Characterization of yeast communities vectored by *Drosophila melanogaster* during post-harvest fermentation of coffee

Caracterización de comunidades de levaduras transmitidas por *Drosophila melanogaster* durante la fermentación postcosecha del café

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ABSTRACT

Drosophila melanogaster, present in coffee processing stages, feeds on mucilage and coffee "cherries", inoculating yeasts that influence coffee bean fermentation. This pioneering study in Colombia evaluated the microorganisms associated with the larvae of this fly, determining their kinetic behavior, observing their morphophysiological structures, and characterizing the types of yeasts present. Understanding the interaction between these microorganisms and coffee bean quality is crucial in the coffee industry, given that in other industries, such as winemaking, they have been shown to directly affect the organoleptic characteristics of the final product. This study was carried out in three coffee-growing municipalities in northern Nariño. The yeasts present in the oral apparatus of *D. melanogaster* larvae were analyzed. These were cultivated in YGC media differentiated by color and growth type. Quantification was performed using colony forming units (CFUs) and lactophenol blue staining. Additionally, the number of CFUs was correlated with spectrophotometric measurements. The results revealed the presence of yeast species such *as Geotrichum, Galactomyces, Trichosporon*, and *Blastoschizomyces*. This research provides an approximation to the microbiome of coffee-growing environments and its biotechnological potential. Moreover, it lays the foundation to formulate methodologies for the morphophysiological classification of yeast strains associated with insects, their role in coffee fermentation processes, and their potential to determine flavor and quality of the final product.

Key words: microbiology, associated yeasts, fermentative microbiome, *Coffea arabica*.

RESUMEN

Drosophila melanogaster, presente en los procesos de beneficio del café, se alimenta del mucílago y la cereza, inoculando levaduras que influyen en la fermentación del grano. Esta investigación, pionera en Colombia, evaluó los microorganismos asociados con las larvas de esta mosca, determinando su comportamiento cinético, observando sus estructuras morfofisiológicas y caracterizando el tipo de levaduras presentes. En la industria cafetera, comprender la interacción entre estos microorganismos y el grano es crucial dado que, en otras industrias, como la vinícola, se ha demostrado que influyen directamente en las características organolépticas del producto final. El estudio se realizó en tres municipios cafeteros del norte de Nariño, donde se analizaron las levaduras presentes en el aparato bucal de las larvas *de D. melanogaster*. Estas se cultivaron en medios YGC, diferenciándose por color y tipo de crecimiento, y se cuantificaron mediante unidades de formación de colonias (UFC) y tinción con azul de lactofenol. Además, se correlacionó el número de UFC con mediciones espectrofotométricas. Los resultados mostraron la presencia de especies como *Geotrichum, Galactomyces, Trichosporon* y *Blastoschizomyces*. Esta investigación ofrece una aproximación al microbioma de los entornos cafetaleros y a su potencial biotecnológico. Además, sienta las bases para formular metodologías de clasificación morfo-fisiológica de cepas de levaduras asociadas a insectos, su papel en los procesos de fermentación del café y su potencial para determinar el sabor y la calidad del producto final.

Palabras clave: microbiología, levaduras asociadas, microbioma fermentador, *Coffea arabica*.

Introduction

Yeasts are vectored by *Drosophila melanogaster* and concurrently constitute a source of nutrients for this species (Ganter, 2006). Indeed, the availability of different yeast species as nourishment influences the developmental process of *D. melanogaster* larvae. In the same vein, the composition of microbial communities within the larval substrate is instrumental in determining the vulnerability of the larvae to parasitic attacks (Anagnostou *et al*., 2010). It has been observed that more complex yeast communities are favored as dietary options (Rohlfs & Kurschner, 2010).

The larvae of *Drosophila* spp. exhibit opportunistic behavior and are usually found in coffee during post-harvest processes and in the decaying fruits. The adult stage of this

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insect is commonly referred to as the 'fruit fly' due to its presence in the aforementioned contexts. While it does not pose a direct threat to coffee crops, the invasion of its larvae during the stages of fermentation, harvesting, and "cherry" pulping is commonplace in the Andean region of the Department of Nariño.

Yeasts play a role in *Drosophila* reproduction. The composition of yeast in the fly's diet affects egg production, particularly in terms of egg size. This relationship indicates the interplay between nutritional availability and reproductive success. In certain *Drosophila* species, yeasts are even presented as nuptial gifts during courtship. Moreover, yeast-colonized substrates emerge as preferred oviposition sites for most fruit-breeding *Drosophila*, surpassing sites dominated by bacteria or molds. In particular, *Drosophila buzzatii* displays a strong affinity for feeding and ovipositing on specific yeast species. Likewise, *Drosophila,* both as larvae and in the adult stage, prefer to feed on particular yeast species. Common synthetic volatiles used to attract *Drosophila* spp. include chemicals released by yeast fermentation of fruits, such as ethanol, acetic acid, methyl acetate, ethyl acetate, acetaldehyde, and *n*-propanol. When offered a choice of pure yeast cultures, the interaction between larvae and yeasts provides essential nutrients such as sterols and, at the same time, produces chemicals that attract scattered insects and favor their migration toward favorable environments (Blackwell, 2017).

The importance of the interaction between insects and yeasts is not clear-cut. However, it has been shown that the presence of *K. ohmeri* increases the invasive and reproductive capacities of insects in environments with ripe fruits (Arbogast *et al*., 2012). This yeast is responsible for producing volatile components in food, which serve as strong attractants for other dipterans. For both larvae and adults, the most frequently identified yeast species is *H. uvarum*, suggesting a potential association between *D. suzukii* and *H. uvarum*. Brix grades, sugar content values, and yeast community profiles were found to be similar for infested and non-infested fruit juices, although fewer yeast colonies were present and identified in non-infested samples (Hamby *et al*., 2023).

Thus, the aim of the present study was to evaluate the microorganisms associated with *Drosophila melanogaster* larvae present in coffee post-harvest processes in Colombia, through the characterization of their kinetic, morphophysiological, and structural behavior, as well as the identification of their geographic origin and the types of yeasts present.

Materials and methods

In this study, coffee "cherries" were collected in the municipalities of Buesaco, Arboleda, and La Unión, in the Department of Nariño. The "cherries" underwent manual pulping, and the epidermis was separated and collected in plastic bags (10 g/bag), which were stored at room temperature (18-20°C). Twenty-four hours post-pulping, the bags were inspected for larvae. Any larvae found were manually collected and placed in 50 ml plastic bottles with 10 ml of distilled water. Subsequently, the bottles were placed in an incubator for 18 h at a temperature of 28°C and a relative air humidity of 85%. For larvae cultivation, a specific culture medium for YGC (Yeast Glucose Chloramphenicol) yeasts was prepared by adding 40 g L^{-1} of water. This culture medium was sterilized along with the Petri dishes and the bacteriological dishes.

Isolation procedure

First, each beetle was individually placed inside YGC agar boxes, allowing it to walk freely across the entire surface. The exterior of the larva was not sterilized in order to keep the insect alive and replicate natural dispersal during decomposition processes. This method increased the probability of isolating yeast from the larvae's mouthparts and digestive systems (Bressani *et al*., 2018). Second, the larvae were macerated and scraped across the entire Petri dish. Third, the residual water from the 50 ml flask was used for further scraping inside the Petri dish.

All Petri dishes were incubated for 24 h at 28°C and a relative air humidity of 85%. After the incubation period, the Petri dishes were examined, and all exhibited microbial growth. Microscopic observation was then conducted, confirming the presence of yeasts.

After the observation, dilutions were prepared in a nutritive broth with the addition of peptone. The purpose was to determine the CFUs, using dilutions ranging from 10^{-1} to 10^{-6} (Tab. 1). For each dilution tube, three replicates were cultured in YGC medium.

Upon incubation, the plates were examined with the following considerations:

1. Colonies of filamentous fungi. All colonies exhibiting a cottony appearance were counted regardless of their size and color;
2. Typical yeast colonies. Creamy, bright colonies of various colors were considered, as these are characteristic of yeasts (Bressani *et al*., 2018).

Based on the criteria above, the colonies on the plates were counted. Two live repetitions were conducted for the same dilution, as shown in Table 1. It is important to highlight that no identification or verification was performed, since this was solely a quantitative sampling of inoculum presence (Mota *et al*., 2020).

To calculate the number of CFUs per milliliter when working with liquid culture media in test tubes, the following equation was used (Madigan *et al*., 2018):

$$N = \frac{\Sigma\, CFU}{V(n_1 + 0.1\ n_2)d} \quad (1)$$

where

N = number of colony-forming units/ml;

V = volume of inoculum applied to each plate in ml;

n_1 = number of plates retained in the first dilution;

n_2 = number of plates retained in the second dilution;

d = dilution number corresponding to the dilution retained.

Absorbance measurement in CFU dilutions

To estimate yeast concentrations in suspension, serial decimal dilutions (1:10) were prepared from liquid cultures previously incubated in YGC medium for 24 h at 28°C with constant agitation. Each dilution was subjected to absorbance measurement using a UV-Vis spectrophotometer at a wavelength of 600 nm, with sterile YGC medium used as the blank. Measurements were performed in quartz cuvettes with a volume of 1 ml, and three replicates were recorded per sample. Subsequently, aliquots of each dilution were plated on solid YGC medium for colony-forming unit (CFU) counting after 48 h of incubation at 28°C. The absorbance values obtained were correlated with CFU counts, allowing for the establishment of regression equations that support the relationship between optical density and microbial concentration, as described by Madigan *et al*. (2018).

Staining yeast with methylene blue

The staining of yeast with methylene blue was conducted under sterile conditions to prevent contamination. A slide was sterilized with 5% hypochlorite, and then a drop of sterile water was added to the slide with a dropper. Each time the spoon was used to transfer the sample from the Petri dish to the slide, it was flamed over high heat for 15 s. A sample of the yeasts to be observed was obtained, collecting a small amount of biomass. This sample was deposited into the drop of water on the slide. Then, it was evenly spread across the entire surface to obtain a single layer of cells, ensuring uniformity in cell distribution when observed under the microscope (Marcos-Zambrano *et al*., 2013). The spoon was flamed again, as it had already been contaminated with the previous sample. After distributing the sample on the slide, a white texture was observed on the upper part. Subsequently, the slide was dried near the top of the burner, avoiding direct contact, until the moisture evaporated. To fix the sample, the slide was passed through the hottest part of the burner to prevent any other type of molecular mass from interfering with its assembly. Then, the sample was stained with methylene blue. The slide was dried by gently heating it near the top of the burner to remove any remaining moisture from the reagent. Finally, the slide was examined under the microscope at 100x magnification (Freydière *et al*., 2001).

Microscopic morphological criteria of classification

The identification of yeast organisms, which are part of the normal flora of the skin and mucous membranes of insects, was conducted to determine the presence or absence of these microorganisms. However, the repeated isolation of yeasts from various insect samples suggests a potential infection by the isolated microorganism, requiring identification of the species. Identification was based on morphological criteria. In this study, however, the focus was on the identification of macro- and micro- characteristics (Freydière *et al*., 2001).

For the identification of yeasts, the following means of identification were employed:

- Germinal tube. The filamentous extension of the yeast is evaluated without narrowing at its origin. Its width is usually half that of the progenitor cell, and its length is three or four times greater than that of the mother cell;
- Formation of hyphae, blastoconidia, chlamydospores, and arthrospores. These morphological characteristics are crucial to identify certain yeast species. When hyphal structures are present, it is important to first determine whether these are pseudohyphae or true hyphae. The former result from the formation of blastoconidia and exhibit regular points of narrowing; the latter fragment into arthroconidia;
- Stains. The microscopic examination of yeasts or related microorganisms (genus *Prototheca*) can be performed using staining techniques, including simple staining or Gram staining. With this technique, yeasts usually behave like Gram-positive organisms.

Test of antagonism and synergism in yeast growth

The *in vitro* evaluation of microbial antagonism was conducted by measuring growth inhibition between microorganisms, using the dual plate tests proposed by Zafra *et al*. (2017).

Results

Following the measurements conducted in the laboratory, CFUs were calculated as detailed in this document. The results indicate the concentrations present in relation to the dilutions applied (Tab. 1).

Correlation between colony-forming units (CFUs) and absorbance in coffee-producing municipalities of Nariño

Figures 1, 2, and 3 illustrate the correlation between yeast concentration, expressed as colony forming units per milliliter (CFU/ml), and optical density (OD) measured at 600 nm in samples collected from three coffee-producing municipalities in the department of Nariño: Arboleda, La Unión, and Buesaco. The positive linear relationships observed in each location support the use of spectrophotometric measurements as a complementary method for estimating microbial density in liquid media. This approach is particularly valuable in studies involving yeast populations, which play a critical role in coffee fermentation processes. The regression equations and R^2 values obtained provide evidence of consistency and reliability in the microbial quantification protocol applied.

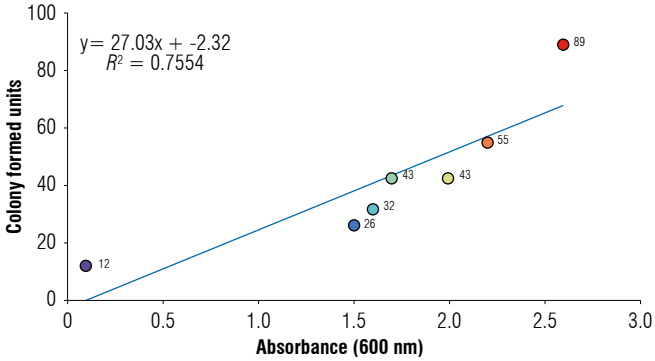

FIGURE 2. Correlation between colony-forming units and absorbance in La Unión.

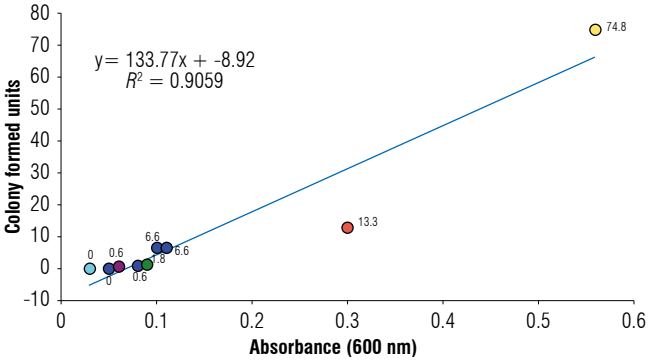

FIGURE 1. Correlation between colony-forming units and absorbance in Arboleda.

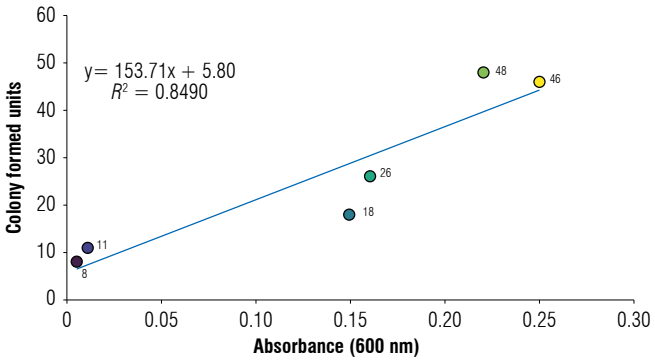

FIGURE 3. Correlation between colony-forming units and absorbance in Buesaco.

TABLE 1. Determination of colony forming units (CFUs) in samples collected from coffee-producing municipalities Arboleda, Buesaco, and La Unión of the department of Nariño.

Dilution	Concentration	Replicate	Filamentous structures	Yeasts structures	Total	Arboleda	Buesaco	La Unión
1	10^1	1	96	76	172	81.90	48	89
		2	77	65	142	67.62	48	88
2	10^2	1	32	24	56	13.33	45	55
		2	34	22	56	13.33	46	55
3	10^3	1	28	18	46	7.30	29	44
		2	20	17	37	5.87	25	43
4	10^4	1	9	3	12	1.43	18	32
		2	10	9	19	2.26	18	33
5	10^5	1	2	5	7	0.67	11	26
		2	2	3	5	0.48	11	26
6	10^6	1	0	0	0	0.00	8	12
		2	0	0	0	0.00	9	10

Test of antagonism and synergism in yeast growth

When performing the culture medium inoculation test with various strains obtained in the laboratory, growth behavior was assessed in the same culture medium. Since insect larvae are common in coffee processing, the yeast Rhodotorula rubra consistently appeared. This yeast was frequently observed in fermentation systems, as previously noted by Reyes Martínez *et al.* (2013). Although their study did not evaluate the distribution of strains across locations, our findings suggest a possible recurrence of similar yeast strains in multiple coffee-processing environments. This finding indicates that this yeast—known to be pathogenic for humans, as it may be the cause of various diseases—is a natural inhabitant of coffee processing environments. Consequently, it needs to be handled with care. The study conducted by Reyes Martínez *et al.* (2013) further revealed that this yeast thrives at a pH value of 4.2, a humidity level of 97%, and environments rich in sugars. Additionally, the highest occurrence of this yeast was observed during fermentation processes between 24 and 48 h.

The genus *Rhodotorula* comprises 34 species, mainly *R. glutinis, R. mucilaginosa*, and *R. minuta*. These red yeast strains are generally healthy, thrive in diverse media, and grow rapidly. *Pseudohyphae* are rare and appear as round or circular cells under the microscope (Jimbo Zapata, 2018). These yeasts produce urease, an enzyme that does not affect food degradation, as reported for *Rhodotorula* species by De Guidi *et al.* (2023). Numerous species of *Rhodotorula* grow in culture media, competing vigorously with other natural inhabitants of fermentation processes, yet without contributing beneficially to such processes. As illustrated in Figure 4, where various strains were cultivated in the same medium, *Rhodotorula* displayed the most extensive spread and inhibited the growth of other yeast colonies, including Saccharomyces, a phenomenon also described by Gomaa (2017) in studies on the antimicrobial activity of *Rhodotorula glutinis.*

FIGURE 4. Results of antagonism and synergism tests between yeast strains isolated from *Drosophila* larvae: white and cream color (*Saccharomyces*), red color (*Rhodotorula*).

The yeast genus examined in this study (*Saccharomyces*) has been associated with sporadic diseases such as endocarditis, meningitis, peritonitis, keratitis, oral ulcers, among others (Tuon & Costa, 2008). According to Reyes Martínez *et al.* (2013), there are no reports of these yeasts being used in biotechnological processes or fermentation. However, they are consistently present in all isolation and purification processes of yeast strains associated with *Drosophila*. Herrera *et al.* (2015) state that these yeasts can be isolated from various natural environments, including air, soil, water, plants, and certain moisture-containing products. These environments are typical of fermentation processes, which may explain the continuous manifestation of this pathogen (Fig. 5). Each microorganism was individually characterized through microscopy to determine its morphophysiological features, as shown in Figure 5. Furthermore, this genus is recognized as an opportunistic fungus and a frequent contaminant in laboratory environments.

A systematic review of *Rhodotorula* infections from the literature revealed that out of 128 cases, 79% were fungemia (103 cases), 7% were ocular infections (9 cases), and 5% (6 cases) were peritoneal dialysis-associated peritonitis. Notably, 87% of *Rhodotorula* infections were associated with underlying immunosuppression or cancer (Tuon & Costa, 2008). This suggests the need for caution when employing this yeast in fermentation processes, as it could serve as a vector for the development of the aforementioned diseases. Furthermore, it was found that *Rhodotorula* was a natural inhabitant in both the larvae and the post-harvest processes of coffee cultivation.

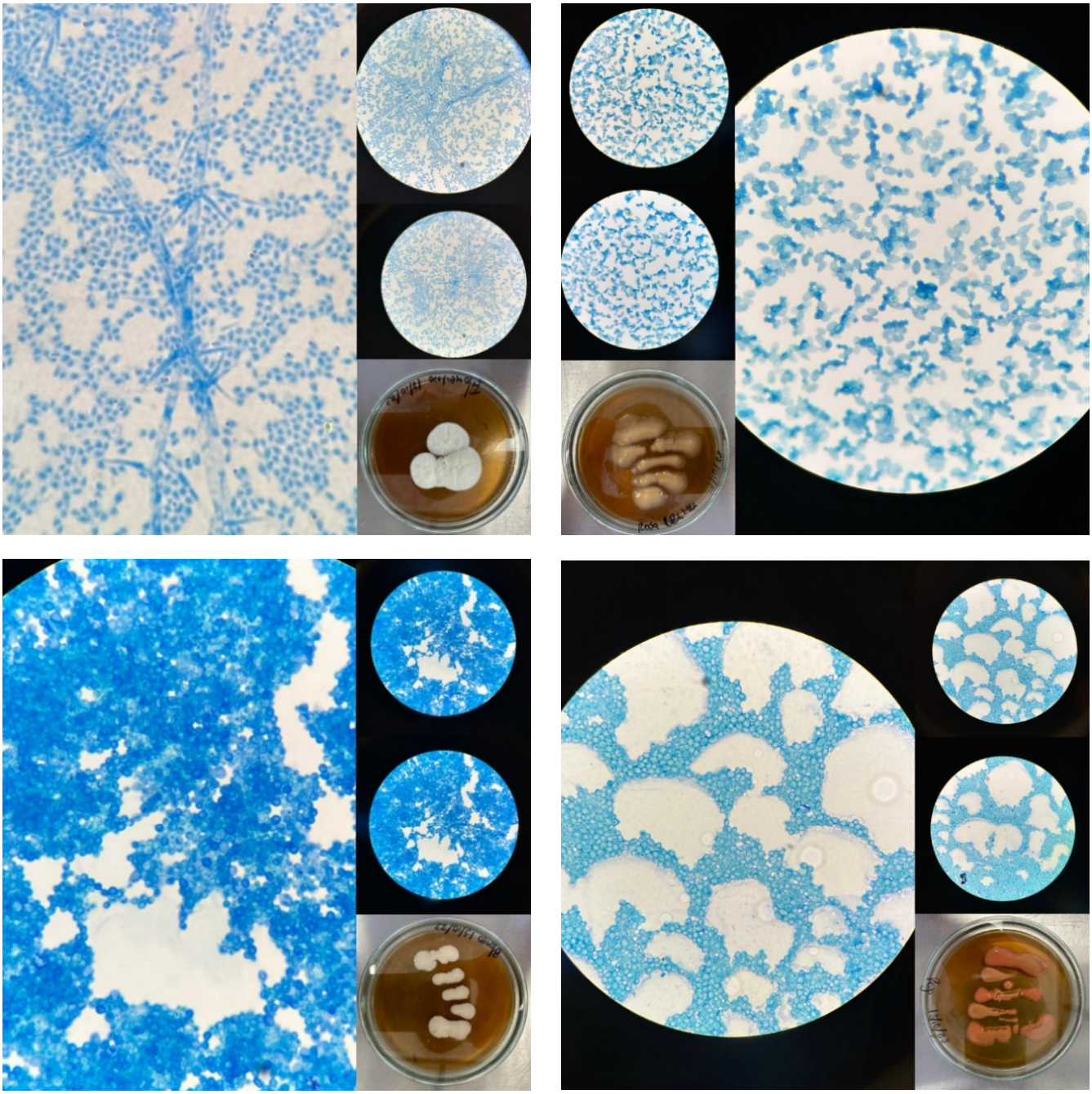

FIGURE 5. Characterization of the isolated strains and the morphophysiological structures observed under a microscope in the different yeast genera.

Discussion

Table 1 illustrates the average colony-forming units (CFUs) across various dilutions. It is important to highlight that the CFUs serve as indicators of the quantity of microorganisms, in this case yeasts, present within a liquid medium (dilution). The values recorded in the columns corresponding to each concentration reflect the number of individual concentrations of cells of this microorganism in liquid medium. These data represent the basis for subsequent analyses related to fermentation hours, which will be conducted in a bioreactor as part of this research project. Considering the coffee post-harvest process, mostly involving liquid fermentation processes, understanding the behavior of these microorganisms under similar conditions is of paramount importance. All observations of the CFUs were evaluated at 12-, 24-, 48- and 96-h post-inoculation in the medium and arranged in Petri dishes for counting. Yeast

growth was observed in the Petri dishes as early as the first 12 h, indicating rapid development in the liquid medium when provided with raw material for consumption—in this case, the Sabouraud liquid medium. This medium was essential to determine yeast growth over different time periods, thus allowing for the assessment of yeast concentration and growth, which will indicate its kinetics.

In the context of coffee growing in Nariño – Colombia, there is an evident absence of technological advancements and knowledge regarding the management of coffee processing methods on farms, whether they involve wet or dry techniques, or a mix between the two. This lack of information leads to significant issues, including defects and poor decision-making in the development of protocols for adequate processing of coffee. This situation impacts the quality of the beverage and the final product, resulting in economic losses and missed market opportunities (Federación Nacional de Cafeteros, 2021).

For the reasons explained above, the project must provide the necessary information from a scientific and analytical perspective, using real data to control both the concentration of the inoculum and its kinetics. This approach will enable precise identification of the active hours and conditions under which microorganisms act. With these results, producers in the region can be trained to implement standardized practices that add value to the beverage, meet market demands and increase profitability for local farmers. In this vein, the project offers a clear and reliable alternative for achieving greater control over all the agro-industrial processes involved in coffee bean processing.

Based on the data obtained from the spectrophotometer and cell count observations, a linear regression analysis was performed to compare absorbance against CFUs. This analysis made it possible to determine kinetic parameters, as illustrated in Figures 1, 2, and 3. An analysis of growth over time was subsequently performed, involving the calculation of generation number and doubling time, following the methodology explained by Ortiz (2012). The results concerning CFU growth aligned with those reported by Chalón *et al.* (2013), who examined the growth of yeast strains in media containing glucose and peptone in a specific medium (YGC). They concluded that these substrates offer favorable conditions for yeast growth. In addition, in the present study, various dilutions up to 10^8 were compared using *Saccharomyces cerevisiae* strains, revealing significantly lower values after concentrations of 10^5. Therefore, the results of this trial corroborate the findings reported by recent studies on microbial dynamics in fermentation systems, particularly regarding growth limitations due to substrate availability and inoculum size. Ioannou *et al.* (2019) and Bruyn *et al.* (2017) have described how microbial interactions and initial biomass concentrations significantly influence fermentation efficiency and microbial succession, supporting the importance of controlling these variables in post-harvest coffee processing. Consequently, in the development of fermentation processes involving microorganisms, the substrate represents a limiting factor for growth. The amount of biomass and initial inoculum must be proportional, as these factors influence the entire action of the fermentation process.

The objective of this study was to assess the yeast species linked to *D. melanogaster* larvae in post-harvest coffee processing, to elucidate their contribution to the fermentation dynamics of coffee cherries (Figs. 1-3). It is essential to determine the concentration and dilutions at which the yeasts remain viable in order to set up subsequent experiments in the bioreactors. This approach is supported by Puerta *et al.* (2012), who demonstrated that adding 60 ml of *Saccharomyces* sp. yeast resulted in enhanced fermentation processes and improved quality during sensory evaluation, reducing fermentation time. In their research, it was observed that a 12-h exposure to the microorganism was sufficient to generate enhanced attributes. It is worth highlighting that in certain fungi, the CFU count depends on three main factors: the handling of coffee fruits during harvesting, the conditions under which the cherries are kept throughout the fermentation process, and the antagonism that occurs between microorganisms when trying to proliferate within a limited growth medium.

Certain microscopic characteristics have proved to be useful for the identification of some species. Among the various identification characteristics, the following stand out:

- Germinal tube. This method involves assessing the filamentous extension of the yeast, without narrowing at its origin. Typically, the width is half that of the progenitor cell, and its length is three or four times greater than that of the mother cell. Only certain genera, such as *C. albicans*, are capable of producing true germ tubes. However, other species such as *C. tropicalis* have been reported to produce precocious *pseudohyphae*. These *pseudohyphae* resemble germ tubes but stand out by a characteristic halo and a constriction zone adjacent to the mother cell. This test is useful for differentiating *C. albicans* from other *Candida* spp., as demonstrated in the germ tube test by Moya-Salazar and Rojas (2018);

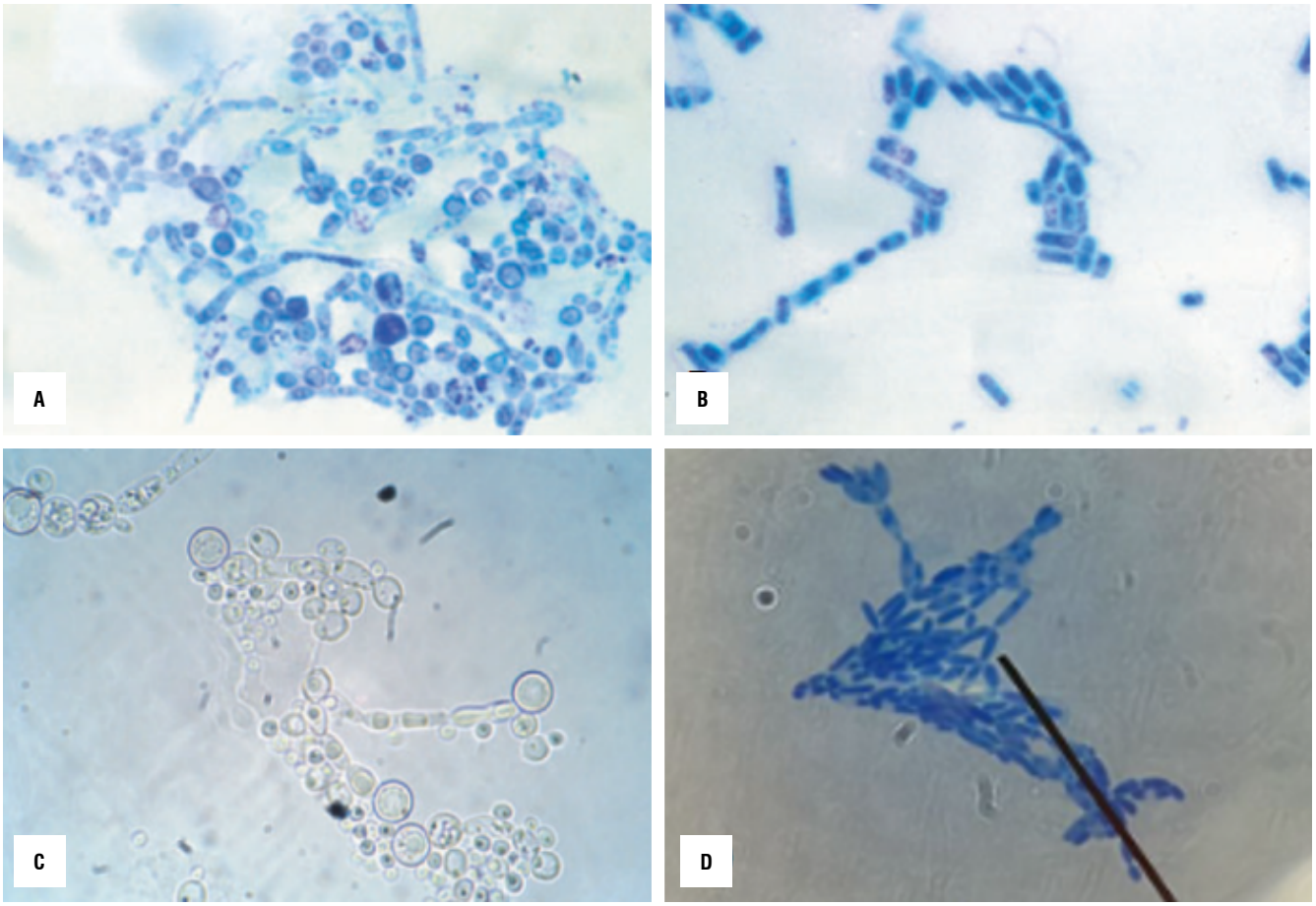

FIGURE 6 (A–D). Morphological diversity of fungal structures observed under light microscopy.

- Formation of Hyphae, Blastoconidia, Chlamydospores, and Arthrospores. The genera *Trichosporon* and *Blastoschizomyces* are characterized by the production of both *Arthroconidia* and *Blastoconidia*. The latter grow from shoots at the angles of the Arthroconidia, acquiring the characteristic rabbit ear shape (Figs. 6A-B). The species *Galactomyces geotrichum* (*G. candidum*) and *Blastoschizomyces capitatus* (*Geotrichum capitatus*) also produce blastoconidia from the angles of the arthroconidia, but in this case, they form a structure known as "hockey stick" (Fig. 6C). Chlamydospores are round or oval, 6-12 µm in diameter, have thick walls, and appear as terminal spores. Chlamydospores are characteristic and diagnostic of *C. albicans* (Fig. 6D), (Navarathna *et al*., 2016);
- Stains. Methylene blue stain was used to differentiate fungal structures, which were subsequently observed under the microscope, as illustrated in Figure 5. This staining technique yielded positive results when comparing the findings reported by Navarathna *et al.* (2016) and Kauffman *et al.* (2011) (Figs. 6A and 6C) with those obtained in this research (Figs. 6B and 6D). Based on morphological characteristics and staining results, it is possible to affirm that the isolated microorganisms are indeed yeasts.

In the laboratory, most yeasts grow easily across various culture media. However, the YGC medium appeared to be particularly conducive to their development due to its greater specificity. Typically, the majority of yeast colonies are slightly oval or flat with a buttery consistency that can be smooth or rough. Generally, it is uncommon for yeast colonies to develop mycelium. However, there have been cases in which spider-shaped projections can be seen on the periphery of fungal rings. Most white and creamy colonies are identified as yeasts from the genus *Prototheca*, a group of achlorophyllic algae, which develop at an incubation temperature of 28°C (Satoh *et al*., 2010). That was the case in this research, in which white and creamy colonies, resembling those of the genus *Candida,* were observed.

The identification of the structure of microorganisms in fermentation processes based on morphological characteristics has its limitations. Therefore, it is necessary to characterize their metabolic and molecular profiles for

proper classification (Hood, 2013). The characterization of microbiomes in specific environments requires a molecular diagnosis that involves the isolation and extraction of genetic material for sequencing and further bioinformatic analysis (Behjati & Tarpey, 2013). This methodology facilitates the creation of a taxonomic classification of the individuals present in such habitat and their relative abundance within the population (Berg *et al*., 2020).

This study demonstrated that *D. melanogaster* larvae present in coffee post-harvest processes act as vectors of various fermentative yeast species, including *Geotrichum, Galactomyces, Trichosporon, Blastoschizomyces*, and *Rhodotorula*. Through isolation techniques, staining, cultivation on YGC media, and colony-forming unit (CFU) counts, the kinetic and morphophysiological behavior of the strains found in the larvae's oral apparatus and digestive tract was characterized, revealing rapid colonization in liquid media within the first 12 h. Regression analyses between CFUs and absorbance enabled the determination of kinetic parameters essential for designing controlled fermentation processes. Furthermore, in vitro antagonism and synergism assays showed that certain yeasts, especially *Rhodotorula rubra,* can inhibit the growth of other species, which is critical when optimizing microbial interactions in fermentation. These findings highlight the biotechnological potential of these microorganisms to enhance coffee bean quality and the importance of implementing robust morphological and molecular classification methods to ensure their safe and effective application. This research lays the groundwork for future studies aimed at regulating fermentative microbiomes in coffee-growing environments to increase the added value of the final product.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author´s contributions

JJAM: writing, methodology testing, and project development; AJCF and JGSG: style correction and content revision. All authors approved the final version of the manuscript.

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