

PRELIMINARY NOTES ON YEASTS ASSOCIATED WITH NECROTIC CACTUS STEMS FROM DIFFERENT LOCALITIES IN BRAZIL

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ABSTRACT

The yeast species found in necrotic stems of three columnar cacti (*Pilosocereus machrisii*, *Pilosocereus vilaboensis*, and *Praecereus euchlorus*) at eight localities in Brazil were described and a similarity analysis using Sorensen distances was used to compare the composition of yeast species at these localities. Of 56 necrotic cactus stems sampled, 32 produced yeast colonies. Ten species of yeast or yeast-like microorganisms were identified from 53 isolates, with *Pichia cactophila*, *Candida sonorensis*, *Geotrichum* sp., and *Sporopachydermia cereana* being the most common. The remaining species occurred in low proportions in the cacti surveyed. The similarity analysis provided a dendrogram (UPGMA) that clustered the yeast communities from different cactus species and indicated that host cactus species was unimportant in this clustering.

Key words: cactus-yeast-*Drosophila* system, *D. buzzatii* cluster, columnar cactus.

RESUMO

Notas preliminares sobre leveduras associadas com cactos necrosados em diferentes localidades no Brasil

As leveduras encontradas em cladódios necrosados de três cactos colunares (*Pilosocereus machrisii*, *Pilosocereus vilaboensis* and *Praecereus euchlorus*) em oito localidades no Brasil foram identificadas e uma análise de similaridade baseada na distância Sorensen foi usada para comparar as comunidades nas diferentes localidades. Dos 56 cladódios necrosados amostrados, 32 produziram colônias de leveduras. Dez espécies de leveduras foram identificadas a partir de 53 isolados, com as espécies *Pichia cactophila*, *Candida sonorensis*, *Geotrichum* sp. e *Sporopachydermia cereana* sendo as mais comuns. As espécies restantes ocorreram em baixas frequências. O dendograma (UPGMA) construído a partir da análise de similaridade agrupou comunidades de leveduras obtidas em diferentes espécies de cactos e indicou que as espécies de cactos hospedeiros não foram determinantes nesse agrupamento.

Palavras-chave: sistema cacto-levedura-*Drosophila*, cluster *D. buzzatii*, cacto colunar.

INTRODUCTION

The cactus-yeast-*Drosophila* system represents a three-way ecological association in which necrotic cactus tissue serves as a substrate for a diverse and often highly specific yeast flora. These yeasts, in turn, constitute an important food source for larval and adult stages of *Drosophila*, which often have specific relationships with the substrate used for breeding and feeding (Sang, 1978; Fogleman, 1982; Phaff *et al.*, 1985; Ganter *et al.*, 1986; Starmer *et al.*, 1990). The interdependence of yeast and *Drosophila* on decaying tissues of cactus also occurs because the flies serve to disperse yeasts to new cactus habitats (Gilbert, 1980). This interactive system provides an interesting scenario for ecological and evolutionary studies.

Previous studies on yeasts associated with cacti have revealed a spectrum of host plant specificities. For instance, *Pichia heedii* is specific to only one cactus species in the North American Sonoran desert (Phaff *et al.*, 1978), *Candida deserticola* is restricted to several species of the genus *Stenocereus* (Phaff *et al.*, 1985), and *Pichia cactophila* and *Candida sonorensis* are found in almost all cactus species from which yeasts have been collected (Lachance *et al.*, 1988; Starmer *et al.*, 1990). Studies on the spatial heterogeneity in species composition and relative abundance in yeast communities associated with necrotic cacti have shown differences within and among geographic areas (Starmer *et al.*, 1990; Barker *et al.*, 1983).

Studies of the cactus-yeast-*Drosophila* system in Brazil have used mainly the *Drosophila buzzatii* species cluster and the cactus genera *Opuntia*, *Cereus*, and *Pilosocereus* (Sene *et al.*, 1988; Rosa *et al.*, 1994, 1995). In this work we describe the yeast species found in the necrotic stems of three columnar cactus species at eight localities in Brazil and report a similarity analysis for these localities based on the species composition. This survey is part of an extensive study on the evolutionary history of *D. gouveai*, a member of the *D. buzzatii* cluster that breeds exclusively in decaying cactus stems and inhabits most of the geographical areas discussed here.

METHODS

Fifty-eight necrotic cactus stems were sampled from eight localities in Brazil (Table 1), but only

32 provided yeast colonies. Three columnar cacti distributed in South America, *Pilosocereus machrisii*, *Pilosocereus vilaboensis*, and *Praecereus euchlorus*, occurred at these localities, with no overlap in distribution. *P. machrisii* and *P. vilaboensis* are typical of quite barren rocky outcrops associated with savannah and *campos rupestres* vegetation, whereas *Praecereus euchlorus* occurs in slopes with dry, shallow soil amidst gallery forest.

Necrotic stems were sampled aseptically by suspending about one gram of material (from various positions within the necrosis, and only one necrotic stem per plant) in 5 ml of sterile distilled water. The samples were vortexed in aseptic conditions and one loop full was streaked onto YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, and 2% agar) supplemented with 100 mg of chloramphenicol/L. The plates were incubated at room temperature ($27 \pm 3^\circ\text{C}$) for three days. Individual yeast colonies were purified and characterized using standard methods (van der Walt & Yarrow, 1984; Lachance *et al.*, 1988). The yeast species were identified according to the keys of Kurtzman & Fell (1998). When two or more cultures isolated from one sample were identified as the same species, they were considered as a single isolate.

The similarity between pairs of yeast communities from different localities was calculated by using the Sorensen distance (Ludwig & Reynolds, 1988) based on the absence or presence of each species at each locality. Clustering was done using the unweighted pair-group arithmetic average (UPGMA) procedure (Sneath & Sokal, 1973).

RESULTS AND DISCUSSION

A total of 53 isolates were obtained and ten species of yeast or yeast-like microorganisms were identified (Table 2). *Pichia cactophila* was the most commonly isolated yeast species, followed by *Candida sonorensis*, *Geotrichum* sp., and *Sporopachydermia cereana*. The PIR locality was the only one where *P. cactophila* was not isolated. The yeast-like microorganism *Geotrichum* sp. was the prevalent species at Altinópolis, SP (FOR) and Brotas, SP (BRO), where the host cactus was *P. machrisii*.

P. cactophila is the most widely distributed cactophilic yeast (Lachance *et al.*, 1988) and the most frequent yeast in necrotic cactus samples

(Starmer *et al.*, 1990). This species is rarely found in habitats other than necrotic cactus stems (Starmer *et al.*, 2003). *C. sonorensis* has only been found in cacti and occurs in rotting somatic tissue more or less independently of the host species or geographic location (Starmer *et al.*, 1990). The species of *Geotrichum* are prevalent in the yeast community associated with the cactus *Pilosocereus arrabidaei* in southeastern Brazil (Rosa *et al.*, 1994; Morais *et al.*, 1994; Rosa *et al.*, 1995). *S. cereana* is a complex of 10 related species (Lachance *et al.*, 2001) and, in Brazil, occurs as two species: *S. cereana* "australis" in *P. arrabidaei* (Rosa *et al.*, 1994) and *S. cereana* "brasiliensis" in *Cereus pernambucensis* (Rosa *et al.*, 1995). *P. cactophila*, *S. cereana*, and *C. sonorensis* are the principal species of most cactus yeast communities (Starmer *et al.*, 1990; Starmer *et al.*, 2003). *P. cactophila*, *Geotrichum* spp., and *C. sonorensis* are among the most prevalent yeast species present in the intestinal tract of *Drosophila serido* larvae collected in necrotic stems of *P. arrabidaei* (Morais *et al.*, 1994). *D. serido* is a cactophilic species of the *D. buzzatii* cluster in South America.

The yeast species found in lowest proportions in the cacti surveyed included *Clavispora lusitanae*, which was found only at Furnas, MG (FUR) and

has frequently been isolated from *Pachycereinae* cacti species in southern Mexico (Starmer *et al.*, 1990). This species may also occur in habitats other than cactus (Lachance *et al.*, 1986). This is the first published record of *C. lusitanae* isolated from Cactaceae in Brazil. *Cryptococcus humicola* also was found only at FUR, and species of this genus generally occur at a low frequency in cacti (Lachance *et al.*, 1988; Rosa *et al.*, 1994). *Pichia membranifaciens*, a yeast species that is not strictly cactophilic (Lachance *et al.*, 1988), was found only at FOR. This species has been found in necrotic stems of *P. arrabidaei* from southeastern Brazil (Rosa *et al.*, 1994). *Pichia novergensis* is an exogenous component of the yeast flora of necrotic cactus tissues (Lachance *et al.*, 1988). In Brazil, *P. novergensis* has been found in stems of *Opuntia* spp. (Rosa *et al.*, 1995) and of *P. arrabidaei* (Rosa *et al.*, 1994), as well as in flowers of *C. pernambucensis* (Rosa *et al.*, 1995). *Rhodotorula* spp. have been isolated at very low frequencies from yeast communities associated with cactus (Lachance *et al.*, 1988), and have been found on the stems and flowers of *P. arrabidaei* and *Opuntia* spp. from Brazil (Morais *et al.*, 1994; Rosa *et al.*, 1995). *Pichia guilliermondii* occurred only at Pirenópolis, GO (PIR), and was the sole species isolated at this locality.

TABLE 1
Localities studied and the host cactus and vegetation type present.

Locality	Geographic coordinates	Identification	Host cactus present	Vegetation type
Altinópolis, São Paulo State	21°02'S, 47°10'W	FOR	<i>Pilosocereus machrisii</i>	<i>campos rupestres</i>
São Simão, São Paulo State	21°27'S, 47°32'W	SAS	<i>Praecereus euchlorus</i>	gallery forest
Dois Córregos, São Paulo State	22°21'S, 48°21'W	DOC	<i>Praecereus euchlorus</i>	gallery forest
Furnas, Minas Gerais State	20°18'S, 46°18'W	FUR	<i>Pilosocereus machrisii</i>	<i>campos rupestres</i>
Brotas, São Paulo State	22°24'S, 47°59'W	BRO	<i>Pilosocereus machrisii</i>	<i>campos rupestres</i>
São Carlos, São Paulo State	22°03'S, 47°50'W	ITA	<i>Pilosocereus machrisii</i>	<i>campos rupestres</i>
Pirenópolis, Goiás State	15°50'S, 48°57'W	PIR	<i>Pilosocereus vilaboensis</i>	<i>campos rupestres</i>
Cristalina, Goiás State	16°46'S, 47°35'W	CRI	<i>Pilosocereus machrisii</i>	<i>campos rupestres</i>

This species has been observed only sporadically in the somatic tissue and fruit of cactus (Lachance *et al.*, 1988). In previous surveys, only the anamorph of this species, *Candida guilliermondi*, has been isolated from necrotic cactus tissues (Rosa *et al.*, 1994; Morais *et al.*, 1994; Starmer *et al.*, 1990).

Figure 1 shows the dendrogram of similarity among the yeast communities based on the composition of yeast species at each locality. The yeast communities from FOR, BRO (host cactus *P. machrisii*), and São Carlos, SP (SAS) (host cactus *P. euchlorus*) formed a cluster with high similarity. The yeast community from FUR clustered with that from Cristalina, GO (CRI), mainly because of the presence of the yeast species *P. novergensis* and *Rhodotorula* sp., which occurred only at these localities. The PIR locality was isolated on a branch by itself because of the presence of the yeast *P. guilliermondii*, which was the only species isolated from this locality. Because yeast communities from different cactus species were clustered together in the dendrogram, the host cactus species appeared to be unimportant in this clustering. This result

agrees with the wide cactus niche occupied by the most of the species isolated (Starmer *et al.*, 2003). We have made extensive collections of cactophilic *Drosophila* from the localities studied in this survey and, with the exception of SAS and Dois Córregos, SP (DOC), all of the other sites had *D. gouveai* and *D. buzzatii*, two members of the *D. buzzatii* cluster. *P. euchlorus* is the only host cactus found in São Simon, SP (SAS) and DOC. The absence of cactophilic *Drosophila* at these two sites suggests that *P. euchlorus* is an unsuitable breeding site for these *Drosophila* species. The lack of *D. gouveai* and *D. buzzatii* in necrotic stems of *P. euchlorus* could reflect the absence of suitable nutrients provided by the yeast species associated with this host. However, based on the similarity in the composition of the yeast species at the different localities (Fig. 1), this hypothesis appears to be unlikely. The yeast communities at SAS and DOC were similar to those of other localities where the cacti present belonged to the genus *Pilosocereus*, a well-defined breeding site for the *D. buzzatii* species cluster.

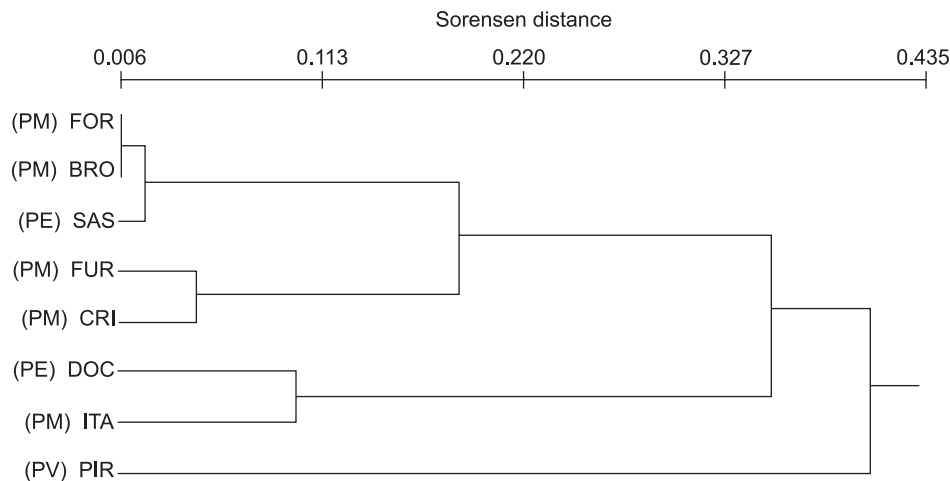


Fig. 1 — UPGMA dendrogram showing the similarity in the composition of cactus yeast species at different localities (see Table 1 for locality identification). Similarity was calculated using the Sorensen distance (Ludwig & Reynolds, 1988) based on the presence or absence of yeast species at each locality. The host cactus present at each locality is shown in parentheses. Abbreviations: PM, *Pilosocereus machrisii*; PE, *Praecereus euchlorus*; PV, *Pilosocereus vilaboensis*.

TABLE 2
Yeast species and number of isolates from necrotic cactus stems from eight sites in Brazil.
 See Table 1 for locality identification.

Yeast species	Locality (sample size)							
	FOR (6)	SAS (2)	DOC (3)	FUR (9)	BRO (2)	ITA (2)	PIR (1)	CRI (5)
<i>Pichia cactophila</i> Starmer, Phaff, Miranda and Miller	4	2	1	8	1	2	–	4
<i>Pichia guilliermondii</i> Wickermam	–	–	–	–	–	–	2	–
<i>Pichia novergensis</i> Least and Yarrow	–	–	–	1	–	–	–	2
<i>Pichia membranifaciens</i> Hansen	1	–	–	–	–	–	–	–
<i>Candida sonorensis</i> (Miller, Phaff, Miranda, Heed and Starmer) Meyer and Yarrow	2	1	–	2	2	–	–	2
<i>Rhodotorula</i> sp.	–	–	–	1	–	–	–	1
<i>Sporopachydermia cereana</i> Rodriguez de Miranda	1	1	–	–	1	–	–	1
<i>Clavispora lusitaneae</i> Rodriguez de Miranda	–	–	–	1	–	–	–	–
<i>Cryptococcus humicola</i> (Dazewska) Golubev	–	–	–	1	–	–	–	–
<i>Geotrichum</i> sp.	4	–	–	1	2	–	–	1
Total number of isolates	12	4	1	15	6	2	2	11

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