

Yeast Potential for the Biological Control of *Colletotrichum musae*

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Received: August 2, 2020

Accepted: September 2, 2020

Online Published: September 15, 2020

doi:10.5539/jas.v12n10p301

URL: <https://doi.org/10.5539/jas.v12n10p301>

This research was financed import by the Coordination for the Improvement of Higher Education Personnel (CAPES) with the Finance Code 001.

Abstract

One of the factors that cause the greatest loss of fruit in post-harvest are diseases, especially rotting such as anthracnose. Therefore, this work aimed to test the potential of the yeasts *Candida albicans*, *Pichia guilliermondii*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, *Cryptococcus laurentii* and *Zygoascus hellenicus* in the control of *Colletotrichum musae* in bananas in post-harvest period. To test the potential of these yeasts, the effect of volatile and non-volatile compounds, culture pairing and spore germination of the fungus *C. musae* *in vitro* was evaluated. In post-harvest fruits, the area below the mycelial growth curve (AACCM) and the area below the disease progress curve (ACPD) were evaluated. The yeasts *C. albicans*, *R. glutinis*, *S. cerevisiae* and *P. guilliermondii* produced volatile compounds with antifungal action, reducing the development of the fungus *in vitro*. The yeast *R. glutinis* was shown to be more efficient in reducing mycelial growth *in vitro* of the fungus through the production of non-volatile compounds. The yeasts *C. albicans* and *P. guilliermondii* showed the presence of an inhibition halo. All yeasts induced the germination of *C. musae* conidia and were not efficient in controlling anthracnose *in vivo*.

Keywords: antagonism, anthracnose, banana fruits, *in vitro*, *Rhodotorula glutinis*

1. Introduction

Banana (*Musa* spp.) is the most produced fruit in the world, reaching approximately 127.3 million tons in 2018 (FAO, 2020). Due to its great economic importance, to preserve the phytosanitary quality, chemical products are used in order to increase yields by improving the fruits quality.

Anthracnose caused by *Colletotrichum musae* is the disease that causes most post-harvest losses in banana (Maqbool et al., 2010; Alemu, 2014). When phytosanitary treatment is not carried out on the fruits, losses can reach up to 80% of the total production (Bill et al., 2014).

Infection caused by *C. musae* in field occurs in the initial stage of fruit development, remaining inactive until ripening (Costa & Erabadupitiya, 2005; Sivakumar & Bautista-Baños, 2014). Its control is an essential component in the post-harvest of fruits, and the main management strategies used through chemical control are responsible for favoring the selection of resistant breeds, causing damage to the environment and human health (Cruz et al., 2010).

The growing demand for chemical-free foods has influenced the research development with alternative control substances. Among the viable alternatives, biological control with the use of yeasts presents itself as a promising biocontrol agent, as they are present in the epiphytic and endophytic microbiota, competing with pathogens (Mello et al., 2011).

Yeasts are organisms that do not need special nutrients to proliferate quickly and do not produce metabolites that cause damage to human health (Ruiz-Moyano et al., 2016), have as their main mode of action competition for space and nutrients, production of antibiotics and / or parasitism (Machado & Bettioli, 2010).

According to Spadaro and Gullino (2004), the use of yeasts as biocontrol agents is due to the good characteristics presented by them, such as a good use of nutrients, multiplying rapidly, production of extracellular polysaccharides that increase their survival, reducing the space for pathogen expansion. Therefore, understanding the action mode of these antagonists is necessary for the development of formulations and methods of application that are appropriate and satisfactory for the pathogens control (Spadaro & Droby, 2016).

Therefore, this work aimed to evaluate the potential of yeast isolates in the control of *C. musae* *in vitro* and the effect on the anthracnose control in bananas in the post-harvest period.

2. Materials and Methods

The experimental design used was completely randomized (CRD) with seven treatments, six yeast isolates and the control (distilled water), with four replicates each.

The isolation of the pathogen was performed using the direct isolation method (Alfenas et al., 2016) of symptomatic banana fruits kept in Petri dishes containing Potato Dextrose Agar (PDA) culture medium. The tested yeasts were obtained from the collection belonging to the university, being: *Rhodotorula glutinis* (AH 14-3), *Pichia guilliermondii* (AH 16-2), *Zygoascus hellenicus* (AH 14-1), *Saccharomyces cerevisiae*, *Cryptococcus laurentii* (AH 04-1) and *Candida albicans* (AH 05-3), preserved in test tubes containing GYMP agar, covered with sterile mineral oil and kept in the refrigerator. They were grown in petri dishes containing solid Agar-YEPG medium for their maintenance.

2.1 Production of Volatile and Non-volatile Compounds by Yeast

The evaluation of the production of volatile and non-volatile compounds by yeasts followed the method proposed by Romeiro (2007). During the incubation period, the growth diameter of the fungus was measured daily by determining two diametrically opposed measures.

After determining the mean diameter of the mycelial growth, the mycelial growth curve was drawn up and the area below the mycelial growth curve (AACCM) was calculated with the aid of Equation 1, adapted from Shaner and Finney (1977).

$$AACCM = \sum_{i=1}^n \left[\frac{(Y_{i+1} + Y_i)}{2} \right] [X_{i+1} - X_i] \quad (1)$$

where, Y_i = is the severity of the mycelium (per unit) in each observation; X_i = is the time (days) of each observation; n = total number of observations.

2.2 Crop Matching Test

The direct confrontation test was performed using the methodology proposed by Romeiro (2007). The fungus growth was evaluated until the control growth reached the other edge of the plate. After this period, the inhibition zone was measured, which consists of the distance between the yeast and the fungal mycelium.

2.3 Germination of *Colletotrichum musae* Conidia Treated With Yeast Cells

In the germination test of pathogen conidia treated with yeast cells, optical microscope slides containing 1% agar-water medium were used, where 30 μ L of conidia suspension of the pathogen was deposited in the Neubauer chamber for 1×10^6 conidia mL^{-1} , subsequently, 30 μ L of cell suspension of each yeast was deposited with concentration adjusted in the Neubauer chamber for 1×10^8 cells mL^{-1} .

The slides were placed in a gerbox containing filter paper soaked in water at the bottom and kept at room temperature for 12 h, after which blue lactophenol cotton was added to stop the growth of germ tube and bluish the fungal structures. Counting up to 100 conidia in sequence under an optical microscope. Germinated conidia was considered to be one whose germinal tube was twice its size.

2.4 Banana Fruits Treated With Cells and Yeast Culture Filtrate

Organic fruits were used for *in vivo* testing, where they underwent asepsis with 70% alcohol and 1% sodium hypochlorite. Subsequently, they were washed in distilled water and packed in plastic gerbox lined with moistened filter paper. Each experimental plot consisted of a box containing two fruits.

In the treatment of fruits with yeast cells, a suspension of yeast cells containing 2 g of cell mass per liter of water was prepared, and for the treatment of fruits with yeast filtrate, the yeast filtrate was prepared using a 45 μ m

syringe filter. The suspension of cells and yeast filtrate was sprayed on the fruits and these were stored in BOD for 24 h, afterwards the fruits were stored on a shelf at room temperature.

The pathogen inoculation was carried out 24 h after the treatments application, with 100 μL of the inoculum in the concentration of 1×10^4 conidia mL^{-1} , inoculating in wounds previously performed in two points of the fruit with a needle.

The injured area was evaluated by measuring two perpendicular diameters performed every 24 h for 8 days. With the values, the disease progress curve was drawn up and the area below the disease progress curve (AACPD) was calculated, according to the methodology of Shaner and Finney (1977).

2.4 Statistical Analysis

The statistical analysis of the experiments was performed with the aid of the SISVAR software (Ferreira, 2014). Analysis of variance of the averages was performed and when significant, the averages were compared using the Tukey test ($p < 0.05\%$).

3. Results and Discussion

3.1 Production of Volatile and Non-volatile Compounds by Yeast and Conidia Germination

The statistical analysis demonstrated that there was a significant effect ($p < 0.05\%$) for the production tests of volatile and non-volatile compounds (Table 1).

Regarding the production of volatile compounds, the yeasts *C. albicans*, *R. glutinis*, *S. cerevisiae* and *P. guilliermondii* reduced the development of the fungus *in vitro* by 81.5, 72.5, 68.4 and 40.7%, respectively. The other yeasts did not differ from the control (Table 1), as they do not produce volatile compounds with antimicrobial characteristics capable of reducing or inhibiting the fungus growth under the experiment conditions.

Table 1. Area under the mycelial growth curve (AACCM) and percentage of growth inhibition due to the production of volatile compounds (CV) and non-volatile compounds (CNV), inhibition halo and non-germinated conidia for the fungus *Colletotrichum musae* submitted crop matching and conidia germination tests with different yeasts

Yeast	AACCM (CV)	AACCM (CNV)	Growth inhibition (%)		Inhibition halo (mm)	Non-germinated conidia
			CV	CNV		
<i>C. albicans</i>	45.06 a	509.56 b	81.76	7.91	42.25 a	2.50 b
<i>R. glutinis</i>	67.97 ab	81.64 a	72.49	85.25	23.75 ab	8.50 b
<i>S. cerevisiae</i>	78.18 ab	454.92 b	68.35	17.78	7.75 ab	4.00 b
<i>P. guilliermondii</i>	146.51 b	490.25 b	40.70	11.40	36.50 a	9.25 b
<i>Z. hellenicus</i>	243.91 c	485.15 b	1.27	12.32	1.50 ab	3.25 b
<i>C. laurentii</i>	261.75 c	503.77 b	-	8.95	19.00 a	3.50 b
Control	247.05 c	553.32 b	-	-	0.00	61.50 a
Mean	155.77	439.80	-	-	20.11	13.21
C.V.%	23.75	20.26	-	-	44.91	30.98

Note. C.V (coefficient of variation); Means followed by different letters in the column, differ by Tukey's test ($p < 0.05$).

França et al. (2015) evaluating the post-harvest control of anthracnose in peppers by the use of *R. glutinis* observed that this was more effective in controlling the disease when using yeast in post-harvest fruits, assuming that the success of this yeast in the anthracnose control in peppers may be due to the yeast's ability to produce enzymes such as pectinases, chitins and glucanases (Saravanakumar et al., 2009; Bauermaister et al., 2010), responsible for depolymerizing the cell wall of certain fungi, as well as competition for nutrients. According to Silva et al. (2014), among the treatments based on use of antagonists, *R. glutinis* also presented a satisfactory result in the control of the sweet rot of pepper.

Fialho et al. (2010) concluded that *S. cerevisiae* produces volatile organic antimicrobial compounds belonging mainly to the alcohol group, the presence of esters, ethyl acetate, ethyl octanoate, which act in reducing protein synthesis and that the enzymatic activity involved in morphogenesis triggers oxidative stress processes in fungi when exposed to these compounds. The use of *C. albicans* yeast has been little reported in the literature, probably because this genus is pathogenic to humans.

The yeast *R. glutinis* proved to be promising, producing non-volatile compounds capable of reducing the mycelial growth of *C. musae* *in vitro*. The other yeasts do not differ significantly from the control. França et al. (2015), found that the most efficient responses in the control of *Colletotrichum gloeosporioides* in pepper culture were from isolates belonging to the species *R. glutinis*, with growth inhibition up to 47.99%, being the control mechanisms competition for space and nutrients, production of antibiotics and also for parasitism.

Regarding the culture matching test, the yeasts *C. albicans* and *P. guilliermondii* showed the ability to inhibit the mycelial growth of *C. musae* *in vitro*. Lahlali et al. (2005) seeking to develop a biological control method against citrus post-harvest diseases, reported significant results of these yeasts in the control of *Penicillium italicum* and *Penicillium digitatum*.

For the germination of *C. musae* conidia, all yeasts induced the conidia germination, and the control treatment showed the least amount of conidia germinated, demonstrating that the compounds produced by the yeast cells did not interfere on the spore.

3.2 Yeasts Efficiency in the Control of Anthracnose in Fruits

There was no significant difference between treatments for fruits treated with yeast culture filtrate. For fruits treated with yeast cell suspension, *C. albicans* was shown to be inferior to the control, helping in the development of the disease (Table 2).

Table 2. Area Below the Disease Progress Curve (AACPD) in fruits treated with yeast culture filtrate and yeast cell suspension

Yeast	AACPD ^{NS} (Yeast filtrate)	AACPD (Cell suspension)	Variation (%) ¹
<i>C. albicans</i>	50.50	69.50 b	85.33
<i>R. glutinis</i>	68.00	54.00 ab	44.00
<i>S. cerevisiae</i>	68.25	56.00 ab	49.33
<i>P. guilliermondii</i>	59.25	44.00 ab	17.33
<i>Z. hellenicus</i>	66.50	43.75 ab	16.67
<i>C. laurentii</i>	80.50	42.50 ab	13.33
Control	51.50	37.50 a	0.00
Mean	63.93	49.61	-
C.V.%	28.25	23.35	-

Note. C.V. (coefficient of variation); ^{NS} not significant at 5% by Tukey test; Means followed by different letters in the column, differ by Tukey's test ($p < 0.05$). ¹ Variation (%) compared with the control treatment.

In this work, no yeast has shown promise in reducing anthracnose in banana fruits, which may be due to the injury caused artificially to inoculate the pathogen, however, in other studies these yeasts have shown a good result against several pathogens.

Hoffmann et al. (2012) found the severity of common bacterial blight to be approximately 8% lower in common bean plants treated with *S. cerevisiae*. Antonioli et al. (2011) also concluded that *S. cerevisiae* showed a potential for reducing post-harvest rot in raspberries, with a reduction of approximately 22.3% in the incidence of rot caused by *Botrytis cinerea*, in addition to not interfering in quality product, contradicting the results of this work.

Other studies using *R. glutinis* to control post-harvest diseases have shown satisfactory results, such as Zhang et al. (2009) who reported the effectiveness of *R. glutinis* in the control of *Botrytis cinerea* and *Penicillium expansum* in pear fruits, reducing the incidence of the disease in stored fruits by approximately 14.6%. Parello et al. (2002) using *R. glutinis* and *Cryptococcus* sp. also verified the efficacy of these yeasts in controlling *Septoria tritici* and *Bipolaris sorokiniana*.

The difference in the results *in vitro* and *in vivo* can be explained by the change of environment in the development of these microorganisms (Mari et al., 2012). The conditions of *in vitro* development do not reflect the conditions of development in the phylloplane of plants. The mechanical injury performed to inoculate the pathogen may have caused a much more favorable environment, facilitating the penetration and establishment of the pathogen in the fruits. Many antagonistic microorganisms are successful in a controlled environment, yet fail when subjected to adverse conditions (El-Ghaouth et al., 2000).

4. Conclusions

Among the tested yeasts, *C. albicans*, *R. glutinis*, *S. cerevisiae* and *P. guilliermondii*, produced volatile compounds with antifungal action, reducing the *in vitro* development of the fungus *C. musae*.

R. glutinis was more efficient in reducing the mycelial growth of *C. musae* through the production of non-volatile compounds.

C. albicans and *P. guilliermondii* presented an inhibition halo, inhibiting the mycelial growth of *C. musae in vitro*.

The yeasts induced the germination of *C. musae* conidia *in vitro* and were not efficient in controlling anthracnose *in vivo*.

Acknowledgements

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the State University of Western Paraná for their support in the development of the research.

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