

Evaluation of Yeast Potential in the Biological Control of *Colletotrichum orbiculare* in Cucumber

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Abstract

This work aimed to evaluate the effect of the yeasts *Candida albicans*, *Pichia guilliermondii*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* and *Zygoascus hellenicus* in the control of anthracnose in cucumbers. The influence of volatile and non-volatile compounds on mycelial growth and the effect of cell suspension and culture filtrate on conidial germination were evaluated. In a greenhouse, yeasts were tested on cucumber cotyledons, one cotyledon being treated and the other not; afterwards both cotyledons were challenged with *C. orbiculare*. The severity of the disease in both cotyledons was assessed by determining biological control and resistance induction. The production of volatile compounds from the yeasts *R. glutinis* and *C. albicans* reduced the pathogen growth by 90.7 and 90.0%, respectively. The production of non-volatile compounds stimulated the pathogen development. Conidia germination was affected when exposed to cell suspension of all tested isolates, ranging from 43 to 75%. For the culture filtrate from *Z. hellenicus*, it reduced the conidia germination by 11.4%. In the *in vivo* test, on the treated cotyledon, the yeasts *Z. hellenicus*, *R. glutinis* and *S. cerevisiae* reduced the anthracnose severity by 52.5, 50.0 and 42.5%, respectively.

Keywords: antagonism, anthracnose, *Cucumis sativus*, resistance induction

1. Introduction

Cucumber (*Cucumis sativus* L.) is an annual crop belonging to the Cucurbitaceae family of great economic expression; China has been the country with the largest cucumber production in the world, reaching approximately 56.3 million tons (Filgueira, 2008). The importance of its phytosanitary quality makes it necessary to use chemical products in order to increase yields by improving plant health (Trybom, 2010; FAO, 2018).

Anthracnose, caused by the fungus *Colletotrichum orbiculare*, is one of the main diseases that cause damage to the cucumber culture. The control of this disease is carried out with the use of fungicides. However, the use of these products has toxicity to the environment, residual effects and promotes the resistance of fungi to fungicides. Therefore, it is necessary to develop alternative management methods, such as biological control with the use of microorganisms and natural products with antimicrobial activity (Schwan-Estrada et al., 2003).

Therefore, the objective of this work was to evaluate the effect of the yeasts *Candida albicans*, *Pichia guilliermondii*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* and *Zygoascus hellenicus* on *Colletotrichum orbiculare* in the control of anthracnose in cucumber.

2. Materials and Methods

The *in vitro* experiment was conducted in a laboratory with a completely randomized design (CRD), containing six treatments (five yeasts and one control) with four replications. The *in vivo* experiment was conducted in a greenhouse with CRD containing six treatments (five yeasts and one control).

The pathogen *C. orbiculare* was obtained through the indirect isolation method (Alfenas et al., 2016). The tested yeasts *Candida albicans*, *Pichia guilliermondii*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* and *Zygoascus hellenicus*, were obtained from the collection belonging to the Phytopathology Laboratory of the University.

2.1 Production of Volatile and Non-volatile Antimicrobial 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 fungus growth diameter was measured daily by determining two diametrically opposite measures.

After determining the mean diameter, the mycelial growth curve was elaborated and the area below the mycelial growth curve (AACCM) adapted from Shaner and Finney (1977) (Equation 1).

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

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

2.2 Germination of *Colletotrichum orbiculare* Conidia Treated With Yeast Cells or Filtrate

To verify the effect on conidia germination, two tests were conducted using yeast cells or culture filtrate from the isolates. For this purpose, optical microscope slides containing 1% agar-water medium were used, where 30 μL of the pathogen's conidia suspension was deposited in the Neubauer chamber by 1×10^6 conidia mL^{-1} . For the first experiment, 30 μL of cells suspension was deposited for the first assay with concentration adjusted in the Neubauer chamber by 1×10^8 cells mL^{-1} , subsequently, for the second experiment, 30 μL of filtrate from isolates of *C. albicans*, *P. guilliermondii*, *R. glutinis*, *S. cerevisiae* and *Z. hellenicus*.

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 cotton wool of lactophenol was added to stop the growth of germ tube and paint the fungal structures. Counting up to 100 conidia in a light microscope in sequence. Germinated conidia was considered to be one whose germinal tube was twice its size.

2.3 Bioassay With Cucumber Plants

For the preparation of *in vivo* test, the cultivar *Pioneiro* hybrid was used, sown in a tray containing commercial substrate. The five yeast isolates were tested against the *C. orbiculare*. The treatments were applied to only one of the cotyledons of each plant, so that it was possible to observe the occurrence of resistance induction by yeast in the untreated cotyledon.

When the cotyledons were approximately 3 cm long, at the end of afternoon, yeast cells were suspended in a concentration of 1×10^8 cells mL^{-1} . After two days, the suspension containing the pathogen conidia was applied at 1×10^6 conidia mL^{-1} , on the surface of the cotyledons and kept in a humid chamber.

When cotyledons showed symptoms of the disease, they were collected separately, placed on a white surface next to a graduated ruler to be photographed. With the aid of the QUANT program (Vale et al., 2003), the healthy and sick leaf area of the cotyledons was quantified to determine the percentage of injured leaf area.

2.4 Statistical Analysis

The statistical analysis of *in vitro* and *in vivo* experiments were performed with the aid of the SISVAR software (Ferreira, 2011). Analysis of variance was performed and when significant the averages were compared using the Tukey test, at the level of 5% probability of error.

3. Results and Discussion

3.1 Production of Volatile and Non-volatile Antimicrobial Compounds by Yeasts and *in vitro* Germination of Conidia

Regarding the production of volatile antimicrobial compounds, the yeasts *R. glutinis* and *C. albicans* demonstrated greater efficiency in reducing the fungus growth, reducing by 90.70 and 90.01%, respectively, followed by *Z. hellenicus* and *S. cerevisiae* with reduction of fungus growth by 69.07 and 61.78%, respectively. On the other hand, the volatile compounds produced by *P. guilliermondii*, did not significantly interfere in the growth of *C. orbiculare* (Table 1).

Considering these results, it is possible to verify that these yeasts produced volatile compounds and have the potential to reduce the development of *C. orbiculare*, however, at differential levels, depending on the species, ranging from 61.78 to a reduction of 90.70%. Thus, these yeasts have the potential to control organisms of agronomic importance through the production of volatile organic compounds.

Table 1. Area under the mycelial growth curve (AACCM), number of spores present and percentage of growth inhibition for the fungus *Colletotrichum orbiculare* due to the production of volatile compounds (CV) and non-volatile compounds (CNV) from different yeasts

Yeasts	AACCM (CV)	AACCM (CNV)	Number of spores		Growth inhibition (%) ¹	
			CV	CNV ($\times 10^5$)	CV	CNV
<i>Rhodotorula glutinis</i>	3.36 a	28.50 ab	0	8.462 c	90.70	-35.71
<i>Candida albicans</i>	3.61 a	28.75 ab	0	0.156 a	90.01	-36.90
<i>Zygoascus hellenicus</i>	11.17 ab	32.50 ab	0	0.306 a	69.07	-54.76
<i>Saccharomyces cerevisiae</i>	13.21 ab	32.75 b	0	2.231 b	61.78	-55.95
<i>Pichia guilliermondii</i>	20.60 bc	34.50 b	0	0.256 a	42.99	-64.29
Control	36.13 c	21.00 a	0	0.075 a	-	-
Average	14.68	29.67	-	1.91	-	-
CV%	22.72	16.69	-	21.29	-	-

Note. CV: coefficient of variation. Averages followed by the same letter in the column do not differ statistically from each other by the Tukey test at the level of 5%. ¹Negative values indicate growth stimulus.

França et al. (2015), observed in a similar work that the yeast *R. glutinis* showed efficacy in the postharvest control of anthracnose in pepper, caused by *Colletotrichum* sp. According to Silva et al. (2014), among the treatments based on use of antagonists, the yeast *R. glutinis* also revealed a significant result in relation to the control of sweet rot of pepper. In studies on the production of volatile antimicrobial compounds, Fialho et al. (2010) found that *S. cerevisiae* also has the ability to produce volatile organic antimicrobial compounds, consisting mainly of alcohols.

For the test of non-volatile compounds the tested yeasts do not reduce the fungus development, on the contrary, they induced mycelial growth, this may have occurred because the yeast filtrate created a favorable environment for its development, possibly providing nutrients for the pathogen. It is possible to see that in addition to inducing the fungus growth, it was also induced to sporulation, in which *R. glutinis* had the largest amount of spores present, followed by *S. cerevisiae*, which presented spore production greater than the other yeasts and control.

Therefore, it can be determined that yeasts that produce volatile compounds are able to reduce the development of the pathogen *C. orbiculare* in *in vitro* tests, being considered as conducive to biological control.

The results regarding the conidia germination test treated with cells and yeast filtrate can be seen in Table 2.

Table 2. Percentage of germination and reduction in the germination of *Colletotrichum orbiculare* conidia submitted to spore germination tests with filtrate and cell suspension of different yeasts

Yeasts	Germination of conidia (%)		Reduction (%)	
	Cells	Filtered	Cells	Filtered
<i>Pichia guilliermondii</i>	33.75 ab	90.00 ab	62.91	4.51
<i>Zygoascus hellenicus</i>	39.75 b	83.50 a	56.32	11.41
<i>Saccharomyces cerevisiae</i>	34.75 ab	87.25 ab	61.81	7.43
<i>Rhodotorula glutinis</i>	22.75 a	93.25 b	75.00	1.06
<i>Candida albicans</i>	31.50 ab	88.75 ab	67.38	5.84
Control	91.00 c	94.25 b	-	-
Average	42.25	89.50	-	-
CV (%)	17.32	4.30	-	-

Note. CV: coefficient of variation. Averages followed by the same letter in the column do not differ statistically from each other by the Tukey test at the level of 5%.

The germination of *C. orbiculare* conidia was suppressed by 75.00% when they were in contact with *R. glutinis* cell suspension, being more efficient than *Z. hellenicus*, which reduced germination by 56.32%. This reduction may be related to the production of inhibitory compounds. Sansone et al. (2005) attributed the production of

rodotorulic acid to the ability to reduce the germination of *Botrytis cinerea* spores. In the same way, Sipiczki (2006) attributed to rodotorulic acid the ability of *R. glutinis* to reduce the conidia germination of *Penicillium expansum* and *B. cinerea*.

On the other hand, yeast filtrates did not significantly interfere in the conidia germination (Table 2), *Z. hellenicus* significantly reduced germination by 11.41% while the yeasts *S. cerevisiae*, *C. albicans* and *P. guilliermondii*, did not reduce germination.

Considering the effective reduction of germination by cell suspension, but not being expressive with culture filtrate, it can be inferred that it is rodotorulic acid or another compound that interferes with germination, its synthesis is only stimulated in the presence of the competitor, in this case, *C. orbiculare*, making the use of microorganism itself more interesting than its filtrate as an alternative product.

Thus, it is possible to affirm that those yeasts that presented potential for reduction in mycelial growth by the production of volatile and non-volatile antimicrobial compounds also interfering in the germination of *C. orbiculare* conidia can be considered as suitable for use in biological control.

3.2 Effect of Yeasts on the Protection of Cucumber Plants From Anthracnose

Cotyledons treated with cell suspension from yeasts *Z. hellenicus*, *R. glutinis* and *S. cerevisiae* reduced the severity by 52.5, 50.0 and 42.5%, respectively. However, in cotyledons without treatment, there was no difference (Figure 1). Since there is no significant difference in untreated cotyledons, it is possible to state that there was no systemic resistance induction in the plant, that is, yeasts applied to cotyledons did not activate defense mechanisms in a systemic way, therefore, they did not send signals to the cotyledon without treatment, showing that these yeasts act basically through direct biological control against the pathogen.

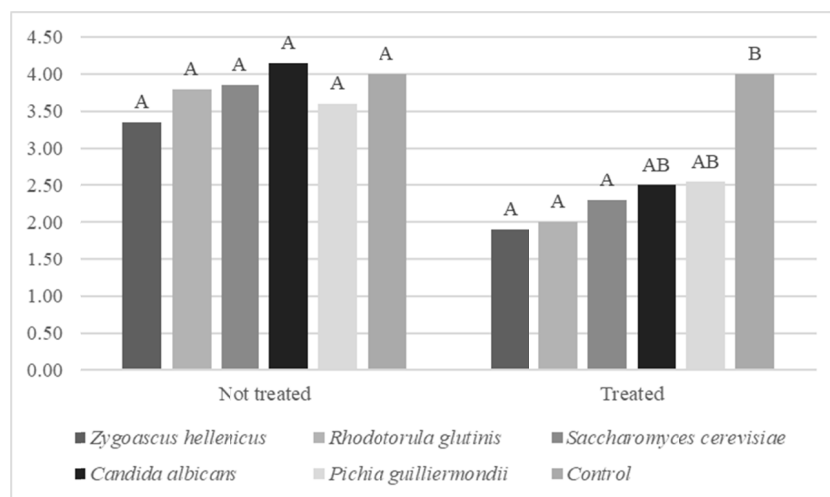


Figure 1. Severity of anthracnose caused by *Colletotrichum orbiculare* in cucumber cotyledons treated or not with yeast cell suspension

Note. Averages followed by the same letter do not differ statistically from each other by the Tukey test at the level of 5%.

Studies carried out using the yeast *R. glutinis* reported significant results, such as the effectiveness of this yeast in controlling *B. cinerea* and *P. expansum* in apple fruits, where the results showed that the higher the concentrations of the antagonist, the lower the incidence of diseases (Zhang et al., 2009) and reduction of the fungus *B. cinerea* that causes gray mold in strawberry culture, when the fruits were stored at a temperature of 4 °C, the reduction in the incidence of the disease was approximately 80% (Zhang et al., 2010).

Among treatments based on the use of antagonists, Silva et al. (2014) demonstrated that *R. glutinis* revealed significant results in relation to the control of soft rot of peppers, reducing the severity of the disease by 82.55%, indicating that it is a possible alternative in the post-harvest management of this disease.

In evaluating the effects of *S. cerevisiae* on phytopathogens and on the manifestation of symptoms in different plant-pathogen interactions, under field conditions, some researchers showed results in which *S. cerevisiae* when

applied weekly, reduced the speed of disease progression, in magnitude similar to the treatment with fungicide, causing an increase in productivity, generating data similar to the treatment with fungicide (Piccinin et al., 2005). Zanardo et al. (2010), in studies on the resistance of cucumber seedlings to *C. orbiculare* induced by fractions of *S. cerevisiae* extract concluded that this yeast contains fractions inducing resistance to this disease, where cotyledons treated with crude extracts of this yeast autoclaved by 4 or 10 h showed a significant reduction in the severity of the disease, with 86.0 and 82.2%, respectively.

4. Conclusion

The use of filtrate for testing non-volatile compounds stimulated the growth and sporulation of the pathogen *in vitro*.

Among all treatments, the yeast *R. glutinis* has the ability to reduce the growth of *C. orbiculare* both *in vitro* and *in vivo* under greenhouse conditions.

The yeasts used did not have the capacity to induce systemic resistance in the plant under greenhouse conditions.

For local protection, the yeasts *Z. hellenicus*, *R. glutinis* and *S. cerevisiae* proved to be efficient in reducing the disease caused by *C. orbiculare* under greenhouse conditions.

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