

Application of Antagonistic Yeasts for Postharvest Disease Control on Chili Fruits

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Abstract--The effective antagonistic yeasts, PFN6 and SS7 isolated from chili field, North East, Thailand. This result based on disease incidence to control *Colletotrichum capsici* by *in vivo* test. Both isolates can reduce disease incidence at 5×10^8 cell/ml when infected with all spore concentrations of pathogen. The disease incidence on chili fruits could be reducing when treated with high spore concentrations of the pathogen. We found isolate PFN6 at 5×10^8 cells/ml showed the best result with 37.27% of disease incidence at 5×10^4 spores/ml of *C. capsici*. These concentrations used to control anthracnose disease on chili fruits. The survival percentage of chili fruit was recorded at 20 days preservation. The result found, antagonistic yeast PFN6 presented the great efficacy of fruit survives with 66.25%. Both of efficacy yeasts were characterized to morphological characteristics. They had white and smooth colony, ovoid shape and single or pairs of cell arrangement. The sequence analysis of D1/D2 domain of 26s ribosomal DNA showed high identity with *Candida haemulonii*.

Keyword--*Candida haemulonii*, postharvest disease control, anthracnose disease.

I. INTRODUCTION

CHILI (*Capsicum* spp.) is considered an important tropical and subtropical crop on the basis of its high consumption, nutritional value, and economic value to farmers [1]. Thailand is the largest producer and exporter of chili in the international market and exports to United State of America, Australia, England, Philippines, Japan, Singapore, Taiwan and the Netherlands, amongst others. In the Northeast of Thailand chili production is distributed between Nakhon Ratchasima, Loei, Chaiyaphum and Ubon Ratchathani provinces [2]. One of the most common diseases in chili plants and a lot of other tropical vegetables is anthracnose and it is caused by *Colletotrichum acutatum*, Simmonds ex Simmonds, *C. capsici* (Syd) Butl & Bisby, *C. coccodes* (wallr.) Hughes and *C. gloeosporioides* Penz & Sacc, which is widespread during the rainy season. Anthracnose is however, more conspicuous as it causes severe damage to mature fruits in the field as well as during transit and storage [3]. Control of the anthracnose disease on chili fruits still relies mainly on the use of synthetic fungicides such as Carboxin, Synap, Benomyl and Carbendazin, etc. This may result in fungicide resistant pathogens such as those that occur in copper fungicides.

This means that there is an increasing need for alternative control strategies. Several research projects have shown success in the use of yeasts antagonism to control plant pathogens in chili fruits [4]. Our objectives were investigated their capabilities to control anthracnose disease caused by *C. capsici* in postharvest conditions.

II. MATERIALS AND METHODS

1. Antagonistic yeasts

A total 60 isolates were screened for their capabilities to control *C. capsici* by dual culture method. Two isolates, PFN6 and SS7 were showed high inhibition percentage up to 40%. These effective isolates were kept at 4 °C on YM medium for further study.

2. Effect of yeast cells and *C. capsici* concentration on biocontrol efficacy

Thirty wounded chilies were used for each treatment on the biocontrol efficacy of various concentrations of antagonistic yeasts. The wounds were inoculated with 10 µl of each antagonistic yeast isolate at 5×10^6 , 5×10^7 , 5×10^8 and 5×10^9 cells/ml and treated with *C. capsici* at 5×10^4 , 5×10^5 and 5×10^6 spore/ml as counted by a hematocytometer. After air drying and cleaning for 1-2 h, 10 µl of *C. capsici* spores/ml were added to each wound. The chili fruits were placed on plastic boxes and stored in a ventilate cabinet in the dark at 28 °C for 5 days. All treatments were measured of biocontrol efficacy and disease incidence. The data were transformed into a percentage of biocontrol efficacy (%BC = [(T-A)/T]x100 and disease incidence (%DI = (A/T)x100), where T is the number of infected wounds inoculated with *C. capsici* only, and A is the number of infected wounds inoculated with yeast antagonists and *C. capsici*.

3. Efficacy of antagonistic yeasts in postharvest disease control on chili fruits

Intact chili fruits (no visible wounds or scar on the surface) were surface sterilized with 0.6% sodium hyper chloride for 5 min, washed with the tap water. After air drying, chili fruits were treated with 70% ethanol. Each treatment was sprayed with 300 ml of cell suspension (5×10^8 cells/ml) and control sprayed with 300 ml of distilled water. Chili fruits were placed on plastic boxes and kept at 28 °C for 2 h and followed by

storage at 28 °C for 20 days. The percentages of chili survival in each group were recorded.

4. Identification of antagonistic yeasts

Antagonistic yeasts were studies morphological characteristics that according to the method described about yeast and taxonomy [5]. These isolates were identified to species by nucleotide sequencing method. The sequence of D1/D2 domain of 26S rDNA was compared by BLASTn Homology.

5. Statistical analysis

Data analysis of disease incidence and survival percentages of chili fruits were done by using compare means with one-Way ANOVA of IBM SPSS statistics (version 19.0), SPSS Inc., Chicago, IL USA). The least significant difference (LSD) test at $P<0.05$.

III. RESULTS

1. Effect of yeast cells and *C. capsici* concentration on biocontrol efficacy

The result of antagonistic yeast to control *C. capsici* on chili fruits at various cell concentrations. The result shown, all isolates can reduced disease incidence when yeast cell concentration increasing and spores concentration of the pathogen were decreased. Isolates PFN6 and SS7 at 5×10^8 cells/ml were presented high effective to control the pathogen in disease incidence than the other concentration of yeast. In addition, isolate PFN6 could be reduced the anthracnose disease more than isolate SS7 at the same concentration. Antagonistic yeast PFN6 showed highest performance to reduce the disease incidence at 37.27% when treated with the spore of the pathogen at 5×10^4 spores/ml. (Fig 1 and 2).

2. Efficacy of antagonistic yeasts in postharvest disease control on chili fruits

The antagonistic yeasts PFN6 and SS7 showed great efficacy to control mycelium of *C. capsici* on chili fruits at all storage times. From the result, the anthracnose disease was happened at 10 days after treated with spore of the pathogen. Isolate PFN6 showed high of survival percentage on chili fruits more than isolate SS7 in the same time. At final storage time, antagonistic yeast PFN6 had highest of the survival percentage of chili fruits at 66.25% (Table 1).

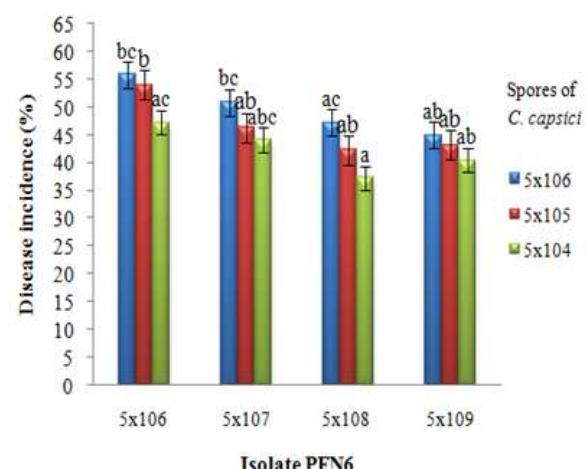


Fig 1. Disease incidence on chili fruits of yeast PFN6. Bars represented the means \pm standard error. The letters on the top of each bar indicate significant difference ($P<0.05$).

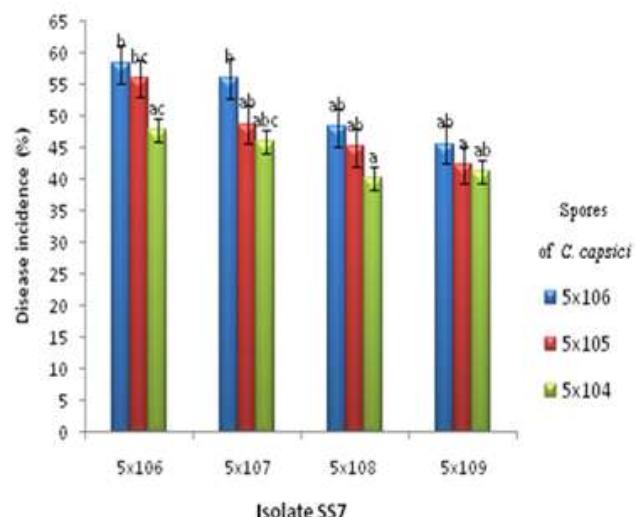


Fig 2. Disease incidence on chili fruits of yeast SS7. Bars represented the means \pm standard error. The letters on the top of each bar indicate significant difference ($P<0.05$).

TABLE1 SURVIVAL PERCENTAGE OF CHILI FRUITS IN POSTHARVEST DISEASE CONTROL

Yeast isolate	Survival of chili fruit (%)			
	5 days	10 days	15 days	20 days
PFN6	100a	90.00a	81.25a	66.25a
SS7	100a	62.50b	58.75b	43.75b
Control	100a	6.25c	0.00c	0.00c
%CV	-	6.63	8.48	10.32

The results are as mean of percentage. Value of each column followed by a different letter indicate significant differences ($P<0.05$) according to LSD test.

3. Identification of antagonistic yeasts

The antagonistic yeasts PFN6 and SS7 were found white and smooth colony, ovoid and single or pair shape and they can produce ascospore. Each strain was analyzed for D1/D2 domain of 26s ribosomal DNA sequencing. The sequence

analysis of yeast isolates PFN6 and SS7 showed the same species as *Candida haemulonii* (Table 2).

TABLE II

IDENTIFICATION OF ANTAGONISTIC YEASTS BY 26S rDNA AND MORPHOLOGICAL CHARACTERISTICS.

Yeast isolate	Identity (%)	rDNA ¹ region	Species	Colony morphology	Cell morphology	Type of spore
PFN6	100	D1/D2	<i>Candida haemulonii</i>	White, light beige, smooth and butyrous	ovoid, ellipsoidal (2-7x2-7 µm), single or pairs	Ascospore
SS7	100	D1/D2	<i>Candida haemulonii</i>	White, light beige, smooth and butyrous	ovoid, ellipsoidal (2-7x2-7 µm), single or pairs	Ascospore

IV. CONCLUSION AND DISCUSSION

The antagonistic yeasts PFN6 and SS7 have been successfully identified *Candida haemulonii* by using 26S ribosomal DNA. Both strains were presented the morphological characteristic similarly *Candida haemulonii* Type II (Van Uden&Kolipinski) S.A. Meyer&Yarrow that located at *Metschnikowia* clade [6]. These antagonistic yeasts can control *C. capsici* mycelium in disease incidence and postharvest disease control on chili fruits. Several antagonistic yeasts in *Candida* have previously been used as biocontrol agents to control the anthracnose disease caused by *C. capsici* in postharvest crops such as *C. tropicalis*, *C. famata* and *C. membranifaciens* [7; 8]. However, there is no report on use of *C. haemulonii* to control *C. capsici* in chili fruits. These studies presented the first evidence of antagonistic yeast *C. haemulonii* to control anthracnose disease. This strain can reduced disease incidence and can preserved chili fruits from the pathogen at 20 days. From the result of disease incidence of *C. haemulonii* shown as 37.27%. This efficacy is similar with the studies of [9] used yeasts *C. musae* R6 and *C. quercitrusa* L2 to control *C. capsici* on chili fruits showed the disease incidence at 6.70% and 33.60% respectively. Yeast PFN6 demonstrated great action to inhibition the pathogen mycelium in postharvest disease control. The result in survival percentage of chili fruits of this strain was showed at 66.25% and 43.75% at 20 days. This investigated that the antagonistic yeast *C. haemulonii* had efficacy to control anthracnose disease over than 20 days. This result indicated the mode of action involes space and nutrient competition and lytic enzyme action. Because of, the antagonistic yeast can rapidly growth more than *C. capsici* mycelium, so, yeast can compete the nutrient and space from the pathogen as a result in eventually death of the pathogen. In addition, the yeast cell produce lytic enzymes such as chitinase and β-1,3-glucanase that could enhance the attaching ability of yeast to hyphae of the pathogen [10]. This finding provide a powerful stimulant for development of the yeast *C. haemulonii* as an alternative to control anthracnose disease caused by *C. capsici* in chili fruit.

Nevertheless, more studies including field trials need to be performed.

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