BIODIVERSITY OF YEASTS ASSOCIATED TO BROMELIADS IN ITAPUÃ PARK, VIAMÃO/RS

Melissa Fontes Landell[1,2]
Juliana Nunes Mautone[1,2]
Patrícia Valente[1,2]

ABSTRACT

Bromeliads are tropical and typically abundant plants in environments of the Atlantic Forest, and sustain a great diversity of organisms. The objective of the present study was to analyze the biodiversity of yeasts associated to bromeliads. Fifty samples of bromeliad leaves were sampled in Itapuã Park/RS (Pedreira Beach and Fora Beach). Leaf pieces were submitted to successive washings with 0.5%Tween 20. Flower samples were processed as above or macerated. Samples of water from the bromeliad tanks were collected with sterile pippetes and placed inside sterile tubes. Endophytes were isolated after surface sterilization of leaves with ethanol and sodium hypochlorite, followed by maceration. Aliquots of 0.1mL of serial dilutions from the last washing of leaves, samples of water from tanks, flowers, and macerated leaves were inoculated in acidified YM medium, and incubated at 25 ºC for 5-7 days. Representatives of the different morphotypes were purified and identified by the conventional methodology. The analysis of biodiversity was done by means of the Shannon-Weaver index. Of the 138 isolates obtained, 13% had ascomycetic affinity, and 87% were basidiomycetic. Twelve isolates belonged to a yet non described species of the genus *Rhodotorula*, while four isolates were identified as *Cryptococcus* sp. nov. Yeast diversity and richness were higher at Pedreira Beach (H = 3.102 and S = 34) than Fora Beach (H = 2.820 and S = 26). The bromeliad phylloplane had a great biodiversity of yeasts, demonstrating to be a good substrate for the isolation of new species of these microorganisms.

Key words: Bromeliads, Atlantic Forest, phylloplane, yeast, biodiversity.

RESUMO

Biodiversidade de leveduras associadas a bromélias do Parque de Itapuã, Viamão/RS

Bromélias são plantas tipicamente tropicais e abundantes em ambientes de Mata Atlântica e abrigam uma grande diversidade de organismos. O objetivo do presente trabalho foi analisar a biodiversidade de leveduras associadas a bromélias. Foram coletadas 50 amostras de folhas de bromélias no Parque Estadual de Itapuã/RS (Praia da Pedreira e Praia de Fora). Fragmentos das folhas foram submetidos a lavagens sucessivas com 0,5% Tween 20. Amostras de flores foram processadas da mesma forma que as folhas ou maceradas. Amostras de água dos tanques de bromélias foram coletadas com pipetas estéreis e colocadas em tubos de ensaio. Endófitos foram isolados após esterilização superficial das folhas com etanol e hipoclorito de sódio, seguida por maceração. Alíquotas de 0.1 mL das diluições decimais da última lavagem das folhas, amostras de água dos tanques, flores e folhas maceradas foram inoculadas em meio YM acidificado e incubadas a 25 °C por 5-7 dias. Representantes dos diferentes morfotipos foram purificados e identificados pela metodologia convencional. A análise da biodiversidade foi realizada através do índice de Shannon-Weaver. Dos 138 isolados obtidos, 13% são leveduras de afinidade ascomicética e 87% de afinidade basidiomicética. Doze isolados pertencem a uma espécie ainda não descrita do gênero *Rhodotorula,* enquanto quatro isolados foram identificados como *Cryptococcus* sp. nov. A diversidade e a riqueza de leveduras foram maiores na Praia da Pedreira (H = 3,102 e S = 34) que na Praia de Fora (H = 2,820 e S = 26). O filoplano das bromélias apresentou uma grande biodiversidade de leveduras, demonstrando ser um bom substrato para o isolamento de novas espécies desses microrganismos.

Palavras-chave: Bromélias, Mata Atlântica, filoplano, leveduras, biodiversidade.

Recebido em: 30.05.06; aceito em: 20.11.06.

[1] Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente, UFRGS.
[2] Departamento de Microbiologia, Instituto de Ciências Básicas da Saúde – ICBS-UFRGS. Rua Sarmento Leite, 500, sala 158, CEP 90050-170, Porto Alegre, RS, Brasil. Fone: (51) 3316-3935 – Fax: (51) 3316-3445. <patricia.valente@ufrgs.br>.

INTRODUCTION

The Atlantic Forest is one of the richest forests in terms of biodiversity on the Planet, with many species that have never been cataloged (Simões & Lino, 2003). Bromeliads are typically abundant tropical plants in environments of the Atlantic Forest, which has been considered the ecosystem with greater diversity of this family (Nunes, 2003). Their leaves are spiral with ample and flexible hens, that frequently form a receptacle in which they accumulate organic debris and water, and can be rich in microorganisms (Leme, 1994). Yeasts are generally found in fruits, cereals and other substrata that contain sugars. But they can also be isolated from air, ground, water, skin and intestine of animals, including associations with insects (Do Carmo-Souza, 1969; Takashima & Nakase, 2001; Lachance et al., 2003; Rosa et al., 2003; Pimentel et al., 2005). Besides, it is known that yeasts can be associated with plants (Lindow & Brandl, 2003). The nutrients for the sustenance of the yeast populations on the phylloplane (surface of leaves) can result from secretions of leaves (Tukey, 1971), or be carried by the wind or insects, among other organisms (Andrews & Harris, 2000).

Yeasts present a great biodiversity, playing crucial functions in the nature, as in ecosystem maintenance. Studies focused on yeasts associated with the Atlantic Forest habitats have indicated the presence of many different biotypes and putative new species (Morais et al.,1992; Rosa et al., 1994; Araújo et al., 1995; Morais et al., 1996; Prada & Pagnocca, 1997; Abranches et al., 1998, Rosa et al., 1999, Abranches et al., 2000; Buzzini & Martini, 2000, Pimenta, 2001; Carreiro et al., 2004), and were mainly done in the Southeast region of Brazil. Studies focusing other regions are practically non-existent. The present work aims at filling up this gap.

MATERIAL AND METHODS

1 Sampling and isolation of yeasts

Samples of bromeliads were collected in tracks at two distinct sites in the Itapuã Park: Pedreira Beach and Fora Beach, located in the city of Viamão, State of Rio Grande do Sul, Brazil. All samples were collected between April 2004 and January 2005. Twenty five samples of mature leaves of the bromeliads *Aechmea recurvata*, *Bilbergia nutans*, *Dyckia* sp., *Vriesea friburgensis*, *Vriesea gigantea*, *Vriesea procera*, *Tillandsia stricta*, *Tillandsia crocata*, *Tillandsia gardneri, Tillandsia geminiflora* and *Bromelia antiacantha* were aseptically collected from each site, placed in individual plastic bags, and transferred to the laboratory. Samples consisted of one leaf per plant, and all bromeliads were found at shaded places. After washing with sterile distilled water for removal of the accumulated dust and other devices eventually present, pieces of 9-10 cm^2 were aseptically cut with a sterile scalpel, placed in Erlenmeyers with 50 mL of sterile distilled water and kept in the shaker for 10 min. After that, water was discarded and two consecutive washings with 30 mL of 0.5% Tween 20 were done, keeping the Erlenmeyers in the shaker for 30 minutes each. Aliquots of 0.1 mL of serial dilutions of the last washing were spread in duplicate on acidified YM agar medium (1% glucose, 0.3% malt extract, 0.3% yeast extract, 0.5% peptone, 2% agar, 400 mg/L chloramphenicol, pH 4.0). Flowers were aseptically collected in individual plastic bags and mostly processed as the leaves. Some flowers were macerated, diluted in distilled water, and spread on acidified YM plates. Samples of water from the bromeliad tanks were collected with sterile pippetes and placed inside sterile tubes. Aliquots of 0.1 mL of serial dilutions were spread on the same medium.

Isolation of endophytic yeasts was done for 5 bromeliad samples (1 *Vriesea gigantea*, 2 *Vriesea procera*, and 2 *Tillandsia gardneri*). Leaves were washed with distilled water, cut in 10 cm^2 fragments, and dried in the laminar flow bench. Leaves were surface sterilized in 70% ethanol for 1 minute, 2% sodium hypochlorite for 3 minutes, 70% ethanol for 1 minute again, and distilled water twice. The last washing in distilled water was kept in the shaker for 30 minutes. After the washings, leaves were macerated, and 0.1 mL aliquots of decimal dilutions were spread on acidified YM agar plates. Aliquots of the last washing before maceration (0.1 mL) were spread on the same medium in order to verify if the isolated yeasts were really endophytic.

After incubation at 22-25ºC for 3-7 days, representative colonies of each morphological type were isolated and purified in YEPG agar medium (0.5% yeast extract, 2% glucose, 1% peptone, 2% agar). The strains were maintained in GYMP medium (0.5% glucose, 2% malt extract, 0.5% yeast extract, 0.2% monobasic sodium phosphate, 2% agar) slants covered with a layer of sterile mineral oil, and kept in the refrigerator. Sampling effort was calculated for the phylloplane samples correlating the number of samples and the number of different species isolated.

2 Yeasts identification

The isolates were phenotypically characterized by means of macro/micro-morphological and physiological features, as fermentation of glucose, maltose and sucrose; assimilation of the following carbon sources: D-Glucose, D-Galactose, D-Ribose, D-Xylose, L-Arabinose, D-Arabinose, L-Rhamnose, Sucrose, Maltose, Trealose, Cellobiose, Salicin, Melibiose, Lactose, Raffinose, Inulin, Starch, Glycerol, Erythritol, Ribitol, D-Glucitol, D-Mannitol, **myo**-Inositol, Lactate, Citrate, Tween 20, N-acetylglucosamine; assimilation of the following nitrogen sources: Nitrate, Nitrite, Ethylamine, Lysine, Creatine and Creatinine; starch formation; urea hydrolysis; Diazonium Blue B reaction; growth on 50% D-Glucose, 10% NaCl/16% NaCl and in different temperatures (Yarrow, 1998). Identification was performed according to Barnett et al. (2000) and the computer program YEASTCOMPARE (C. Ciriello and M. A. Lachance, copyright 1999-2001).

3 Analysis of biodiversity

Species richness was calculated as the number of different species per sampling site. The analysis of yeast biodiversity was done by means of the Shannon-Weaver index (Shannon & Weaver, 1963).

RESULTS

One hundred and thirty eight strains were isolated and identified in about 40 species, at least 10 with ascomycetic affinity and 30 basidiomycetes (Table 1). The genera more commonly found were *Candida* and *Debaryomyces* within the ascomycetous group and *Cryptococcus*, *Rhodotorula* and *Sporobolomyces/ Sporodiobolus* within the basidiomycetes. The predominant species were *Debaryomyces hansenii* (n = 4) among the ascomycetes, and *Sporobolomyces roseus* (n = 16), *Cryptococcus laurentii* (n = 15), *Cryptococcus albidus* (n = 14), *Rhodotorula* sp. nov. (n = 12), and *Sporobolomyces salmonicolor* (n = 9) among the basidiomycetes. All these species were found in both sampling sites, although with minor differences in the frequencies. *S. roseus* and *C. laurentii* were more common in Fora Beach, while *S. salmonicolor* presented a slightly higher frequency in Pedreira Beach (Table 1). The other dominant species had a similar frequency value at both sampling sites. Ballistosporogenic yeasts comprised 31.7% of the basidiomycetous isolates. Twelve isolates belonged to a yet non described species of the genus *Rhodotorula*, while four isolates were identified as *Cryptococcus* sp. nov. Strains named as *Candida* spp., *Bullera* spp., *Cryptococcus* spp., and *Fellomyces* sp. could only be identified at the genus level. The species frequency in each sampling site can also be seen in Table 1.

The value of the Shannon-Weaver (H) index of biodiversity was higher at the Pedreira Beach (H = 3.102) than at the Fora Beach (H = 2.820). The same occurred with the species richness (S), with the Pedreira Beach presenting greater richness (S = 34) compared to the Fora Beach (S = 26).

DISCUSSION AND CONCLUSION

There was a predominance of basidiomycetic yeasts in the bromeliad samples from Itapuã Park (87%), corroborating the reports that yeast populations on most plant surfaces are dominated by basidiomycetes (Bab'Eva & Chernov, 1995; Santos et al., 1996; Azeredo et al, 1998; Jager et al., 2000; Inácio et al., 2002). Although bromeliads are widespread in tropical ecosystems, there are few studies of microbial diversity associated with them, mostly dealing with samples of tank water. The yeast community of tanks of *Neoregelia cruenta* was typical of plant surfaces, with prevalence of basidiomycetous anamorphs and *Aureobasidium pullulans*, while *Quesnelia quesneliana* had a substantial proportion of ascomycetous yeasts and their anamorphs (Hagler et al., 1993). Araújo et al. (1998) found that yeast communities in the water tanks of bromeliads exposed to intense sunlight were dominated by basidiomycetous species, while ascomycetous yeasts were prevalent in bromeliads located in shaded habitats. Valente (2000), studying yeasts isolated from the phylloplane of bromeliads in Rio de Janeiro, obtained 32% of yeasts with ascomycetic affinity, 54.6% of basidiomycetous, and 13.4% of yeast-like fungi, including an ascomycetic non described new yeast species phenotypically similar to *Candida tenuis*. Ruivo et al. (2005) recently described two new yeast species isolated from the tank of the bromeliad *Canistropsis seidelii*, *Candida bromeliacearum* and *Candida ubatubensis*. These reports, together with the isolation of *Rhodotorula* sp. nov. and *Cryptococcus* sp. nov. from bromeliads in Itapuã Park, suggest that these plants are a good substrate for the isolation of new yeast species. Futhermore, as identification of yeasts based on phenotypic characters is often incomplete and misleading, there is possibility that more new yeast species are found if molecular methods are used for

confirming the identification of our isolates (Fell et al., 2000; Kurtzman and Robnett, 1998).

Although the low sampling number of each bromeliad precludes the use of statistic evaluation of data, some interesting conclusions can be drawn. The number of different yeast species isolated from each bromeliad varied. In general, few different yeast species were isolated from *A. recurvata*, *B. nutans*, *B. antiacantha*, and *Dickia* sp. Species of *Tillandsia*, except *T. stricta*, and *Vrisea* had many different yeast species, although their sampling effort was mostly similar to the other bromeliads. The highest number of different yeast species was isolated from *T. geminiflora* and *V. friburgensis,* but this was a consequence of their high sampling numbers, 9 and 11, respectively. If we analyze the number of different yeast species in relation to the number of samples, the bromeliads that presented the highest number of different species were *T. gardneri*, with an average of 3.75 yeast species for each sample, *T. crocata* and *V. procera*, with 3 species each, and *V. gigantaea*, with 2.25 different yeast species per sample. Only one yeast species was isolated from *T. stricta*, *S. roseus*, in contrast to the high number of yeast species isolated from the other *Tillandsia*, but it may be a consequence of the few samples of this bromeliad.

Concerning the isolation of endophytic yeasts, *D. hansenii* and *R. glutinis* were obtained from *V. procera* leaves, and *Candida* sp. and *C. albidus* from *T. gardneri*. This methodology resulted in the isolation of few yeast strains per sample, and was applied in just one sampling. In spite of this, it is worthwhile mentioning that *D. hansenii* isolates were obtained from flower, phylloplane and by the endophytic methodology, indicating that this species is a common inhabitant of the bromeliad environment, although with lower frequency than the basidiomycetes. Araújo et al. (1998) isolated *D. hansenii* from bromeliad water tanks, corroborating our conclusion. Camatti-Sartori et al. (2005) found *Sporobolomyces*, *Rhodotorula*, *Debaryomyces* and *Cryptococcus* as the most representative endophytic yeast genera in apples in Brazil, confirming that these genera are common endophytic fungi, while Pirttilä et al. (2003) isolated *Rhodotorula minuta* as an endophyte of *Pinus sylvestris*.

It was expected that Fora Beach had a greater biodiversity than Pedreira Beach, as it is a place with little anthropic influence, closed to the public at the time of samplings. Pedreira Beach, on the other hand, was a place exploited for granite extraction, and receives several visitors in its tracks. Nevertheless, yeast biodiversity and richness at Pedreira Beach were higher. This is explained by the more equitable distribution of the isolates among the species in this beach. In Fora Beach, there was a predominance of *C. laurentii* and *S. roseus*, with 11 and 12 isolates respectively. Both species have a very variable assimilative profile, and could represent a complex of phenotypically similar species, as was reported for *C. albidus* (Fonseca et al., 2000). If this is the case, the greater biodiversity of Pedreira Beach is a fallacy.

Studies describing the diversity of yeasts from tropical habitats are still required. It seems likely that we have discovered endophytic yeasts in bromeliads and their role needs to be better understood. Here we have described a very diverse yeast collection associated with one family of plants on two beaches in Southern Brazil. Future molecular analysis of this collection may reveal new species and with them new biotechnologies. These data provide the foundations for yeast biodiversity studies in Itapuã Park and on which further studies will follow.

ACKNOWLEDGMENTS

The authors thank the financial support from Fundação de Amparo à Pesquisa do Rio Grande do Sul (grant no. 02/1754-0) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant no. 477528/2003-1). M.F. Landell received a fellowship from CNPq.

REFERENCES

ABRANCHES J. et al. Yeast diversity and killer activity dispersed in fecal pellets from marsupials and rodents in a Brazilian tropical habitat mosaic. **FEMS Microbiology Ecology**, Amsterdam, v. 26, p. 27-33, 1998.

ABRANCHES J. et al. The yeast community and mycocin producers of guava fruit in Rio de Janeiro, Brazil. **Mycologia**, Philadelphia, v. 92, p. 16-22, 2000.

ANDREWS J. H.; HARRIS R. F. The ecology and biogeography of microorganisms on plant surfaces. **Annual Review of Phytopathology**, Palo Alto, v. 38, p. 145-180, 2000.

ARAÚJO, F. V. et al. Ascomycetous yeast communities of marine invertebrates in a southeast Brazilian mangrove ecosystem. **Antonie van Leeuwenhoek**, Amsterdam, v. 68, p. 91-99, 1995.

ARAÚJO, F. V. et al. A preliminary note on yeast communities of bromeliad-tank waters of Rio de Janeiro, Brazil. **Revista de Microbiologia**, São Paulo, v. 29, p. 118-121, 1998.

AZEREDO, L. A. I. et al. Yeast communities associated with sugarcane in Campos, Rio de Janeiro, Brazil. **International Microbiology**, Barcelona, v. 1, p. 205-208, 1998.

BAB'EVA, I. P., CHERNOV, I. Y. Geographic aspects of yeast ecology. **Physiology and General Biology Reviews**, [s.l], v. 9, p. 1-54, 1995.

BARNETT J. A. et al. **Yeasts, Characteristics and Identification**, 4rd ed. Cambridge: Cambridge University Press, 2000. 811p.

BUZZINI, P.; MARTINI, A. Biodiversity of killer activity in yeasts isolated from Brazilian rain Forest. **Canadian Journal of Microbiology**, Ottawa, v. 46, p. 607-611, 2000.

CAMATTI-SARTORI et al. Endophytic yeasts and filamentous fungi associated with southern Brazilian apple (*Malus domestica*) orchards subjected to conventional, integrated or organic cultivation. **Journal of Basic Microbiology**, Weinheim, v. 45, p. 397-402, 2005.

CARREIRO, S. C. et al. *Sympodiomyces attinorum* sp. nov., a yeast species associated with nests of the leaf-cutting ant *Atta sexdens*. **International Journal of Systematic and Evolutionary Microbiology**, Berks, v. 54, p. 1891-1894, 2004.

CIRIELO, C. J.; LACHANCE, M. A. **YeastCompare.** London: University of Western Ontario, 2001. Software.

DO CARMO-SOUZA, L. Distribution of yeasts in nature. In: ROSE, A. H.; HARRISON, J. S. (Eds.). **The Yeasts.** London: London Academic Press, 1969. p. 79-105.

FELL et al. Biodiversity and systematics of basidiomycetous yeasts as determined by large-sbunit rDNA D1/D2 domain sequence analysis. **International Journal of Systematic and Evolutionary Microbiology**, v. 50, p. 1351-1371, 2000.

FONSECA et al. Diversity in the yeast Cryptococcus albidus and related species as revealed by ribosomal DNA sequence analysis. **Canadian Journal of Microbiology**, Ottawa, v. 46, p. 7-27, 2000.

HAGLER, A. N. et al. Yeasts and coliform bacteria of water accumulated in bromeliads of mangrove and sand dune ecosystems of southeast Brazil. **Canadian Journal of Microbiology**, Ottawa. v. 39, p. 973-977, 1993.

INÁCIO, J. et al. Estimation and diversity of phylloplane mycobiota on selected plants in a Mediterranean-type ecosystem in Portugal. **Microbial Ecology**, New York, v. 44, p. 344-353, 2002.

JAGER, E.S. et al. Microbial ecology of the mango phylloplane. **Microbial Ecology**, New York, v. 42, p. 201-207, 2000.

KURTZMAN, C. P., ROBNETT, C. J. Identification and phylogeny of the ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. **Antonie van Leeuwenhoek**, Amsterdam, v. 73, p. 331-371, 1998.

LACHANCE, M. A. et al. *Metschnikowia santaceciliae*, *Candida hawaiiana*, and *Candida kipukae*, three new yeast species associated with insects of tropical morning glory. **FEMS Yeast Research**, Amsterdam, v. 3, p. 97-103, 2003.

LEME, E. M. C. Bromélias. **Ciência Hoje**, Rio de Janeiro, v. 3, p. 66-72, 1994.

LINDOW, S. E.; BRANDL, M. T. Microbiology of the phyllosphere. **Applied and Environmental Microbiology**, Washington, v. 69. p. 1875-1883. 2003.

MORAIS P.B, et al. Yeast associated with Drosophila in tropical forests of Rio de Janeiro, Brazil. **Canadian Journal of Microbiology**, Ottawa, v. 38, p. 1150-1155, 1992.

MORAIS P.B, et al. Yeasts vectored by *Drosophila quadrum* (*calloptera* group) in tropical rain forests. **Revista de Microbiologia**, São Paulo, v. 27, p. 87-91, 1996.

NUNES, J. V. C. Bromélias. In: SIMÕES, L. L.; LINO, C. F. (Eds.). **Sustentável Mata Atlântica**. São Paulo: Senac São Paulo, 2003. p. 117-130.

PIMENTA, R. S. **Levantamento de leveduras em dois fragmentos de Mata Atlântica no estado de Minas Gerais**. 2001. 70f. Dissertação (Mestrado) – Programa de Pós-Graduação em Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte, 2001.

PIMENTEL, M. R. C. et al. *Candida riodocensis* and *Candida cellae*, two new yeast species from the *Starmerella* clade associated with solytary bees in the Atlantic rain forest of Brazil. **FEMS Yeast Research**, Amsterdam, v. 5, p. 875-879, 2005.

PIRTILLÄ, A. M. et al. Two endophytic fungi in different tissues of scots pine buds (*Pinus sylvestris* L.). **Microbial Ecology**, New York, v. 45, p. 53-62, 2003.

PRADA, G. M. M.; PAGNOCCA, F. C. Ascomycetous yeasts associated with naturally occuring fruits in a tropical rain forest. **Folia Microbiology**, [s.l.], v. 42, p. 39-46, 1997.

ROSA, C.A. et al. Yeast communities of the cactus *Pilosocereus arrabidae* and associated insects in sandy coastal plains in southeastern Brazil. **Antonie van Leeuwenhoek**, Amsterdam, v. 65, p. 55-62, 1994.

ROSA, C. A. et al. *Kodamaea nitidulidarum*, *Candida restingae* and *Kodamaea anthophila*, three new related yeast species from ephemeral flowers. **International Journal of Systematic Bacteriology**, London, v. 49, p. 309-318, 1999.

ROSA, C. A. et al. Yeast communities associated with stingless bees. **FEMS Yeast Research**, Amsterdam, v. 4, p. 271-275, 2003.

RUIVO, C. C. et al. *Candida bromeliacearum* sp. nov. and *Candida ubatubensis* sp. nov., two yeast species isolated from the water tanks of *Canistropsis seidelli* (Bromeliaceae). **International Journal of Systematic and Evolutionary Microbiology**, Berks, v. 55, p. 2213-2217, 2005.

SANTOS, F. A. et al. Yeast associated with flowers and fruits from a semi-arid region of northeastern Brazil. **Revista de Microbiologia**, São Paulo, v. 27, p. 33-40, 1996.

SIMÕES, L. L., LINO, C. F. **Sustentável Mata Atlântica:** a exploração de seus recursos florestais. 2. ed. São Paulo: Editora Senac, 2003.

SHANNON, C. E.; WEAVER, W. **The mathematical theory of communication**. Urbana, IL: University of Illinois Press, 1963.

TAKASHIMA, M.; NAKASE, T. *Tilletiopsis derxii*, *Tilletiopsis oryzicola* and *Tilletiopsis penniseti*, three new species of the ustilaginomycetous anamorphic genus *Tilletiopsis* isolated from leaves in Thailand. **Antonie van Leeuwenhoek**, Amsterdam, v. 80, p. 43-56, 2001.

TUKEY Jr, H. B. Leaching of substances from plants. In: PREECE, T. F.; DICKINSON, C. H. (Eds.). **Ecology of leaf surface microorganisms**. London: Academic Press, 1971.

VALENTE, P. **Identificação convencional e molecular de leveduras isoladas de filoplano de bromélias**. 2000. 92f. Tese (Doutorado) – Programa de Pós-Graduação em Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2000.

YARROW, D. Methods for the isolation, maintenance, and identification of yeasts. In KURTZMAN, C. P.; FELL, J. W. (Org.). **The Yeasts:** a taxonomic study. Amsterdam: Elsevier Science B.V., 1998. p. 77-100.

TABLE 1 – Frequency of the yeast isolates associated with bromeliads in Itapuã Park, Viamão, RS.

Yeasts	Total number of isolates	Sampling sites		Bromeliads										
		Pedreira Beach	Fora Beach	*Achmeae recurvata*	*Billbergia nutans*	*Bromelia antiacantha*	*Dyckia* sp.	*Tillandsia crocata*	*Tillandsia gardneri*	*Tillandsia geminiflora*	*Tillandsia stricta*	*Vrisea fribugensis*	*Vrisea gigantaea*	*Vrisea procera*
		[a]n=25	n=25	n=7	n=2	n=5	n=1	n=2	n=4	n=9	n=2	n=11	n=4	n=3
Ascomycetes														
Candida diddensiae	1	0	1										1[b]	
Candida etchellsii-like	1	0	1	1										
Candida spp.	6	2	4			1			2[d]			2		1
Debaryomyces hansenii	4	1	3	1[c]	1				1[c]					1[d]
Debaryomyces melissophilus	1	1	0						1					
Debaryomyces vanrijiae	1	1	0						1					
Dipodascus albidus	1	0	1	1[c]										
Metschnikowia fructicola	1	1	0							1				
Metschnikowia hawaiiensis	1	1	0						1					
Zygossacharomyces bailli	1	1	0						1					
Basidiomycetes														
Bullera spp.	2	1	1				1					1		
Bulleromyces albus	3	1	2			1				2				
Cryptococcus albidus	14	8	6	1		3			4[d]	4		1	1	
Cryptococcus albidus ?	1	0	1		1									
Cryptococcus amylolentus ?	1	0	1			1								
Cryptococcus flavus-like	1	1	0							1				
Cryptococcus humicolus	1	1	0									1		
Cryptococcus hungaricus	1	1	0									1		
Cryptococcus laurentii	15	4	11			1		3[c]	3	3		3	1	1
Cryptococcus luteolus	3	2	1						1[c]	1			1	
Cryptococcus sp. nov.	4	4	0						2			1		1
Cryptococcus spp.	7	3	4					1	2	2			1	1
Fellomyces fuzhouensis	1	0	1											1
Fellomyces polyborus	1	0	1				1							
Fellomyces sp.	1	1	0							1				
Rhodotorula aurantiaca	7	5	2	1					2	2			1	1
Rhodotorula aurantiaca-like	1	0	1							1				
Rhodotorula bacarum	1	1	0					1						
Rhodotorula glutinis	2	1	1					1[c]						1[d]
Rhodotorula lactosa	1	0	1							1				
Rhodotorula lactosa-like	1	1	0					1						
Rhodotorula lignophila-like	1	1	0									1		
Rhodotorula minuta	4	3	1						1			1	2	
Rhodotorula sonckii	1	1	0									1		
Rhodotorula sp. nov.	12	5	7				1		1	2		7		1
Sporidiobolus pararoseus	5	2	3			2		1[c]		1		1		
Sporidiobolus pararoseus-like	2	1	1									2		
Sporobolomyces roseus	16	4	12	2[c]		1			3	7[c]	1		2	
Sporobolomyces salmonicolor	9	7	2			3				4		1	1	
Sporobolomyces salmonicolor-like	1	0	1			1								

[a] Number of samples.
[b] At least one of the isolates was obtained from the water accumulated inside the bromeliad tank.
[c] At least one of the isolates was obtained from the bromeliad flower.
[d] At least one of the isolates is endophytic.