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Yeasts associated with algarrobo trees (*Prosopis* spp.) in northwest Argentina: a preliminary report

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SUMMARY

Yeasts were isolated from exudates from trees at three sites in northwest Argentina, two between the towns of Amaicha del Valle and Cafayate and one in the Quebrada de Cafayate, a deep river valley north of Cafayate. The majority of the yeasts were identified as *Candida famata* and *Rhodotorula graminis*, though isolates of other species of *Rhodotorula, Candida boidinii, Pichia membranaefaciens*, and occasional isolates of other species were obtained. None of the species was the same as those isolated in Crete, from pods of the carob (European algarrobo). Of 96 cultures investigated, 26 utilized methanol as sole carbon source. The frequency of isolation of methylotrophic yeasts from this habitat may prove to be of considerable scientific and technological interest.

INTRODUCTION

The yeast microflora associated with trees of several types has been investigated by numerous workers for a number of years. These yeasts are mostly associated with exudates of sap (slime fluxes) and are part of a varied population of yeasts, bacteria, worms, larvae of numerous insects, especially *Drosophila* spp., and others [9]. However, there are very few references to yeasts associated with leguminous plants. There are some references to yeasts in association with *Acacia* species (*Pichia acaciae*, associated with Buprestidae larvae infesting *Acacia nilotica* var. *kraussiana* [13]). Lachance and Starmer [6] discussed the evolutionary significance of the association of 38 species of yeasts with trees belonging to both Angiosperms and Gymnosperms, but included only two species of Leguminosae (*Acacia koa* and *Prosopis juliflora*), and concentrated mostly on yeasts associated with 10 species of giant cactus and 12 species of pines. It is interesting that while Boidin [2] isolated a number of yeasts from tanning liquors, which were prepared from a number of tree species including *Acacia* [3], many of these yeasts have not been found in association with the actual trees where they presumably originated. However, they are thought to be associated with the bark of the tree species, used in production of tanning liquors, where they proliferate preferentially in this habitat [9]. Similarly, the yeast species in slime fluxes of leguminous trees may have a selective advantage favoring their development in these habitats. Boidin *et al.* [3] also isolated yeasts from trees in the Cameroons, which included some leguminous species.

There is a wide variety of leguminous trees in South America, which occur over a wide geographic range [1]. Leguminous trees which form slime fluxes are relatively rare (M.-A. Lachance, pers. commun.), but there are several species of algarrobo (*Prosopis* spp.), (family Leguminosae, subfamily Mimosoidea) which are common in northwestern Argentina, and which form slime fluxes readily. A related species, *Ceratonia siliqua*, the European algarrobo or carob, like the species of *Prosopis*, bears edible fruits having a high sugar and tannin content, and is an agricultural crop in Greece [7]. Numerous species of yeasts have been isolated from carob pods and associated soils, and tested for utilization of carob sugars and tannins, and biomass and protein production from them.

We have collected material from fluxes on algarrobo trees and isolated numerous yeast species from them. Here we present a preliminary report of the species isolated and discuss the unusual features of these yeast populations.

MATERIALS AND METHODS

Habitat and isolation

Species of algarrobo in this part of Argentina are not forest trees, but grow as individuals or in rather small groups, often as several trunks arising from the same root. The region is arid to semi-arid and can expect approximately 360 days of sunshine per year, which does not encourage dense tree growth. We took samples in March (autumn in Argentina) from a set of three or four small trunks arising from one root, near a pre-Columbian Indian town at Quilmes, Provincia de Tucumán; from a small grove of older trees outside a cemetery just south of Colalao del Valle, Provincia de Tucumán; and from two isolated trees in the Quebrada de Cafayate, approximately 10 and 20 miles north of the city of Cafayate.

Samples of slime fluxes were obtained from the white algarrobo, *Prosopis alba*, and transported in sterile Whirlpack bags.

This paper is dedicated to Professor Herman Jan Phaff in honor of his 50 years of active research which still continues.

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Slime fluxes on algarrobos differ in consistency from those on species such as poplar or willow, since they are much higher in sugar content and in the hot, dry conditions of their environment, become very viscous, black and sticky. Eventually they are completely solidified and brittle. Samples were moistened if necessary and streaked on acidified malt extract agar, approximately pH 3.7. The inoculated plates were incubated at approximately 24 °C for 3 or 4 days, until growth was observed. Colonies were restreaked and picked to YEP-glucose agar.

Media

Cultures were maintained on yeast extract 1%, peptone 1%, glucose 2%, agar 1.5%. Fermentation tests were done according to the methods given in 'The Yeasts, a Taxonomic Study' [5,14] in culture tubes containing a Durham tube and 5 ml of a solution containing 0.5% yeast extract and 1% of the sugar to be tested. All cultures except those of red yeasts were tested for the ability to ferment glucose, and for the ability to ferment other sugars, if these were assimilated. *Phaffia rhodozyma* forms red carotenoid pigments and ferments glucose, but does not grow at temperatures higher than 27 °C [8,9]. It has not been found in the region near Cafayate. Assimilation tests for carbohydrates and related compounds were done on plates of Wickerham's Yeast Nitrogen Base agar (with amino acids; Difco, Detroit, MI, USA) containing 1% of the compound to be tested. Ability to assimilate nitrate-nitrogen was determined using Wickerham's Yeast Carbon Base (Difco) containing nitrate as nitrogen source. Utilization of nitrate-nitrogen was confirmed using the auxanographic method. The methods are described by van der Walt and Yarrow [14].

Determination of assimilation patterns

Yeast cultures were grown for 48–72 h on YEP-glucose agar and the cells were suspended in sterile distilled water in the wells of the sample plate of a microinoculation apparatus. The assimilation plates were inoculated with 25 strains per plate of the YNB test media. The plates were incubated at 25 °C and read visually after 3, 7 and 14 days. Growth was registered as nil or positive. Weak growth was disregarded.

Identification of the yeast strains

The yeasts were identified according to their carbohydrate and nitrogen assimilation patterns, using the keys and descriptions in 'The Yeasts' [5].

RESULTS

The cultures isolated from slime fluxes of algarrobo species are listed in Table 1. The majority of the cultures were identified as *Candida famata* and the red yeasts, *Rhodotorula araucariae* (one strain) and *Rhodotorula graminis* (20 strains). Six strains of *Pichia membranaefaciens* were also identified. Other *Candida* species isolated in small numbers were *Candida boidinii* (six strains), *Candida pinus* (two strains), and *Candida cantarellii*. Seventeen strains of the methylotrophic species *Pichia angusta* (synonym *Hansenula polymorpha*) and three strains of *Pichia pastoris* were isolated. One isolate had the same assimilation pattern as *Pichia membranaefaciens*, but had a different colonial morphology. One was classified as *Pichia strasburgensis* according to its assimilation pattern. Four brilliant yellow cultures remained unidentified, which would have been classified as *Cryptococcus laurentii* if they had formed starch, but we were unable to demonstrate this characteristic. The assimilation pattern of *Cryptococcus flavus* is very similar [12], and this is another species which does not form starch-like compounds. However, the morphology of the vegetative cells of this species differs from that of the unknown isolates. *Cr. flavus* is a rather rare species.

TABLE 1

Yeasts isolated from exudates of algarrobo species, Colalao del Valle, 1993

Species	Number of isolates
Candida famata	35
Candida boidinii	6
Candida pinus	2
Candida cantarellii	1
Pichia angusta (synonym *Hansenula polymorpha*)	17
Pichia pastoris	3
Pichia membranaefaciens	6
Pichia strasburgensis	1
Rhodotorula araucariae	1
Rhodotorula graminis	20
Unknown yellow	4
Total methylotrophic yeasts*	26

*Growth very weak in some isolates.

DISCUSSION

The yeast flora of the slime fluxes of the algarrobo trees in northwest Argentina differed greatly from that of the carob fruits of Crete [7], consisting of 17 species identified as *Saccharomyces*, four species of *Pichia*, two of *Candida*, two of *Kloeckera*, three of *Schizosaccharomyces*, and one each of *Hanseniaspora valbyensis, Sporobolomyces gracilis, Candida stellata, Trigonopis variabilis, Rhodotorula glutinis*, and *Pichia bispora*. Note the presence of the rare species *Trigonopsis variabilis*, isolated from storehouse soil. It is also interesting to note that out of 40 yeasts tested, 28 reduced the tannin content of the carob extract with *Schizosaccharomyces versatilis* (see Marakis and Karagouni, [7]) giving the greatest reduction.

None of the yeasts isolated from the algarrobo trees we sampled in Argentina were isolated from the Cretan carobs, though we isolated two other species of *Rhodotorula*.

Leguminous plants produce a wide range of tannins, alkaloids and other nitrogenous compounds, besides sugars, and some of these probably display different degrees of toxicity to yeasts and other organisms. These characteristics will undoubtedly influence the composition of the microflora of the slime fluxes. Although algarrobo beans are not widely used in

Argentina as a food product, the beans and pods are high in sugar like those of the European carob. The sugars produced by the algarrobo are used in cottage industries to produce a fermented beverage (F. Siñeriz, pers. commun.), and the fluxes are attractive to several species of insects, including Lepidoptera, large numbers of which were observed around the trees which were sampled.

The frequency of isolation of methylotrophic yeasts was high, amounting to more than 15% of the isolates. The growing importance of methylotrophic yeasts in the production of heterologous proteins of high commercial value makes this observation of considerable interest. We have isolated other strains of methylotrophic yeasts during investigations in the Tucumán region, but this has required enrichment cultures in mineral medium containing methanol. Phaff et al. [10,11] observed that *Pichia pastoris* was the most common yeast found in tree exudates in the Pacific northwest in North America, and that *Candida sonorensis*, another methylotrophic yeast, was common in rotting cactus tissue [9].

We did not find the latter species in the present work, nor have we found any new species of methylotrophic yeasts as yet, although the proposed extension of the survey may reveal some. We found three strains of *P. pastoris*, but the methylotrophic species we encountered most frequently was *P. angusta* (17 strains), and the next most frequent, *C. boidinii* (six strains). The relatively high frequency of isolation of methylotrophic yeasts from this habitat suggests that further investigation of the nature of the yeast microflora of leguminous trees in this region may be profitable, especially in the search for new and valuable strains of methylotrophic yeasts. These species are becoming increasingly valuable for use in the production of heterologous proteins [4].

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