii	3	Lagoa Santa	2–4	0.25–0.5	0.25–1
Pichia guilliermondii	2	Olhos D'Água	0.5–4	0.25	0.5–1
Pichia japonica	1	Lagoa Santa	4	0.25	0.5
Pichia manshurica	3	Lagoa Santa	1–32*	0.25–**1**	0.25–**2**
Pichia manshurica	1	Olhos D'Água	32*	0.25	1
Pichia rabaulensis	1	Olhos D'Água	0.25	0.25	0.25
Pichia rhodanensis	3	Olhos D'Água	0.25–32*	0.25–0.5	0.5–**4**
Saccharomyces cerevisiae	5	Lagoa Santa	0.5–16*	0.25–**16**	0.031–0.5
Saccharomyces cerevisiae	2	Olhos D'Água	0.5–32*	0.062	0.25–**2**
Torulaspora globosa	1	Lagoa Santa	0.062	0.125	0.031
Trichosporon coremiiforme	2	Olhos D'Água	4	0.25–0.5	**2–8**

*Indicates that susceptibility is dependent on dose values; bold numbers indicate the resistance values.

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for amphotericin B and itraconazole. Medeiros *et al.* (2008) reported that most of the yeasts isolated from two lakes of an Atlantic rainforest site in Brazil presented low resistance to fluconazole and amphotericin B. The occurrence of yeast strains resistant to common antifungal drugs suggests that these aquatic environments can pose potential health risks for people using the contaminated waters for recreation. Fluconazole, itraconazole and amphotericin B are considered to be active against a wide number of opportunistic fungal pathogens belonging to the genus *Candida* (Sanglard 2003). However, we found a relatively high percentage of resistance to these drugs among environmental yeast strains.

Three isolates each of *C. glabrata* and *C. krusei,* two of *C. guilliermondii* and one isolate of *C. tropicalis* were simultaneously resistant to itraconazole and amphotericin B. The isolates of *C. krusei* presented 13.8 and 27.7% of resistance to itraconazole and amphotericin B, respectively; 42.8% of the *C. glabrata* isolates presented simultaneous resistance to itraconazole and amphotericin B. This species was isolated from lakes Sumidouro and Olhos D'Água. No opportunistic *Candida* strain was simultaneously resistant to all three antifungal drugs. According to Cury *et al.* (2007), antifungal sensibilities of opportunistic *Candida* isolates are different for each species, and isolates from the same species could present different sensitivity profiles.

Many authors have observed an increase in the number of non-*albicans* species in opportunistic fungal infections (Sandaven 1990; Saballs *et al.* 2000). Ubiquitous fungi previously thought to be merely colonizers or contaminants–the so-called emerging fungal pathogens, some of which are resistant to all antifungal drugs available–are now known to cause invasive and life-threatening infections (Canuto & Rodero 2002). In the present study, many *Candida* species able to grow at 37°C and associated with plant material or insects, such as *C. atlantica, C. sonorensis* and *C. stellimalicola*, were resistant to amphotericin B and fluconazole. One isolate of *C. incospicua* from Lagoa Santa Lake was resistant to amphotericin B, and this yeast is considered to be an emergent pathogen associated with fungal infections (D'Antonio *et al.* 1998). Four isolates of *K. apis* were resistant to itraconazole, two were resistant to amphotericin B and four isolates presented dose-dependent susceptibility to fluconazole. One isolate each of *Pichia fermentans* and *P. galeiformis,* three of *P. manshurica* and two of *P. rhodanensis* showed dose-dependent sensitivity to fluconazole. One isolate each of *P. rhodanensis* and *P. manshurica* were resistant to amphotericin B, and another isolate of *P. manshurica* was resistant to itraconazole. Medeiros *et al.* (2008) had found resistance of one isolate of *P. membranifaciens* and one of *P. anomala* to itraconazole and fluconazole. Additionally, these authors had found dose-dependent sensitivity to itraconazole for *P. rhodanensis*. In our study, two isolates of *T. coremiiforme* were resistant to amphotericin B, with high MIC values. *Trichosporon* infections are associated with a wide spectrum of clinical manifestations, ranging from superficial cutaneous involvement in immunocompetent individuals to severe systemic disease in immunocompromised patients (Walling *et al.* 1987). One isolate of *S. cerevisiae* was resistant to amphotericin B and two were resistant to itraconazole. Recent reports about the involvement of *S. cerevisiae* in superficial and life-threatening systemic diseases (Munoz *et al.* 2005; de Llanos *et al.* 2006*a*,*b*) suggest that this yeast can be pathogenic under certain circumstances due to its ability to proliferate, persist and disseminate in the body and invade different organs (de Llanos *et al.* 2006*a*; Klingberg *et al.* 2008). The presence of yeast species which are known to be involved in human mycoses and additionally display high resistance to the antifungal drugs tested here is a serious concern for the people who use these aquatic environments for recreational purposes.

CONCLUSION

Most of the yeast species isolated in our study are common in aquatic environments with high levels of organic matter and probably originated from terrestrial environments, such as soil and plant debris, and from waste contamination. We have found a positive correlation between yeast densities and the counts of a standard indicator (*E. coli*). This result suggests that yeasts may be also used as indicators, in addition to the fecal coliform group, of organic pollution in recreational waters. The high incidence of yeast species involved in human mycoses and resistant to the antifungal drugs tested shows that these microorganisms could pose a health risk to the people who use these lakes for recreation.

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