Journal of Agriculture and Environment for International Development - JAEID 2025, 119 (2): 25-40
DOI: 10.36253/jaeid-15073

Antagonistic yeasts isolated from bioferments of mountain microorganisms (MM): an alternative for the control of fruit anthracnose caused by *Colletotrichum tamarillo*

JUAN ALBERTO TRUJILLO-SALAZAR[1], MARCELA MORA-LÓPEZ[1], DANIEL ANDRÉS LÓPEZ-RESTREPO[1], JULIÁN ESTEBAN PINEDA-MONTOYA[1], JOHANA PATRICIA RAMÍREZ-OLIER[2*], LILIANA ROCÍO BOTERO-BOTERO[1*]

[1] *Faculty of Engineering, Research Group on Biodiversity, Biotechnology and Bioengineering GRINBIO, Medellin University, Antioquia, Colombia*

[2] *Faculty of Economic and Administrative Sciences. Research Group on Biodiversity, Biotechnology and Bioengineering GRINBIO, Medellin University, Antioquia, Colombia*

**Correspondence details: lbotero@udemedellin.edu.co, jpramirez@udemedellin.edu.co*

Submitted on: 2023, 19 September; accepted on: 2025, 12 December. Section: Research Papers

Abstract: Antagonistic yeasts emerge as a friendly option for the control of anthracnose, a postharvest disease caused by phytopathogenic fungi of the genus *Colletotrichum*. This study evaluated, under laboratory conditions, the antagonistic activity of 24 native yeasts isolated from fermentations of mountain microorganisms (MM) against *Colletotrichum tamarillo* through dual confrontation tests, production of volatile organic compounds (VOCs) in mouth-to-mouth tests and the evaluation of the ability to control the development of rotting wounds infected with *Colletotrichum tamarillo* in tree tomato fruits (*Solanum betaceum*) in preventive and curative treatments. In this study, 5 of the 24 isolated yeasts showed antagonistic potential, *Pichia kudriavzevii* GRB-LB11 presented the highest antagonistic potential in dual culture (PICR of 41.3 ± 2.1%), and Pichia kudriavzevii GRB-LB02 presented the highest production of VOCs in mouth-to-mouth tests (67.3 ± 2.6%). For the tests on tree tomato fruits, *Pichia kudriavzevii* GRB-LB11 and *Candida haemulonis* GRB-LB06 controlled 100% of the incidence of the disease in the preventive treatments, reducing the diameters of the wounds caused by Colletotrichum tamarillo to 0 ± 0 cm. In the curative tests, *Candida haemulonis* GRB-LB06 managed to reduce the incidence of the disease by 96.8 ± 5.2, reducing the diameters of the wounds caused by Colletotrichum tamarillo to 0.1 ± 0.16. The results support the agroecological benefits of MM ferments and the biotechnological potential of isolated yeasts for anthracnose control.

Keywords: Antagonistic yeasts, Colletotrichum, Anthracnose, Fruit rot, Mountain microorganisms

Introduction

Anthracnose, or fruit rot disease, is a postharvest disease caused by fungi of the phytopathogenic genus *Colletotrichum* that colonizes the flesh of weak fruits or those with wounds resulting from the harvesting, transport, and storage processes, generating circular necrosis (Joshi, 2018; Campos-Martínez et al., 2016; Ramírez-Olier et al., 2019) causing estimated losses between 25 and 100% of the production of fruits such as tree tomato (Caicedo

et al., 2017), papaya (Hernandez-Montiel et al., 2018), mango (Cabrera et al., 2018), strawberry (Ventura-Aguilar, Bautista-Baños, Flores-García, & Zavaleta-Avejar, 2018), citrus (Guarnaccia et al., 2017), avocado (Sharma, Maymon, & Freeman, 2017), among others.

Chemical synthesis products such as Antracol, Curacarb, WP, Captan, Funcloraz, Carbendazim, etc. (Arias Rivas & Carrizales, 2007) have traditionally been used to control this disease. These compounds are absorbed into the fruit, generating a risk of consumption (Agyekum et al., 2015), International laws restrict the trade of fruits containing compounds derived from synthetic fungicides. These restrictions added to the effects of these fungicides on the environment and human health have forced farmers to reduce their use and implement new alternatives with lower toxicity, stimulating the development of new products (Agyekum et al., 2015; FAO & World Health Organization, 2017).

Agroecology has developed simple techniques that generate products to replace chemical synthesis products reducing their use in crops, one of them is known as Mountain Microorganisms (MM) proposed by Higa in 1991. This technique proposes anaerobic fermentation of the organic layer of the forests to obtain Efficient Microorganisms (EM) that include bacteria, molds, and yeasts with metabolic characteristics that contribute to plant health and nutrition (Hart, 1985; Guzñay, 2016).

The isolation, characterization, and selection of Colombian antagonist yeasts, preselected in the MM fermentation processes, is an alternative that can be explored for the control of anthracnose caused by fungi of the genus *Colletotrichum* (Fan et al., 2018). Antagonistic yeasts possess a wide variety of mechanisms that include the production of secondary metabolites, enzymes, and volatile organic compounds (VOCs) that inhibit the growth of other microorganisms, and form protective biofilms, making them useful as control agents for fungal diseases (Liu, et al., 2013; D. Spadaro & Droby, 2016). This study evaluates the antagonistic activity of 19 native yeasts isolated from MM liquid fermentation against *Colletotrichum* sp. using laboratory tests such as dual confrontation, VOC production in mouth-to-mouth assays, and confrontation in tree tomato fruits (*Solanum betaceum*).

Materials and methods

Microbial isolation

Yeast isolation: To obtain the antagonistic yeasts, two fermentative processes of MM were used, initially with cultures in solid state (MMS) (Guzñay D. (2016) and, later, in liquid state (MML) (Higa & Par, 1994). The MMS was carried out by manually mixing 12 kg of organic cornmeal with 3 kg of organic matter in the process of decomposition, previously collected from the wooded areas of Cerro de las Tres Cruces (Medellín, Antioquia - Colombia).

The mixture was incubated at room temperature (25±3°C) and 80% relative humidity for 90 days for maturation; After this time, the mass obtained was placed in a natural fiber bag, and then the bag was sealed and submerged in 100 L of nutrient solution previously prepared by mixing 3 Kg of molasses as a carbon source and 115 liters of non-chlorinated water. This fermentative process was maintained under anaerobic conditions for 20 days, during which periodic sampling of 250 mL every 5 days was carried out to measure pH and microbial isolation. In the microbial isolation processes, serial dilutions were made using the methodology proposed by Liu et al. (2017), to achieve the growth of independent colonies, 100µl of the 10-4, 10-6, 10-7, and 10-8 dilutions were inoculated into Petri dishes containing Yeast Extract Peptone Dextrose agar (YPDA) composed by yeast extract (10 g L-1), peptone (20 g L-1), D-glucose (20 g L-1), Bacto-agar (15 g L-1) at pH 4.5 supplemented with Chloramphenicol at 0.04 g L-1 and incubated at 25°C for 48 hours. Finally, the colonies of the formed yeasts were characterized by microscopy using fresh smears with lactophenol blue staining using a 40X magnification. The yeasts isolated were maintained on YPDA and subcultured every 30 days.

Colletotrichum isolation: To evaluate the antagonistic potential of isolated yeasts in control of the causal agent of anthracnose, a strain of Colletotrichum sp. (GRB-HP18) was isolated on Sabouraud agar from infected tree tomato fruits with anthracnose symptoms, which were obtained from plantations located in the municipality of Santo Domingo (Antioquia - Colombia). The pathogenic activity of the strain was previously tested by infecting fruits of tree tomatoes under in vitro conditions. The pathogenic fungus was maintained on Sabouraud agar with subcultures every 30 days. The characterization of the fungus was carried out following the keys of Bailey et al., (1996).

Evaluation of antagonistic potential in vitro

Dual confrontation: The modified methodology proposed by Zazzerini & Tosi (1985) was implemented, for which 90 mm Petri dishes with Potato Dextrose Agar (PDA) were used. 5 mm diameter disks of the pathogenic fungus *Colletotrichum* were placed at one end of the Petri dish (with 7 days of growth on PDA agar) and discs of each of the isolated yeasts (with 48 hours of growth on PDA) were placed at 5 cm away to evaluate their antagonistic potential. As a control, discs of 5 mm diameter were impregnated with the systemic fungicide Chlorothalonil (Control 500® brand) or sterile distilled water, and discs of the pathogen were placed into the Petri dishes. The dual cultures were incubated for 10 days at room temperature.

The Radial Growth Inhibition Percentage (PICR) was determined by measuring the microbial growth radius of the pathogens with a vernier caliper. Measurements obtained in the dual cultures were compared to the control cultures using Equation 1, where PICR is the Percent Inhibition of Radial Growth; *C* is the diameter of the colony of the control culture and *T* is the diameter of the colony treated with the antagonist.

Equation 1. $PICR = \left(C - \frac{T}{C}\right) * 100$

Evaluation of the production of volatile organic compounds (VOCs): Yeasts with positive PICR in dual culture tests were additionally evaluated for antifungal activity by production of VOCs under *in vitro* conditions using the mouth-to-mouth methodology in pairs (yeast-phytopathogen) proposed by Parafati *et al*., (2015). For the test, Petri dishes with PDA were used for the cultivation of *Colletotrichum* sp. and, YPDA for the cultivation of yeasts. The inoculation of the antagonistic yeasts was carried out by surface culture, spreading, on the YPDA agar, 20 µl of a yeast solution at a concentration of $1x10^7$ cells mL^{-1} with the help of the Digralsky loop. For the inoculation of the pathogenic fungus, a 5mm diameter disc obtained from 7-day cultures was placed in the central part of the Petri dish with PDA agar. The inoculated Petri dishes were placed face to mouth and sealed with parafilm to allow gas exchange, finally, they were incubated for 10 days at 25 ± 2°C. The inhibitory action of VOCs on the phytopathogenic fungus *Colletotrichum* sp. was calculated by determining the effect on the radial growth of the pathogen using Equation 1.

Infection tests on tree tomato fruits (Solanum betaceum)

Disinfection of tree tomato fruits: For this stage of the study, the 3 yeasts with the highest antagonistic *in vitro* activity were selected. The tests were carried out using ripe tree tomato fruits produced without pesticide applications in the Eco- Garden of the University of Medellín to avoid the presence of systemic fungicides that could affect microbial growth. For the infection tests, the modified methodology described by Hernandez-Montiel *et al*., (2018) was used, for which, initially the fruits were disinfected with 2% (v/v) sodium hypochlorite for 3 minutes, subsequently, washed individually with abundant sterile distilled water and finally, dried for 2 hours under aseptic conditions inside the laminar flow chamber.

Determination of safety of preselected yeasts: To determine the harmlessness of the preselected yeasts over the tree tomato fruits three 2-cm cross-shaped wounds were made on each dry tree tomato using a sterile scalpel. Each tree tomato was inoculated with a 10µl suspension of one of the yeasts to evaluate (10^7 cells mL^{-1}). As a control, fruits inoculated with sterile distilled water were used. Tree tomato fruits were incubated for 10 days at room temperature (25 ± 2°C) in a sterile humid chamber. The state of the wounds made on the fruits was periodically monitored in search of symptoms of infection, finally, after 10 days of cultivation, the diameters of the damage (cm) around the cuts were measured.

Determination of the antagonistic potential of each yeast in vivo: The yeasts that showed harmlessness on the fruits were subjected to two types of tests to determine their antagonist potential against the pathogen *Colletotrichum* sp., one preventive and one curative. In the preventive treatments, three 2-cm cross-shaped wounds were made on each dry tree tomato using a sterile scalpel and, in each wound, 10µL of the suspension (10^7 cells mL^{-1}) of one of the yeasts evaluated was inoculated. The inoculated fruits were then incubated for 4 days at room temperature (25 ± 2°C) in a humid chamber to allow the yeasts to grow and establish themselves on the fruit, finally, after the incubation, the fruits were inoculated with 10µl of the conidia suspension of the phytopathogen *Colletotrichum* sp (10^4 con mL^{-1}; Tween 80, 0.1% v/v). In the curative treatments, the yeast was not allowed to establish itself and the colonization with the phytopathogen was carried out simultaneously, in these tests, the wounds were inoculated with 10µl of the yeast suspension (10^7 cells mL^{-1}), and after drying, the wounds were inoculated with 10µl of the conidia suspension of the phytopathogen *Colletotrichum* sp (10^4 con mL^{-1}; Tween 80, 0.1% v/v). For both treatments, fruits inoculated with sterile distilled water were used as a control and, as an infection control, tree tomatoes were inoculated with 10µl of suspension of conidia of the phytopathogen *Colletotrichum* (10^4 con mL^{-1}; Tween 80, 0.1% v/ v). In all cases, to determine the antagonist potential, after 10 days of cultivation in a humid chamber of the treated fruits, the diameter of the damage (cm) was measured.

Molecular identification

At the beginning of the study, the molecular identification of the phytopathogenic fungus GRB-HP18 isolated from tree tomato crops selected for its ability to generate symptoms of infection and anthracnose in tree tomato fruits was carried out, which was classified as belonging to the genus *Colletotrichum* using taxonomic keys. After *in vitro* and *in vivo* assays, the 3 yeasts isolated from the MML cultures that showed the highest antagonistic potential validated *in vivo* tests on tree tomato fruits (GRB-LB02, GRB-LB06, and GRB-LB11) were also subjected to molecular identification. The identification of the microorganisms was carried out by the university institution Colegio Mayor de Antioquia through processes of extraction, quantification, and evaluation of DNA purity in NanoDrop 2000, Thermo Fisher Scientific, Wilmington, DE. For the identification, the amplification of the ITS region was carried out by polymerase chain reaction (PCR) with the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') recommended for fungi. Purified PCR products were sequenced by Macro Gen Inc. (Seoul-Korea). The sequences of each primer were refined and aligned using the Geneious® program version 9.1.5 and compared in the GenBank database of the "National Center for Biotechnology Information" (http://www.ncbi.nlm.nih.gov/), using BLASTN nucleotides to determine the possible identity of the isolates.

Statistical analysis

In the study, a completely randomized design (DCA) was used for the evaluation of the antagonistic potential of the yeasts on the phytopathogenic fungus *Colletotrichum* sp. evaluating the PICR in the dual confrontation essays and by the production of VOCs *in vitro* cultures mouth to mouth. The effects of the treatments were initially analyzed through descriptive statistics and later using parametric tests. Each test was performed in triplicate and all cases the variables were

expressed as the mean ± standard deviation. To determine the effects of the treatments on the response variables, a one-way ANOVA analysis of variance was implemented, followed by a Tukey test. Before proceeding with the analysis of variance, Levene tests were performed to determine the homogeneity of variance and Shapiro-Wilk tests to determine normality. In all cases, $p<0.05$ was used as the statistical criterion to determine significant differences between treatments, taking into account a 95% confidence interval. All data were analyzed using the statistical program IBM SPSS version 25, using an exclusive programming routine for DCAs.

Results

Molecular Identification of pathogenic fungi

For the identification of the fungus *Colletotrichum* sp. GRB-HP18, isolated from tree tomato fruits with anthracnose symptoms, ITS-PCR amplification of the DNA extractions was performed, allowing its identification as *Colletotrichum tamarillo* with a similarity percentage of 99% and whose access number was MH865701.

Preparation of bioferments of Mountain Microorganisms (MM)

After 20 days of MML culture, 24 microorganisms compatible with yeasts and 12 with filamentous molds were isolated. The diversity of molds and yeasts decreased with culture time, on day 0, 11 microorganisms were isolated (8 consistent with yeasts and 3 with molds), by day 5, 11 were isolated (7 consistent with yeasts, and 4 with filamentous molds), and finally, on days 10, 15 and 20, 9 microorganisms were isolated (3 consistent with yeasts and no filamentous molds).

In vitro evaluation of antagonist potential

Dual challenge (DC): Despite their ability to survive in liquid bioferment, after 10 days of culture, only 5 of the yeasts isolated from the MML showed a positive response in the PICR in DC (Fig.1), the highest inhibition of *C. tamarillo* GRB-HP18 was achieved in the confrontations in DC with the isolate GRB-LB11 (PICR: 41.3 ± 2.1%), which reached a value similar to that obtained with chlorothalonil used as chemical control (Control 500®, PICR: 40.0± 2.1%), for the yeasts GRB-LB06 (PICR: 12.6 ± 2%), GRB-LB05 (11.6 ± 1%), GRB- LB01 (10.3 ± 2%) and GRB-LB02 (9.7 ± 0.3%), the PICR in DC values were significantly lower.

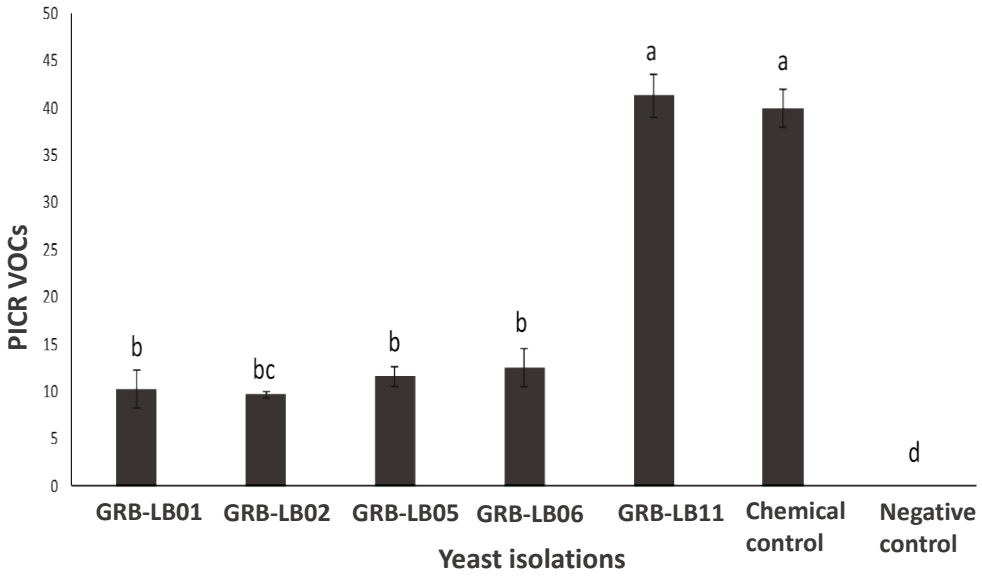

Figure 1. The Radial Growth Inhibition Percentages (PICR) obtained after 10 days of dual culture of the isolates corresponding to yeasts vs. the fungus C. tamarillo GRB-HP18. The data followed by different letters in the column are significantly different (p≤ 0.05) according to Tukey's test.

VOCs production and antagonistic activity: It was shown that the yeasts GRB-LB01, GRB-LB02, GRB-LB05, GRB-LB06, and GRB-LB11, preselected for their antagonistic activity in dual culture, were able to inhibit radial growth as evidence of the production of VOCs *in vitro* mouth-to-mouth tests against the phytopathogenic fungus *C. tamarillo* GRB-HP18 (Fig. 2). In this study, the yeast GRB-LB02 was the one that presented the best inhibition results by VOCs (PICR: 67.3 ± 2.6%), despite not being the one with the best control capacity in dual cultures (PICR: 9.7 ± 0.3%) evidencing the inhibitory effect of the biochemical encounter of the yeast with the phytopathogenic fungus and the potential antagonist of this yeast due to the movement of gases in solid fermentations in confined spaces. In the mouth-to-mouth tests carried out with the yeasts GRB-LB01, GRB-LB11, GRB-LB06 and GRB-LB05, PICRs were lower than those reached by GRB-LB02 with values of 45.6 ± 2.5%, 44.4 ± 6.5%, 44.0 ± 5.1% and 39.0 ± 2.3% respectively.

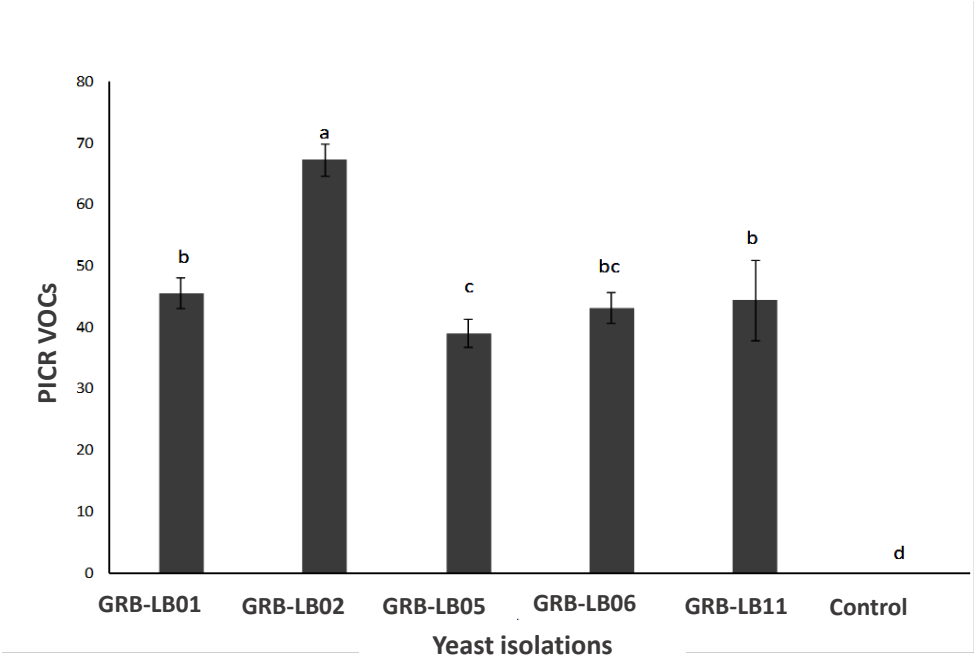

Figure 2. Percentage of inhibition of VOCs of corresponding isolates with yeasts against the phytopathogenic fungus C. tamarillo GRB-HP18 analyzed at 10 days. Data followed by different letters in the column are significantly different (p≤ 0.05).

Safety and antagonism of yeasts in fruits of tree tomatoes (Solanum betaceum)

To evaluate the innocuousness and antagonist activity *in vivo* in tree tomato fruits, the 3 yeasts with the best results in *in vitro* tests were chosen, the yeast GRB-LB11 for standing out in dual culture (41.3 ± 2.1%) and good response in the mouth-to-mouth tests (44.4 ± 6.5%), the GRB-LB02 for presenting the highest PICR in mouth-to-mouth tests (67.3 ± 2.6%) and finally the yeast GRB-LB06, which, although it was not the best, presented antagonism in both the dual culture (12.6 ± 2%) and the mouth-to-mouth tests (44.0 ± 5.1%).

Determination of innocuousness: The innocuousness of the 3 yeasts could be evidenced after 10 days of cultivation in a humid chamber of the injured tree tomatoes and inoculated with the selected yeasts, in no case were there any damages or an increase in the damage of the fruits (diameter of the wound 0±0 cm), in addition, for the three yeasts, the same type of healing was observed as that found in the negative control inoculated with sterile distilled water, without generating rotting problems that affected the quality of the fruits.

Antagonist potential in preventive treatment: When analyzing the diameters of the wounds in the preventive treatments, in which the yeasts were allowed to establish themselves and generate biofilms before the attack of the phytopathogen, it was possible to see that the best results were those obtained with the yeasts GRB-LB06 and GRB-LB11 since these yeasts decreased the incidence of anthracnose infection by 100% and obtained wound diameters with values of 0 ± 0 cm (Table 1).

Antagonist potential in curative treatments: The results for the curative treatments made it possible to demonstrate the antagonistic capacity, all the yeasts evaluated presented a decrease in the incidence of the disease, reducing the damage between 38-97%. The curative treatment carried out with the yeast GRB-A06 was the one that achieved the greatest reduction of the incidence of the disease (96.8 ± 5.2%). Regarding the severity of the infection, after 10 days of cultivation in a humid chamber, it was observed that the yeast with the greatest capacity for curative control of anthracnose was GRB-LB06 (0.1 ±0.2 cm), followed by GRB-LB11 (1.1±0.3

cm) and GRB-LB02 (1.9±0.3 cm) about infection control (3.1±0.3 cm), the latter being, again, the least effective, presenting active mycelium and rot in the inoculated tree tomatoes.

Table 1. Percentage of incidence of and diameter of the infection of C. tamarillo. regarding the different treatments. The data followed by different letters in the column are significantly different (P≤ 0.05) according to Tukey's test.

TREATMENT		INCIDENCE CONTROL (%)	WOUND DIAMETER (CM)
Preventive	*C. tamarillo.* + GRB-LB02	51.6 ± 7.1 [bc]	1.5 ± 0.22 [bc]
	C. tamarillo. + GRB-LB06	100 ± 0.0 [a]	0.0 ± 0.0 [d]
	C. tamarillo. + GRB-LB011	100 ± 0.0 [a]	0.0 ± 0.0 [d]
Curative	*C. tamarillo.* + GRB-LB02	38.7 ± 10.6 [c]	1.9 ± 0.33 [b]
	C. tamarillo. + GRB-LB06	96.8 ± 5.2 [a]	0.1 ± 0.16 [d]
	C. tamarillo. + GRB-LB11	64.5 ± 8.4 [b]	1.1 ± 0.26 [c]
Negative control	Distilled water	99.3 ± 1.4 [a]	0.3 ± 0.29 [d]
Infection control	*C. tamarillo.* + distilled water	0.0 ± 11 [d]	3.1 ± 0.34 [a]

Molecular identification of preselected antagonistic yeasts

The molecular identification of the yeasts preselected for their antagonistic potential, evidenced *in vitro* by the production of metabolites in the dual culture assays and by the production of VOCs in the mouth-to-mouth tests, making it possible to determine the presence of two species: *Pitchia kudriavzavii* (yeasts GRB-LB02 and GRB-LB11) and *Candida haemulonis* (yeast GRB-LB06) (Table 2).

Table 2. Identity of the preselected yeasts GRB-LB2, GRB-LB6 and GRB-LB11. Including access number and similarity percentage.

YEAST	MOLECULAR IDENTIFICATION	ACCESS NUMBER	SIMILARITY PERCENTAGE
GRB-LB02	*Pichia kudriavzevii*	KJ706301.1	94 %
GRB-LB06	*Candida haemulonis*	MG637448.1	99 %
GRB-LB11	*Pichia kudriavzevii*	KP674518.1	99 %

Discussion

Molecular Identification of pathogenic fungi

The identification of the fungus *Colletotrichum* sp. GRB-HP18 as *Colletotrichum tamarillo* is consistent with the area in which this strain was isolated and as the causal agent of anthracnose in tree tomato or tamarillo (*Solanum betaceum*) fruits, as it is known in several countries (Caicedo et al., 2017).

Preparation of bioferments of Mountain Microorganisms (MM)

The solid anaerobic fermentation MMS behaved as reported (Guzñay, 2016), after 24 hours of culture a putrefactive odor product of microbial activity was evidenced, over time, the odor became less intense with the time culture and, after the 90 days recommended to finish the

fermentation, a light brown material with a pasty texture moist to the touch with characteristics like those reported was obtained.

This behavior agrees with the reports of Higa & Par (1994) and Zeballos Heredia (2017) who describe this phenomenon stating that microbial biodiversity is affected by environmental demands within the bioferment. According to previous studies, in the fermentation processes, microorganisms carry out reactions that include hydrolysis, acidogenesis, acetogenesis, and methanogenesis, these processes generate organic compounds and secondary metabolites that transform the bioferment in an environment with extreme conditions for many microorganisms that, consequently, decrease their proliferation or die (Liu et al., 2013; Wilfrido et al., 2016; Zeballos Heredia, 2017).

During the 20 days of LMM liquid anaerobic fermentation, the pH dropped from 5 to 3.7 at a rate of 0.062 pH units/day (R2=0.91), this condition favored the growth of molds and yeasts (Pushpa et al, 2016). The acidification originates due to different processes, according to Pushpa *et al.,* (2016), this pH reduction is a consequence of the process of mineralization of nitrogen into nitrites and nitrates. Other authors indicate that acidification is the result of microbial metabolism that generates a wide range of organic acids that allow fungi to solubilize nutrients (López et al., 2020). According to David (2011), the microbial community responsible for the decomposition of organic matter residues of plant origin are the fungi belonging to the Phylum Ascomycota, which dominate the process both in abundance and in activity. According to the author, in the process, acidification allows microorganisms to hydrolyze complex molecules such as lignin and cellulose; these results justify the presence of experimentally isolated molds and yeasts in this study. However, some authors indicate that lactic acid bacteria and yeasts form part of the microbial consortia that actively participate in the acidification process (Sigstad, Schabes, & Tejerina, 2013; Zeballos Heredia, 2017; Pushpa et al., 2016).

In vitro evaluation of antagonist potential

Dual challenge (DC): The highest inhibition of *C. tamarillo* GRB-HP18 was achieved in the confrontations in DC with the isolate GRB-LB11 (PICR: 41.3 ± 2.1%) (Fig. 1) were higher than those reported by Campos-Martínez *et al.*, (2016), who faced various yeasts against *Colletotrichum gloeosporioides* under in DC and evaluated the PICR. In their reports, Campos-Martínez *et al.*, (2016) indicate that the yeasts *Candida intermedia* KP238317 (PICR: 27.3%), *Candida intermedia* KP238318 (PICR: 23.5%) and *Wickerhamomyces anomalus* KP238319 (PICR: 35.4%) were the most effective and, in their analyzes, they indicated that the PICR values found were promising to carry out control activities. The abundance of potentially antagonistic yeasts isolated in this study supports the agronomic potential reported for the microbial consortia formed during the MMS and MML processes for the control of fungal diseases (Higa & Par, 1994; Zeballos Heredia, 2017) and evidence of the biotechnological potential of the yeast GRB-LB11 (PICR: 41.3 ± 2.1%) for the control of anthracnose in fruits such as tree tomatoes. After MML cultivation, 14 yeast-compatible isolates did not show antagonistic activity in dual cultures challenged with *C. tamarillo* GRB-HP18, even being, in some cases, colonized by the pathogenic fungus. The survival of these 14 yeasts, which did not present an antagonistic response, indicates that other strategies could facilitate their saprophytic action, such as high growth speed, abundant sporulation, and the wide range of substrates on which they can grow (Hjeljord, Tronsmo, 1998; Pérez, 2004), making it possible for them to survive in difficult environments such as those generated in MML bioferments.

VOCs production and antagonistic activity: The results of antagonism by the production of VOCs achieved by GRB-LB02 (PICR: 67.3 ± 2.6%) were higher than those reported by Oro *et al.*, (2018) and Zhou, *et al*., (2018) doubling the effectiveness of the inhibition response reported for *Saccharomyces cerevisiae* on *Colletotrichum* sp. (reported PICR of 32%) and exceeding the control response achieved by yeast by *Debaryomyces nepalensis* by 1.4 times

(reported PICR of 41.6%). In general, all the yeasts included in this study exceeded the results reported for *Saccharomyces cerevisiae*, only the yeast GR-LB08 did not reach the control results reported for *D. nepalensis*, evidencing the biotechnological potential of the yeasts isolated after progressive MMS culture and MML, and supporting the beneficial results in the control of diseases reported in agroecology for the application of these kind of ferments.

Safety and antagonism of yeasts in the fruits of tree tomatoes (Solanum betaceum)

Similar results to those obtained in the antagonist potential in preventive treatment with the GRB-LB06 and GRB-LB11 yeasts, with which the incidence of anthracnose infection was reduced by 100% and with which the wound diameters were reduced to values of 0 ± 0 cm (Table 1), were reported by Hernandez-Montiel *et al.*, (2018) who evaluated the antagonistic activity of the yeast *Debaryomyces hansenii* against anthracnose in papaya fruits (*Carica papaya*), managing to reduce the diameters of the wounds by 0 cm. In this study, the preventive treatments carried out with GRB-LB02, however, managed to reduce the incidence of the disease to wounds of 1.5 ± 0.2 cm in diameter in the tree tomato fruits, although GRB-LB02 showed the least control capacity, it was able to reduce the anthracnose incidence compared to the infection control that obtained a wound diameter of 3.1±0.3 cm, for this yeast, even though the results were not as effective, they are better than those reported by Campos-Martínez *et al*., (2016) who evaluated the yeast *Wickerhamomyces anomalus* H2 to control *C. gloeoporioides* in avocado fruits, managing to reduce the diameter of the wound to 1.7 cm.

When analyzing the antagonistic capacity of the yeasts GRB-LB06, GRB-LB11, and GRB-LB02 against the attack of *C. tamarillo* GRB-HP18, their potential is evidenced, both in preventive and curative inoculations. However, prior yeast inoculation has benefits over simultaneous inoculations. In this study, the yeasts GRB-LB06 and GRB-LB11 did not present statistically significant differences when applied as a preventive treatment, achieving 100% control; however, this capacity is reduced to 64.5 ± 8.4% for GRB-LB11 when the fruit is inoculated simultaneously with the phytopathogen, which suggests that the establishment of the GRB-LB11 isolate in the fruit generated an increase in the potential for antagonism, to the point of equaling the results of the GRB-LB06 isolate in simultaneous application. This condition has already been reported by authors such as Spadaro & Droby, (2016) who mention that the establishment of yeasts within the host is essential so that the mechanisms that allow the inhibition of phytopathogenic fungi such as *Colletotrichum* sp. Furthermore, authors such as Zhou, Li, Zeng, & Shao, (2018) stated that the inoculation time of the yeast affects the antagonistic capacity and that this is directly proportional to the decrease in the diameter of the wounds, these authors generated wounds of 3.58 cm in diameter when simultaneously inoculating the antagonistic yeast *D. nepalensis* to control *C. gloeosporiodes* in mango fruits (*Mangifera indica*), however, the wounds were reduced to 2.2 cm in diameter when the yeast was allowed to establish 24 hours before infection of the pathogen and These were reduced to 1.53 cm in diameter when the yeast was established in the fruits 48 hours before infection with the pathogen, thus doubling the effectiveness of the antagonist activity. According to Yan *et al*., (2018), one of the main mechanisms of action of yeasts is the ability to make a biofilm around the fruits, which could reduce the injury by preventing the phytopathogenic fungus from colonizing the fruit. and thus interrupt the triangle of infection. In addition, Spadaro & Droby, (2016) report that yeasts have various mechanisms of action to control diseases, so if the fruits are inoculated preventively with yeasts, they have more time to act and generate all the mechanisms such as biofilm formation, resistance induction and release of volatile metabolites. Although the findings of this study lead to the same recommendations as Liu *et al.*, (2013) who indicate using yeasts as a disease-preventive method, to give them time to generate their action mechanisms to control the different phytopathogens, the results provided by the GRB-LB06 yeast indicate that it also prolongs the life of the infected fruits since they can contribute to

damage control when the pathogen attacks simultaneously reducing the dimensions of the wounds.

Biotechnological potential of selected yeasts.

In general, it can be said that *C. haemulonis* GRB-LB06 was the yeast that obtained the most promising results in the *in vivo* tests carried out with the pathogen *C. tamarillo* GRB-HP18, achieving the greatest control of the incidence of the disease (%C: 100 ± 0.0%) the highest percentage of reduction in the diameters of the wounds (100%) in the preventive tests and, reaching the highest reduction in the incidence of the disease (96.8 ± 5.2%) and the lowest size of the wounds (0.1 ± 0.2 cm) in the curative tests. It is feasible that for this yeast the control capacity was mainly associated with the production of VOCs (PICR: 44.0 ± 5.1%) (Table 3), since the production of metabolites, as evidenced in DC cultures was low (PICR: 12.6 ± 2.0 %).

Few reports have been found of this yeast, the first ones are associated with studies carried out with microflora of the intestine of the fish *Haemulon scirus* (Van Uden & Kolipinski, 1962), more recent studies indicate that it has been isolated from flowers on the island of Mindanao, Philippines (Sipiczki & Tap, 2016), however, no records were found with information on the beneficial biological activity of this yeast, so this could be the first report of the biotechnological potential of this species, however, reports were found that indicate that *C. haemulonis* may be responsible for nosocomial infections and diseases (Sipiczki & Tap, 2016), therefore, to develop its industrial potential as an efficient microorganism for the control of post-harvest diseases, it would be necessary to carry out pathogenicity tests in humans.

Table 3. Summary of the results of the in vitro and in vivo antagonism tests for the preselected yeasts on the phytopathogenic fungus C. tamarillo. In vitro PICRs in CD cultures and mouth-to-mouth tests and percentage of control of the incidence of the disease (% C) and diameter of the wounds (WD) of the infection caused by C. tamarillo in the preventive and curative treatments carried out in vivo with fruits of tree tomatoes.

Microorganism	In *vitro* PICR		Tests on tree tomato fruits (*S. betaceum*)		
	VOC's (%)	DC (%)	Type of treatment	Incidence control percentage (%C)	Wounds diameter (cm)
C. haemulonis	44.0 ± 5.1	12.6 ± 2.0	Preventive	100.0 ± 0.0 [a]	0 ± 0 [d]
GRB-LB06			Curative	96.8 ± 5.2 [a]	0.1 ± 0.16 [d]
P. kudriavzevii	44.4± 6.5	41.3± 2.1	Preventive	100 ± 0.0 [a]	0 ± 0 [d]
GRB-LB11			Curative	64.5 ± 8.4 [b]	1.1 ± 0.3 [c]
P. kudriavzevii	67.3± 2.6	9.7± 0.3	Preventive	51.6 ± 7.1 [bc]	1.5 ± 0.2 [bc]
GRB-LB02			Curative	38.7 ± 10.6 [c]	1.9 ± 0.3 [b]

The other two yeasts selected for their activity both *in vitro* and *in vivo*, were classified as *P. kudriavzevii*, these, despite being the same species, showed differences in behavior against the pathogen *C. tamarillo* GRB-HP18. In the study, *P. kudriavzevii* GRB-LB11 achieved similar results to *C. haemulonis* in preventive treatment, managing to reduce the incidence of the disease by 100% and the progression of the disease in the wounds by 100% (WD: 0 ± 0 cm). However, its efficacy was lower in the curative tests in which the pathogen *C. tamarillo* GRB-HP18 was applied at the same time as the yeast, in this case, *P. kudriavzevii* GRB-LB11 managed to reduce

the incidence to 64.5 ± 8.4% and to 67% the size of the wounds (1.1 ± 0.3 cm) so, as other authors have said, it could be that this yeast needs to establish itself before the infection (Zhou, Li, Zeng, & Shao, 2018). The efficacy of *P. kudriavzevii* GRB-LB02 did not reach the control of the incidence in the preventive tests achieved by *C. haemulonis* GRB-LB06 or *P. kudriavzevii* GRB-LB011, managing to reduce the incidence of the disease by 51.6 ± 7.1 % and by 55% the size of the wounds (1.9 ± 0.3 cm), additionally, its efficacy was even lower in the curative tests, for these tests, *P. kudriavzevii* GRB-LB02 managed to reduce the incidence by 38.7 ± 10.6%. and in 42% of the size of the wounds caused by the phytopathogenic fungus on the tree tomato fruits, so this strain would require being part of a mixed product to be effective.

A review of the biotechnological potential indicates that *P. kudriavzevii* is a teleomorphic species of *Candida krusei*. According to the records found, this species is widely distributed in the soil, in the skin of fruits, and even in fermented beverages (Corbu & Portocalelor, 2020). Liu *et al*., (2020) were able to significantly inhibit decay rate, weight loss, and delay color change, with no effect on total soluble solids (TSS), titratable acid (TA), or firmness during storage of the cherry tomato using *P. kudriavzevii* (Liu *et al*., 2020). According to reports from some researchers, the mechanism of action of this yeast is associated with the formation of biofilms, production of antioxidant compounds, VOCs, lytic enzymes, and competition for nutrients, and its antagonistic activity has been recorded against *Alternaria alterna*, *Aspergillus nigri*, *Botritys cinerea*, *C. gloeosporioides* (Cabañas et al., 2020; Chi et al., 2015). No evidence was found to indicate that this species is associated with food spoilage, which agrees with the results of this study in which no damage to the fruits was evidenced during the safety tests when it was inoculated on the wounds of the fruits. These findings support the idea that these yeasts can be used as an active ingredient in the development of new products to control fruit rot and thus extend their useful life during the marketing stages, and in the same way they could serve as an agent of biological control of fungal diseases in crops. Finally, the differences between these strains of *P. kudriavzevii* highlight the need to evaluate each strain for each biotechnological activity for which they intend to be recommended since the identification of the species is not a guarantee of equality of response or effectiveness.

Conclusions

The fermentation of MM is a process that allows the isolation of antagonistic yeasts with potential activity for the control of anthracnose that causes the rotting of tree tomato fruits, helping to mitigate the effects of damage during harvesting processes. transport and commercialization of the fruits. The native yeasts *Candida haemulonis* GRB-LB06 and *Pichia kudriavzevii* GRB-LB11 presented the complement of production of metabolites and VOCs with greater potential for the control of *Colletotrichum tamarillo* (GRB-HP18) *in vivo* tests, reducing both the incidence of the disease as the diameter of the wounds. *C. haemulonis* GRB-LB06 (PICR VOCs: 44.0 ± 5.1%; PICR CD: 12.6 ± 2) achieved the greatest control of disease incidence (%C:100 ± 0.0%,) and the greatest reduction of diameters of the wounds (0.1 ± 0.16) in the preventive tests and, in the curative tests, it reached the greatest reduction in the incidence of the disease (96.8 ± 5.2%) and the smallest size of the wounds (0. 1±0.2 cm). *P. kudriavzevii* GRB-LB11 (PICR VOCs: 44.4 ± 6.5%; PICR DC: 41.3 ± 2.1%,) was efficient only for preventive control of the disease (100%), evidencing the agronomic potential of these yeasts. It is recommended to evaluate the effectiveness of these yeasts in the control of other fungal diseases and other types of fruits. It is necessary to continue with the studies for the scaling of EM-based products to treat post-harvest diseases and to develop options that contribute to mitigating the negative effects and dependence on the synthetic products that we use in agriculture.

References

Agrios, G. N. (1991). Fitopatología (1ra ed.). México: LIMUSA.

Akwasi Akomeah Agyekum, George Soda Ayernor, Firibu Kwasi Saalia, & Betty Bediako-Amoa. (2015). Translocation of Pesticide Residues in Tomato, Mango, and Pineapple Fruits. *Journal of Food Science and Engineering*, *5*(3). https://doi.org/10.17265/2159-5828/2015.03.006

Arias Rivas, B., & Carrizales, L. (2007). Control químico de la antracnosis del mango (Mangifera indica l.) en pre y postcosecha en el Municipio Cedeño, Estado Monagas, Venezuela. Bioagro, 19(1), 19–25.

Bautista-Rosales, P. U., Calderon-Santoyo, M., Servín-Villegas, R., Ochoa-Álvarez, N. A., & Ragazzo-Sánchez, J. A. (2013). Action mechanisms of the yeast Meyerozyma caribbica for the control of the phytopathogen Colletotrichum gloeosporioides in mangoes. *Biological Control*, *65*(3), 293–+'301.

Cabañas, C. M., Hernández, A., Martínez, A., Tejero, P., Vázquez-Hernández, M., Martín, A., & Ruiz-Moyano, S. (2020). Control of Penicillium glabrum by Indigenous Antagonistic Yeast from Vineyards. Foods, 9(12), 1864. https://doi.org/10.3390/foods9121864

Cabrera, L., Rojas, P., Rojas, S., Pardo-De la Hoz, C. J., Mideros, M. F., Danies, G., … Restrepo, S. (2018). Most Colletotrichum species associated with tree tomato (Solanum betaceum) and mango (Mangifera indica) crops are not host-specific. Plant Pathology, 67(5), 1022–1030.

Caicedo, J. D., Lalangui, K. P., Pozo, A. N., Cevallos, P. A., Arahana, V. S., & Méndez, K. S. (2017). Multilocus molecular identification and phylogenetic analysis of Colletotrichum tamarilloi as the causal agent of Tamarillo (Solanum betaceum) anthracnose in the Ecuadorian highlands. European Journal of Plant Pathology, 148(4), 983–996.

Campos-Martínez, A., Velázquez-del Valle, M. G., Flores-Moctezuma, H. E., Suárez-Rodríguez, R., Ramírez-Trujillo, J. A., & Hernández-Lauzardo, A. N. (2016). Antagonistic yeasts with potential to control Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. And Colletotrichum acutatum J.H. Simmonds on avocado fruits. *Crop Protection*, *89*, 101–104. https://doi.org/10.1016/j.cropro.2016.07.001

Chi, M., Li, G., Liu, Y., Liu, G., Li, M., Zhang, X., Sun, Z., Sui, Y., & Liu, J. (2015). Increase in antioxidant enzyme activity, stress tolerance and biocontrol efficacy of Pichia kudriavzevii with the transition from a yeast-like to biofilm morphology. Biological Control, 90, 113–119. https://doi.org/10.1016/j.biocontrol.2015.06.006

Choque V., J., 2008. Producción de Humus de lombriz. La Paz: CIPCA. p 24 Diversidad Microbiana y Taxonomía, s/f. Consultado 5 diciembre 2012. Disponible en http://www.diversidadmicrobiana.com/index.php?option=com_content&view=article&id=671&Itemid=79

Corbu, V., & Portocalelor, A. (2020). Biodiversity studies on Pichia kudriavzevii from romanian spontaneous fermented products. AgroLife Scientific Journal, 9(1).

D.B. Wilson. Microbial diversity of cellulose hydrolysis. Curr Opin Microbiol, 14 (2011), pp. 259-263.

De la Cruz-Quiroz, R., Roussos, S., Rodríguez-Herrera, R., Hernandez-Castillo, D., & Aguilar, C. N. (2018). Growth inhibition of Colletotrichum gloeosporioides and Phytophthora capsici by native Mexican Trichoderma strains. *Karbala International Journal of Modern Science*, *4*(2), 237–243. https://doi.org/10.1016/j.kijoms.2018.03.002

Fan, Y. V., Lee, C. T., Klemeš, J. J., Chua, L. S., Sarmidi, M. R., & Leow, C. W. (2018). Evaluation of Effective Microorganisms on home scale organic waste composting. *Journal of Environmental Management*, *216*, 41–48. https://doi.org/10.1016/j.jenvman.2017.04.019

Guarnaccia, V., Groenewald, J. Z., Polizzi, G., & Crous, P. W. (2017). High species diversity in Colletotrichum associated with citrus diseases in Europe. Persoonia - Molecular Phylogeny and Evolution of Fungi, 39(1), 32–50.

Guzñay D, C. (2016). *Guía Agroecológica para una agricultura resiliente en la parte baja de la Subcuenca del río Daula*. Retrieved from https://www.avsf.org/public/posts/2254/guia_agroecologica_agricultura_resiliente_ecuador_avsf_2017.pdf

Habiba, Noreen, R., Ali, S. A., Hasan, K. A., Sultana, V., Ara, J., & Ehteshamul-Haque, S. (2019). Evaluation of biocontrol potential of epiphytic yeast against postharvest Penicillium digitatum rot of stored Kinnow fruit (Citrus reticulata) and their effect on its physiochemical properties. *Postharvest Biology and Technology*, *148*, 38–48. https://doi.org/10.1016/j.postharvbio.2018.10.007

Hart, R. D. (1985). *Conceptos Básicos sobre Agroecosistemas*. Centro Agonomico Tropical de Investigación y Enseñanza.

Higa, T., & Par, J. F. (1994). *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Enviroment*. 25.

Hernandez-Montiel, L. G., Gutierrez-Perez, E. D., Murillo-Amador, B., Vero, S., Chiquito-Contreras, R. G., & Rincon-Enriquez, G. (2018). Mechanisms employed by *Debaryomyces hansenii* in biological control of anthracnose disease on papaya fruit. *Postharvest Biology and Technology*, *139*, 31–37. https://doi.org/10.1016/j.postharvbio.2018.01.015.

Hjeljord L, Tronsmo A. *Trichoderma* and *Gliocladium* in biological control: an overview. In: *Trichoderma* & *Gliocladium*: Enzymes, biological control and commercial applications. Harman GE, Kubice CP. (Eds). Volumen 2. p.131-151. Taylor & Francis. 1998.

Ingram, D. S. & Joachim, I. (1971). The growth of Peronospora farinosa f.sp, betae and sugar beet callus tissues in dual culture. Journal of General Microbiology 69, 211-220.

Joshi, R. (2018). A Review on Colletotrichum spp. Virulence mechanism against host plant defensive factors. *Journal of Medicinal Plants Studies*, *6*(6), 64–67. https://doi.org/10.22271/plants.2018.v6.i6b.02

López Julián E., Gallego Jorge L., A Vargas-Ruiz lejandra, Amny Liceth Peña-Mosquera1 & Arley David Zapata-Zapata2 & Idalia Jacqueline López-Sánchez1 & Liliana Rocio Botero-Botero1 *Aspergillus tubingensis* and *Talaromyces islandicus* Solubilize Rock Phosphate Under Saline and Fungicide Stress and Improve *Zea mays* Growth and Phosphorus Nutrition Journal of Soil Science and Plant Nutrition (2020) 20:2490–2501. https://doi.org/10.1007/s42729-020-00315-w /

Liu, J., Sui, Y., Wisniewski, M., Droby, S., & Liu, Y. (2013). Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *International Journal of Food Microbiology*, *167*(2), 153–160. https://doi.org/10.1016/j.ijfoodmicro.2013.09.004

Liu, X., Gao, Y., Yang, H., Li, L., Jiang, Y., Li, Y., & Zheng, J. (2020). Pichia kudriavzevii retards fungal decay by influencing the fungal community succession during cherry tomato fruit storage. Food Microbiology, 88, 103404. https://doi.org/10.1016/j.fm.2019.103404

Oro, L., Feliziani, E., Ciani, M., Romanazzi, G., & Comitini, F. (2018). Volatile organic compounds from Wickerhamomyces anomalus, Metschnikowia pulcherrima and Saccharomyces cerevisiae inhibit growth of decay causing fungi and control postharvest diseases of strawberries. *International Journal of Food Microbiology*, *265*, 18–22. https://doi.org/10.1016/j.ijfoodmicro.2017.10.027

Pérez N. Manejo Ecológico de plagas. CEDAR: La Habana. Cuba. 2004. 296 pp.

Pushpa, T. B., Sekaran, V., Basha, S. J. S., & Jegan, J. (2016). Investigation on Preparation, Characterization and Application of Effective Microorganisms (EM) Based Composts—An Ecofriendly Solution. *Nature Environment and Pollution Technology*, *15*(1), 7.

Ramírez-Olier, J., Trujillo-Salazar, J., Osorio-Echeverri, V., Jaramillo-Ciro, M., & Botero-Botero, L. (2019). In vitro antagonism of Trichoderma asperellum against Colletotrichum gloeosporioides, Curvularia lunata, and Fusarium oxysporum Antagonismo in vitro de

Trichoderma asperellum contra Colletotrichum gloeosporioides, Curvularia lunata, y Fusarium oxysporum. *Revista UIS Ingenierías*, *18*, 7.

Rungjindamai, N. (2016). Isolation and evaluation of biocontrol agents in controlling anthracnose disease of mango in Thailand. *Journal of Plant Protection Research*, *56*(3), 306–311. https://doi.org/10.1515/jppr-2016-0034

Sharma, G., Maymon, M., & Freeman, S. (2017). Epidemiology, pathology and identification of Colletotrichum including a novel species associated with avocado (Persea americana) anthracnose in Israel. Scientific Reports, 7(1), 15839.

Sharma, V., Salwan, R., Sharma, Prem. N., & Kanwar, S. S. (2017). Elucidation of biocontrol mechanisms of Trichoderma harzianum against different plant fungal pathogens: Universal yet host specific response. *International Journal of Biological Macromolecules*, *95*, 72–79. https://doi.org/10.1016/j.ijbiomac.2016.11.042

Sigstad, E. E., Schabes, F. I., & Tejerina, F. (2013). A calorimetric analysis of soil treated with effective microorganisms. *Thermochimica Acta*, *569*, 139–143. https://doi.org/10.1016/j.tca.2013.07.007

Sipiczki, M., & Tap, R. M. (2016). Candida vulturna pro tempore sp. nov., a dimorphic yeast species related to the Candida haemulonis species complex isolated from flowers and clinical sample. International Journal of Systematic and Evolutionary Microbiology, 66(10), 4009–4015. https://doi.org/10.1099/ijsem.0.001302

Spadaro, D., & Droby, S. (2016). Unraveling the mechanisms used by antagonistic yeast to control postharvest pathogens on fruit. *Acta Horticulturae*, (1144), 63–70. https://doi.org/10.17660/ActaHortic.2016.1144.9

Spadaro, Davide, & Droby, S. (2016). Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends in Food Science & Technology*, *47*, 39–49. https://doi.org/10.1016/j.tifs.2015.11.003

van Uden, N., & Kolipinski, M. C. (1962). *Torulopsis haemulonii* nov. spec. a yeast from the Atlantic ocean. Antonie van Leeuwenhoek, 28(1), 78–80. https://doi.org/10.1007/BF02538724

Ventura-Aguilar, R. I., Bautista-Baños, S., Flores-García, G., & Zavaleta-Avejar, L. (2018). Impact of chitosan based edible coatings functionalized with natural compounds on *Colletotrichum fragariae* development and the quality of strawberries. Food Chemistry, 262, 142–149.

Wilfrido, Y. Y., Alfredo, V.-A. L., Alfredo, L.-G. O., Patricio, V.-E. G., Cristina, L.-V. I., & Eduardo, C.-T. S. (2016). Efectos de un compost enriquecido con microorganismos eficientes sobre la germinación de semillas recalcitrantes de *Artocarpus altilis* (Parkinson) Fosberg y Theobroma cacao L. Effects of enriched compost with efficient microorganisms on the germination of recalcitrant seeds of breadfruit (Parkinson) Fosberg and Theobroma cacao L. *Journal of the Selva Andina Biosphere*, *4*(2), 9.

Zazzerini, A., & Tosi, L. (1985). Antagonistic activity of fungi isolated from sclerotia of *Sclerotinia sclerotiorum*. Plant Pathology, 34(3), 415–421.

Zeballos Heredia, M. F. (2017). Caracterización de microorganismos de montaña (MM) en biofertilizantes artesanales. Escuale Agricola Panamericana, Honduras.

Zhou, Y., Li, W., Zeng, J., & Shao, Y. (2018). Mechanisms of action of the yeast Debaryomyces nepalensis for control of the pathogen Colletotrichum gloeosporioides in mango fruit. *Biological Control*, *123*, 111–119. https://doi.org/10.1016/j.biocontrol.2018.05.014