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Potential of endophytic yeasts as biocontrol of *Phytophthora capsici*, causative agent of foot rot disease on black pepper

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Abstract. *Safitri D, Wiyono S, Purbajanti ED. 2023. Potential of endophytic yeasts as biocontrol of* Phytophthora capsici, *causative agent of foot rot disease on black pepper. Biodiversitas 24: 5847-5853. Phytophthora capsici* is the main pathogen of foot rot disease of black pepper. This disease contributes to a significant decrease in black pepper productivity. Conventional disease management strategies usually require the use of chemical pesticides, which can have harmful environmental and human health consequences. Introducing biological agents such as endophytic yeasts for controlling foot rot disease has minimal effect on human health and is sustainable. One technique to control foot rot disease is to use biological agents such as endophytic yeasts. The aim of this research was to find yeast against *Phytophthora capsici*. The methods used included (i) study development and identification of endophytic yeast and (ii) in vitro and in vivo antagonism screening of yeast against *P. capsici*. The results showed that 49 endophytic yeasts were isolated from the leaves, fruits and roots of three black pepper lines. Five isolates had selected based on the highest antibiosis in antagonism test by in vitro: END6, END21, END66, END87, and END14. Isolate END87 had a mycelium inhibition rate of 64.55%, and a low level of inhibition was found in isolate 21, which had a mycelium size of 42.81%. The molecular characterization based on the sequence partial of NL1 and NL4. Moreover, 5 isolates of endophytic yeast (END6, END21, END66, END87, and END14) were identified as *Rhodotorula mucilaginosa*, *Hannaella oryzae*, *Cryptococcus tephrensis*.

Keywords: Antibiosis, biocontrol, black pepper, endophytic yeast, foot rot disease, molecular identification

INTRODUCTION

Foot rot disease caused by *Phytophthora capsici* is the most important disease in black pepper (*Piper nigrum* L.). Although it infects many parts of black pepper plants, basal stem infection may have the most serious effect on productivity (Vandana et al. 2014). This disease is widespread in many black pepper production area in Indonesia and has caused yield losses up to 40%. This disease also become significant obstacle in black pepper-producing countries such as India, Malaysia, and Brazil. This fungal ability to survive in the soil as saprophytic fungi is one of the difficulties in controlling this disease (Anandaraj et al. 2020). Several control techniques such as the use of resistant varieties or fungicides, have been adopted, but have not been effective in controlling foot rot disease. In the other hand, it needs a very long time to provide the resistant black pepper varieties (Vandana et al. 2014), the use of synthetic chemical fungicides has caused another problems, especially negative effect on the environment. Indonesia has a very high diversity of microbes and many of them have the potential as biocontrol agents such as endophytic yeast (Into et al. 2020). Yeast as unicellular fungi with rapid growing period and many antagonistic mechanisms has not been studied massively in Indonesia. For example, *Aureobasidium pullulans* have been studied to control chili disease (Hartati et al. 2014), and other research is still limited to postharvest disease. One of the critical points of the ability of yeast to control plant disease is the adaptation to the host plants, so the use of yeast from the same family of plants needs to be explored comprehensively. In this research, we isolated endophytic yeast from Piperaceae plants and characterized its antagonistic mechanisms to find the most effective and efficient strategy in controlling foot rot disease.

Endosymbiotic yeast endophytes reside in plant tissues without causing any harm to the host. Yeast endophytes are abundant in plant tissues. Endophytic yeast mostly belong to *Rhodotorula*, *Pichia*, *Candida*, and *Debaryomyces* genera. They are generally found in the stomata and xylem. These species can promote growth and aid host plants in suppressing diseases caused by active metabolites, both directly and indirectly. Because of these advantages, yeast has potential for the suppression of *Phytophthora capsici* foot rot diseases. More studies are required to completely understand the mechanisms affecting yeast's resistance to these diseases. The resistance caused the yeast to have a mechanism against *Phytophthora capsici*. Doty et al. (2013) reported that endophytic for host plants Yeasts provide nutrients to plants that both host and provide biotic relief from tension. These benefits are due to the presence of phytohormones, secondary metabolites, nutrients, and the production of elicitin, all of which are beneficial to plants.

It is important to understand the modes of action of biocontrol agents in the control of foot rot. Antibiosis, production of VOCs, hyperparasitism, competition space and nutrition, the ability to create 1,3-glucanase enzymes

and induce resistance are the key modes of action of yeast. *Aureobasidium pullulans* produces extracellular exochitinase and -1-3-glucanase, as well as synthesize volatile chemicals (VOCs) (Hartati et al. 2014). The production of *volatile organic compounds* (VOCs) was assessed as one of *Aureobasidium pullulans* strains L1 and L8, which are effective against certain fruit postharvest pathogens, as part of their mechanisms of action against five pathogens (*Penicillium expansum*, *P. digitatum*, *P. italicum*, and *Botrytis cinerea*). Both L1 and L8 strains are capable of producing *volatile organic compounds* (VOCs) such as 2-phenyl, 1-butanol-3-methyl, 1-butanol-2-methyl, and 1-propanol-2-methyl. These compounds belong to the alcohol group and were the primary products of both strains. (Choińska et al, 2020).The purpose of this study was to find out the mode of action of yeast endophytes in suppressing *Phytophthora capsici* causing foot rot in black pepper.

MATERIALS AND METHODS

The research was conducted at the Plant Mycology Laboratory and IPB experimental field at the Dramaga Campus of IPB, Indonesia, from September 2018 to January 2020.

Isolation and identification of *Phytophthora capsici*

Phytophthora capsici was isolated according to the method of Anandaraj et al. (2020). Black pepper leaves with foot rot symptoms were cut in 0.5 x 0.5 cm, then surface-sterilized by NaOCl 1% and alcohol 70%. The sterilized piece were inoculated in corn meal agar (CMA) media and incubated at room temperature for 48 hours. The mycelial growth in media were then re-cultured in fresh CMA media and preserved for further study. Microscopic and molecular analysis were used to identify the fungi. The For microscopic observation, a mycelium of fungus was placed on top of the glass slide. A single drop of lactophenol cotton blue solution was uniformly dispersed, subsequent placement of cover slip. The identification key used for the microscopic diagnosis of *Phytophthora* infection was based on Erwin et al. (1996) entitled *Phytophthora* Diseases Worldwide. *Phytophthora capsici* isolate was then identified by *polymerase chain reaction* (PCR) using ITS1 and ITS 4primer. Electrophoresis process was carried out on 1.5% w/v agarose gel (TopVision, Thermo) with 1000 bp GeneRuler marker and stained with ethidium bromide. PCR products were shipped to (DNA sequencing company). The nucleotide sequences were compared with other NCBI GenBank sequences using the Basic Local Alignment Search Tool (BLAST).

Exploration of yeast endophytes from the plants of Piperaceae family

Yeast samples were taken from Balumbang Jaya Village, Bogor Regency and Sukamulya Village, Sukabumi Regency, West Java. Leaves, stem, and fruits of healthy plants of three lines of black pepper were selected for the experiment. The endophytic yeast was isolated as per the methods of Chetia et al. (2019). 10 g of leaves, stems, and fruit from were surface sterilized with distilled water. The sample was subsequently put into 70% alcohol and rinsed three times with distilled water, then shaken for 1 hour rotary shaker for 15 minutes at 120 rpm. The obtained yeast suspension was serially diluted to 10^{-5}, subsequently grown on yeast glucose chloramphenicol agar (YGCA) medium, and incubated at 23-30°C. Pathogenicity test of yeast was performed according to (Nishad and Ahmed 2020) using tobacco leaves. Endophytic yeast isolates were grown on potato dextrose broth (PDB) then incubated using rotary shaker 120 rpm for 48 hours. The incubated yeast suspension was then injected using a 1 mL syringe on tobacco leaves. Only the yeast isolates did not show necrotic symptoms on tobacco leaves were used in further research.

in vitro screening of endophytic yeast as biocontrol agents

Hyperparasitism

48-hour-old yeast was inoculated around water agar (WA) blocks at a 0.5 cm distance from the colony of *P. capsici* and incubated for five days. The yeast affinity in colonizing *P. capsici* mycelium was classified as follows: (i) no affinity; (ii) weak affinity (≤10 cells per hypha); (iii) moderate affinity (10-50 cells per hyphae); and (iv) strong affinity (>50 cells per hyphae) (Köhl et al. 2019).

Antibiosis

For antibiosis performance, yeast isolates were inoculated in the center of PDA media and then *P. capsici* isolates were placed on the left and right side (±2.5 cm) from yeast isolates then incubated for 48 hrs. For control, *P. capsici* isolates were inoculated in PDA media without yeast isolates. Antibiosis performance was determined by measuring relative inhibition by formula as follows: THR = (R2-R1)/R2 x 100% (R2 = the radius of *P. capsici* away from yeast colony; R1 = the radius of *P. capsici* approaching yeast colony) (Perez et al. 2016).

Production of volatile compounds

The volatile compounds produced by yeast were tested using the dual culture method with separate petri dish, which followed the (Choińska et al. 2020) methods. In the center of medium, 0.5 cm specimen of *Phytophthora capsici* was inserted, and yeast was scratched from the entire surface on the YMA medium. Both the yeast and *Phytophthora capsici* were further identified. Each isolate of yeast and *P. capsici* without touch (separated) by isolating yeast on top and *P. capsici* on the bottom, and incubated at room temperature for 48 hrs.

Production of β-glucan

The yeast antagonistic isolate was streaked on glucan medium perpendicularly and incubated at room temperature for 3-5 days. After the incubation, a clear zone formed around the yeast indicates that yeast isolates was the ability to produce β-glucan for degrading the fungal cell wall (Dewi et al. 2016).

Test of endophytic yeast against *Phytophthora capsici* in black pepper seeds *in vivo*

Preparation of yeast isolates and planting media

Selected yeast isolates were cultured on PDB media and incubated at 150 rpm at room temperature for 72 hours. Black pepper seeds were planted using sterile soil media added with compost in a ratio of 2:1. Two-months-old black pepper seedlings were selected for the test. The treatments used were: application of endophytic yeast, fungicide, and control without application of yeast and fungicides, with two replications and ten sub-units of black pepper seeds in each replication. Endophytic yeast inoculation was performed by injecting 10 mL of yeast suspension (density 10^7 cfu mL^{-1}) in the roots of black pepper plant seedlings. Furthermore, fungicide application was carried out by watering the roots according to the recommended dosage. Ten days after applying endophytic yeasts and five days before the application of fungicides, suspension of *P. capsici* (7-day-old) was inoculated with a density of 1.1 $x10^5$ spores ml^{-1} on the roots (Volynchikova and Kim 2022).

The observed variables for disease progression were latent period (LP), disease incidence (DI), disease severity (DS), infection rate (r), area under the disease progression curve (AUDPC). The latency period was calculated from the inoculation of pathogen until the appearance of first symptoms. Disease incidence was calculated using the formula: DI = n/N x 100%, where n = number of infected plants and N = number of plants observed. Measurement of disease severity was carried out according to Salgadoe et al. (2018), with the formula DS= (∑_1^3 〖ni.vi)/(N.V) x100% where, ni = number of infected plants on the i-th score, vi = the i-th score, N = the number of plants observed, and V = the highest score found in the scoring reference.

The calculation of disease incidence, disease suppression, disease severity, and disease progression was observed one day after inoculation of *P. capsici* by observing the leaf spot symptoms in each treatment. The scoring of leaf spot symptoms was predicted based on the method of Salgadoe et al. (2018) with some modification (Table 1).

Identification and characterization of endophytic yeasts

Selected yeast isolates were identified based on macroscopic characterization, including colony size, color, and molecularly using specific primers NL1 and NL4. The initial stage of PCR was yeast DNA extraction. The DNA extraction process involved subjecting the sample to a temperature of 90°C using a heat block, as described by Silva et al. (2012). The yeast cells were suspended in distilled water, subjected to a temperature of 95ºC for a duration of 15 minutes, and subsequently placed in a freezer at a temperature of 75°C. The next step was DNA duplication with a PCR machine using general primers NL 1 and NL 4. A total of 1 µL of DNA solution was amplified with a reaction volume of 25 µL consisting of 12.5 µL master mix, 1 µL forward primer NL 1 (5'- GCA TAT CAA TAA GCG GAG GAA AAG - 3'), 1 µL reverse primer NL 4 (5'- GGT CCG TGT TCA AGA CGG - 3') and 9.5 µL NFW. The initial denaturation cycle was carried out at 95°C for 90 seconds, denaturation at 95°C for 30 seconds, annealing 55°C for 30 seconds and extension at 72°C for 90 seconds. Steps two to four were repeated for 30 cycles and a final extension at 72°C for 3 minutes and 4°C for 10 minutes. Electrophoresis was carried out on 1.5% w/v agarose gel (TopVision, Thermo) with 100 bp GeneRuler markers and stained with ethidium bromide. PCR products were sent to First Base for DNA sequencing. Nucleotide sequences were compared to nucleotide sequences in the NCBI GenBank database, using nucleotide BLAST (Nasanit et al. 2015).

Experiment design and data analysis

In vitro and in planta tests were conducted using a completely randomized design with ten replications and ten sub-units. Tables and graphs were processed by MS Office Excel 2010. The effect of in vitro test treatment, *in planta* disease progression, and significant treatment were further tested by Student-Newman Keul (SNK) test at α = 5% using SAS 9.1 software.

RESULTS AND DISCUSSION

Exploration and pathogenicity test of endophytic yeast

The results showed that a total of 49 yeast isolates were isolated from three lines of black pepper plants. 16 yeast isolates were obtained from the leaves of *Piper betle* plant, while no yeast was isolated from the fruits and stems. Furthermore, 14, 2, 12 isolates were isolated from the leaves, fruit and stem of *Piper nigrum*, respectively. Also, one isolate was isolated from leaves and four isolates from the stem of *Piper ornatum* (Table 2).

Table 1. Scoring of *Phytophthora capsici* leaf spot symptom on black pepper leaves

Score	Symptoms
0	Healthy plants, fresh green leaves
1	Most of the leaves turned yellow and wilt
2	Leaves remained green, but most of the leaves appeared wilted
3	Plants died, the stem base turned black

Table 2. Endophytic yeasts isolated from *Piper nigrum*, *P. retrofractum*, *P. ornatum*

Plant origin	Number of endophytic yeasts		
	Leave	Fruit	Stem
Piper nigrum	14	2	12
Piper betle	16	0	0
Piper ornatum	1	0	4
No. of endophytic yeast	31	2	16

It was observed that population and diversity of endophytic yeasts of the Piperaceae plant were mostly found in the leaves rather than on the stem and fruit. This may be because leaves are a desirable habitat for yeast

growth and contain source of nutrition for yeast development. Following Petkova et al. (2022), yeast is known as endophytic microorganisms that inhabit plant tissue surface, but yeast can also enter plant tissue through stomata, hydathodes, mechanical wounds, cuticles, and epidermis. Also, some yeasts have a lipolytic and pectinolytic activity to enter the plant tissue through the cuticle.

The five yeast isolates were tested for their pathogenicity on blood agar tests, tobacco plants, and black pepper leaves (Table 3). The five yeast isolates that had potential against *P. capsici* were not pathogenic in humans with no clear blood agar zone. Besides that, the test of the ability of the five yeasts on black pepper leaves did not have symptoms of necrosis in black pepper, indicating that the five yeast isolates were not pathogenic in black pepper, besides that in the hypersensitive reaction test and hemolysis test, there were no endophytic yeast isolates that showed symptoms of necrosis. Therefore, most of the yeast isolates obtained were not pathogenic to plants and humans (Table 3).

Mechanism of biocontrol of endophytic yeast against foot rot stem

The research results on inhibition, volatile, and yeast endophytic ability test against *P. capsici* on black pepper plants showed different results in each treatment. END6 and END66 isolate had an inhibitory ability of 13.09 mm and 17.49 mm on PDA media than the control treatment. *P. capsici* isolates can grow well in Petri dish dishes up to 7 days after inoculation. However, inhibition test treatment using Potato Dextrose Broth (PDB) exhibited different results. The suspension of the five isolates grown on PDB media was able to significantly inhibit *P. capsici*, whereas treatment with endophytic yeast inoculated was able to inhibit the growth of *P. capsici*. Furthermore, the treatment with yeast inoculated mycelium wet weight growth ranged from 0.14 g (isolate END87) to 0.16 g (isolate END6) which was different from the control treatment. In control, comparatively significant increase i.e., 6.35 g in biomass of *P. capsici* was observed (Table 4).

Based on the study results, five endophytic yeast isolates had significantly different *P. capsici* mycelium inhibition levels in the control treatment. The result was significantly different from PDA medium grown by endophytic yeast and inoculated by *P. capsici*. This treatment inhibited low mycelium growth and showed that the isolate could produce high volatile compounds.

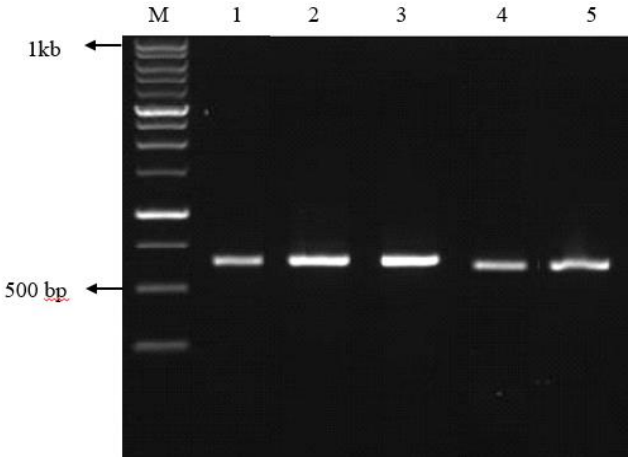

Figure 1. Visualization of isolated yeast total DNA on agarose gel 1.5%. M: Marker 1 kb, 1-5: samples of isolated yeast antagonism in black pepper leaves

Table 3. Screening for endophytic yeasts

Tests	No. of yeast isolates with a positive reaction	No. of yeast isolates with negative reactions
Hypersensitivity	2	47
Hemolysis	1	46
Phytopathogenicity	0	46

Table 4. Biocontrol-related characters of antagonistic yeast in inhibiting *P. capsici*

Isolates code	Antibiosis inhibition zone on PDA media (mm)[a]	Decrease of biomass mycelium *P. capsici* on PDB media (g)[a]	Relative inhibition rate of volatile compound (%)[a]	Hyperparasitism (No. of affinities yeast to *P. capsici* (colony of yeast)	β-glucanase production on glucan media (mm)[a]
Control	0.00b	6.35a	0.00d	0	90
END6	13.09a	0.16b	31.62c	>50	90
END21	0.00b	0.18b	42.81a	>50	-
END66	17.49a	0.16b	20.12c	>50	-
END87	0.00b	0.14b	24.24c	>50	80
END14	0.00b	0.18b	35.64b	>50	-

Note: [a]Mean with the same letter is not significantly different on SNK (Student-Newman-Keul) α=5%, [b]RIR: the relative resistance levels

Morphological and molecular characteristics of endophytic yeasts

The five endophytic yeast isolates were isolated from black pepper, namely END 6, END21, END66, END87, and END14. The five endophytic yeast isolates had multipolar type of budding and oval cell shape with single and paired cell arrangements shown in Figure 1. During the morphological observation, it was recorded that hyphae were not formed. Isolates END21, END87, END14 and END6 had cell sizes ranging from 3.00-4.70 µm x 2.32-4.41 µm. Besides, there were three yeast isolates, namely END21, END87, END14 isolates derived from different plants having various size variations ranging from 2.20-5.40-4.23-11.10 µm (Table 5).

The morphological characteristics of antagonistic yeast cells by microscopic observation showed that the five endophytic yeast isolates had around to cylindrical shape. Microscopic observations showed that yeast isolates END6 were round in shape and had spherical buds, colony of these isolates was light orange, and colony was round convex, besides the surface of two isolates was smooth and shiny. In the present study, three endophytic yeast isolates had either elliptical or oval shape, namely END21, END87, and END14. The yeast surface exhibited a polished and lustrous appearance, along with a raised convex contour. In contrast, the colony of *Hannaella oryza* colony was cream in color and medium in size (Table 5).

The sequence length of five yeast isolates ranged between 600 and 650 bp, based on identification using specific primers NL1 and NL4. Based on DNA sequencing studies, three isolates, END 14, END 21, and END 87, were shown to be 99% identical to *Hannaella oryzae*. Furthermore, the END 6 isolate was 96.45% similar to *Rhodotorula mucilaginosa*, whereas the END 66 isolation was 94.75% similar to *Cryptococcus tephrensis*. Based on the data in Table 6, the endophytic yeasts identified by molecular species identification were Cryptococcus tephrensis (END66), *H. oryzae* (END87), *H. oryzae* (END14), and *Rhodotorula mucilaginosa* (END6). *H. oryzae* (END21) and *Cryptococcus tephrensis* (END66) isolates were supplied by the user. The yeast endophytes generated in this study show nucleotide sequence similarity between 91.85% and 98.15%; the associated gene bank accession codes are KM891575.1 (END66), KM246122.1 (END87), JQ754139.1 (END14), KY109137.1 (END6), and JQ754139.1 (END21).

Effectiveness of antagonistic yeast against *P. capsici* on black pepper seeds *in vivo*

The results showed that black pepper plants treated with antagonistic yeasts had potential biological control agents against basal stem rot disease in black pepper seedlings. It was demonstrated by the more extended incubation period, disease incidence, and low severity of each of the yeast antagonists tested (Table 7).

Table 5. Measurements of biocontrol isolates of yeast endophytic

Isolates code	Plant part	Cell size (µm)	
		Length	Width
END6	*Piper betel* leaf	3.00-4.70	2.32-4.31
END21	*Piper nigrum* leaf	2.20-5.40	4.23-11.10
END66	*Piper nigrum* leaf	2.13-5.18	4.11-11.00
END87	*Piper nigrum* stem	4.21-6.25	3.34-6.51
END14	*Piper nigrum* leaf	2.01-5.19	4.20-11.06

Table 6. The results of molecular identification of biocontrol yeast endophytic isolates

Isolates code	Accession numbers	Source of isolate	Homology (%)	Query length (bp)	Species
END6	KY109137.1	Food	96.45	613	*Rhodotorula mucilaginosa*
END21	JQ754139.1	Endophytic in *Phaseolus vulgaris*	91.85	655	*Hannaella oryzae*
END66	KM891575.1	Floral nectar in *Delphinium tenii*	94.75	651	*Cryptococcus tephrensis*
END87	KM246122.1	Endophytic in *Catuaí Vermelho*	98.15	656	*H. oryzae*
END14	JQ754139.1	Endophytic in *Phaseolus vulgaris*	92.13	651	*H. oryzae*

Table 7. The ability to control yeast antagonists against *P. capsici* based on in planta tests on black pepper

Isolates code	Latent period (day)a	Disease incidence (%)a	Disease severity (%)a	Infection rate (%)a	AUDPC (unit)a	Biocontrol effectiveness (%)
Control	3.1d	100.0a	100.0a	0.8b	468.7a	0.00
END6	4.2abc	100.0a	43.3cd	1.1a	128.7b	56.7
END14	3.8c	60.0ab	36.7de	0.8b	142.0c	63.3
END21	4.0bc	50.0bc	20.0f	1.1a	68.7d	80.0
END66	4.0bc	60.0bc	26.7ef	0.5c	62.0d	73.3
END87	4.6a	20.0c	10.0f	0.7bc	48.7e	90.7
Fungisida	4.2abc	30.0c	10.0f	0.1e	42.0e	90.0

Note: [a] Mean with the same letter is not significantly different on SNK (Student-Newman-Keul) α=5%

The disease incubation period in treatment inoculated with the antagonist yeast required a slower time than the control. The antagonistic yeast treatment showed foot rot symptoms on the leaves on days 4 to 5, while the control treatment showed foot rot symptoms on all black pepper seeds from day 2. The longer the incubation period of the disease, the more resistant the plant was, and vice versa. At the end of observation, the it was observed that the control treatment had the highest disease incidence (100%). The treatment of yeast antagonists had the potential to reduce the incidence of basal stem rot between 20-100% in line with the low infection rates of yeast isolates END21 and END87 ranging from 0.3-1.1%, and AUDPC values ranging from 48.7-142.0 units. The higher the infection rate and AUDPC values, the higher the severity of disease progression in the treatment. The variable of isolates END21 and END87 showed better basal stem rot disease development suppression than other isolates.

Discussion

Endophytic yeast isolates obtained 49 yeast isolates from the Piperaceae family, isolated from leaf, stem, and fruit tissue. Most endophytic yeasts were isolated from the leaves. It is assumed that the leaves contain nutrients that endophytic yeasts prefer. Inácio et al. (2010) reported that the formation of yeast population and diversity in leaves was influenced by microclimate conditions, plant age, leaf morphology, and the number of nutrients. The exploration carried out in this study used three types of plants in the Piperaceae family: *Piper nigrum*, *Piper betle*, and *Piper ornatum*. Differences in the types of plant species affect the type of endophytic yeasts obtained, and differences in plant tissue also affect the type of yeast obtained. This is consistent with Di Francesco et al. (2015), who reported that *A. pullulans* L1 and L8 yeast population tended to decrease at the beginning of application. The population could last up to 10 days on tomato leaves.

The suppression of *P. capsici* mycelium growth caused by antifungal compounds produced by antagonistic yeasts is one of the mechanisms that can inhibit *P. capsici* growth. *P. capsici* mycelium inhibition by yeast antagonists in this study showed several inhibitory mechanisms and produce volatile compounds. Also, the antibiotic properties shown by isolates END21 and END87 were characterized by the formation of an inhibition zone between the antagonistic yeast and *P. capsici*. The inhibition of *P. capsici* by antagonistic yeasts is thought to be due to endophytic yeasts producing antimicrobial compounds that cause inhibition of mycelium growth by forming clear zones (inhibition zones). Zhimo et al. (2016) reported that *Candida tropicalis* YZ 1 (CtYZ 1), *Saccharomyces cerevisiae* YZ 7 (ScYZ 7) and *C. tropicalis* YZ 27 (CtYZ 27) reduced the germination of *Colletotrichum musae, C. capsici, C. gloeosporioides, Aspergillus niger*, *Fusarium oxysporum* conidia at 48 hours by 75.8% compared to control. Li et al. (2016) reported that the interaction antagonism between *Rh. Glutinis* against *Botrytis cinerea* occurs through an antibiotic mechanism that begins with yeast cells' attachment to the fungal hyphae *B. cinerea*.

This study, indicates a mechanism of endophytic yeast hyperparasitism against *P. capsici* mycelium. Yeast colonizes hyphal stems and branches between hyphae. Yeast colonization causes *P. capsici* cells to lysis at several sites. The attachment of yeast to the pathogenic structure is closely related to its biocontrol ability. The destruction of hyphae cells is strongly suspected to be related to the lysis mechanism. When there is contact between yeast cells and *P. capsici*, the cell wall degrading enzymes of the endophytic yeast will stick to the cell walls of *P. capsici*. The attachment of endophytic yeast to the cell wall of *P. capsici* cause damaged to the cell wall Lima et al. (2013) stated that yeast *Wickerhamomyces anomalus* (strain422) and *Meyerozyma guillier-mondii(strain443)* had a higher affinity when tested on *Colletotrichum gloeosporioides*. Another mechanism possessed by the antagonist yeasts was the lysis ability to produce the glucanase enzyme. Two endophytic yeast isolates (END6 and END66) had the potential to produce enzymes β 1.3 glucanase. It was suspected that antagonistic yeast isolates are able to produce cell wall degrading enzymes. Ueki et al. (2020) reported that yeast can produce β 1.3 glucanase enzyme, degrading hyphae's cell walls. The yeast *Rhodotorula glutinis* was reported to have strong hyperparasitic abilities against the conidia of *Botrytis* (Li et al. 2016).

The use of antagonistic yeast isolates to black pepper's growth substrate significantly reduced the severity of foot rot disease by 56.7-90.7%. The foot rot disease severity reached 10.00-43.0%, significantly different from the positive control where the disease severity reached 100%. Yeast antagonists inhibit the growth of *P. capsici* mycelium as they have antibiotic potential, produce VOC compounds, produce β 1.3 glucanase enzymes, and hyperparasitism, besides that it plays a role in inducing plant resistance to produce plant-derived enzymes as a form of plant defense, such as peroxidase and total phenol. Wachowska and Głowacka (2014), reported that yeast *Aureobasidium pullulans* were able to show 30% disease suppression against wilt in wheat caused by *Fusarium culmorum.* Lopes et al. (2015) reported that *S. cerevisiae* isolates exhibiting antifungal activity against *Colletotrichum acutatum* secreted exoglucanases, as did *Pichia guilliermondi* biocontrol isolate.

Five different types of endophytic yeasts, namely END6, END21, END66, END87, and END14 were tested to see if they could fight each other, produce volatiles, and inhibit the growth of *P. capsici in vitro*. The low level of inhibition was found in END21 isolate, which had mycelium size of 42.81%, and yeast and *Phytophthora capsici* were used for an *ad planta* test on black pepper leaves. Isolates END6 and END21 have the potential to decrease the incidence of diseases. Various tests revealed that five yeast isolates might have the potential to be used as biocontrol agents against *P. capsici* foot rot. The five antagonistic yeast isolates were *Rhodotorula mucilaginosa* (END6), *H. oryzae* (END21), *Cryptococcus tephrensis* (END66), *H. oryzae* (END87), and *H. oryzae* (END14). Two isolates (*H. oryzae* (END21) and *Cryptococcus tephrensis* (END66) were able to control the growth of *P. capsici* both *in vitro* and *in vivo*.

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