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PRESENCE OF THE YEAST *CANDIDA TROPICALIS* IN FIGS INFECTED BY THE FRUIT FLY *ZAPRIONUS INDIANUS* (DIP.: DROSOPHILIDAE)

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SHORT COMMUNICATION

ABSTRACT

In the last years, the fruit fly *Zapronius indianus* became the most important plague of Brazilian fig production. A fermentation process is associated with infection of the fruit by this fly. A single yeast species, *Candida tropicalis,* was identified in all the infected figs. The presence of one species and the low genetic variability (RAPD) of the isolates indicates an uncommonly strict interaction between *C. tropicalis* and *Z. indianus.*

Key words: *Zaprionus indianus, Candida tropicalis*, RAPD markers, yeast-insect association

The first register of the occurrence of *Zaprionus indianus* in the American continent was made in Japanese pergammon fruits in São Paulo State, Brazil. In the same region and agricultural season, these flies were observed feeding and laying eggs at the ostiole region of fig fruits in the beginning of the maturation period (14). This fly of African origin, recently introduced in Brazil, is not considered a plague in Africa. However, the loss estimates in fig production in Brazil since its appearance is around 50%, becoming the most important plague of this fruit in less than two years from the first report.

The genus *Zaprionus* is formed by two sub-genera and 56 species (3). *Z. indianus* is the only species observed in tropical regions like the Comores Islands, Madagascar, Reunion Islands, Canary Islands, India, Sauddith Arabia (2), and now in Brazil. *Z. indianus* is a fly of about 2.5 to 3.0 mm length, with light brown body, red eyes, and characteristic longitudinal black and white stripes along the dorsal region of the head and thorax.

Most of the species of the Drosophilidae family are associated to yeast and bacteria communities. *Metschnikowia hawaiiensis* (6), *Candida amapae, Kloeckera* sp., *Hanseniaspora* sp., *Candida guillermondii*, and *C. krusei* (10), *Candida ipomoeae* (8), among several other yeast species (1,11), have been isolated from drosophilids in tropical regions. It has been proposed that living yeasts represent a necessary energetic trade-off between reproductive and somatic functions in *Drosophila melanogaster*, increasing the fecundity of the flies, and decreasing starvation resistance and length of life (12).

The study of the microbial communities associated to the drosophilid *Zaprionus indianus* is especially important considering the plague status of this fruit fly in Brazil. In the present paper, we identified the yeast species associated with *Zaprionus indianus* in the subtropical region of São Paulo State, Brazil.

Four infested and four non infested figs were collected at four different farms at the Valinhos region, São Paulo State, Brazil, giving a total of sixteen infested and sixteen non infested fruits. The figs were opened and samples from the inner part of the fruits were plated on appropriate media.

Yeasts were isolated on YEPD (1% yeast extract, 1% peptone, 2% glucose, and 2% agar) medium with 100 mg/l of ampicilin, 25 mg/l of tetracycline and 100 mg/l of kanamycin. Bacteria were isolated on YEPD medium with 100 mg/l of nystatin. Ten yeast and bacterial colonies from each sample were isolated and purified on the above media.

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The reference yeast strains used were: IZ-300 (*Candida utilis*), IZ-809 (*Candida tropicalis*), ATCC-8655 and IZ-431 (*Candida kefyr*), IZ-1339 (*Kluyveromyces marxianus* isolated from a drosophilid), and FF (*Saccharomyces cerevisiae*). The fermentation or assimilation of sugars was evaluated on YNB with aminoacids (Difco) and 1% of the following sugars: maltose, arabinose, raffinose, celobiose, lactose, xylose, starch, inulin, sucrose, and melezitose.

Two isolates from of each farm were analyzed by RAPD. DNA for RAPD analysis was isolated by the SDS method (4). The amplification reactions (16) were performed in a final volume of 25 µl containing 20 mM Tris-HCl pH 8.0, 50 mM KCl, 3.75 mM $MgCl_2$, 100 µM dNTPs (Gibco-BRL), 5 pmol primer (10bp), 40 ng of genomic DNA and 1.5 units of Taq DNA polymerase (Gibco-BRL). Amplification was performed in a thermal cycler PTC-100 (MI Research Inc.) programmed for 40 cycles of 1min at 92ºC, 1min at 37ºC and 2 min at 72ºC, with a final extension of 3 min at 72ºC. The amplification products were separated by electrophoresis in 1.4% w/v agarose gels and visualized by staining with ethidium bromide. The following decamer primers from Operon Techn. Inc. were used for the analysis: OPB-01 (GTTTCGCTCC), OPB-10 (CTGCTGGGAC), OPB-04 (GGACTGGAGT), OPB-11 (GTAGACCCGT), OPB-12 (CCTTGACGCA), OPB-14 (TCCGCTCTGG) and OPB-17 (AGGGAACGAG). Band sharing analysis (Jaccard´s coefficient) was conducted. Cluster analysis and dendrogram based on UPGMA (Unweighted Pair-Group Method with Arithmetic average) were generated to estimate using NTSYS-pc. To evaluate the reproducibility of the branching patterns, bootstrap probabilities were calculated using 1000 bootstrap resampling data with the program WinBoot.

Yeasts and bacteria were present in high concentrations in all figs infested with *Zaprionus*, and no yeast was found in healthy fruits. Flies obtained from surface-sterilized pupa harbored both yeast and bacteria. However, no yeast or bacteria were isolated from larvae obtained from surface-sterilized eggs. The association of yeast and bacteria to insects, in particular, to drosophilids, has been reported in several opportunities (1,6,7,8,10,11). These microorganisms are part of the diet of these flies, and can interfere with their development (12).

All the bacteria isolated were characterized as Gram positive, rod-shaped, spore forming, motile bacterium of the genus *Bacillus*. Further characterization of these bacteria is in process.

All the yeast colonies were cream-colored, with similar morphology. Fifteen of the colonies isolated from infected figs were characterized. All isolates were imperfect budding yeasts, able to grow at 37ºC. Yeast cells, grown in YEPD liquid medium at 30ºC for 3 days, were ovoid in shape with well visible vacuolar structures.

Ten isolates were further characterized for their ability to assimilate different carbon sources, and to grow on YEPD media supplemented with cyclohexamide. The results were identical for all isolates and they were classified as *Candida tropicalis* (5).

The RAPD pattern of *Candida tropicalis* isolates was compared to those of reference strains. As can be observed in Fig. 1, the pattern obtained for one of the isolates is almost identical to that of the reference strain IZ-809 (*C. tropicalis*), and different from the patterns obtained for the other species. This similarity with the reference strain was also obtained with the other primers and isolates. RAPD markers have been used with success for the identification and characterization of clinical isolates of several species of *Candida* (9,13).

Several *Candida* species, such as *C. lipophila*, *C. drosophilae*, *C. restingae*, *C. sonorensis*, *C. deserticola, C. guillermondii* and *C. krusei* have been isolated from *Drosophila* spp. and other insects (1,5,10,11). *Candida tropicalis*, as well as other species of *Candida, Hanseniospora, Hansenula, Trichosporon, Rhodotorula, Cryptococcus* and *Aureobasidium* have been isolated from the gut of adult *Cotinis nitida* beetles (15). In general, more than one yeast species is associated to each insect, and yeasts communities are significantly influenced by the habitat rather than phylogeny of the flies (7,11).

The multivariate analysis of RAPD data (Fig. 2) of eight yeasts isolated from different infected figs confirmed that all of them belonged to the same species. The eight isolates clustered together with the reference strain IZ-809. The variation observed among the isolates was very low when compared to that obtained with perfect yeast species, but is within the range previously observed for *C. tropicalis* and other imperfect yeasts of the genus *Candida* isolated from clinical samples (9,13).

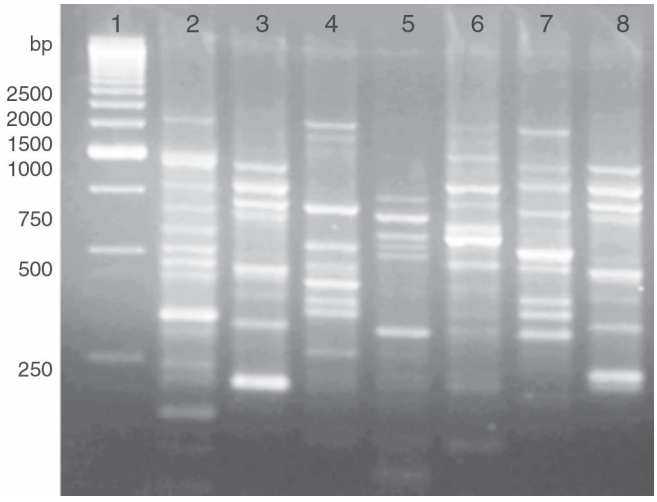

Figure 1. RAPD patterns of six yeast, isolated from figs infected with *Zaprionus indianus*, using primer OPB17. Lines from left to right: 1- DNA ladder, 2- IZ-300 (*Candida utilis*), 3- IZ-809 (*Candida tropicalis*), 4- ATCC-8655 (*Candida pseudotropicalis*), 5- IZ-1339 (*Kluyveromyces marxianus* isolated from a drosophilid), 6- FF (*Saccharomyces cerevisiae*), 7- IZ-431 (*Candida kefyr*), 8- A3 (Yeast isolated from *Z. indianus*).

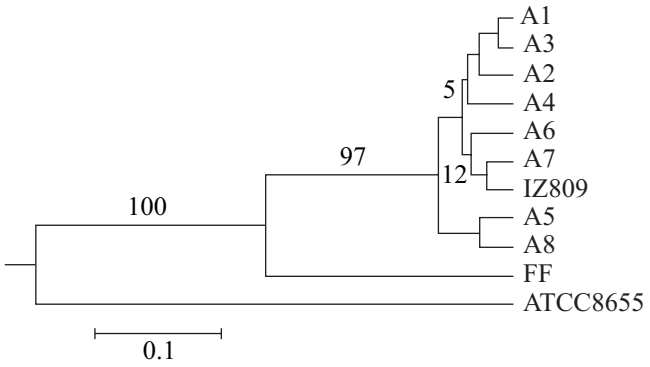

Figure 2. Dendrogram based on UPGMA analysis of the phenetic similarity (Jaccard's coefficient) of eight isolates (A1 – A8) obtained from infected figs and reference strains *C. tropicalis* (IZ 809), *C. kefyr* (ATCC 8655) and *Saccharomyces cerevisiae* (FF), as determined by 64 RAPD markers using seven decameric primers. Numbers above internal branches are bootstrap probabilities (%) based on 1000 bootstrap resampling.

The presence of only one yeast species and the high genetic uniformity observed among the isolates suggest an uncommonly and so far unreported strict yeast-insect interaction between *C. tropicalis* and *Zaprionus indianus.* We are currently working on the further characterization of this association, and the classification of bacterial isolates.

RESUMO

Presença da levedura *Candida tropicalis* em figos infestados pela mosca da fruta *Zaprionus indianus* (Dip.: Drosophilidae)

Nos últimos anos, a mosca-africana-do-figo *Zaprionus indianus* tem se tornado a praga mais importante desta cultura no Brasil. Um processo fermentativo está associado com a infecção dos frutos por esta mosca. Uma única espécie de levedura, *Candida tropicalis*, foi identificada nos figos infectados. A presença de uma única espécie de levedura e a baixa variabilidade genética (RAPD) dos isolados indica uma relação muito estreita entre *C. tropicalis* e *Z. indianus*.

Palavras-chave: *Zaprionus indianus, Candida tropicalis*, marcadores de RAPD, associação inseto-levedura.

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