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Research Article

## Potential of Wild Yeast from Banana to Control *Colletotrichum musae* Fungi Caused Anthracnose Disease and Its Short Antagonistic Mechanism Assay

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### ABSTRACT

Anthracnose caused by *Colletotrichum musae* is one of several important diseases in banana fruit. An effective, cheap, and safe control method is necessary as a postharvest disease control alternative. This research aimed to obtain and identify wild yeast from banana which was isolated from its peel that had antagonist ability effectively in controlling anthracnose disease of banana fruit. This research was started with isolation of *C. musae* and yeast from banana, followed by in vivo assay with count percentage antagonist level, slide culture, and in vivo assay to know pathogen incubation stage and incident disease level. The yeast that isolated from Ambon variety was *Candida* sp., *Pichia* sp., and *Metchnikowia* sp. from Kepok variety respectively.

**Keywords:** *Colletotrichum musae*, *Pichia* sp., *Metchnikowia* sp., antagonist mechanism

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### Introduction

Yeast is a group of unicellular fungi which has many benefits. In agriculture, yeast also has a potency as biocontrol. As a biocontrol yeast can be found on fruit [1]. Banana is one of famous fruits by common people. The disease is anthracnose caused by *Colletotrichum musae* which is considered as one of the most important diseases of banana. It deteriorates the quality and nutritive value of the fruits and renders them unfit for marketing and consumption. It has level damage on banana till 100% [2]. Some banana has different resistance depend on their varieties. Time average that pathogen needed to infect is 3.5 days on un-ripening condition. The damage intensity is different in each variety, it may be caused by thick peel [3].

As far as now, banana anthracnose problem has not been completely overcome yet. Potency of microbial yeast contained in the banana was important. It was necessary to study its ability in controlling anthracnose. Yeast isolated from the ripe banana of susceptible and resistant variety has po-

tency to over-come *C. musae*. To obtain ripe bananas, one way is used to accelerate the ripening of fruit by adding carbide acid that can increase temperature until 33°C [4]. At 20 – 40°C temperature, not all yeast genus can survive. It is because of the environment factors affect microbe's survivability [5, 6]. Diversity of thermotolerant yeast from banana peel needs to be discovered along with its potency as biocontrol agent for *C. musae*. Toward, systematic expiration of yeast is needed for studying more details [7].

Purpose of the research was to find out yeast diversity from peel of Kepok and Ambon banana varieties on 40°C temperature and to study their yeast potencies in controlling anthracnose on banana by *in vitro* and *in vivo* assay.

### Material and Methods

The research was held on January-June, 2016 in Plant Pest and Disease Department, Mycology Laboratory, Agriculture Faculty Brawijaya University. The research was held by exploration and experimental methods. Exploration method was

implemented by isolating yeast from *Ambon* and *Kepok* banana varieties subsequently treated temperature 40°C. Experimental method was carried out by *in vitro* and *in vivo* assays to know the yeast antagonist ability against *C. musae*.

#### **Isolation of thermotolerant yeast and *C. musae***

*C. musae* was isolated by growing spores obtained from bananas that showed anthracnose symptom, the spore was planted on PDA. Yeast isolated from *Kepok* and *Ambon* banana varieties were categorized as 6 index of ripening. 10 grams of banana peel was shaken with 90 mL aquadest with 120 rpm speed for 24 hours. Immersion diluted water, was diluted on  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  respectively, and then it was suspended in 50 mL aquadest and planted on YMA by spread plate method. Yeast then was screened on 40°C temperature for 90 minutes and incubated for 3 – 4 days [8, 9].

Yeast microscopic and macroscopic identifications refer to the Yeast 5th Edition [1]. Macroscopic characteristics consisted of the appearance of yeast colonies on petri dish which were the shape of colony, color, texture, margin, and elevation. Microscopic characteristics consisted of the appearance of cell shape, size, reproduction type, budding type, and pseudo hyphae existence. Microscopic identification was held after yeast incubated for 24 hours under microscope with 400× magnifications.

#### ***In vitro* assay thermotolerant yeast against *C. musae***

##### **Percentage of relative inhibition level**

The assay was done by planting thermotolerant yeast with *C. musae* in petri dish with  $\Phi$  9 cm diameter. We used PDA medium. 1 loop yeast stretched crossway on PDA, *C. musae* laid between the yeast inoculation line for 3 cm [10]. Observation had been done for 6 days by counting *C. musae* colony growth wide, then percentage relative inhibition level was counted by formula:

$$THR = \frac{dk - dp}{dk}$$

Note:

THR: Percentage of relative inhibition level formed by pathogen

dk: Number of colonies radius (r1 + r2) pathogens without treatment (control)

dp: Number of colonies radius (r1 + r2) pathogens

with yeast treatment

#### **Slide culture method**

Slide culture method was used to know antagonist mechanism between yeast and *C. musae* under microscopic observation. Slide culture method was done by putting 1 cm<sup>2</sup> PDA on glass, yeast and pathogen stretched on PDA with 5 mm distance, then covered by cover glass. This culture then incubated for 4 days with moist and aseptic conditions. After 4 days, the yeast and *C. musae* viewed by observing the interactions [11].

#### ***In vivo* assay thermotolerant yeast against *C. musae***

All the fruits used were surface cleaned with flowing water then air dried at room temperature and disinfected with alcohol 70%. Every fruit syringed with yeast suspension ( $10^7$  cell/mL) until wet. Fruits' surfaces were wounded by sterilized pin ( $\pm$  1 mm depth from the surface). Three wounds were made per fruit and the wounds were inoculated with 1 drop of *C. musae* suspension ( $10^6$  cell/mL). The inoculated fruits were placed on plastic trays and enclosed in clean plastic wrap to maintain the humidity and incubated at room temperature for 6 days. Observation had been done every day until the first symptom appeared. After 6 days, the wound with symptom observed by counting the percentage of incidence level by the formula:

$$ID = \frac{n}{N} \times 100\%$$

Note:

ID: Incidence of disease

n: Number of points inoculations that shown symptoms of illness

N: Number of points of inoculation were observed

#### **Statistical analysis**

Data from antagonist test had been analyzed using Analysis of Variance (ANOVA) and continued with Least Significant Different (LSD) test at 5% level for post hoc test.

#### **Results and Discussion**

##### **Identification of the isolated fungi from postharvest diseased fruit**

*C. musae* spore obtained by spore isolation from banana with the symptom of anthracnose on

Table 1. Macroscopic and microscopic appearances of thermotolerant yeasts obtained

No.	Species	Macroscopic Characteristic (Colony)				
		Colour	Texture	Surface	Elevation	Margin
1.	<i>Candida</i> sp.	White bone	Thick	Smoth	Curved	Irregular
2.	<i>Pichia</i> sp.	White bone	Thick	Smoth	Curved	Irregular
3.	<i>Metchnikowia</i> sp.	White bone	Thick	Slippery	Curved	Flat

No.	Species	Microscopic characteristic (Cell)				
		Shape	Size (µm)	Reproduction	Budding type	Pseudohyphae
1.	<i>Candida</i> sp.	Oval	4.48 – 5.02	Budding	Multilateral	Present
2.	<i>Pichia</i> sp.	Bulat	4 – 9	Budding	Multilateral	Absent
3.	<i>Metchnikowia</i> sp.	Bulat	4.13 – 4.62	Budding	Multilateral	Absent

Table 2. Average percentage relative inhibition level

No.	Yeast treatment	Percentage relative inhibition level (%)					
		1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI
1.	Control	0	0 a	0 a	0 a	0 a	0 a
2.	<i>Candida</i> sp.	0	20.89 b	6.42 ab	13.85 ab	10.41 b	0 a
3.	<i>Pichia</i> sp.	0	18.48 b	14.15 b	30.11 b	10.83 b	8.33 b
4.	<i>Metchnikowia</i> sp.	0	14.39 ab	9.31 ab	6.73 ab	4.58 ab	0 a

Note: values with different letters within columns are significantly different (LSD 5%)

PDA. The isolated fungal colony was identified as *C. musae*. On PDA plate it formed white aerial which turned pinkish in color with aged, smooth surface colony. Microscopic appearance showed hyaline and septate hyphae. Cilindrical conidia, aseptate, and the conidial length range 9.73 – 14.5 × 3.08 – 5 µm.

Another research reported that on PDA, *C. musae* showed blackish white, pink, and orange colony, it had irregular margin and the rest of isolated yeasts were having smooth margin [2]. Conidia of *C. musae* isolations were aseptate, hyaline, mostly elipsoid, with length ranging for 10 – 18 µm and 5 – 9 µm.

#### Identification of the isolated thermotolerant yeasts from Kepok and Ambon varieties

Thermotolerant yeasts obtained from Kepok banana was *Pichia* sp. and *Metchnikowia* sp. and from Ambon banana was *Candida* sp. The result of identification thermotolerant yeasts was performed on Table 1.

#### In vitro assay thermotolerant yeast against *C. musae*

##### Percentage of relative inhibition level

A total of three isolations of thermotolerant yeasts were selected by temperature treatment

40°C for 90 minutes. The percentage of relative inhibition level showed on Table 2. Yeast has inhibited pathogen for 5 days, started at 2 until 6 DAI. *Candida* sp. and *Pichia* sp. treatments showed fluctuate inhibition level, and *Metchnikowia* sp. inhibition level had reduced every day until 6 DAI. In 6 DAI, percentage inhibition level of control treatment, *Candida* sp., and *Metchnikowia* sp. showed percentage inhibition 0% and, *Pichia* sp. percentage was 8.3%.

#### Slide culture method

Antagonist mechanism caused by yeast from slide culture method was antibiosis and competition for nutrition and space. On *Candida* sp. treatment did not show antagonist mechanism (Figure 1b), whereas *Pichia* sp. treatment showed antibiosis mechanism (Figure 1c) and *Metchnikowia* sp. with competition mechanism (Figure 1d).

#### In vivo assay of thermotolerant yeast against *C. musae*

Thermotolerant yeast treatment did not give effect for percentage incidence disease level and pathogen incubation phase (Table 3). Whereas variety treatment gave effect for percentage incidence disease and incubation pathogen phase (Table 4). Combination of yeast and banana variety

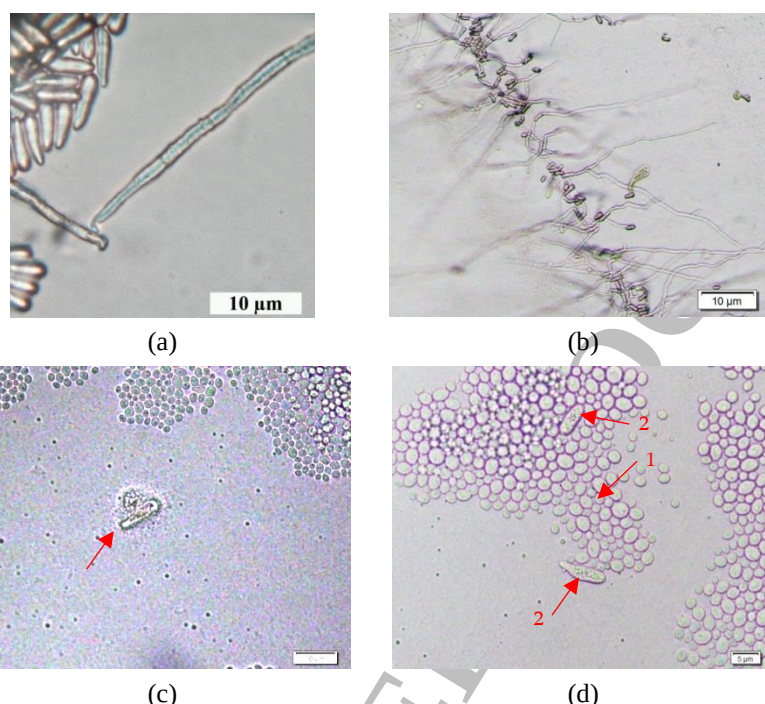


Figure 1. Antagonist assay by slide culture method: Control (a), *Candida* sp. (b), *Pichia* sp. (c) (Lysis of pathogen cell), and *Metchnikowia* sp. (d) (1. Pathogen cell, 2. Yeast cell)

Table 3. Yeast effect against disease incubation phase and incidence disease level

No.	Yeast Treatment	Incidence Disease Level (%)	Disease Incubation Phase (Day)
1.	Control	95.23 a	2.35 a
2.	<i>Candida</i> sp.	90.47 a	2.42 a
3.	<i>Pichia</i> sp.	90.47 a	2.50 a
4.	<i>Metchnikowia</i> sp.	95.23 a	2.42 a

Note: Values with different letters within columns are significantly different (LSD 5%)

Table 4. Banana variety effect against disease incubation phase and incidence disease level

No.	Variety Treatment	Incidence Disease Level (%)	Disease Incubation Phase (Day)
1.	Ambon	100 a	2.03 a
2.	Kepok	85.70 b	2.82 b

Note: Values with different letters within columns are significantly different (LSD 5%)

treatments did not affect it (Table 5).

Banana anthracnose starts to show symptom after ripening condition. As far as now, it was informed that *C. musae* has not been restrained yet, research has been needed to reduce pathogen infection. In unripe condition, symptom has not showed yet because of the unavailability of nutrition for pathogen. It was different with yeast isolation from avocado. Yeast isolation from avocado was effective to reduce anthracnose on avocado [12]. Yeast *Rhodotolura* sp. and *Metchnikowia* sp. can reduce *Colletotrichum* sp. on strawberry and chili [13].

The percentage relative inhibition level and

slide culture method showed antibiosis and competition for nutrition and space against *C. musae* growth. Antibiosis mechanism was occurred toxic compound by disturbing permeability cell membrane and destroying cytoplasm of cell pathogen [14]. It showed in *Pichia* sp. treatment that destroyed cell pathogen close to them (Figure 1c). Not only toxic compound, yeast also produced enzyme like chitinase, protease, and glutanase [15].

*Metchnikowia* sp. treatment indicated competition for antagonist mechanism. It showed on Figure 1d, that the yeast cell attached to the pathogen cell. The speed growth of yeast was faster than pathogen in this medium, so it affected competi-

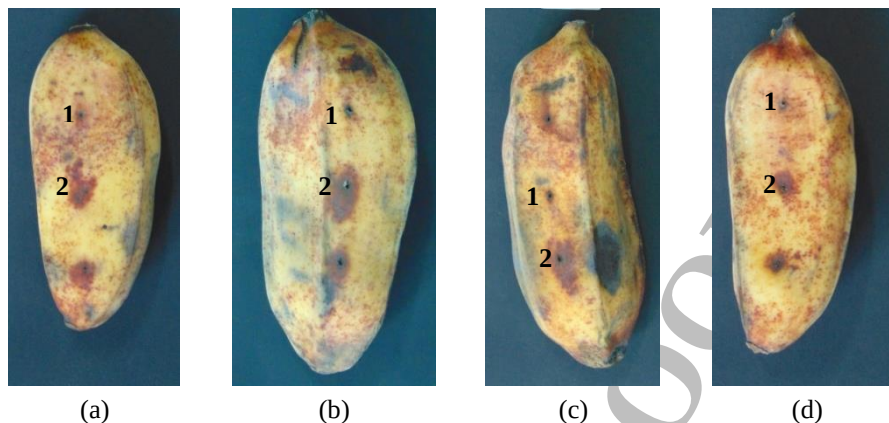


Figure 2. Incidence disease level on *Kepok* variety: Control (a), *Candida* sp. (b), *Pichia* sp. (c), and *Metchnikowia* sp. (d) (Note: uninfected (1) and infected (2))



Figure 3. Incidence disease level on *Ambon* variety: Control (a), *Candida* sp. (b), *Pichia* sp. (c), and *Metchnikowia* sp. (d) (Note: the sign showed infection of *C. musae*)

tion for nutrition and space growth. The increased of yeast population decreased nutrition and space for pathogen, and decreased germination ability. Epiphyte yeast has antagonist ability, made it easier to grow and adapt. This advantage made yeast growing faster in nature habitat [16].

Application of yeast earlier than pathogen has advantages on adaptation. Pathogen that inoculated after yeast application prone to lack in nutrition and spaces for growth. Antagonist yeast was applied 8 hours earlier, showed higher antagonist ability to reduce width pathogen colony [17]. *Candida famata* applied 24 hours earlier can induct plant resistance against pathogen attack. It happened because yeast inducted chitinase enzyme activity and damaged pathogen compound [15]. In this observation, yeast and pathogen application had been done almost at the same time. It indicated that yeast cell had not been adapted with the new environment, then disease incidence level and incubation phase had not reduced.

Infection advanced by the ripening phase. It caused by nutrition availability for pathogen. The ripening fruit has rapid infection phase and it influence incubation pathogen phase [3]. Conidia can germinate for 4 hours and obtained appressorium for 20 hours. Direct infection was occurred for 24 – 48 hours and caused the closed cells to be hypersensitive and obtained red-brown spot. It would be on latten condition until ripening. Latten condition caused by the host compound that made dormant appressoria [18, 19, 20].

On Figure 2 and 3 showed different incidences disease level. Incubation phase on *Kepok* variety was longer than *Ambon* variety. *C. musae* infection depends on ripening phase and banana variety. Bananas used in this research were fruits with ripening index 6. On this condition, banana has character yellow peel and smooth. On ripening condition, biochemistry changes play on important role for pathogen. On banana, insoluble starch has changed to soluble starch. Then, glu-

cose compound concerned resistance of host against pathogen colonization. It caused postharvest disease. In the other way, unripe banana has its own resistance against *C. musae*. It forms anti-fungal component, that could restrain and resist pathogen infection attack [18]. Banana variety, differentiated based on nutrition compound and the thickness of peel. Nutrition on *Kepok* variety is higher than *Ambon* variety, but *Kepok* peel is thicker than *Ambon* variety in ripen condition. Infection will be slow on *Kepok* variety because *Kepok* has thick and tight peel under ripening condition.

## Conclusion

According to this observation, a conclusion is derived that thermotolerant yeasts can be obtained from *Kepok* and *Ambon* banana varieties. From *Ambon* obtained *Candida* sp., and from *Kepok* obtained *Pichia* sp. and *Metchnikowia* sp.

Banana varieties treatment gave significant effect for disease incubation phase and incidence disease level, whereas yeast treatment and combination of yeast and banana variety did not have any effect on disease incubation phase and incidence disease level.

## Acknowledgment

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