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Research Article

Saccharomyces cerevisiae found in the Crop of a Neotropical *Drosophila* Species Fly Collected in a Natural Forest Remnant

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Abstract

Background: Hoang et al. [1] questioned the use of commercial *Saccharomyces cerevisiae* as a model for investigating *Drosophila* – yeast association, since this approach "may not be fully representative of host-microbe interactions as they operate in nature". They also claimed: "*S. cerevisiae* is rarely found with natural populations of *D. melanogaster* or other *Drosophila* species". Indeed, previous choice experiments found that *Sophophora* subgenus flies (including invasive species *D. melanogaster*) are more attracted to banana baits inoculated with apiculate yeasts such as *Hanseniaspora uvarum* over *S. cerevisiae* inoculated baits. Yet, the *forest interior dwelling species* (FIDS) *D. tripunctata* group flies choose preferentially *S. cerevisiae* inoculated baits over *H. uvarum* in a natural forest environment.

Aim and Methods: Our objective was to carry out a pilot experiment to examine yeast species associated with *Drosophila* in a natural Atlantic Rainforest fragment, especially examining, the yeast found with FIDS of the *D. tripunctata* group. We sampled *Drosophila* in a natural population from a Neotropical forest fragment. Males were dissected for isolating yeast colonies from their crops and to use their genitalia for species identification. Yeast species were identified by sequencing the D1/D2 domains of the 26S rRNA gene.

Results and Conclusion: We isolated five yeast species from crops of *Drosophila* species of *tripunctata* group, including one strain of *S. cerevisiae* (from *D. paraguayensis*), confirming a previous record of *S. cerevisiae* isolates from a few *tripunctata* group species. Thus, their contention that "the results from *D. melanogaster–S. cerevisiae* laboratory experiments may not be fully representative of host–microbe interactions in nature" is probably right, but because *D. melanogaster* is an invasive species that is preferentially attracted in forests to apiculate yeasts, yet *S. cerevisiae* may be associated with FIDS *Drosophila* such as *D. paraguayensis*.

Keywords: Crop; Natural Forest Remnant; Yeast-*Drosophila* association; *Drosophila* species

Introduction

The symbiotic association between yeast and *Drosophila* in natural environments has long been assessed with experiments investigating *Drosophila* species attraction to baits inoculated with different yeast species as well as isolating yeasts from *Drosophila* crops [2-4]. A number of differential attractivity experiments have used baits inoculated with various yeast

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species isolated from *Drosophila* crops and also commercial *Saccharomyces cerevisiae*, as a control treatment (e.g.: Da Cunha et al. [5]; Klaczko et al [6]; Becher et al. [7]).

Hoang et al. [1] criticized this approach, first, claiming that: "*S.cerevisiae* is rarely found with natural populations of *D. melanogaster* or other *Drosophila* species". To explain the finding of *D. simulans* associated with *S. cerevisiae* in a single study from New Zealand, they argued that it could be due to the unnatural environment (vineyard) where the flies were collected. Furthermore, they carried out a feeding preference experiment in the laboratory with *D. melanogaster*, when they allowed flies to choose between *S. cerevisiae* and another species taken from five natural yeast species. In no case, did the flies prefer *S. cerevisiae* over the other species. Finally, they questioned the overuse of *S. cerevisiae* as a model for studying the fly-yeast relationship, since it "may not be fully representative of host-microbe interactions as they operate in nature."

We collected specimens of *Drosophila tripunctata* species group within an Atlantic Rainforest fragment. This group encompasses 80 species [8] and is widely distribute over the Neotropical region [9,10]. Several species that belong to D. *tripunctata* group are *forest interior dwelling species* (FIDS) of flies and use naturally-occurring fruits for feeding and breeding [11,12]. Our objective was to carry out a pilot experiment to examine yeast species associated with *Drosophila* species in a natural Atlantic Rainforest fragment, especially examining, the yeast found with FIDS of the D. *tripunctata* group.

Materials and Methods

We sampled yeast of *Drosophila* crops from an Atlantic Rainforest fragment located at Itatiba, SP, Brazil (23º 00.073' S, 46º 52.917' W; altitude = 740 m) on June 29, 2015. We collected drosophilids by sweeping entomological nets over baits of mashed banana inoculated with commercial *S. cerevisiae* and covered with sterile tulle cloth. Flies were brought to the laboratory and dissected within one hour as suggested by Phaff et al. [13]. Wild males were identified by their external morphology and genitalia [14,15].

Before dissected in a drop of *Drosophila* Ringer's solution, flies were immersed in distilled water and in alcohol 70%, following the procedures described by Hamby et al. [16]. Next, crops were streaked in formulated YM medium (1.0% glucose, 0.5% peptone A, 0.3% yeast extract, 0.3% malt extract, 2.0% agar with Chloramphenicol 1.0%) and incubated at 30°C for 48 hours. Then, genomic DNA of the colonies was extracted as described by Rosa et al. [17]. Regions ITS-D1/D2 of the 26S rRNA gene sequences were amplified according to PCR conditions and protocol described in Rosa et al. [17].Yeast species were identified submitting the sequences to GenBank database and comparing them to entries for yeast.

Results

Twenty males of different *Drosophila* species had their crop dissected, but only five yeast strains were isolated from five fly specimens sampled of the Itatiba population (Table 1). From two different *D. mediopunctata* males two *Candida* sp. strains were isolated (top BLAST identity was 97% to *Candida sake* strain K2.6.1 and 96% to *Candida sake* strain NRRL Y-1622). A not yet identified yeast species was isolated from *D. frotapessoai*; from *D. unipunctata* a *Starmerella bacillaris* strain was identified with 100% identity to reference strain CBS 13663. Finally, from *D. paraguayensis* crop, *Saccharomyces cerevisiae* was isolated and identified with 100% identity to reference strain NRRL Y-12632.

Discussion and Conclusion

Several reports show the diversity of substrates where *Saccharomyces cerevisiae*, *Starmerella bacillaris* and *Candida sake* have already been found. Particularly, they were found in fruits, grains and in the soil of natural environments [18]. Barbosa et al. [19] reported the occurrence of natural populations of *S. cerevisiae* associated with bark trees in several Brazilian forest ecosystems, including Atlantic Rainforest. The results of this work show that yeast populations of this species are available to *Drosophila* in these ecosystems. Moreover, *Drosophila paraguayensis*, *D. mediopunctata* and its cryptic sibling species *D. unipunctata* have been collected repeatedly in the interior of forests, and adults have emerged from naturally collected fruits [11,12]. These are good evidences that they occur naturally within the forest environment. Experiments of differential attractiveness in the field are important for characterizing the feeding habit differentiation of *Drosophila* species. For example, Klaczko et al. [6] collected *Drosophila* over baits inoculated with *S. cerevisiae*, *Kloeckera apiculata* (=*Hanseniaspora uvarum*) and other yeasts in James Reserve, San Jacinto Mountains, USA.

Table 1: Yeast strains isolated from crops of *Drosophila* species belonging to the *tripunctata* group, yeast species with top identity compared to sequences submitted in BLAST, with identity and percentage identity to reference accession number.

Yeast strains	*Drosophila* species	Yeast species –BLAST top identity (identity – % identity to reference)
BTC-L1	*Drosophila frotapessoai*	Not identified
BTC-L2	*Drosophila paraguayensis*	*Saccharomyces cerevisiae* (499/499 – 100% to NG042623)
BTD-L1	*Drosophila mediopunctata*	*Candida* sp. (467/483 – 97% to KC485459)
BTD-L2	*Drosophila unipunctata*	*Starmerella bacillaris* (405/405 – 100% to KP346913)
BTD-L3	*Drosophila mediopunctata*	*Candida* sp. (460/478 – 96% to U45728)

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They collected fewer specimens of *D. obscura* group and *D. melanogaster* group over baits inoculated with *S. cerevisiae* than *K. apiculata* over baits (796 to 1243 respectively). Yet, flies from subgenus *Drosophila*, such as *D. occidentalis*, were more collected over *S. cerevisiae* baits (295 over 194). We found a similar pattern in the Itatiba population [20,21]. More flies from subgenus *Sophophora* (including invasive species such as *D. melanogaster* and *D. suzukii*, among others) were collected over baits inoculated with *H. uvarum* (68 in a total of 81 =84%) than over *S. cerevisiae* (13 in 81 = 16%); while flies of the *tripunctata* group (subgenus *Drosophila*) were more attracted to baits inoculated with *S. cerevisiae* (93 in 121 = 77%) than to *H. uvarum* (23%). Da Cunha et al. [22] sampled yeasts from crops of *Drosophila* collected in Serra da Mantiqueira, Brazil. They found 58.9% out of 17 *S. cerevisiae* isolates were obtained from *tripunctata* species crops, while only 9% out of 24 *H. uvarum* isolates were isolated from flies of the same group. However, the opposite pattern is observed for *willistoni* group (subgenus *Sophophora*), with 58% out of 24 *H. uvarum* isolates obtained and 11.8% of 17 *S. cerevisiae* isolates. Altogether, there are evidences in support of the natural association between *S. cerevisiae* and FIDS of the D. *tripunctata* group; while species of subgenus *Sophophora* such as *D. melanogaster*, may be naturally associated with apiculate yeasts (for reviews on non-*Saccharomyces* yeasts and *Saccharomyces* see, respectively: Jolly et al. [23]; and Meriggi et al. [24]). Thus, Hoang et al. [1] contention that "the results from *D. melanogaster–S. cerevisiae* laboratory experiments may not be fully representative of host–microbe interactions in nature" is probably right, but because *D. melanogaster* is an invasive species that is preferentially attracted in forests to apiculate yeasts, yet *S. cerevisiae* may be associated in natural environments with FIDS *Drosophila* such as *D. paraguayensis*.

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