Proceedings of the 10th International Conference on Environmental Science and Technology
Kos island, Greece, 5 – 7 September 2007

LEAF YEASTS AS BIOINDICATORS OF AIR POLLUTION IN SOUTHEASTERN BRAZIL

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ABSTRACT

Phylloplane yeasts are susceptible to a wide variety of environmental fluctuations such as urban and industrial air pollution. Surveys of air quality using leaf yeasts as bioindicators have never been carried out in Brazil. By using the spore-fall method, leafyeast populations were surveyed from the phyllosphere of *Bauhinia forficata*, *Tabebuia* sp., and *Terminalia catappa*, wide-spread tree species in urban environments of Brazil. Two cities from São Paulo State, representing high pollution level (Cubatão) and air good quality (Águas de São Pedro), were leaf tree sampled in winter and summer seasons of 2005 and 2006. The results demonstrated lower counts of leafyeast for urban area of Cubatão indicating that such yeasts are sensitives to pollutants of the air, specifically to sulphur dioxide of which level reached around 50 ug/m^3 in the winter, concentration of which the yeasts respond. Between trees, there was significant difference ($p<0.1$) for ipê (*Tabebuia* sp.) and chapeu de sol (*Terminalia catappa*) at the winter term for Águas de São Pedro. Air pollution seems to affect leafyeast populations for all trees in the same way. The most common yeast isolated was *Sporobolomyces roseus*, a ballistosporous basidiomycete species. Other ballistosporous yeasts, colonies of which were typically white, were infrequently isolated by the used method (< 5%). A small subset of isolates exhibiting variation in colony morphology was identified as *Aureobasidium pullulans*, *Pseudozyma antarctica*, *Cryptococcus albidus*, *Candida guillermondii* and *Candida* sp. The data may have validity for other tropical areas where studies should be addressed to the practical use of leafyeasts as bioindicators for monitoring the air quality.

1. INTRODUCTION

The plant leaf surface, termed phyllosphere, supports the growth of a diverse microbiota of bacteria and fungi through the utilization of the resources available in this habitat (1; 2; 3; 4; 5). Therefore, phylloplane microorganisms occupy prominent positions in plant canopies. Among of this epiphytic phyllosphere community, yeasts are found in a wide variety of trees in temperate and tropical regions (6; 7; 8). They exist both as small colonies and single cells concentrated on the junctions between epidermal cells of leaves. The colonies increase in size by budding, as do true yeasts, but also actively discharge spores, mainly at night (6). Since the leaves of higher plants have evolved as gas-exchange organs, in their relatively exposed position may act as effective pollutant traps. Leafyeasts are, therefore, in a very exposed position concerning to the physico-chemical effects of gaseous and particulate pollutants of the air such as ozone, heavy metal and sulphur dioxide (9; 10; 11). Dowding and Carvill (12) were the first to report the negative influence of urban atmospheres on *Sporobolomyces* spp. inhabiting leaves of ash plants growing in a series of urban areas of Ireland. Further studies revealed that fungi and leafyeast populations were particularly sensitive to air pollutants, mainly sulphur dioxide (6; 7; 13; 14). Nakase *et al.* (8; 15), in the course of a survey of yeasts in the phyllosphere from plants in Taiwan, found yeast strains belonging to the genus

Sporobolomyces. However, no reports have been published on pattern of air quality revealed by the impact of pollution over leafyeast populations or another related to the counting of yeasts from the phyllosphere of trees, in Brazil. Our purpose was isolate ballistosporous yeasts from the phyllosphere of *Bauhinia forficata* (pata de vaca), *Tabebuia* sp. (ipê), and *Terminalia catappa* (chapeu de sol), wide-spread tree species in urban environments of Brazil. Two cities from São Paulo State (southeast of Brazil) were chosen to be sampled in winter and summer seasons of 2005/2006, one representing high pollution level from industrial activity (Cubatão) and the other, represented high quality of the air (Águas de São Pedro).

2. MATERIALS AND METHODS

Study sites: Leaf samples were collected from *Bauhinia forficata* (pata de vaca), *Tabebuia* sp. (ipê), and *Terminalia catappa* (chapeu de sol), grown in urban area of Águas de São Pedro (22°35' S, 47°52' W) at 470 m above sea level and Cubatão at sea level, cities of São Paulo State, southeast of Brazil. The climate of both cities is warm-humid at summer with scarce precipitation during the winter months. Mean annual precipitations are about 500 mm and 2541 mm to Águas de São Pedro and Cubatão, respectively. At the leaf sampling, the temperature/air humidity were 23°C/56% and 22°C/66% for winter season and 28°C/62% and 24°C/81% for summer season to Águas de São Pedro and Cubatão, respectively. The mean concentrations, over 2005 year, of the most air pollutants, in µg/m^3, from Cubatão sampled sites were: SO_2, 39; NO_2, 89; O_3, 87 and inhalant particulate >10 (PM10), 89. These data were extracted from the CETESB database (16), the air quality management bureau for São Paulo State.
Leaf sampling: Four individuals of *Bauhinia forficata*, five of *Tabebuia* sp., and eighteen of *Terminalia catappa*, grown in urban area of Águas de São Pedro and Cubatão, were tagged and mapped using GPS system. In each tree, three green leaves from N,S,E,W directions were picked on a random basis at July 2005 (winter sampling) and January 2006 (summer sampling). The leaves were transferred, under cooling, to the laboratory to survey phylloplane yeasts.
For isolating the ballistosporous phylloplane yeasts, it was used the spore-fall method according to Dowding and Peacock (7). Thus, six 1-cm diameter discs were cut around the rib of leaves of each of the sampled trees. Using a template, these discs were stuck by their adaxial (upper) surface to Petri dish lids, using a blob of petroleum jelly. The lids were replaced on the Petri dishes containing 2.5% malt agar. The plates were incubated, lid-uppermost, at room temperature and after 24h the plates were inverted and incubated for a further 72h. After the incubation time, the number of colonies, pink colour in their majority, under each leaf disc were counted, recorded and identified according to method described below. Each different yeast morphotype was purified and maintained on YM slants or liquid nitrogen for later identification. The yeasts were characterized by standard methods (17). Identifications followed the keys of Kurtzman & Fell (18).
Data were analyzed as split plot randomized design, where plots represented the trees and split plot were directions (N,S,E,W). Statistical analysis was performed using appropriate model in PROC MIXED from Statistical Program SAS 8.02 (19). Leafyeast count means were compared using Tukey-Kramer test ($p<0.1$). Since the counts of ballistosporous yeast are non-normally distributed, data were log (count+1) transformed.

3. RESULTS AND DISCUSSION

3.1 Leafyeast survey

This survey was carried out for urban zones of Águas de São Pedro and Cubatão, where maximum concentrations of pollutants, over the year, in µg/m^3, were 50, 105, 80 and 120 for SO_2, NO_2, O_3 and PM10, respectively. SO_2 and NO_2, the most yeast deleterious pollutants, showed increasing trend for mean monthly concentrations at the dry cold season (May to September). According to CETESB, the bureau on air quality management of São Paulo State, this seasonal pattem reflects the meteorological conditions that difficult the pollutant dispersion. Tables 1 and 2 summarize the analysis of leafyeast counts for winter and summer periods, respectively. Significant variation in counts from different sites was observed. According to the pooled analysis, counts are significantly higher ($p<0.01$) on host trees from Águas de São Pedro at both seasons. Among the tree species, there was significant difference ($p<0.1$) in the yeast counts only for *Tabebuia* sp. and *Terminalia catappa* at the winter term for the location of Águas de São Pedro. It was possible to discern some collect sites of Cubatão where leaves yielded no colonies; this cleary reflected the industrial activity (refinery, chemical industry and port) of the zone. Curiously, some of these sites, where the recorded leafyeast counts were so low, were located not so close to the industrial area. This demonstrated the influence of the wind direction on carrying and spreading air pollutants across the environment, changing the pattern and level of pollution close to the source. Interference of cardinal points on sporing leafyeast enumeration between leaves derived from the same tree was observed only for Cubatão, specially for northern side of plant canopies showing that they are susceptible to environmental fluctuations of air pollutants. Thus, it would suggest to be unnecessary to record different sides of trees from unpolluted areas as the balneary Águas de São Pedro. Concerning to the influence of the solar radiation, Newsham *et al.* (2) demonstrated that *Sporobolomyces roseus* was one of the most sensitive yeast to u.v-B radiation. According to CETESB, the pollution level, specially for sulphur dioxide for which leafyeasts are particularly sensitive, was higher in winter season. Unlike to temperate zone, an advantage in using leafyeast survey in tropical species is that they are throughout the year evergreen trees. Given that ballistosporous yeasts rely upon spore discharge for dispersal on leaf surfaces, these organisms show strategic exposure to environmental fluctuations, specially air pollutants.

3.2 Leafyeast identification

The most common yeast isolated by the spore-fall method was *Sporobolomyces roseus*, a ballistosporous basidiomycete. Other ballistosporous yeasts, colonies of which were typically white, were infrequently isolated by this method (< 5%). A small subset of isolates exhibiting wide variation in colony morphology was identified as *Aureobasidium pullulans*, *Pseudozyma antarctica*, *Cryptococcus albidus*, *Candida guillermondii* and *Candida* sp.

Table 1: Pooled analysis of count means of leafyeast colonies isolated from the trees *Bauhinia forficata*, *Tabebuia* sp. and *Terminalia catappa* collected at urban zones of Águas de São Pedro and Cubatão (SP) at the winter period.

		Águas de São Pedro		Cubatão			
		n		n		**n**	**Mean**
Tree	CS	28	2.17 ±0.15[1] ab (183 ±15)[2]	44	1.35 ±0.12a (42 ±12)	**72**	**1.76 ±0.09a (112 ±10)**
	IP	4	2.44 ±0.39a (313 ±40)	16	1.53 ±0.19a (62 ±20)	**20**	**1.99 ±0.22a (187 ±22)**
	PV	8	2.05 ±0.28b (138 ±28)	8	1.10 ±0.28a (12 ±28)	**16**	**1.57 ±0.19a (75 ±20)**
Direction	N	10	2.19 ±0.20a (170 ±34)	17	1.54 ±0.14a (75 ±24)	**27**	**1.86 ±0.12a (123 ±21)**
	S	10	2.33 ±0.20a (285 ±34)	17	1.44 ±0.14ab (39 ±24)	**27**	**1.88 ±0.12a (162 ±21)**
	E	10	2.32 ±0.20a (232 ±34)	17	1.23 ±0.14bc (27 ±24)	**27**	**1.78 ±0.12ab (129 ±21)**
	W	10	2.06 ±0.20a (158 ±34)	17	1.09 ±0.14c (15 ±24)	**27**	**1.58 ±0.12b (86 ±21)**
Mean			**2.22 ±0.17A (211 ±17)**		**1.32 ±0.12B (39 ±12)**		

[1] Mean transformed in log (count+1); [2] Original mean; n, observation number. Values followed by a different small letter are significant by Tukey-Kramer test (p<0.1) and values followed by a different capital letter are significant by F test (p<0.01). CS, *Terminalia catappa*; IP, *Tabebuia* sp.; PV, *Bauhinia forficata*.

Table 2: Pooled analysis of count means of leafyeast colonies isolated from the trees *Bauhinia forficata*, *Tabebuia* sp. and *Terminalia catappa* collected at urban zones of Águas de São Pedro and Cubatão (SP) at the summer period.

		Águas de São Pedro		Cubatão			
		n		n		**n**	**Mean**
Tree	CS	28	2.18 ±0.14[1] a (182 ±11)[2]	44	1.42 ±0.11a (41 ±9)	**72**	**1.80 ±0.09a (111 ±7)**
	IP	4	2.40 ±0.37a (260 ±29)	16	1.57 ±0.19a (43 ±14)	**20**	**1.99 ±0.21a (151 ±16)**
	PV	8	2.08 ±0.26a (136 ±20)	8	1.19 ±0.26a (15 ±20)	**16**	**1.64 ±0.19a (76 ±14)**
Direction	N	10	2.11 ±0.18a (156 ±25)	17	1.46 ±0.13a (35 ±18)	**27**	**1.79 ±0.11a (96 ±15)**
	S	10	2.22 ±0.18a (185 ±25)	17	1.26 ±0.13b (22 ±18)	**27**	**1.74 ±0.11a (104 ±15)**
	E	10	2.33 ±0.18a (227 ±25)	17	1.38 ±0.13ab (31 ±18)	**27**	**1.86 ±0.11a (129 ±15)**
	W	10	2.23 ±0.18a (202 ±25)	17	1.48 ±0.13a (43 ±18)	**27**	**1.85 ±0.11a (123 ±15)**
Mean			**2.22 ±0.16A (193 ±12)**		**1.40 ±0.11B (33 ±9)**		

[1] Mean transformed in log (count+1); [2] Original mean; n, observation number. Values followed by a different small letter are significant by Tukey-Kramer test (p<0.1) and values followed by a different capital letter are significant by F test (p<0.01). CS, *Terminalia catappa*; IP, *Tabebuia* sp.; PV, *Bauhinia forficata*.

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