USE OF YEAST ANTAGONIST *CRYPTOCOCCUS TERREUS* TO CONTROL PETAL BLIGHT OF *DENDROBIUM* CAUSED BY *CURVULARIA PALLESCENS*

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**ABSTRACT**

This research sought to obtain a superior yeast antagonist as a biological control agent of petal blight disease of dendrobium, to study the mechanism involved in biocontrol activity of yeasts and to study the effectiveness of yeast antagonists under field conditions. Five yeast isolates were investigated for their antagonistic activity *in-vivo* on detached petals, physiological characteristics *in vitro* and subsequently tested under field conditions. Among the yeasts tested, *Cryptococcus terreus* YSW1 was the most effective against petal blight of dendrobium caused by *Curvularia pallescens*. Antibiosis partly contributed to the biocontrol mechanism of *C. terreus*. The yeast was able to control petal blight disease under field conditions at the effective rate of 63-64%. Optimum concentration for application of *C. terreus* was 10 cc L\(^{-1}\), or approximately 10\(^{5}\) cfu L\(^{-1}\).

**Key words:** biological control, phyllosphere, tropical floriculture

**INTRODUCTION**

Petal blight caused by *Curvularia pallescens* is the most important disease of dendrobium orchid in Indonesia. Indonesian growers mostly use synthetic fungicides to control the disease with side negative effects such as intoxication and environmental pollution. Environment awareness of people all over the world requires more environmentally-sound control measure.

A promising technique is biological control using antagonistic yeast. Yeast has advantages for biological control of diseases of aerial plant parts because it is dry tolerant (Elmer and Reglinski 2006). Most previous studies on biocontrol using yeast worked on post harvest diseases (Benbow and Sugar 1999). There is few record of success stories of yeast in controlling plant diseases in the field (Core et al. 2003; El-Tarabily 2004; Khalimi 2010). Some yeasts isolates collected by the first author has promising as an antagonist against disease of aerial plant parts. *Cryptococcus albidus* var *aerius* WSW1 is effective against *Botryodiplodia theobromae*, *Cryptococcus terreus* YSW1 effective against *Alternaria solani* on tomato (Sugiprihatini et al. 2011). There are no reports of the use of yeasts in controlling petal blight of dendrobium.

This research sought: to test biocontrol activity of yeasts isolates against petal blight, to study the mechanism involved in biocontrol activity, and to study the effectiveness of the yeast under field conditions.
 MATERIALS AND METHODS

The research was carried out in the Laboratory of Plant Mycology, Department of Plant Protection, Bogor Agricultural University. Four yeasts isolates were Cryptococcus albidus var. aerius WSW1, Cryptococcus albidus var. aerius WSW2, Candida edax OSW1, Cryptococcus terreus YSW1. One yeast-like fungus was Aureobasidium pullulans ASW1. These microbes were obtained from the Collection of Plant Clinic, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University.

Microbes and Fungicide Preparation

All of the yeasts and yeast-like fungus isolates were cultured in potato dextrose broth (PDB) pH 5.5, shaken at 100 rpm for 72 h (early stationary phase). All of these were centrifuged at 8000 rpm and washed with sterilized distilled water and, the density was determined by spectrometer. The density of each yeast isolate was used, with the estimation of 10^6 cfu cc⁻¹. The cell suspension was directly used for in vivo test and field tests. Standard fungicide used was mancozeb 45% prepared as a suspension at 3 g formulated material L⁻¹.

Antagonistic Activity Test of Yeasts on Detached Petals

Five detached petals of dendrobium orchid cultivar Sonia were used for the test. Treatments were Aureobasidium pullulans ASW1, Cryptococcus terreus YSW1, C. albidus var. aerius WSW1, C. albidus var. aerius WSW2, Candida edax OSW1 Candida edax OSW1 Candida edax OSW1, mancozeb and untreated (water). Each treatment had 5 replications, in which one replication consisted of one petri dish with 4 petals inside. The petals were laid on moistened towel tissue, supported by plastic straw to avoid direct contact with moistened paper. For yeast treatments, the petals were dipped in yeast suspension which had 0.05% (v/v) Triton X-7 as wetting agent. All of the petals were then air dried for 3 hours. Suspension of C. pallescens as much as 50 µL and concentration of 10^4 cc⁻¹ was dropped on the petal using an automatic pipette. After inoculation the petals were placed on moistened Petri dishes and incubated in the dark for 24 hours. The disease was assessed by estimating diseased severity (%) at 7 days after inoculation.

Biocontrol effectiveness of yeasts was calculated using formulae of Chanchaichaovivat et al. (2007) as follows:

$BE = \frac{(dc-dt)}{dc} \times 100\%$

Where BE, biocontrol effectiveness (%); dc, disease severity of control; dt, disease severity of treatment.

Antibiosis Assay

Dual culture bio-assay was carried out to determine mechanism of antibiosis (Wisniewski et al. 2007). All yeasts were tested against Curvularia pallescens. The yeast suspension was streaked by forming line in the middle of 9-mm petri dish containing PDA. An agar plug of Curvularia pallescens Ø 3 mm-7-day old was seeded on the right and left of the yeast streak, therefore the line connecting two fungal colony centers was perpendicular to the yeast streak. The inhibition zone was assessed after 3 days of incubation and expressed in mm. The size of inhibition zone indicated antibiosis of yeasts against the tested fungus (Spadaro 2002; Indriatmi 2008). All of the treatments were arranged in randomized complete design and replicated three times.
Chitinolytic activity

All the yeast isolates were grown on chitin agar, containing 0.5 % colloidal chitin for 3 days (Shanmugiah et al. 2008). Chitinolytic activity was indicated by the presence of a clear zone along the yeast colony (Brzezinska and Donderski 2001).

Field Test of Effective Antagonist Yeasts

Yeast effective in bioassay then tested in the field in Cikampek – West Java Indonesia, in September 2007- February 2008. Treatment consist of C. terreus 10 cc/l, 5 cc/liter and 2.5 cc/liter, untreated and mancozeb 3 g formulation/l as standard. Dendrobium used is cultivar Sonia at the age of 3 years and cultivated under standard agronomical technique except pesticide application. Yeast application was conducted by spraying canopy using hand sprayer at one week interval, starting at flowering. Observation on disease incidence was conducted weekly on ten inflorescence per replications for four week or five observation points. Experiment was arranged in randomized complete design with five replications and each replication consists of 20 potted plants. Each flower inflorescence per pot was taken as sample. Disease incidence was applied in this study instead of disease severity because one disease spot makes a flower unmarketable. Disease incidence was then analyzed using ANOVA.

To compare disease progress among treatments, two approach were applied, first by calculation rate of disease as Vanderplank (1963) formulae as follow:

\[ r = \frac{1}{t_2-t_1} \left[ \ln x_2 - \ln x_1 \right] \]

Where:
- \( r \) = disease progress rate
- \( t_1 \) = time 1 (initial observation)
- \( t_2 \) = time 2 (final observation)
- \( x_1 \) = disease severity at \( t_1 \)
- \( x_2 \) = disease severity at \( t_2 \)

and by calculating effectiveness rate of disease with different initial rate by formulae of Henderson and Tilton (Puntener 1981) as follows:

\[ EI = [1 - \frac{Ta}{Ca} \times \frac{Cb}{Tb}] \times 100\% \]

Where:
- \( EI \) = effectiveness rate
- \( Ca \) = disease severity of untreated after treatments
- \( Cb \) = disease severity untreated before treatments
- \( Ta \) = disease severity of treatments after treatment
- \( Tb \) = disease severity of treatments after treatment

RESULTS AND DISCUSSION

Cryptococcus terreus YSW1 was the most effective yeast based on in vivo test using detached petals (Table 1), showing complete inhibition on petal blight (0% disease severity), even better control
Use of yeast antagonist Cryptococcus terreus.

than the standard commercial fungicide (6.25% disease severity). Some Cryptococcus genera were reported as biocontrol agents (Qing and Shipping 2000; Fan and Tian 2001; Core et al. 2003; Yu et al. 2006; Zhang et al. 2007).

**Table 1.** Disease severity of detached petals of dendrobium orchids treated by yeasts and inoculated by *C. pallescens* and incubated in moistened petri dish for 5 days

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease Severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>43.75 c</td>
</tr>
<tr>
<td><em>Cryptococcus terreus</em> YSW1</td>
<td>0 a</td>
</tr>
<tr>
<td><em>C. albidus</em> var. <em>aerius</em> WSW1</td>
<td>37.50 c</td>
</tr>
<tr>
<td><em>C. albidus</em> var. <em>aerius</em> WSW2</td>
<td>37.50 c</td>
</tr>
<tr>
<td><em>Candida edax</em> OSW1</td>
<td>56.25 d</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>6.25 b</td>
</tr>
<tr>
<td>Control (water)</td>
<td>62.50 d</td>
</tr>
</tbody>
</table>

Note: Values in the same column followed with the same letters were not significantly different at p<0.05.

The mechanism of biocontrol is necessary to be defined before a biocontrol agent can be further developed and optimized. Various mechanisms may be involved in biocontrol using yeasts *i.e.* competition, antibiosis, lysis and resistance induction (Janisiewicz and Korsten 2002; De Ingeniis et al. 2009; Wisniewski et al. 2007; Chanchaichaovivat et al. 2008). Antibiosis contributed to the effectiveness of yeast, in which *C. terreus* also provided antibiosis activity (Table 2). On the contrary, chitinolytic activity had no relation to effectiveness (Table 3). Weak correlation of antibiosis activity *in vitro* and biocontrol effectiveness *in vivo*, such as depicted in Table 2 and Table 3 explain that antibiosis is not the main mechanism underlying biocontrol using the tested yeasts. Indriatmi (2008) also noted that there is no antibiosis of biocontrol yeasts *Debaryomyces* sp. against anthracnose caused by *Colletotrichum gloeosporioides* on chili.

**Table 2.** Inhibition zone produced by tested yeasts against *C. pallescens* in dual culture test

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>Inhibition Zone Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>12.22 ab</td>
</tr>
<tr>
<td><em>C. terreus</em> YSW1</td>
<td>9.10 a</td>
</tr>
<tr>
<td><em>C. albidus</em> var. <em>aerius</em> WSW1</td>
<td>11.43 a</td>
</tr>
<tr>
<td><em>C. albidus</em> var. <em>aerius</em> WSW2</td>
<td>14.00 b</td>
</tr>
<tr>
<td><em>Candida edax</em> OSW1</td>
<td>11.52 a</td>
</tr>
</tbody>
</table>

Note: Values in the same column followed with the same letters were not significantly different at p<0.05.

**Table 3.** Formation of clear zone by tested yeasts on chitin agar in chitinolytic test

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Formation of clear zone</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>-</td>
</tr>
<tr>
<td><em>C. terreus</em> YSW1</td>
<td>-</td>
</tr>
<tr>
<td><em>C. albidus</em> var. <em>aerius</em> WSW1</td>
<td>-</td>
</tr>
<tr>
<td><em>C. albidus</em> var. <em>aerius</em> WSW2</td>
<td>-</td>
</tr>
<tr>
<td><em>Candida edax</em> OSW1</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: + showing clear zone, - no clear zone
Even though other previous researchers reported that chitinolytic and other lytic enzyme activity involve in mechanism of biocontrol using yeast (Spadaro 2002; El-Masih and Paul 2002), this study showed no relation between chitinolytic activity and biocontrol effectiveness of yeasts. Nutrient competition and induced resistance which involve in biological control using yeast (Droby et al. 2002; Janisiewicz and Korsten 2002; El-Masih and Paul 2002; El-Tarabily 2004; Yao and Tian 2005) were not investigated in this study.

Many yeasts were reported as effective antagonists against various post harvest diseases (El-Ghaouth et al. 2000; Chanchaichaovivat et al. 2007; Tan and Fuan 2007; Gholamnejad et al. 2010). However, Candida guilliermondii and Candida oleihphila were applied in the field to control botrytis blight of tomato (Saligkarias et al. 2002). This experiment showed that C. terreus was effective in controlling petal blight of dendrobium caused by Curvularia pallescens under field conditions.

Experimental results showed that initial disease incidence was not the same (Table 4 and Table 5), therefore the infection rate and effectiveness rate were applied for further comparison. C. terreus, 10 cc L⁻¹, provided highest control performance as indicated by high effectiveness rate (33-62%) and low infection rate (0.15-0.28), was even better than mancozeb treatment (15 % - 18% effectiveness rate and an infection rate of 0.32-0.34 (Table 4 and 5). Based on infection rate and effectiveness rate, C. terreus at 10 cc L⁻¹ provided best results to control petal blight.

Table 4. Disease incidence of petal blight of dendrobium in the field treated by various concentration of of Cryptococcus terreus YSW1 – experiment I (November –December 2007).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease Incidence (%)</th>
<th>Infection Rate (r)</th>
<th>Effectiveness Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>Y 10 cc/l</td>
<td>20.25 b</td>
<td>36.73 b</td>
<td>40.38 b</td>
</tr>
<tr>
<td>Y 5 cc/l</td>
<td>20.25 b</td>
<td>23.34 a</td>
<td>0.25 a</td>
</tr>
<tr>
<td>Y 2.5 cc/l</td>
<td>10.50 a</td>
<td>26.73 a</td>
<td>26.73 a</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>10.50 a</td>
<td>36.73 b</td>
<td>27.75 b</td>
</tr>
<tr>
<td>Untreated</td>
<td>10.50 a</td>
<td>31.97 b</td>
<td>30.50 a</td>
</tr>
</tbody>
</table>

Note: First yeast application was at week 1
Values in the same column followed with the same letters were not significantly different at p<0.05

Table 5. Disease incidence of petal blight of dendrobium in the field treated by various concentration of Cryptococcus terreus YSW1 – experiment II (January-February2008).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease Incidence (%)</th>
<th>Infection Rate (r)</th>
<th>Effectiveness Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>Y 10 cc/l</td>
<td>20.25 b</td>
<td>38.50 b</td>
<td>52.50 c</td>
</tr>
<tr>
<td>Y 5 cc/l</td>
<td>20.25 b</td>
<td>38.50 b</td>
<td>52.50 c</td>
</tr>
<tr>
<td>Y 2.5 cc/l</td>
<td>24.73 b</td>
<td>40.00 b</td>
<td>46.73 b</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>11.73 a</td>
<td>33.38 a</td>
<td>40.00 b</td>
</tr>
<tr>
<td>Untreated</td>
<td>15.73 a</td>
<td>33.12 a</td>
<td>42.18 a</td>
</tr>
</tbody>
</table>

Note: First yeast application was at week 1
Values in the same column followed with the same letters were not significantly different at p<0.05.

Biological control of plant diseases using yeasts sometimes provide better control than standard chemical control as shown by this research, and other previous research (Sugiprihatini et al.
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2011), because antagonistic yeast is able colonize growing plants causing more continuous protection against pathogen attack and this not possible for chemicals. The field test I gave different value of disease incidence compared to field test II, due to the higher rainfall in field test II that may cause higher yeast leaching from the dendrobium phyllosphere. This effectiveness rate was comparable to other results using yeast antagonists, i.e. 50-80 % (Kefialew and Ayalew 2008; Gholamnejad et al. 2010). The highly effective yeast antagonist C. terreus obtained from this study, should be further developed as a biocontrol agent of petal blight of dendrobium in terms of formulation, bio-safety aspects, and application technique.

CONCLUSION

Cryptococcus terreus YSW1 is proven to be an effective yeast antagonist to control petal blight of dendrobium caused by Curvularia pallescens under field conditions. Antibiosis partly contributes to the biocontrol mechanism of the yeast antagonist.

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REFERENCES


