RESEARCH ARTICLE

Yeasts from *Macrobrachium amazonicum*: a focus on antifungal susceptibility and virulence factors of *Candida* spp.

Raimunda S.N. Brilhante[1], Manoel A.N. Paiva[1,2,3], Célia M.S. Sampaio[2,3], Carlos E.C. Teixeira[1], Débora S.C.M. Castelo-Branco[1], João J.G. Leite[1], Camila A. Moreira[1], Liliane P. Silva[3], Rossana A. Cordeiro[1], André J. Monteiro[4], José J.C. Sidrim[1] & Marcos F.G. Rocha[1,2]

[1]Department of Pathology and Legal Medicine, Medical Mycology Specialized Center, College of Medicine, Post-Graduation Program in Medical Microbiology, Federal University of Ceará, Fortaleza, Ceará, Brazil; [2]College of Veterinary Medicine, Post-Graduation Program in Veterinary Sciences, State University of Ceará, Fortaleza, Ceará, Brazil; [3]Laboratory of Carciniculture of the State University of Ceará, Fortaleza, Ceará, Brazil; and [4]Department of Statistics and Applied Mathematics, Federal University of Ceará, Fortaleza, Ceará, Brazil

Correspondence: Raimunda S.N. Brilhante, Rua Barão de Canindé, 210, Montese. CEP 60.425-540, Fortaleza, CE, Brazil. Tel.: +55 85 3295 1736; fax: +55 85 3366 8303; e-mail: brilhante@ufc.br

Received 21 June 2010; revised 20 December 2010; accepted 27 December 2010.
Final version published online 14 February 2011.

DOI:10.1111/j.1574-6941.2011.01050.x

Editor: Philippe Lemanceau

Keywords
Yeast diversity; antifungal susceptibility; *Macrobrachium amazonicum*; virulence factors.

Abstract

In the present study, it was sought to compare yeast microbiota of wild and captive *Macrobrachium amazonicum* and evaluate the antifungal susceptibility and production of virulence factors by the recovered isolates of *Candida* spp. Additionally, cultivation water was monitored for the presence of fungi. Overall, 26 yeast isolates belonging to three genera and seven species were obtained, out of which 24 were *Candida* spp., with *Candida famata* as the most prevalent species for both wild and captive prawns. From cultivation water, 28 isolates of filamentous fungi were obtained, with *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. as the most frequent genera. Eight out of 24 *Candida* spp. isolates were resistant to azole derivatives, out of which four were recovered from wild-harvested prawns. As for production of virulence factors, three (12.5%) and eight (33.3%) isolates presented phospholipase and protease activity, respectively. This is the first comparative study between wild and captive prawns and the first report on yeast microbiota of *M. amazonicum*. The most relevant finding was the high percentage of resistant *Candida* spp., including from wild individuals, which suggests the occurrence of an environmental imbalance in the area where these prawns were captured.

Introduction

Macrobrachium amazonicum is a continental prawn (Martin & Davis, 2001) that is widely distributed in South America, from the Orenoco basin, through the Amazon River, to the Paraguay basin (Holthuis, 1952). Among all Brazilian native species, the Amazon river prawn (*M. amazonicum*) is the preferred one for cultivation, due to its rapid growth and easy maintenance in captivity (Moraes-Valenti & Valenti, 2010).

There are reports that emphasize the importance of yeasts and filamentous fungi for aquaculture, as pathogens of different groups of economic interest (Gatesoupe, 2007). *Candida* species represent the greatest number of isolates from prawn farming, with *Candida sake* as the most common one (Johnson & Bueno, 2000). Additionally, yeasts have been found in water and sediment from lakes and ponds inhabited by these crustaceans (Lu *et al.*, 1998).

Potentially pathogenic yeast species have been isolated from freshwater environments (Medeiros *et al.*, 2008) and animals (Brito *et al.*, 2009; Brilhante *et al.*, 2010). It is noteworthy that among these fungi, resistant strains have also been found. In a research work on lake water from Southeastern Brazil, resistance phenomena to itraconazole and amphotericin B in 50% of the isolated yeasts and 6% of the isolated *Candida* spp., respectively, were observed (Medeiros *et al.*, 2008). Additionally, the occurrence of resistance to ketoconazole, fluconazole and itraconazole in *Candida albicans* and *Candida tropicalis* obtained from dogs (Brito *et al.*, 2009) and to fluconazole and itraconazole among *C. albicans* recovered from cockatiels (*Nymphicus hollandicus*) (Sidrim *et al.*, 2010) was observed.

Until now, no studies comparing microbiota composition, antifungal susceptibility profile and virulence factors of yeasts recovered from *M. amazonicum* from natural

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environment and captivity have been performed. Therefore, this study sought to compare yeast microbiota of captive and wild *M. amazonicum* and evaluate *in vitro* antifungal susceptibility and phospholipase and protease activities of recovered *Candida* spp. In addition, the presence of fungi in cultivation water was investigated.

Materials and methods

Wild-harvested prawns

Adults of *M. amazonicum* were harvested from Catu Lake (03°49′44,8″S and 38°29′9″W), in the municipality of Aquiraz, 35 km from Fortaleza, Ceará, Northeastern Brazil. The animals were captured by covos, a traditional trap used to capture this species of prawn, and were properly taken to Laboratory of Crustacean Culture of the State University of Ceará, where healthy individuals, presenting all thoracic and abdominal appendices, were randomly selected for this study.

Captive prawns

Experiment set-up and obtention of larvae

Larvae, juveniles and adults *M. amazonicum* that were cultivated at the Laboratory of Carciniculture of the State University of Ceará were used in this study. For the obtention of larvae, ovigerous females, in good health conditions, presenting all thoracic and abdominal appendices and eggs at the final stage of embryonary development, were used (Valenti *et al.*, 1998). These females were collected from Catu Lake, as described for wild-harvested prawns, and then maintained in a 60-L glass fiber tank, containing water with salinity of 4000 mg L^{-1}, under controlled temperature and abundant aeration, to which a biofilter was attached. These females were not fed while they were kept in the eclosion tank.

Set-up and maintenance of larviculture

Newly hatched larvae of *M. amazonicum* were stored in three rectangular tanks, each with a capacity of 70 L, filled with 60 L of water with different salinity, equipped with closed water circulation and heating systems. During larviculture, the experiments were performed in three different tanks, each one of them with different salinities: 2000, 4000 and 6000 mg L^{-1} for tanks T1, T2 and T3, respectively. A density of 20 larvae L^{-1} was used. Water temperature, pH and ammonia and nitrite concentrations were monitored daily.

Larvae were fed *ad libitum* with *Artemia* sp. nauplii, in the morning, from stages II to IX. Starting at stage V, larvae were also fed in the afternoon. After observing the first post-larva, polyvinyl chloride pipe substrates were placed into the tanks, in order to avoid cannibalism.

Larval health

Every 3 days, four larvae of *M. amazonicum* were taken from each tank and placed into a clock glass, containing water from the respective salinity, in order to observe larval stage and larval health status. Such evaluation was based on the criteria described previously for *Macrobrachium rosenbergii* larvae (Tayamen & Brown, 1999). Briefly, natatory behavior, presence of parasites and/or necrosis, intestinal conditions, pigmentation and phototaxis were evaluated.

Set-up and maintenance of grow-out

The post-larvae obtained during larviculture were stored in three different tanks, T4, T5 and T6, which contained the animals from the tanks with salinities of 2000 mg L^{-1} (T1), 4000 mg L^{-1} (T2) and 6000 mg L^{-1} (T3), respectively. The new tanks were circular, with a capacity of 400 L, filled to 360 L, equipped with closed water circulation and heating systems and populated at a density of 2 post-larvae L^{-1}. Only fresh water was used in the grow-out tanks, after filtering by activated charcoal filter. Water temperature, pH and ammonia and nitrite concentrations were monitored daily.

During 3 weeks, post-larvae were fed *ad libitum* with *Artemia* sp. nauplii and pelleted ration (Coyle *et al.*, 2010) only in the morning. After this period, an all-ration diet was instituted and offered in the morning and in the afternoon.

Collection of biological material for fungal isolation

After randomly selecting healthy wild adults from Catu Lake, the digestive tracts of 10 individuals were removed by making a dorsal transverse incision, and were placed in sterile slants containing sterile saline and were treated as one single sample. Overall, 18 collections were performed, with a total of 180 adult prawns.

After starting larviculture, 10 larvae from each tank (T1, T2 and T3) were collected, once a week, for 3 weeks, totaling 30 larvae per tank. After collection, larvae were ground and suspended in 1 mL of sterile saline (NaCl 0.9%). During the grow-out phase, 10 individuals (juveniles and/or adults) were collected, weekly, from each tank (T4, T5 and T6) and were treated as described for larvae, until reaching 2 cm in length. When the cultivated animals were bigger than 2 cm, their digestive tracts were removed, as described for wild-harvested adults. Overall, 15 collections were performed during this phase, with a total of 150 individuals per tank. After each collection, microbiological processing was immediately performed, as described in the following section.

Water samples from cultivation tanks were collected with 5 mL syringes. Aliquots of water were obtained from different regions of each tank (bottom, substrate, surface and

walls). Then, samples were taken to the laboratory, where they were homogenized in vortex and an aliquot of 100 μL was streaked onto microbiological media. All samples from animal and water were taken to the Specialized Medical Mycology Center (CEMM) of Federal University of Ceará, in Fortaleza, Ceará, Brazil.

Microbiological processing

Yeast isolation

For each sample, two culture media were used for primary isolation: 2% Sabouraud agar with chloramphenicol (0.5 g L^{-1}), and birdseed (*Guizotia abyssinica*) agar. Larvae and post-larvae suspensions were streaked onto the media, using a microbiological loop.

Digestive tracts were opened and homogenized in sterile Petri dishes, and approximately 1 g was added to a saline solution (NaCl 0.9%) containing chloramphenicol (0.4 g L^{-1}). The suspension was homogenized in a vortex for 3 min, and then left to decant for 30 min at 25 °C. Afterwards, aliquots of 100 μL from the supernatant of each sample were streaked onto both media (Brilhante *et al.*, 2010). Petri dishes containing the cultured media were incubated at 25 °C for 10 days, and were observed daily.

Identification

Initially, colonies that were suggestive of yeasts were observed microscopically (× 40), after Gram staining, in order to verify the presence of blastoconidia, hyphae or pseudohyphae, as well as to discard the occurrence of bacterial contamination. Then, yeasts were identified by performing specific morphological and biochemical tests and, when necessary, through VITEK 2 (bioMérieux®) (Brilhante *et al.*, 2010).

Briefly, identification of *Candida* species was based on phenotypical characteristics, such as macromorphology, colony observation, and micromorphology, after growth on Cornmeal-Tween 80 agar. Additionally, biochemical tests were performed, such as carbohydrate and nitrogen assimilation and urease production (De Hoog *et al.*, 2002). All *Candida* spp. isolates were grown on chromogenic medium (HiCrome Candida Differential Agar, HiMedia Laboratories, Mumbai, India), for the identification of mixed colonies (Brilhante *et al.*, 2010).

Cryptococcus spp. isolates were initially grown onto cornmeal Tween-80 agar and on Christensen's urea agar for microscopic and biochemical evaluation, which suggested the genus. Afterwards, an automated analysis was performed using VITEK 2 (bioMérieux®) in order to determine the species.

For *Rhodotorula* spp., colonies were initially identified based on their color. Then, the microorganism was submitted to urease production tests and grown on 2% malt extract agar for morphologic evaluation. Sugar assimilation tests were performed for each isolate and these were crucial for species identification (De Hoog *et al.*, 2000).

Identification of fungi from cultivation water

In order to identify filamentous fungi obtained from cultivation water, slide cultures on potato dextrose agar were performed. Micromorphological analysis was interpreted according to the identification keys (De Hoog *et al.*, 2000). Additionally, yeasts that were obtained from water were identified as described above.

In vitro antifungal susceptibility test

Twenty-four isolates of *Candida* spp. were tested: three *C. albicans* (one from larvae, one from the digestive tract of captive adults and another from the digestive tract of wild-harvested adults), three *C. tropicalis* (one from the digestive tract of captive adults and two from the digestive tract of wild-harvested adults), four *Candida parapsilosis* (three from the digestive tract of captive adults and one from the digestive tract of wild-harvested adults), 10 *Candida famata* (two from the digestive tract of captive adults, two from the cultivation water and six from the digestive tract of wild-harvested adults) and four *Candida guilliermondii* (one from the cultivation water and three from the digestive tract of wild-harvested adults).

The minimum inhibitory concentration (MIC) for these microorganisms was determined by a broth microdilution method as described by Clinical and Laboratory Standard Institute (CLSI, 2002) and in other researches of our group (Brito *et al.*, 2009; Sidrim *et al.*, 2010). As quality control for each test performed, *C. parapsilosis* ATCC 22019 was included. The strains were tested against four drugs: amphotericin B, caspofungin, itraconazole and fluconazole. Stock solutions of amphotericin B (Sigma Chemical Corporation) and itraconazole (Janssen Pharmaceutica, Belgium) were prepared with dimethyl sulfoxide and caspofungin (Merck Sharp & Dohme, Brazil) and fluconazole (Pfizer, Brazil) were diluted with distilled water.

Final concentrations of drugs (amphotericin B, caspofungin, itraconazole and fluconazole) were obtained according to some studies (CLSI, 2002; Pfaller *et al.*, 2006; Brito *et al.*, 2009). All drugs were diluted and resuspended in RPMI 1640 (HiMedia Laboratories). The concentration range tested for amphotericin B, caspofungin and itraconazole was 0.03125–16 μg mL^{-1} and for fluconazole was 0.125–64 μg mL^{-1} (Pfaller *et al.*, 2006; Brito *et al.*, 2009). The criteria for resistance and sensitivity were established according to some authors (CLSI, 2002; Pfaller *et al.*, 2006). Isolates with MICs > 1, ≥ 2, ≥ 1 and ≥ 64 μg mL^{-1} were

considered resistant to amphotericin B, caspofungin, itraconazole and fluconazole, respectively.

Inocula of all tested isolates were prepared from 1-day-old cultures grown on potato dextrose agar at 35 °C. Sterile 0.9% saline (5 mL) was added to sterile glass slants and a sample of the colony was added to the saline solution, adjusting its concentration to 0.5 on McFarland Scale (CLSI, 2002). Afterwards, inocula were diluted 1 : 100 and then 1 : 20, in RPMI 1640 medium, with L-glutamine (HiMedia Laboratories), buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid. The final concentration of the inocula was 0.5–2.5 $\times 10^3$ cells mL^{-1} (CLSI, 2002; Brito *et al.*, 2009).

Susceptibility testing was performed on 96-well microdilution trays, which were properly prepared and incubated at 35 °C, for 48 h (Brito *et al.*, 2009). For each isolate, drug-free and yeast-free controls were included and all the isolates were tested in duplicate. For the azole derivatives and caspofungin, the MIC was defined as the lowest drug concentration inhibiting 80% growth when compared with the growth in the control well, and for amphotericin B the MIC was the lowest concentration at which no growth was observed (CLSI, 2002; Brito *et al.*, 2009; Sidrim *et al.*, 2010).

Minimum fungicidal concentration (MFC)

After obtaining MIC values, microdilution trays were agitated in order to homogenize the inocula and 100-μL aliquots of each isolate, from three consecutive wells with increasing drug concentration, starting at the MIC, were subcultured into slants containing 2% Sabouraud dextrose agar, which were incubated for 48 h at 35 °C. The MFC was defined as the lowest drug concentration at which subcultures presented negative results or produced less than three colonies, indicating the death of $> 99\%$ of the original inoculum (Tawara *et al.*, 2000).

Phospholipase production

The same isolates that were tested for antifungal susceptibility were evaluated for phospholipase activity. The test was performed according to the previously described methodology (Price *et al.*, 1982), with some modifications. Briefly, the medium used was 2% Sabouraud dextrose agar, to which 1 mol L^{-1} sodium chloride, 0.05 mol L^{-1} calcium chloride and 8% sterile egg yolk emulsion, at a concentration of 30%, were added. The emulsion was heated up to 40 °C and incorporated into the sterile medium, after it reached a temperature of 50 °C. Then, the medium was poured into 90-mm Petri dishes, forming a 4-mm film. Yeast inocula were prepared in sterile saline at a final concentration of 4 on McFarland scale. A 5-μL drop of each inoculum was placed on a 5-mm sterilized filter paper disk, which was then placed on the agar. The plates were incubated at 35 °C, for 7 days (Sidrim *et al.*, 2010).

Phospholipase activity (Pz) was determined by calculating the ratio between the diameter of the fungal colony and the total diameter, including the colony and the precipitation zone. Thus, when $Pz = 1$, the isolate is phospholipase negative; when $1 > Pz \geq 0.64$, the isolate is positive for phospholipase activity; and when $Pz < 0.64$, the isolate is strongly positive for this enzyme (Sidrim *et al.*, 2010).

Protease production

The 24 isolates of *Candida* spp. were also screened for protease production. The protease test was performed according to a previously described methodology (Charney & Tomarelli, 1947; Cenci *et al.*, 2008) with modifications. Briefly, yeast inocula were prepared from 1-day-old cultures in a sterile saline solution, reaching a final concentration of 4 on McFarland scale. Then, the inocula were cultured in RPMI medium in a 1 : 1 dilution, by adding 2 mL of each inoculum to 2 mL of RPMI. After 48 h of incubation in a shaker at 150 r.p.m., the inocula were centrifuged at 805 ***g*** for 15 min, and the supernatant was divided into three slants containing 1 mL each. The content from one of the slants was considered the reference substance (blank) and the content from the other two slants was used for testing in duplicate. To the blank content, 1 mL of trichloroacetic acid and 1 mL of azoalbumine were added, while only 1 mL of azoalbumine was added to the other slants. All slants were incubated in 37 °C bath for 48 h, after which the reaction was stopped by adding 1 mL of trichloroacetic acid to the tested slants. Then, the tubes were centrifuged for 30 min at 805 ***g***, and 2 mL of the supernatant were added to 2 mL of 5% NAOH for posterior spectrophotometric analysis at an absorbance of 530 nm.

Statistical analysis

In order to compare yeast prevalence, Fisher's exact test was applied. Pearson's correlation coefficient was used to calculate the correlation between MICs and MFCs. Paired-samples Student's *t*-test was used to compare MICs with MFCs. For all cases, a significance level of 5% was adopted for significant conclusions.

Results

From 126 samples of wild-harvested adults, captive animals and tank water, 26 yeast isolates belonging to three genera and seven species were recovered. Fourteen (54%), eight (31%) and four (15%) isolates were obtained from wild-harvested prawns, captive prawns and cultivation water, respectively (Table 1).

From the digestive tract of wild-harvested adult prawns, 14 (14/26; 53.85%) yeast isolates were recovered: one *C. albicans*,

Table 1. Yeast species isolated from *Macrobrachium amazonicum* and water from cultivation

	Collection site													
	Wild prawns		Captive prawns						Water				Total	
			Larvae		Juvenile and adult				Larviculture		Grow-out			
			T2		T4		T5		T2		T6			
Yeast species	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Candida albicans	1	3.85	1	3.85	–	–	1	3.85	–	–	–	–	3	11.54
Candida famata	6	23.08	–	–	1	3.85	1	3.85	1	3.85	1	3.85	10	38.46
Candida guilliermondii	3	11.54	–	–	–	–	–	–	1	3.85	–	–	4	15.38
Candida parapsilosis	1	3.85	–	–	–	–	3	11.54	–	–	–	–	4	15.38
Candida tropicalis	2	7.69	–	–	–	–	1	3.85	–	–	–	–	3	11.54
Cryptococcus laurentii	1	3.85	–	–	–	–	–	–	–	–	–	–	1	3.85
Rhodotorula mucilaginosa	–	–	–	–	–	–	–	–	1	3.85	–	–	1	3.85
Total	14	53.84	1	3.85	1	3.85	6	23.07	3	11.54	1	3.85	26	100

T2, larviculture tank (salinity 4 mg L^{-1}); T4, grow-out tank (fresh water) corresponding to T1 (salinity 2 mg L^{-1}); T5, grow-out tank (fresh water) corresponding to T2 (salinity 4 mg L^{-1}); T6, grow-out tank (fresh water) corresponding to T3 (salinity 6 mg L^{-1}).

Table 2. Filamentous fungus species isolated from cultivation tanks of *Macrobrachium Amazonicum*

	Collection site											
	Larviculture water				Grow-out water						Total	
	T2		T3		T4		T5		T6			
Species	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Penicillium sp.	1	3.57	1	3.57	2	7.15	–	–	3	10.71	7	25.00
Penicillium decumbens	–	–	–	–	1	3.57	1	3.57	1	3.57	3	10.71
Penicillium chrysogenum	–	–	–	–	1	3.57	–	–	–	–	1	3.57
Penicillium griseofulvum	–	–	–	–	1	3.57	1	3.57	–	–	2	7.15
Cladosporium sp.	1	3.57	–	–	2	7.15	1	3.57	–	–	4	14.29
Cladosporium sphaerospermum	–	–	–	–	–	–	1	3.57	–	–	1	3.57
Cladosporium cladosporioides	–	–	–	–	1	3.57	–	–	1	3.57	2	7.15
Aspergillus sp.	–	–	–	–	1	3.57	1	3.57	1	3.57	3	10.71
Aspergillus niger	–	–	–	–	–	–	1	3.57	–	–	1	3.57
Hortae werneckii	2	7.14	1	3.57	–	–	–	–	–	–	3	10.71
Mucor racemosus	–	–	–	–	0	0	0	0	1	3.57	1	3.57
Total	4	14.28	2	7.15	9	32.15	6	21.42	7	25.00	28	100

T2, larviculture tank (salinity 4 mg L^{-1}); T3, larviculture tank (salinity 6 mg L^{-1}); T4, grow-out tank (fresh water) corresponding to T1 (salinity 2 mg L^{-1}); T5, grow-out tank (fresh water) corresponding to T2 (salinity 4 mg L^{-1}); T6, grow-out tank (fresh water) corresponding to T3 (salinity 6 mg L^{-1}).

six *C. famata*, two *C. tropicalis*, one *C. parapsilosis*, three *C. guilliermondii* and one *Cryptococcus laurentii* (Table 1).

Out of eight yeast isolates from captive prawns, one was obtained from larvae (1/26; 3.85% – *C. albicans*) and no isolates were recovered from post-larvae. On the other hand, seven isolates were obtained from the digestive tract of adult prawns (7/26; 26.92% – one *C. albicans*, two *C. famata*, one *C. tropicalis* and three *C. parapsilosis*) (Table 1). *Candida famata* ($P = 0.0311$) and *C. guilliermondii* ($P = 0.0599$) were more frequently isolated from wild-harvested prawns, when compared with those from captivity.

No yeast isolates were recovered from larviculture water with salinities of 2000 and 6000 mg L^{-1} (T1 and T3, respectively) or from grow-out tanks T4 and T5. Three isolates (3/26; 11.54% – one C. *famata*, one *C. guilliermondii* and one *Rhodotorula mucilaginosa*) were obtained from water with a salinity of 4000 mg L^{-1}. (T2) and one water sample from grow-out tank T6 was positive for yeast growth (1/26; 3.85% – *C. famata*) (Table 1).

It was possible to recover filamentous fungi from 42.6% (23/54) of cultivation water samples. These fungi belonged to five genera, with a total of 28 isolates (Table 2). Four (4/28; 14.29%) and two (2/28; 7.14%) isolates were obtained from larviculture tanks T2 and T3, respectively. No filamentous fungi were recovered from the larviculture tank with a salinity of 2000 mg L^{-1} (T1). Nine (9/28; 32%), six (6/28;

Table 3. MIC and MFC distribution of amphotericin B, caspofungin, itraconazole and fluconazole against 24 isolates of *Candida* spp.

Candida spp.		Amphotericin B ($\mu g\,mL^{-1}$)		Caspofungin ($\mu g\,mL^{-1}$)		Itraconazole ($\mu g\,mL^{-1}$)		Fluconazole ($\mu g\,mL^{-1}$)	
	n	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans	3	0.125 (1)*	0.125 (1)	0.03125 (2)	0.03125 (1)	≥16 (3)	≥16 (3)	≥64 (3)	≥64 (3)
		0.5 (2)	0.5 (2)	0.0625 (1)	0.0625 (1)				
					0.25 (1)				
C. famata	10	0.03125 (5)	0.03125 (5)	0.03125 (1)	0.03125 (1)	0.03125 (2)	0.03125 (1)	0.5 (2)	2 (2)
		0.0625 (3)	0.0625 (3)	0.0625 (2)	0.0625 (2)	0.0625 (3)	0.0625 (1)	2 (3)	4 (1)
		0.125 (1)	0.125 (1)	0.125 (1)	0.125 (1)	0.125 (2)	0.125 (2)	4 (1)	16 (2)
		0.5 (1)	0.5 (1)	0.25 (2)	0.25 (1)	0.5 (1)	0.25 (1)	8 (1)	32 (2)
				0.5 (4)	1 (1)	≥16 (2)	0.5 (3)	32 (1)	≥64 (3)
					2 (3)		≥16 (2)	≥64 (2)	
					4 (1)				
C. parapsilosis	4	0.0625 (1)	0.0625 (1)	0.25 (1)	2 (2)	0.03125 (1)	0.625 (1)	8 (2)	4 (1)
		0.125 (2)	0.125 (2)	0.5 (1)	4 (1)	0.0625 (1)	0.5 (1)	4 (2)	8 (2)
		0.25 (1)	0.25 (1)	1 (2)	8 (1)	0.5 (1)	2 (1)		32 (1)
						≥16 (1)	≥16 (1)		
C. tropicalis	3	0.125 (3)	0.125 (3)	0.125 (2)	0.5 (1)	0.03125 (2)	0.0625 (2)	8 (2)	8 (1)
				0.5(1)	1 (1)	4 (1)	≥16 (1)	≥64 (1)	32 (1)
					2 (1)				≥64 (1)
C. guilliermondii	4	0.03125 (1)	0.03125 (1)	0.03125 (2)	0.03125 (1)	0.03125 (3)	0.03125 (1)	0.5 (1)	4 (1)
		0.0625 (2)	0.625 (2)	0.0625 (1)	0.25 (1)	≥16 (1)	0.0625 (1)	1 (1)	8 (2)
		0.25 (1)	0.25 (1)	1 (1)	1 (1)		0.125 (1)	2 (1)	16 (1)
					4 (1)		≥16 (1)	8(1)	

*Represents the number of isolates for each indicated MIC and MFC.

21.43%) and seven (7/28; 25%) isolates were obtained from water samples from grow-out tanks T4, T5 and T6, respectively.

Penicillium sp. was the most isolated genus (13/28; 46.42%), followed by *Cladosporium* sp. (7/28; 25%) and *Aspergillus* sp. (4/28; 14.29%). Other obtained isolates were *Hortaea werneckii* (3/28; 10.71%) and *Mucor* sp. (1/28; 3.57%) (Table 2).

Concerning *in vitro* antifungal susceptibility tests of *Candida* spp., the MIC values for all tested isolates are described in Table 3. Briefly, for *C. famata* (10/24), the MIC for amphotericin B varied from 0.03125 to 0.5 $\mu g\,mL^{-1}$, and for caspofungin, itraconazole and fluconazole, MICs ranged from 0.03125 to 0.5 $\mu g\,mL^{-1}$, 0.03125 to ≥16 $\mu g\,mL^{-1}$ and 0.5 to ≥64 $\mu g\,mL^{-1}$, respectively. For *C. parapsilosis* (4/24), MIC for amphotericin B, caspofungin, itraconazole and fluconazole ranged from 0.0625 to 0.25 $\mu g\,mL^{-1}$, 0.25 to 1 $\mu g\,mL^{-1}$, 0.03125 to ≥16 $\mu g\,mL^{-1}$ and 4 to 8 $\mu g\,mL^{-1}$, respectively. For *C. guilliermondii* (4/24), MICs varied from 0.03125 to 0.25 $\mu g\,mL^{-1}$, 0.03125 to 1 $\mu g\,mL^{-1}$, 0.03125 to ≥16 $\mu g\,mL^{-1}$ and 0.5 to 8 $\mu g\,mL^{-1}$ for amphotericin B, caspofungin, itraconazole and fluconazole, respectively. For *C. tropicalis* (3/24), the MIC value for amphotericin B was 0.125 $\mu g\,mL^{-1}$, while those for caspofungin, itraconazole and fluconazole ranged from 0.125 to 0.5 $\mu g\,mL^{-1}$, 0.03125 to 4 $\mu g\,mL^{-1}$ and 8 to ≥64 $\mu g\,mL^{-1}$, respectively. Finally, for *C. albicans* (3/24), MIC values for amphotericin B and caspofungin varied from 0.125 to 0.5 $\mu g\,mL^{-1}$ and 0.03125 to 0.0625 $\mu g\,mL^{-1}$, respectively, while those for itraconazole and fluconazole were ≥16 and ≥64 $\mu g\,mL^{-1}$, respectively. A positive correlation was observed when comparing MICs for amphotericin B and itraconazole ($P = 0.0005$), amphotericin B and fluconazole ($P = 0.0020$), and fluconazole and itraconazole ($P = 0.0007$), but not for caspofungin, when compared with the other tested drugs.

MFC values for all *Candida* spp. isolates varied from 0.03125 to 0.5 $\mu g\,mL^{-1}$, 0.03125 to 8 $\mu g\,mL^{-1}$, 0.03125 to ≥16 $\mu g\,mL^{-1}$ and 2 to ≥64 $\mu g\,mL^{-1}$ for amphotericin B, caspofungin, itraconazole and fluconazole, respectively (Table 3). A positive correlation was also observed when comparing MFCs for amphotericin B and itraconazole ($P = 0.0001$), amphotericin B and fluconazole ($P = 0.0104$), and fluconazole and itraconazole ($P = 0.0013$), but not for caspofungin, when compared with the other tested drugs. When comparing MIC and MFC, a positive correlation was observed for caspofungin ($P = 0.0000$), fluconazole ($P = 0.0000$) and itraconazole ($P = 0.0000$). For amphotericin B, MICs were equal to MFCs; for itraconazole, MICs and MFCs were not statistically different ($P = 0.1108$), and for caspofungin ($P = 0.0007$) and fluconazole ($P = 0.0013$), MICs and MFCs were statistically different.

Concerning phospholipase activity, only three (12.5%) isolates presented positive results ($Pz < 1$). The obtained Pz

values were 0.64, 0.67 and 0.71 for *C. famata*, *C. tropicalis* and *C. parapsilosis*, respectively. *Candida famata* was isolated from the larviculture water (T1) and *C. tropicalis* and *C. parapsilosis* were isolated from the digestive tract of captive adults (T5).

As for protease production, eight isolates presented positive results (8/24; 33.3%), which ranged from 1.0 to 27.0 U μL^{-1}. These isolates were four *C. famata* (4/10; 40%; one from T3 water and three from wild-harvested adults), two *C. albicans* (2/3; 66.7%; one from wild-harvested adults and one from captive adult from T5), one *C. parapsilosis* (1/4; 25%; from captive adult from T5) and one *C. guilliermondii* (1/4; 25%; from wild-harvested adult).

Discussion

The larval health index was satisfactory for all analyzed tanks during this experiment, with values > 1 (Tayamen & Brown, 1999). The water temperature varied from 28 to 30 °C and ammonia and nitrite concentrations were maintained at zero throughout the research, within ideal management conditions for *M. amazonicum* (Moraes-Valenti & Valenti, 2010).

Candida was the most isolated genus and *C. famata* the most frequently isolated species, with 10 isolates (38.46%), followed by *C. guilliermondii* (15.4%) and *C. parapsilosis* (15.4%). Other recovered species were *C. albicans*, *C. tropicalis*, *C. laurentii* and *R. mucilaginosa*. *Candida albicans* was recovered from larvae and adult prawns, but not from water, corroborating the generally accepted idea that ecological niches for this yeast species do not exist in the environment. Medeiros *et al.*, 2008 recovered several *Candida* species from water and sediment samples from two unpolluted lakes, but *C. albicans* was not recovered, supporting this idea.

Candida famata was the most commonly isolated species from wild-harvested adult prawns and cultivation water (42% and 50%, respectively). From the digestive tract of cultivated prawns, *C. tropicalis* and *C. famata* were the most frequently recovered species (28.6% each). The digestive tract of wild-harvested adult *M. amazonicum* from Catu Lake represented the type of sample where the highest number of species was found, followed by digestive tracts of cultivated animals, cultivation water and larvae. Considering that the intestinal microbiota of aquatic invertebrates resembles that of the environment where they are inserted (Hagler *et al.*, 1995; Kutty & Philip, 2008), this observation was expected, once environments under natural conditions are subjected to several influencing factors, including natural and anthropic ones, harboring a greater diversity of microorganisms (Medeiros *et al.*, 2008), when compared with environments under controlled conditions, commonly used in prawn farming.

In a study with *M. rosenbergii* cultivated in Taiwan, the greatest percentage of the isolates (86%) were represented by *Candida* species, out of which 70% were *C. sake* and 16% were *C. famata* (Lu *et al.*, 1998). However, 61% of the animals presented clinical alterations, which may explain the high prevalence of *C. sake*, a well-known pathogen of freshwater prawns. In our study, only healthy prawns were assessed and *C. sake* was not recovered, while *C. famata* was the most frequently isolated species, demonstrating the presence of this yeast species in these animals.

In our study, although the number of yeasts recovered from the cultivation water was small, *Candida* spp. were the predominantly isolated ones, similar to that observed by Lu *et al.*, 1998 and Leaño *et al.*, 2005 in studies with captive giant river prawns and tiger prawns, respectively. The water-sampling methodology used in this research differed from those mentioned by other authors (Lu *et al.*, 1998; Leaño *et al.*, 2005; Medeiros *et al.*, 2008), which could have accounted for the low recovery rate of yeasts, including the inability to obtain *C. albicans* from cultivation water. However, these authors studied the quality of water from natural sources or ponds, but not from tanks equipped with closed water circulation system, which justifies their need to work with larger volumes of water.

The three predominant genera of filamentous fungi found in cultivation water of this study, *Penicillium*, *Cladosporium* and *Aspergillus*, were also found in an investigation of filamentous fungi in cultivation water of tilapia and tiger prawns by Leaño *et al.*, 2005. *Penicillium* sp. and *Aspergillus* sp. were the predominant genera found by these authors, but *Cladosporium* sp. was isolated from only one sample of the tiger prawn cultivation. In contrast, *Cladosporium* sp. represented 25% of the isolates in our study, which probably reflects the environmental condition under which this research was conducted, considering that the species belonging to this genus, along with *Penicillium* spp. and *Aspergillus* spp., represent common airborne fungi in indoor and outdoor environments (Solomon *et al.*, 2006; Pantoja *et al.*, 2009; Miao *et al.*, 2010).

In our study, the fungus *H. werneckii* (3/28) was isolated from water samples from tanks T2 and T3 (salinities of 4000 and 6000 mg L^{-1}, respectively). This fungus inhabits tropical and subtropical regions (De Hoog *et al.*, 2000; Varga & Godoy, 2004) and it is the predominant species in hypersaline waters, acting as a saprobic microorganism in these environments (Gunde-Cimerman *et al.*, 2000; Mbata, 2008). *Hortaea werneckii* can inhabit a wide range of salinities, varying from 0 to 25 000 mg L^{-1}, but optimal growth occurs between 3000 and 7500 mg L^{-1} of NaCl (Petrovic *et al.*, 2002), which includes the salinity of the samples from which this fungus was isolated in our study.

Although some filamentous fungi are identified as pathogens for prawns, such as *Aspergillus* sp., their isolation was not harmful to the health of cultivated prawns in this study, considering that no clinical alterations were observed. Most

pathogenic fungi for prawns and shrimps belong to the microbiota of cultivation water and are secondary or opportunistic invaders. These microorganisms cause cultivation problems only when the animals are submitted to inadequate management conditions, which favor the dissemination of other diseases (Leaño *et al.*, 2005). Hence, the animals were kept under adequate management conditions

In this study, all *Candida* strains were susceptible to amphotericin B, as observed in other researches performed by our group (Brito *et al.*, 2009; Sidrim *et al.*, 2010). Eight out of 24 *Candida* spp. isolates (33.3%) were resistant to itraconazole and/or fluconazole, with particular attention given to the isolates of *C. albicans*, which were resistant to both drugs simultaneously. The MFC results obtained in our study were similar to those for clinical isolates of non-*albicans Candida* species (Tawara *et al.*, 2000).

Additionally, it is noteworthy that 28.6% (4/14) of the isolates of *Candida* spp. obtained from wild-harvested *M. amazonicum* were resistant to these azole derivatives. The observed resistance phenomenon to this class of drugs arose the curiosity to seek the causes that may be associated with this phenomenon in the environment. Catu Lake is a freshwater source that has been used for human consumption and supply, animal consumption, agriculture, industries and leisure activities. Considering that industrial wastes and pollutants are reported as causes of mutagenesis in yeasts (Keenan *et al.*, 2007) and that the occurrence of gene mutation (Feng *et al.*, 2010) may result in antifungal resistance, we believe that the resistance phenomenon observed in these *Candida* strains is related to anthropic activities developed in the studied area, such as pollution with industrial, agricultural and farming wastes.

As mentioned before, the intestinal microbiota of aquatic invertebrates is similar to that of the environment where they are inserted (Hagler *et al.*, 1995; Kutty & Philip, 2008); thus, yeasts isolated from the digestive tract of wild-harvested *M. amazonicum* may reflect the environmental conditions of Catu Lake. Based on this, crustaceans, including *M. amazonicum*, and fishes can be used as sentinels for the occurrence of resistant yeasts in the environment. Several animal species have been reported as sentinels for the occurrence of resistant bacteria in the wild, including predatory fishes (Blackburn *et al.*, 2010) and terrestrian mammals (Routman *et al.*, 1985; Costa *et al.*, 2008; Mariano *et al.*, 2009). Interestingly, all of these authors reported that the occurrence of resistant bacteria was associated with the close relationship between these wild animals or their environment and human beings and that water was the main vehicle for spreading chemical compounds involved in the development of resistance.

Classically, bacteria have been used as bioindicators of environmental imbalance related to pollution. However, more recently, the use of yeasts has been encouraged, especially because of the structural and molecular similarities between fungal and mammalian cells (Keenan *et al.*, 2007). Several pollutants are capable of altering gene expression or sequence composition (Wegrzyn & Czyz, 2003; Keenan *et al.*, 2007; Müller *et al.*, 2007). Considering that azole resistance is basically associated with changes in gene expression or in gene nucleotide sequence, the presence of pollutants in the water of this lake, including azole derivatives commonly used in agricultural practices, may be responsible for the observed resistance. Based on these observations, we propose the use of *M. amazonicum* and other aquatic species as sentinels for the occurrence of resistant yeasts in the environment and the use of the isolated yeast as bioindicators for the presence of pollutants in natural water sources.

Additionally, the risk of yeast infections occurring in humans should be considered when manipulating these animals or the water they inhabit, considering that potentially pathogenic species were isolated from these sources. Three species should be highlighted, *C. albicans*, *C. parapsilosis* and *C. tropicalis*, for being the most commonly involved in human candidiasis in Latin America (Palacio *et al.*, 2009). Besides, special attention must be given to *C. albicans*, considering that animals can represent a source of infection for humans (Edelmann *et al.*, 2005) and that the recovered isolates of this species were all azole resistant, which is not common among human strains (Kanafani & Perfect, 2008). Thus, studies on genetic diversity of this *Candida* species could help to elucidate whether or not the recovered isolates are commonly distributed among humans.

There are several researches concerning phospholipase activity of fungi isolated from humans (Fotedar & Al-Hedaithy, 2005; Zeng *et al.*, 2008). However, researches with yeasts isolated from animals are scarce (Sidrim *et al.*, 2010) and no reports have been found for yeasts from prawns or shrimps. For many years, it was believed that only *C. albicans* was able to produce phospholipase. However, now it is known that other species of *Candida* also produce this enzyme, usually in smaller amounts (Ghannoum, 2000), as demonstrated by our research. Paradoxically, the isolates of *C. albicans* obtained in this study did not present phospholipase activity, while 14.29% (3/21) of the non-*albicans Candida* species were positive for this enzyme.

Some studies have evaluated the protease activity of *Candida* species; however, in those researches, the strains were obtained from humans with candidiasis (Kantarcioglu & Yucel, 2002; Mohan & Ballal, 2008; Ombrella *et al.*, 2008). In our study, the production of protease by *Candida* spp. strains isolated from wild-harvested and captive crustaceans was demonstrated for the first time.

Finally, this work represented the first study on yeast microbiota of *M. amazonicum*, comparing wild and captive populations. Additionally, the most relevant finding was the

isolation of azole-resistant *Candida* spp. from wild-harvested animals, showing the role of these animals as sentinels for the occurrence of resistant yeasts in the natural environment.

Acknowledgements

The authors thank the financial support of FUNCAP (Ceará State Research Funding; Proc. 071.02.00/09), CAPES/PNPD (Proc. 23038.027637/2009-68) and CNPq (National Counsel for Technological and Scientific Development; Brazil, Proc. 473881/2008-0).

References

Blackburn JK, Mitchell MA, Blackburn MCH, Curtis A & Thompson BA (2010) Evidence of antibiotic resistance in free-swimming top-level marine predatory fishes. *J Zoo Wildlife Med* **41**: 7–16.

Brilhante RS, Castelo-Branco DSCM, Soares GD, Astete-Medrano DJ, Monteiro AJ, Cordeiro RA, Sidrim JJ & Rocha MF (2010) Characterization of the gastrointestinal yeast microbiota of cockatiels (*Nymphicus hollandicus*): a potential hazard to human health. *J Med Microbiol* **59**: 718–723.

Brito EHS, Fontenelle ROS, Brilhante RSN, Cordeiro RA, Monteiro AJ, Sidrim JJC & Rocha MFG (2009) The anatomical distribution and antimicrobial susceptibility of yeast species isolated from healthy dogs. *Vet J* **182**: 320–326.

Cenci E, Francisci D, Belfiori B, Pierucci S, Baldelli F, Bistoni F & Vecchiarelli A (2008) Tipranavir exhibits different efects on opportunistic pathogenic fungi. *J Infect* **56**: 58–64.

Charney J & Tomarelli RM (1947) A colorimetric method for the determination of the proteolytic of duodenal juice. *J Biol Chem* **23**: 501–505.

Costa D, Poeta P, Sáenz Y, Vinué L, Coelho AC, Matos M, Rojo-Bezares B, Rodrigues J & Torres C (2008) Mechanisms of antibiotic resistance in *Escherichia coli* isolates recovered from wild animals. *Microb Drug Resist* **14**: 71–77.

Coyle SD, Alston DE & Sampaio CMS (2010) Nursery systems and management. *Freshwater Prawns: Biology and Farming* (New MB, Valenti WC, Tidwell JH, D'abramo LR & Kutty MN, eds), pp. 108–126. Wiley-Blackwell, Oxford.

De Hoog GS, Guarro J, Gené J & Figueiras MJ (eds) (2000) *Atlas of Clinical Fungi.* Centraalbureau voor Schimmslcultures, The Nederlands.

De Hoog GS, Guarro J & Gené J (eds) (2002) *Atlas of Clinical Fungi*. Centraalbureau voor Schinmelculture/Universitat Rovira i Virgilli, Delf.

Edelmann A, Krüger M & Schmid J (2005) Genetic relationship between human and animal isolates of *Candida albicans*. *J Clin Microbiol* **43**: 6164–6166.

Feng L, Wan Z, Wang X, Li R & Liu W (2010) Relationship between antifungal resistance of fluconazole resistant *Candida albicans* and mutations in *ERG11* gene. *Chin Med J* **123**: 544–548.

Fotedar R & Al-Hedaithy SSA (2005) Comparison of phospholipase and proteinase activity in *Candida albicans* and *C. dubliniensis*. *Mycoses* **48**: 62–67.

Gatesoupe FJ (2007) Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture* **267**: 20–30.

Ghannoum MA (2000) Potential role of phospholipases in virulence and fungal pathogenesis. *Clin Microb Rev* **13**: 122–143.

Gunde-Cimerman N, Zalar P, De Hoog S & Plemenitas A (2000) Hypersaline waters in salterns – natural ecological niches for halophilic black yeasts. *FEMS Microbiol Ecol* **32**: 235–240.

Hagler AN, Mendonça-Hagler LC, Rosa CA & Morais PP (1995) Yeast as an example of microbial diversity in brazilian ecosystems. *Oecologia brasiliensis* **1**: 225–244.

Holthuis LB (1952) A general revision of the Palemonidae (Crustacea, Decapoda, Natantia) of the Americas. *Allan Hancock Foundation* **12**: 1–396.

Johnson SK & Bueno SLS (2000) Health management. *Freshwater Prawn Culture: The Farming of Macrobrachium Rosenbergii*, *Vol. 1* (New MB & Valenti WC, eds), pp. 18–40. Blackwell Science, London.

Kanafani ZA & Perfect JR (2008) Resistance to antifungal agents: mechanisms and clinical impact. *Clin Infect Dis* **46**: 120–128.

Kantarcioglu AS & Yucel A (2002) Phospholipase and protease activities in clinical Candida isolates with reference to the sources of strains. *Mycoses* **45**: 160–165.

Keenan PO, Knight AW, Billinton N, Cahill PA, Dalrymple IM, Hawkyard CJ, Stratton-Campbellc D & Walmsleya RM (2007) Clear and present danger? The use of a yeast biosensor to monitor changes in the toxicity of industrial effluents subjected to oxidative colour removal treatments. *J Environ Monitor* **9**: 1394–1401.

Kutty SN & Philip R (2008) Marine yeasts – a review. *Yeast* **25**: 465–483.

Leaño EM, Lio-Po GD, Nadong LA, Tirado AC, Sadaba RB & Guanzon NG Jr (2005) Mycoflora of the 'green water' culture system of tiger shrimp *Penaeus monodon* Fabricius. *Aquac Res* **36**: 1581–1587.

Lu CC, Tang KFJ & Chen SN (1998) Identification and genetic characterization of yeasts isolated from freshwater prawns, *Macrobrachium rosenbergii* de Man, in Taiwan. *J Fish Dis* **21**: 185–192.

Mariano V, McCrindle CME, Cenci-Goga B & Picard JA (2009) Case–control study to determine whether river water can spread tetracycline resistance to unexposed impala (*Aepyceros melampus*) in Kruger National Park (South Africa). *Appl Environ Microb* **75**: 113–118.

Martin JW & Davis GE (2001) An update classification of the recent Crustacea. *Nat Hist Mus Los Angeles* **39**: 1–124.

Mbata TI (2008) Isolation of fungi in hyper saline Dead Sea water. *Sudanese J Public Health* **3**: 170–172.

Medeiros AO, Kohler LM, Hamdan JS, Missagia BS, Barbosa FAR & Rosa CA (2008) Diversity and antifungal susceptibility of

yeasts from tropical freshwater environments in Southeastern Brazil. *Water Res* **42**: 3921–3929.

Miao Z, Chai T, Qi C, Cai Y, Liu J, Yuan W & Yao M (2010) Composition and variability of airborne fungi in an enclosed rabbit house in China. *Aerobiologia* **26**: 135–140.

Mohan V & Ballal M (2008) Proteinase and phospholipase activity as virulence factors in *Candida* species isolated from blood. *Rev Iberoam Micol* **25**: 208–210.

Moraes-Valenti P & Valenti WC (2010) Culture of the Amazon river prawn *Macrobrachium amazonicum*. *Freshwater Prawns: Biology and Farming* (New MB, Valenti WC, Tidwell JH, D'abramo LR & Kutty MN, eds), pp. 485–501. Wiley-Blackwell, Oxford.

Müller FMC, Staudigel A, Salvenmoser S, Tredup A, Miltenberger R & Herrmann JV (2007) Cross-resistance to medical and agricultural azole drugs in yeasts from the oropharynx of human immunodeficiency virus patients and from environmental bavarian vine grapes. *Antimicrob Agents Ch* **51**: 3014–3016.

National Committee for Clinical Laboratory Standards (2002) Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. NCCLS, document M27-A2.

Ombrella AM, Racca L & Ramos L (2008) Actividades proteinasa y fosfolipasa de aislamientos de *Candida albicans* provenientes de secreciones vaginales con distintos valores de pH. *Rev Iberoam Micol* **25**: 12–16.

Palacio A, Villar J & Alhambra A (2009) Epidemiología de las candidiasis invasoras em población pediátrica y adulta. *Rev Iberoam Micol* **26**: 2–7.

Pantoja LDM, Moreira-Filho RE, Brito EHS, Aragão TB, Brilhante RSN, Cordeiro RA, Rocha MFG, Monteiro AJ, Quinet YP & Sidrim JJC (2009) Ants (Hymenoptera: Formicidae) as carriers of fungi in hospital environments: an emphasis on the genera *Tapinoma* and *Pheidole*. *J Med Entomol* **46**: 895–899.

Petrovic U, Gunde-Cimerman N & Plemenitas A (2002) Cellular responses to environmental salinity in the halophilic black yeast *Hortaea werneckii*. *Mol Microbiol* **45**: 665–672.

Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S & Diekema DJ (2006) *In vitro* susceptibilities of *Candida* spp. To caspofungin: four years of global surveillance. *J Clin Microbiol* **44**: 760–763.

Price MF, Wilkinson ID & Gentry LO (1982) Plate method for detection of phospholipase activity of *Candida albicans*. *Sabouraudia* **22**: 201–207.

Routman E, Miller RD, Phillips-Conroy J & Hartl DL (1985) Antibiotic resistance and population structure in *Escherichia coli* from free-ranging African yellow baboons. *Appl Environ Microb* **50**: 749–754.

Sidrim JJC, Castelo-Branco DSCM, Brilhante RSN, Soares GDP, Cordeiro RA, Monteiro AJ & Rocha MFG (2010) Candida species isolated from the gastrointestinal tract of cockatiels (*Nymphicus hollandicus*): *in vitro* antifungal susceptibility profile and phospholipase activity. *Vet Microbiol* **145**: 324–328.

Solomon GM, Hjelmroos-Koski M, Rotkin-Ellman M & Hammond K (2006) Airborne mold and endotoxin concentrations in New Orleans, Louisiana, after flooding, October through November 2005. *Environ Health Persp* **114**: 1381–1386.

Tawara S, Ikeda F, Maki K *et al.* (2000) *In vitro* activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi. *Antimicrob Agents Ch* **44**: 57–62.

Tayamen M & Brown JH (1999) A condition index for evaluating larval quality of *Macrobrachium rosenbergii* (De Man, 1879). *Aquac Res* **30**: 917–922.

Valenti WC, Mallasen M & Silva CA (1998) Larvicultura em sistema fechado dinâmico. *Carcinicultura de água doce: Tecnologia para Produção de Camarões*, *Vol. 1* (Valenti WC, ed), pp. 115–143. FAPESP/IBAMA, S ã o Paulo, Brazil.

Varga VES & Godoy P (2004) *Tinea Nigra*. *Micologia médica à luz de autores contemporâneos*, *Vol. 1* (Sidrim JJC & Rocha MFG, eds), pp. 124–128. Guanabara Koogan, Rio de Janeiro.

Wegrzyn G & Czyz A (2003) Detection of mutagenic pollution of natural environment using microbiological assays. *J Appl Microbiol* **95**: 1175–1181.

Zeng X, Hou X, Wang Z, Jiang L, Xiong C, Zhou M & Chen Q (2008) Carriage rate and virulence attributes of oral *Candida albicans* isolates from patients with oral lichen planus: a study in an ethnic Chinese cohort. *Mycoses* **52**: 161–165.