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Clavispora opuntiae and other yeasts associated with the moth *Sigelgaita* sp. in the cactus *Pilosocereus arrabidae* of Rio de Janeiro, Brazil

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Summary

Clavispora opuntiae was the prevalent yeast associated with the feeding sites of *Sigelgaita* sp. larvae in the cactus *Pilosocereus arrabidae*. Also associated with this habitat were *Candida sonorensis*, *Pichia cactophila*, *Pichia barkeri*, *Candida* sp. A, *Geotrichum* sp., *Geotrichum sericeum* and the yeast like organisms *Prototheca zopfii* and *Acremonium* sp. Atypical yeast biotypes that may represent new species of *Pichia*, *Sporopachydermia* and *Candida* were isolated. Mating types of *Clavispora opuntiae* were at a ratio 70 h^+ to 3 h^- and reduced levels of sporulation suggested low pressure for sexual reproduction in this habitat. *Sigelgaita* sp. probably was not an important vector for *Clavispora opuntiae* because it was not isolated from an adult or eggs of this moth.

Introduction

Most yeasts depend on insects or other vectors for their dispersal, and various examples of yeast-insect associations have been described (Phaff & Starmer 1987). The necrotic tissues of cacti are among the most studied models of yeast communities and their associations with insect vectors. The yeast community isolated from necrotic cactus tissue is mostly specific for this substrate, and recognized as cactophilic. Some of these yeasts are generalists associated with various species of cacti but others occur only in certain cacti (Lachance et al. 1988; Starmer et al. 1990). One cactophilic yeast, *Clavispora opuntiae* Phaff et al., has been reported as specifically associated with the moth *Cactoblastis cactorum* Berg and prickly pear cacti of the genus *Opuntia* (Tournefort) Miller. This moth has been used world-wide for the biological control of *Opuntia,* and it has been suggested as the primary vector of *Cl. opuntiae* for both intraspecific and interspecific distribution in *Opuntia* cacti. Other vectors may carry this yeast less specifically and in lower numbers to other cacti (Lachance et al. 1988; Starmer et al. 1988).

Another association similar to that between *Opuntia* and *Cactoblastis* exists between a new species of moth *Sigelgaita* (Heinrich) sp. (Lepidoptera: Phycitidae), and *Pilosocereus arrabidae* (Lem.) Byles et Rowl., the prevalent columnar cactus of a coastal sand dune area (Restinga ecosystem), in Rio de Janeiro (R.F. Monteiro, unpubl.). The larvae of this moth are solitary internal cactus feeders, each forming a linear tunnel with a single external exit. The feces are deposited at the tunnel exit forming a plug that obscures access to the tunnel.

Fig. 1. Infestation of the cactus *Pilosocereus arrabidae* by the moth *Sigelgaita* sp. (1) External fecal deposit. (2) Tunnel entrance with fecal deposit removed. (3) Moth larva (3 cm length) and tunnel.

Recent deposits are light-colored but darken and become black in time (Fig. 1). This moth lays eggs individually at the bases of cactus spines and flower buds, and occasionally on fruits resulting in the presence of a few larvae in each plant. Each larva appears to remain in a single tunnel for about 30 days and the pupa incubates in the soil during 20 to 30 days. The larvae are observed most frequently during spring and summer, but are present throughout the year. *Sigelgaita* sp. is apparently one of the principal causes of mechanical damage resulting in formation of necroses in stems of *P. arrabidae*. In this paper we have shown *Cl. opuntiae* to be the prevalent yeast associated with *Sigelgaita* sp. larvae and tunnels in *P. arrabidae*, and have noted the occurrence of some other yeast species in this habitat.

Methods

Samples from *P. arrabidae* stems infested with *Sigelgaita* sp. were collected during the months of January and February of 1991 from Restinga da Barra de Maricá, about 50 km north of the city of Rio de Janeiro, Brazil. Samples included 36 of larvae, 37 of feces and rotting tissue from within tunnels, and 34 of external fecal deposits, and were transported to the laboratory on ice and processed within a maximum of 5 hours after collection. Most samples were taken in sets of 3 including one of each type of material from the same plant. Thirty-three eggs of *Sigelgaita* sp. were collected aseptically from the needles and flowers of about 10 *P. arrabidae* individuals. Various samples of feces from within and outside the tunnels of *Sigelgaita* sp. containing larvae of other insects were collected and reared in the laboratory to allow the adults to emerge.

Tunnel material or light-colored fresh fecal deposit from the tunnel entrance was removed and 0,5 g suspended in 4,5 ml of sterile distilled water. This suspension was shaken vigorously with a vortex mixer, and one loopful was streaked out or 0,1 ml of sucessive decimal dilutions were spread on acidified YM agar (yeast extract 0,3%, malt extract 0,3%, peptone 0,5%, glucose 1% and agar 2%, adjusted to pH 3,7–4,0 by addition of 0,7% of 1N HCl). Plates were incubated five days at room temperature (26 ± 3°C). Eggs of *Sigelgaita* sp. were broken with a sterile glass rod on surface of the acidified YM agar and streaked directly on plates. Moth larvae were surface-disinfected in the field by imersion for 1 min in 70% ethanol, and each was placed in a tube with sterile distilled water. In the laboratory, larvae were homogenized in a hand-held glass-teflon tissue homogenizer, 0,1 ml aliquots spread on acidified YM agar and incubated as noted above.

Representatives of each distinct colony morphology were picked from the plates and pure cultures isolated from them. Characterization was done by standard methods as recommended by van der Walt & Yarrow (1984) and Lachance et al. (1988), and the DBB test as described by Hagler & Mendonça-Hagler (1991). Tests for assimilation of ethyl acetate were done as described by Lachance et al. (1988), except that plates were supplimented with 0.1 ml volumes of ethyl acetate placed asepticaly in the inverted petri dish lids 1 and 2 weeks after inoculation. The formation of zygotes and asci to determine mating types of *Clavispora opuntiae* were done by pair-wise mixing with the type culture NRRL Y–11820(h^+) and mating type NRRL Y–11821(h^-) on 1 and 2% malt extract agar and 2% agar containing 1% ground dry *P. arrabidae* tissue (Phaff et al. 1986). Identifications were done according to the keys of Kreger-van Rij (1984), Barnett et al. (1990), and Lachance et al. (1988). Two or more cultures identified as one species from the same sample were considered as a single isolate.

Results

The 257 isolates were identified as 23 different species of yeast and two yeast-like organisms. These and their respective frequencies of isolation are presented in Table 1. The yeasts isolated from larvae were also present in the interior of tunnels of *Sigelgaita* sp., and the external fecal deposit had more yeast species than other materials sampled. *Clavispora opuntiae* was the prevalent species with an occurrence in about 70% of the samples and had populations of about 10^4 to 10^5 colony forming units per gram in tunnel material and external fecal deposits. The mating types of 76 *Cl. opuntiae* isolates were found to be highly unbalanced with 70 h^+ to only 3 h^- and 3 strains with undetermined mating type. However, no zygotes or asci were observed from mating between our strains, and all *Cl. opuntiae* ascospores were formed in matings of our strains with the type and mating type cultures. Counts of *Candida sonorensis*, *Candida* sp. A, *Geotrichum* sp., '*Pichia amethionina* var. *fermentans*', *Pichia barkeri*, *Pichia cactophila*, and *Prototheca zopfii* reached levels in the range of 10^3 to 10^4 per gram when present. *Geotrichum* sp. and a yeast like fungus tentatively identified as *Acremonium* sp. were isolated from about 35% and 25% of the samples, respectively. Most isolates of *P. cactophila* and *P. barkeri* were isolated from tunnels or external fecal deposits. *Geotrichum sericeum* and the yeast like alga *P. zopfii* were frequently isolated from the larvae and tunnel material. *Aureobasidium pullulans*, *Candida famata*, and *Cryptococcus albidus* var. *albidus*, were only isolated from external fecal deposits or eggs. Yeasts were isolated from 16 of 33 eggs, but *Cl. opuntiae* was not isolated from any of them. An adult moth yielded an isolate of *Candida antarctica* and molds.

Insects other than *Sigelgaita* sp. were also frequent in these samples. The flies *Drosophila serido* Vilela et Sene and a species of Milichiidae, two beetle species belonging to the genera *Camptodes* (Nitidulidae) and *Trycorynus* (Anobiidae) were found in samples from the tunnels. Feces from outside the tunnels had two diptera species of the families Psychodidae and Milichiidae, and the same beetle species noted above and a species of Cryptophagidae.

Discussion

Lachance et al. (1988) and Starmer et al. (1988) suggested that *Clavispora opuntiae* is mostly specific for cacti of the tribe Opuntieae. Our results have shown that this yeast has a wider spectrum of host plants within the family Cactaceae, and could be more generally associated with the larvae of cactus-feeding moths. *Cactoblastis cactorum* may be the primary vector or principal habitat (or both) of

Cl. opuntiae, but the association of this yeast with moth eggs and adults has not been verified (Lachance 1990; Lachance et al. 1988; Starmer et al. 1988). Since *Cl. opuntiae* was not associated with *Sigelgaita* sp. eggs, and larvae of this moth appear to be restricted to individual plants, other insects present in this habitat were probably the principal vectors of the yeast community associated with the feeding sites of *Sigelgaita* sp. larvae. In contrast to its prevalence in the association with the moth larvae habitat, the frequency of occurrence of *Cl. opuntiae* was less than five percent in necrotic tissues from *P. arrabidae* (C.A. Rosa & A.N. Hagler, unpubl.). The high frequency of isolation of *Cl. opuntiae* suggested that this yeast is intimately associated with the feeding site of *Sigelgaita* sp. larvae, and it probably has a competitive advantage over other cactophilic yeasts in this habitat.

Cl. opuntiae is a haploid heterothallic yeast with mating compatibility determined by two alleles of a single locus (Phaff et al. 1986; Lachance 1990). According to Lachance (1990), sexual recombination appears to prevail in natural populations of *Cl. opuntiae* because the overall mating type distribution does not deviate significantly from the 1:1 ratio expected. However, the low level of fertility

Table 1. Yeasts and yeast like organisms and their frequency of isolates associated with the feeding site of *Sigelgaita* sp. larvae in the cactus *Pilosocereus arrabidae*.

Yeasts	Larvae (n=36)[1]	Tunnels (n=37)	External fecal deposits (n=34)	Eggs (n=33)
Acremonium sp.[2]	10	9	8	–
Aureobasidium pullulans (de Bary) Arnaud	–	–	2	7
Candida antarctica (Goto, Sugiyama et Iizuka) Kurtzman, Smiley, Johnson et Hoffman	–	–	–	2
Candida famata (Harrison) Meyer et Yarrow	–	–	1	–
Candida guilliermondi (Castellani) Langeron et Guerra	1	1	1	–
'*Candida ingens*-like'	–	–	1	–
Candida sonorensis (Miller, Phaff, Miranda, Heed et Starmer) Meyer et Yarrow	4	8	4	1
'*Candida terebra*-like'	–	1	1	–
Candida sp. A	1	4	2	–
Candida sp. B	–	–	1	–
Candida sp. C	–	–	1	–
Candida sp. D	–	–	–	1
Clavispora opuntiae Phaff, Miranda, Starmer, Tredick et Barker	26	25	25	–
Cryptococcus albidus var. *albidus* (Saito) Skinner	–	–	1	–
Cryptococcus laurentii (Kufferath) Skinner	–	–	1	1
Cryptococcus luteolus (Saito) Skinner	1	1	–	4
Cryptococcus sp.	–	–	–	3
Geotrichum sericeum (Stautz) Hoog, Smith et Gueho	6	8	2	–
Geotrichum sp.	14	14	12	–
'*Pichia amethionina* var. *fermentans*'[3]	2	2	–	–
Pichia barkeri Phaff, Starmer, Tredick-Kline et Aberdeen	–	2	4	–
Pichia cactophila Starmer, Phaff, Miranda et Miller	2	4	4	–
Pichia sp. A	2	–	–	–
Prototheca zopfii Kruger[4]	8	6	3	–
Rhodotorula rubra (Demme) Lodder	–	–	–	2
Total	77	85	74	21

[1] Number of samples collected.

[2] Yeast like fungus.

[3] Undescribed variety now being described as a new species *Pichia caribaea* (H. J. Phaff, unpubl.).

[4] Yeast like colorless *Chlorella*.

and lack of balance between the mating types of our isolates suggested reduced selective pressure for sexual reproduction in the feeding site of *Sigelgaita* sp. larvae. A similar unbalance of *Cl. opuntiae* mating types with a ratio of 156 h^+ to 5 h^- has been noted for Australian isolates from *Opuntia* cacti (Phaff et al. 1986). Plants from Argentina infected with the moth *C. cactorum* were introduced into Australia as a biological control for eradication of introduced cacti. Other shipments of insect-infested cacti were also made to Australia from the Southern United States (Dodd 1940 cited in Starmer et al. 1988). In North America both mating types of *Cl. opuntiae* occur together, and frequently in the same host plant (Phaff et al. 1986). *Cactoblastis* and *Sigelgaita* are indigenous to South America and both seem to be associated with high h^+ mating type ratios of *Cl. opuntiae*. This high proportion of h^+ mating type was not found in North American isolates from cacti but there have been no studies of *Cl. opuntiae* or other yeasts associated specifically with North American cactus-feeding moths. The high proportion of the h^+ mating type in *Cactoblastis cactorum* and *Sigelgaita* sp. supported an Argentine origin of the Australian population of *Cl. opuntiae* as suggested by Phaff et al. (1986).

Some other yeasts and yeast like organisms including *Acremonium* sp., *Candida* sp. A, *Candida sonorensis*, *Geotrichum* spp., '*Pichia amethionina* var. *fermentans*', *Pichia cactophila, Pichia barkeri*, and *Prototheca zopfii* were also clearly associated with the feeding site of *Sigelgaita* sp. larvae but in lower frequency. The isolates identified as *Candida* sp. A were similar to *Cryptococcus cereanus*, the anamorph of *Sporopachydermia cereana*. We have not isolated this yeast from habitats other than cacti so *Candida* sp. A probably represented the anamorphic state of a new cactophilic species of *Sporopachydermia*. They were DBB negative, and assimilated trehalose, D-xylose, erythritol and inositol, but differed from the standard description of *Cr. cereanus* by failing to assimilate cellobiose, L-arabinose, D-arabinose, D-mannitol, D-glucitol, salicin, glucono-δ-lactone and DL-lactic acid, and by having oval to elongate but not curved cells. *Cr. cereanus* is a geographically widespread complex of two or three species based on G + C content and DNA-DNA reassociation experiments, and are difficult to separate phenotypically (Lachance et al. 1988; Starmer et al. 1990). *P. barkeri* has been suggested to be a cactophilic yeast of South American origin and mostly restricted in habitat to *Opuntia stricta* Haworth (Phaff et al. 1987). Our data supports a South American origin of *P. barkeri* but it aparently has a wider host range than was previously thought. The isolates of *Pichia amethionina* were of an undescribed variety refered to as '*fermentans*' by Lachance et al. (1988) and Starmer et al. (1990). This variety is geographically the most widespread of the amethionina complex. Although *Geotrichum* have not always been considered in other studies of yeasts associated with columnar cacti, Lachance et al. (1988) suggested that this fungus may be associated with initiation of necrosis formation in cacti. The high frequency we observed for *Geotrichum* suggested that this genus is important in the *Sigelgaita* sp. habitat. The lower incidence of these cactophilic yeasts and yeast like organisms may be the result of dispersal by cactus-associated insect vectors that visit the feeding site of *Sigelgaita* sp. larvae less frequently than the vectors of *Cl. opuntiae* or may have been less frequently carried by the vectors than *Cl. opuntiae* in this habitat.

The remaining yeast isolates were probably incidental contaminants from other habitats of this region. The isolate identified as '*Candida ingens*-like' fits the species description well, but Phaff & Blue (1990) have noted that isolates from cacti previously identified as *Candida ingens* are a species complex specific for cacti. An unidentified species of *Pichia* (*Pichia* sp. A in Table 1), with characteristics similar to *Pichia ohmeri* but failing to assimilate trehalose and with latent and weak assimilation of D-xylose, and failed to form zygotes or ascospores when crossed with the type strain and mating type of *P. ohmeri*. *Pichia* sp. A was the most frequently yeast isolated from decaying flowers of *P. arrabidae* (C. A. Rosa & A. N. Hagler, unpubl.), and also occurred in fruits of this cactus. Penetration of *Sigelgaita* sp. larvae through of the flowers was observed and could have allowed inoculation of *Pichia* sp. A into the tunnels. Both larval and adult forms of one beetle of the genus *Camptodes* were found in the interior of the tunnels of *Sigelgaita* sp. The adult of this beetle was also encountered feeding in decaying flowers of *P. arrabidae* and also may have carried *Pichia* sp. A to the tunnels. The isolates identified as '*Candida terebra*-like' differed from its species by not assimilating L-rhamnose and by forming ovoidal rather than elongate cells. These strains may also represent a new biotype of the *Pichia mexicana* species complex that has been noted by Lachance et al. (1988) to exbihite some phenotypic heterogeneity. With the exception of one isolate of *C. sonorensis*, yeasts from moth eggs were not cactophilic, but species more associated with other habitats. *Aureobasidium pullulans*, *Cryptococcus albidus* var. *albidus*, *Cryptococus laurentii*, *Cryptococcus luteolus* and *Rhodotorula rubra* are widely associated with plant surfaces and isolated from air (Phaff & Starmer 1987; Robbs et al. 1989). Such yeasts may have come to this habitat by accidental visits of insects not specifically associated with cacti or other less important dispersal mechanisms such as air currents.

This study has shown the species *Cl. opuntiae* to be more widely distributed than previously thought and has yielded several possible new species of yeasts. Sand dune ecosystems are widely distributed along the coast of Brazil and have well-defined plant populations associated with a distinct community of potential insect vectors of microorganisms (Lacerda et al. 1984). The Brazilian Sand Dune ('Restinga') ecosystems offer a good model for studies in yeast ecology.

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