International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Mode of Action of Yeast-Like Fungus *Aureobasidium pullulans* in Controlling Anthracnose of Postharvest Chili

Sri Hartati[a]*, Suryo Wiyono[b], Sri H. Hidayat[c], Meity S. Sinaga[d]

[a]*Postgraduate Student, Department of Plant Protection, Bogor Agricultural University, Jl Kamper Kampus IPB Darmaga, Bogor 16680, Indonesia, Universitas Padjadjaran, Jl Raya Bandung-Sumedang Km 21 Jatinangor, Sumedang 45363, Indonesia*

[b,c,d]*Bogor Agricultural University, Jl Kamper Kampus IPB Darmaga, Bogor 16680, Indonesia*

[a]*Email: shartatifito21@gmail.com*

[b]*Email: suryow@hotmail.com*

[c]*Email: srihendrastutihidayat@gmail.com*

[d]*Email: mssinaga@yahoo.com*

Abstract

Biological control emerged as a promising alternative in pre- and postharvest diseases control, such as chili anthracnose caused by *Colletotrichum acutatum*. Potency of *Aureobasidium pullulans* as antagonist in chili anthracnose has been demonstrated. Three isolates of *A. pullulans* were evaluated in vitro to determine its antagonism activity against *C. acutatum*, as causal of anthracnose on chili. Bioassay was performed through antibiosis test, formation of volatile compounds, chitinolytic activity, and hyperparasitism. Three isolates of *A. pullulans* were able to significantly inhibit the growth of *C. acutatum* through the formation of volatile compounds (VOCs). Two isolates of *A. pullulans* had chitinolytic activity. Microscopic observation showed that chitinolytic activity of the isolates causes the hyperparasitism in the form of lysis of the cell walls of *C. acutatum* hyphae.

Keywords: *Colletotrichum acutatum*; biological control; antibiosis; VOCs; chitinolytic; hyperparasitism

---

* Corresponding author.

E-mail address: shartatifito21@gmail.com

1. Introduction

Anthracnose is a very destructive disease of chili and becomes production limiting factor in many countries [26]. Yield loss due to anthracnose infection can reach up to 50% [27]. Anthracnose in chili is caused by several species of *Colletotrichum* such as *C. acutatum, C. gloeosporioides*, and *C. capsici* [8].

Generally, anthracnose is controlled by spraying synthetic fungicides. However, the use of synthetic fungicides may cause negative effects on the environment, such as resistance development of pathogen to fungicides, and residual effect on postharvest product. Considering such negative effects, biocontrol agents has been developed as an alternative control method. Some antagonists microorganisms have been reported effective in controlling anthracnose disease on chili, among others are *Trichoderma harzianum* [11, 19] and *Pseudomonas fluorescens* [11]. Recently, yeasts were also reported effective in controlling this disease [2, 3, 26]. Some species of yeasts such as *Pichia guilliermondii, Candida musae, Issatchenkia orientalis* and *Candida quercitrusa* were able to reduce the incidence of anthracnose disease on chili fruit caused by *C. capsici* [2].

Understanding the mode of action of biocontrol agents in suppressing pathogens are important to know. The main modes of action are competition, parasitism, antibiosis, and induced resistance [32]. *Aureobasidium pullulans* (de Bary) Arnaud is a yeast-like fungus with a good potential as biocontrol agents against various plant pathogens. *A. pullulans* have been reported has a good ability in nutrients and space competition [35], formation of extracellular exochitinase and β-1-3-glucanase [29], and formation of volatile compounds (VOCs) [5]. Results of previous studies showed that three isolates of *A. pullulans* isolated from chili leaf surface was potential as antagonist agents for anthracnose caused by *C. acutatum* [34]. However, mode of action of *A. pullulans* in controlling pre- and postharvest pathogens is still not widely understood. This study aimed to determine the mode of action of three isolates of *A. pullulans* in controlling anthracnose pathogen *C. acutatum* on chili.

2. Materials and Methods

Three isolates of yeast-like fungi were isolated from chili leaf surfaces (*C. annuum*) collected from plants grown in Darmaga, Bogor, West Java, Indonesia. The isolates has been molecularly identified as *A. pullulans. C. acutatum* was isolated from chili fruit showing anthracnose symptom from Panjiwangi, Garut, West Java, Indonesia.

2.1. Antibiosis Test

Dual culture method was used to analyze antibiosis mechanism [24]. One loop of 5 days old yeast-like fungus was streaked transversely on the middle of petridish containing the potato dextrose agar (PDA). Then 6 mm of 10 days old *C. acutatum* culture was grown at 3 cm on the left and 3 cm on right side of the streak, in the same petridish. As comparison, in a separate petridish pieces of *C. acutatum* was placed on the same position without yeast-like fungus.

The width of clear zone indicated antibiosis of yeast-like fungus against tested fungus.

2.2. Formation of Volatile Compounds

Volatile compound test was performed according to method of Huang [31] with some modification. One loop of 5 days old yeast-like fungus isolate was streaked right in the middle of PDA in a petridish, and a 6 mm piece of 10 days old *C. acutatum* culture was placed in the middle of PDA in another petridish. Both petridishes (without lids) were stacked facing each other, the one with yeast-like fungus on top, and the one with *C. acutatum* on the bottom. They were then plastic wrapped. Petridish containing *C. acutatum* without yeast-like fungus as a control was stacked with PDA. The growth of *C. acutatum* on the treatment was compared with the growth on the control plates. The relative inhibition percentage was assessed at 3 to 10 days incubation and was calculated using the formula:

$$HR = [(Øk – Øp)/Øk] \times 100\%$$

Where HR = relative inhibition percentage

Øk= diameter of *C. acutatum* of control

Øp= diameter of *C. acutatum* of treatment

2.3. Chitinolytic Activity

The chitinolytic activity test was performed on colloidal chitin agar media 0.2% [18]. Twenty gram chitin (C8H13NO5)n from shrimp skeleton (C717O practical grade sigma) was diluted in 400 ml HCl. The solution was left for 24 hours in low temperature, and then it was strained with glass wool. The filtrate was added with 200 ml cold distilled water and + 500 ml 10 N NaOH, to the pH 7.0. The filtrate was then centrifuged at 7.000 rpm for 10 min, and suspended again in cold distilled water and re-centrifuged. The pellet of the colloidal chitin obtained was placed at 4^{o}C.

The method of Shurtleff & Averre [21] was adapted to make colloidal chitin agar media. Colloidal chitin (1-2.5 g) and agar (20 g) were diluted in 1000 ml distilled water or mineral salt. The mineral salts composed of 0.7 g K2HPO4; 0.5 g KH2PO4; 0.5 g MgSO4.7H2 crystalline; 0.001 g FeSO4; and 0.001 g ZnSO4.

To test chitinolytic potency, 3-5 days old yeast-like fungus isolate was grown on colloidal chitin agar media, and incubated at room temperature. The growth was observed every day. Clear zone area was considered to chitinolytic yeast-like fungus activity. The chitinolytic index was calculated using the formula:

$$\Delta Y = y2/y1$$

Where ΔY = chitinolytic index

y2 = width clear zone and the colony

y1 = width colony

2.4. Hyperparasitism Test

Hyperparasitism was tested using water agar by agar block method (0.6 cm in size). Five days old of yeast-like fungus isolate was grown in one side of the agar block and 10 days old of C. *acutatum* was grown on the other side. The agar blocks were placed in a sterile object glass and covered with sterile cover glass, and then the object glass placed in sterile petridish. Observations were carried out under photomicrograph multi eyepiece (Zeiss Axiocam) at 4 to 6 days of incubation.

2.5. Statistical Analysis

All treatments arranged in a completely randomized design with three replication. Data of antibiosis test, formation of volatile compounds and chitinolytic activity were analyzed with SPSS software (version 16.0 for Windows) and subsequently tested by Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

Previous studies showed that three isolates of *A. pullulans i.e*. Dmg 11 DEP, Dmg 29 DEP, and Dmg 30 DEP was potential as antagonist for anthracnose disease caused by *C. acutatum*. Inhibitory effect on anthracnose disease by these three isolates was 78.04%, 82.87% and 79.65%, respectively [34].

Antibiosis test showed that three isolates of *A. pullulans* did not produce clear zone (Table 1). The three isolates of yeast-like fungus were not significantly affecting relative inhibition values.

Antibiosis has an important role in the mechanism of biological control by microorganisms producing antibiotic. Antibiotic is mostly produced by antagonistic bacteria. Yeast-like fungi has never been reported to produce antibiotics. However, yeasts-like fungi produced antibiotics in a broad sense, which consists of simple compounds known as killer toxin. *Williopsis mrakii, Saccharomyces cerevisiae* and *Pichia anomala* were able to produce antimycotic killer toxin [16]. The ability of yeast-like fungus to inhibit the growth of witches' broom caused by *Moniliophthora perniciosa* by producing killer toxin has been reported [4]. Killer toxin was also reported for the first time produced by *Torulaspora globosa* [23].

In this study, *A. pullulans* was suspected to produce killer toxin only at low concentrations that do not affect the relative inhibition values of *C. acutatum*. Killer toxin is different from antibiotics produced by bacteria which might affect human. Therefore, yeasts-like fungus are relatively safe to use for plant disease control particularly for postharvest products.

Three isolates of *A. pullulans* produced volatile compounds. This was indicated by growth inhibition of *C. acutatum* colonies by the isolates. Growth inhibition of *C. acutatum* by *A. pullulans* occured without physical

contact among the microbes, therefore it was concluded that the inhibition was caused by the volatile compounds produced by *A. pullulans*. The isolates of *A. pullulans* affected on relative inhibition of *C. acutatum* with a range between 32.55% to 45.54% (Table 1).

Table 1: Clear zone, growth inhibition of *C. acutatum*, and index chitinolytic by the three isolates of *A. pullulans* at 10 days after treatment

Isolates of *A. pullulans*	Clear zone	Growth inhibition of *C. acutatum* (%) by VOCs	Chitinolytic index
Control	-	0.00 c	0.00 d
Dmg11DEP	-	32.55 ab	1.10 ab
Dmg29DEP	-	45.42 a	0.00 d
Dmg30DEP	-	37.52 ab	0.38 cd

Note : Clear zone, growth inhibition, chitinolytic index was measured by antibiosis test, volatile compound test, and chitinolytic test, relatively; - = not forming clear zone, values in the same column followed by the same letter were not significantly different by Duncan's Multiple Range Test ($P < 0.05$)

Further test showed that the isolates of *A. pullulans* had significantly different ability in inhibiting the mycelial growth of *C. acutatum* compared to control, i.e. by forming volatile compounds. This result indicated that three isolates of *A. pullulans* had antibiosis mechanism. However, the relative inhibition among the three isolates were not significantly different (Table 1). The relative inhibition by the three isolates were high compared with other 19 yeast isolates which were isolated from the surface of chili leaf and fruit (Data are not shown).

The volatile organic compounds (VOCs) composed of simple hydrocarbons, heterocycles, aldehydes, ketones, alcohols, phenols, thioalcohols, thioesters and their derivatives, derivative of benzene and cyclohexanes [6]. VOCs have low molecular weight (less than 300 Da), low polarity and high vapor pressure [7]. Component of these volatile compounds have important roles in biocontrol of plant pathogens.

Both bacteria and yeasts produce ketones, dimethyl disulphide and dimethyl trisulphide which control sapstain fungi [1]. Yeasts produce VOCs on the surface of the cell wall [25]. Yeast-like fungus, *Tilletiopsis pallescens*, produces fatty acid ester, the antifungal compound, which inhibited the growth of mildew fungi and other competitor when they were applied on leaves [14]. *Candida intermedia* strains C410 produces 49 types of volatile compounds (esters, alcohols, alkenes, alkanes, alkynes, organic acids, ketones, and aldehydes) which reduced the disease incidence and intensity of fruit rot caused by *Botrytis cinerea*. Two of the 49 volatiles i.e. 1,3,5,7- cyclooctatetraene and 3-methyl-1-butanol were obtained abundantly [31].

VOCs produced by *A. pullulans* strain L1 and L8 play important antagonistic activity on five postharvest pathogens i.e. *B. cinerea, C. acutatum, Penicillium expansum, Penicillium digitatum* and *Penicillium italicum*

[5]. *A. pullulans* L1 and L8 produced alcohols i.e. 2-phenyl, 1-butanol-3-methyl, 1-butanol-2-methyl and 1-propanol-2-methyl at the first 96 hours of growth [5]. VOCs active to all tested pathogens was 1-propanol-2-methyl. The EC50 was more than 0,8 ml ml-1. The most active VOCs was alcohol-2-phenethyl with the EC50 lower than 0,8 ml ml-1, except for *C.acutatum* 1.97 ml ml-1 [5]. The result of this study was another evidences that *A. pullulans* produced VOCs to inhibit the growth of *C. acutatum*. The compounds produced were assumed as simple compound of alcohols.

VOCs produced by the three isolates of *A. pullulans*, were toxic on *C. acutatum*. The isolates were able to greatly reduce the disease incidence of anthracnose on chili caused by *C. acutatum* (78.04 to 82.87%). VOCs produced by *A. pullulans* significantly controlled pathogen infection by inhibiting the conidia germination and reducing lesions [5].

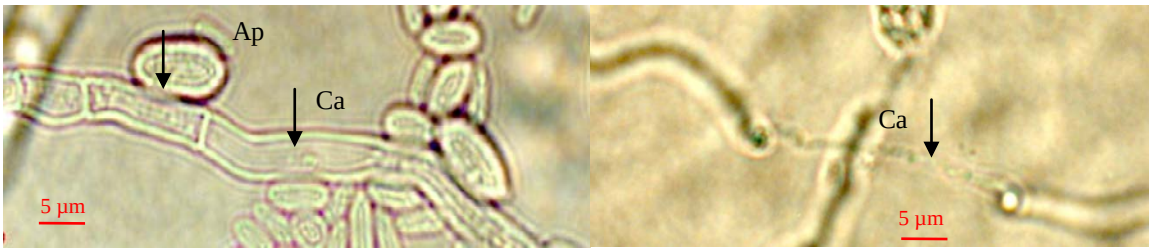
Chitinolytic activity test showed that all isolates of *A. pullulans* produced chitinolytic enzyme, except Dmg 29 DEP (Table 1). The potency of the chitinolytic activity was shown by clear zone around the colony, which indicated the ability of this yeast-like fungus to degrade chitin on the growth media. Based on the differences in the chitinolytic activity, the three isolates might be a different strain of *A. pullulans*.

In the previous studies, *A. pullulans* isolates Dmg 11 DEP and Dmg 30 DEP showed high inhibition ability which in turn caused suppression on disease incidence [34]. This high inhibition ability of *A. pullulans* might be caused by volatile compounds and chitinolytic activity. Chitinase is able to degrade cell wall of fungi and inhibit the growth of the hyphal tips [15]. Chitinolytic enzymes produced by *A. pullulans* (isolates Dmg 11 DEP and Dmg 30 DEP) disrupted the hyphal growth of *C. acutatum* and caused lysis of cell wall, so the fungus would not be able to infect the chili fruit. Chitinolytic activity is an important mechanism in the biological control. Antagonistic activity in the biological control of plant pathogens was based on extracellular secretion of lytic enzymes [20]. Chitinolytic activity and other lytic enzymes involved in the mechanism of yeasts biocontrol [13].

Yeast spesies of *Bulleromyces albus* and *Pichia guilliermondii* were reported to produce chitinolytic enzymes [12]. Yeast-like fungus *Tilletiopsis pallescens* ATCC96155 was reported to produce β -1,3-glucanase and chitinase in controlling powdery mildew [14]. *A. pullulans* was known to produce various enzymes such as amylase, protease, lipase, cellulose, xylanase, glucanase, and others [30], but report on chitinase production by *A. pullulans* is still limited. Extracellular activity of exochitinase and β-1,3-glucanase of *A. pullulans* was reported involved in antagonistic activity against several postharvest pathogens such as *Botrytis cinerea*, *Penicillium expansum*, *Rhizopus stolonifer* and *Aspergillus niger* [29]. Such enzyme was detected in the primary site of postharvest fungal pathogens penetration in wounds on apples.

Microscopic observations showed that *A. pullulans* of isolates Dmg 11 DEP and Dmg 30 DEP causes lysis on *C. acutatum* cell wall. Such lysis indicated by the destruction of hyphae wall and whole hyphae (Figure 1). This was related to the presence of chitinolytic activity of the yeast-like fungi isolates. *A. pullulans* isolate Dmg 11 DEP showed the mode of parasitism. Parasitism known with empty hyphae without contents caused by the nutrients-taking process of yeast-like fungi. Observations by light microscopy showed that cells of *A. pullulans*

was in contact on the surface of the hyphae of *C. acutatum*, lyse occurs in the cell walls of hyphae and taking nutrients from the hyphae.



(a) (b)

Figure 1: Hyphae of *C. acutatum* were parasite by *A. pullulans* isolates Dmg 11 DEP (a), Dmg 30 DEP (b) (Ap: *A. pullulans* cell, Ca: *C. acutatum* hyphae) (magnification 1000x)

Plant pathogenic fungus has been known to be infected by mycoparasitic fungi, by the mechanism called hyperparasitism [36]. Mycoparasitic take nutrients from its host through 3 ways i.e. producing haustoria in the host cell, degradation of the host cell wall at the contact site between the parasite and its host (in this case, the nucleus of the host cell walls migrate through the hole into specific basal cells of the parasite), and producing contacts cells at the end of mycoparasitic hyphae, called as cell buffer [22]. *Cladosporium cladosporioides* was able to parasite *Puccinia striiformis* f. sp.*tritici* (Pst), causing uredinial discoloration and reduction of urediniospora production. Observation with SEM showed that *C. cladosporioides* spores get in contact at the surface of urediospora, germination, and germ tube directly penetrates urediospora. After successfully invade and colonize urediospora, causing urediospora collapse and lose their viability [17]. Parasitism can be observed through the fixation of pathogen mycelium. *Rhodotorula glutinis* was able to fix pathogen mycelium at 12 h after treatment and remained constant at 24 and 48 h after treatment [28]. *A. pullulans* was reported did not have the ability to fix the pathogen mycelium [29]. Some studies suggest that functional proteins of antagonist and pathogen involved in the adhesion process [36]. Mode of parasitism is also shown through the secretion of hydrolytic enzymes antagonist agents. *Rhodotorula mucilaginosa* 2 has high hydrolytic enzyme activity (β-1,3-glucanase, nagase and chitinase) to inhibit *C. gloeosporioides* [10].

4. Conclusion

The results of this study indicated that the mode of action of yeast-like fungus, *A. pullulans*, in controlling anthracnose disease (*C. acutatum*) was through the formation of volatile compounds, chitinolytic activity, and hyperparasitism. The three isolates of *A. pullulans* may involve other modes of action to inhibit chili anthracnose by *C. acutatum*, such as formation of another hydrolysis enzyme, induce resistance and competition for space and nutrients. This might need further investigation.

Acknowledgements

The authors acknowledge the financial support of the Lembaga Penelitian dan Pengabdian kepada Masyarakat (Institute of Research and Community Outreach), Universitas Padjadjaran, under research grant scheme

Penelitian Hibah Kompetitif.

References

[1] A. Bruce, S. Verrall, C. A. Hackett, and R. E. Wheatley. "Identification of volatile organic compounds (VOCs) from bacteria and yeast causing growth inhibition of sapstain fungi." *Holzforschung*, Vol. 58, pp. 193-198, Jun 2005.

[2] A. Chanchaichaovivat, P. Ruenwongsa, and B. Panijpan. (2007, Jun.) "Screening and identification of yeast strains from fruits and vegetables: Potential for biological control of postharvest chilli anthracnose (*Colletotrichum capsici*)." *Biological Control*. [On-line]. 42, pp. 326-335. Available: www.sciencedirect.com. [May, 24, 2013].

[3] A. Chanchaichaovivat, P. Ruenwongsa, and B. Panijpan. "Putative modes of action of *Pichia guilliermondii* strain R13 in controlling chili anthracnose after harvest." *Biological Control*, Vol. 47, pp. 207-215, 2008.

[4] A.d.S.Cabral, P.M.B. de Carvalho, T. Pinotti, A.N. Hagler, L.C.S. Mendonca-Hagler, A. Macrae. "Killer yeast inhibit the growth of the phytopathogen *Moniliophthora perniciosa*, the causal agent of witches'broom disease." *Brazilian Journal of Microbiology*, Vol. 40, pp. 108-110, Feb. 2009.

[5] A.D. Francesco, L. Ugolini, L. Lazzeri, and M. Mari. "Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens." *Biological Control*, Vol. 81, pp. 8-14, Feb. 2014.

[6] A. Korpi, J. Jarnberg, and A.L. Pasanen. "Microbial volatile organic compounds." *Critical Reviews in Toxicology*, Vol. 39, pp. 139-193, 2009.

[7] A. Vespermann, M. Kai, and B. Piechulla. "Rhizobacterial Volatiles Affect the Growth of Fungi and *Arabidopsis thaliana*." *Applied and environmental microbiology*, Vol. 73, pp. 5639-5641, Sept. 2007.

[8] AVRDC. "Evaluation of phenotypic and moleculer criteria for the identification of *Colletotricum* spesies causing pepper anthracnose in Taiwan". In *AVRDC Report* 2003. AVRDC, Taiwan, 2003, pp. 58-59.

[9] B. Stankova, J. Vichova, and R. Pokorny. "Virulence of *Colletotrichum acutatum* isolates to several host plants." *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis*, Vol. 59, pp. 161-170, 2011.

[10] C. G. Magallon-Andalon, G. Luna-Solano, J. A. Ragazzo-Sanchez, M. Calderon-Santoyo. "Parasitism and Substrate Competitions Effect of Antagonistic Yeasts for Biocontrol of *Colletotrichum gloeosporioides* in papaya (*Carica papaya* L.) var Maradol." *Mexican Journal of Scientific Research*, Vol. 1(1), pp. 2-9, 2012.

[11] C. Srinivas, S.R. Niranjana, L. Praveen Kumar, S. Chandra Nayaka, and H.S. Shetty. "Effect of fungicides and bioagents against *Collectotrichum capsici* on seed quality of chilli." *Indian Phytopathology*, Vol. 59, pp. 62-

67, 2006.

[12] D. Sugiprihatini, S. Wiyono, and Widodo. (2011, Dec.). "Selection of yeasts antagonists as biocontrol agent of mango fruit rot caused by *Botryodiplodia theobromae*." *Microbiology Indonesia*. [On-line]. 5(4), pp. 154-159. Available: www.permi.or.id/journal/index.php/mionline [Dec. 17, 2014] .

[13] E.I. Masih, B. Paul. "Secretion of ß-1,3 glucanases by the yeast and Its possible role in biocontrol of causing grey mold disease of the grapevine." *Current Microbiology*, vol. 44, pp. 391-395, 2002.

[14] E. J. Urquhart and Z. K. Punja. "Hydrolytic enzymes and antifungal compounds produced by *Tilletiopsis* species, phyllosphere yeasts that are antagonists of powdery mildew fungi." *Canadian Journal Microbiology*, Vol. 48(3), pp. 219-29, Mar., 2002.

[15] F. Mauch, B. Mauch-Mani, and T. Boller. "Antifungal hydrolases in pea tissue: II. Inhibition of fungal growth by combination of chitinase and β-1,3-glucanase." *Plant Physiology*, Vol. 88, pp. 936-942, 1988.

[16] G. M. Walker, A. H. Mcleod, and V. J. Hodgson. (2006, Jan.). "Interactions between killer yeasts and pathogenic fungi." *FEMS Microbiology Letters*. [On-line]. 127(3), pp. 213-222. Available: www.onlinelibrary.wiley.com/doi/10.1111 [Feb. 22, 2014].

[17] G. Zhan, Y. Tian, F. Wang, X. Chen, J. Guo, M. Jiao, L. Huang, Z. Kang. (2014, Nov.). "A Novel Fungal Hyperparasite of *Puccinia striiformis* f. sp. *tritici*, the Causal Agent of Wheat Stripe Rust." *PLoS ONE*. [On-line]. Vol. 9(11), pp. e111484. Available: www.plosone.org [Dec. 18, 2014].

[18] L. D. Arnold and N. A. Solomon. "Manual of Industrial Microbiology and Biotechnology." *Washington: American Society for Microbiology*, 1986.

[19] L. T. K. Oanh, V. Korpraditskul, C. Rattanakreetakul, and S. Wasee. "Influence of biotic and chemical plant inducers on resistance of chili to anthracnose." *Kasetsart Journal (Natural Science)*, Vol. 40, pp. 39-48, 2006.

[20] M. Carstens, M.A. Vivier, P. Van Rensburg, and I. S. Pretorius. "Overexpression, secretion and antifungal activity of the *Saccharomyces cerevisiae* chitinase." *Annals of Microbiology*, Vol. 53, pp. 15-28, 2003.

[21] M. C. Shurtleff and C. W. Averre. "The Plant Diseases." Minnesota: *The American Phytopatological Society* St Paul, APS Press, 1997.

[22] M. G. Boosalis. " Hyperparasitism." *Annual Review of Phytopathology*, Vol. 2, pp. 363-376, 1964.

[23] M.M. Rosa-Magri, S.M. Tauk-Tornisielo, and S.R. Ceccato-Antonini. "Bioprospection of Yeasts as Biocontrol Agents Against Phytopathogenic Molds." *Brazilian Archives of Biology and Technology*, Vol. 54, pp. 1-5, Feb. 2011.

[24] M. Wisniewski, C. Wilson, S. Droby, E. Chalutz, A. El Ghaouth, and C. Stevens. "Postharvest Biocontrol: New Concepts and Applications," in *Biological Control*: a Global Perspective, C. Vincent, M. S. Goettel, G. Lazarovits, Ed. Wallingford (UK):CAB International, 2007, pp. 262-273.

[25] N. Jiménez-Moreno and C. Ancín-Azpilicueta. "Sorption of volatile phenols by yeast cell walls." *International Journal of Wine Research*, Vol. 1, pp. 11-18, May 2009.

[26] N. Nantawanit, A. Chanchaichaovivat, B. Panijpan, and P. Ruenwongsa. "Induction of defense response against *Colletotrichum capsici* in chili fruit by the yeast Pichia guilliermondii strain R13." *Biological Control*, Vol. 52, pp. 145-152, 2010.

[27] P. Pakdeevaraporn, S. Wasee, P.W.J. Taylor, and O. Mongkolporn. (2005, Apr.) "Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*." *Plant Breeding*. [On-line]. 124(2), pp. 206-208. Available: www. onlinelibrary.wiley.com/doi/10.1111/j.1439-0523.2004.01065.x [Apr. 18, 2013].

[28] R. Castoria, F. De Curtis, G. Lima. V. De Cicco. "β-1,3-Glucanase activity of two saprophytic yeasts and possible mode of action against postharvest diseases". *Postharvest Biology and Technology*, Vol. 12, pp. 293–300, 1997.

[29] R. Castoria, F.D. Curtiis, G. Lima, L. Caputo, S. Pacifico, and V. D. Cicco. "*Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: study on its modes of action." *Postharvest Biology and Technology*. Vol. 22 (1), pp. 7-17, March 2001.

[30] R. Gaur, R. Singh, M. Gupta, and M. K. Gaur. (2010, Nov.). "Review *Aureobasidium pullulans*, an economically important polymorphic yeast with special reference to pullulan." *African Journal of Biotechnology*. [On-line]. 9(47), pp. 7989-7997. Available: www.academicjournals.org/AJB [Dec. 18, 2014].

[31] R. Huang, G. Q. Li, J. Zhang, L. Yang, H. J. Che, D. H. Jiang, and H. C. Huang. "Disease Control and Pest Management Control of Postharvest Botrytis Fruit Rot of Strawberry by Volatile Organic Compounds of *Candida intermedia*." *Phytopathology*, Vol. 101, pp. 859-869, Jul. 2011.

[32] R.R. Sharma, D. Singh, and R. Singh. "Biological Control of Postharvest Diseases of Fruits and Vegetables by Microbial Antagonists: A review." *Biological Control*, Vol. 50, pp. 205-221, 2009.

[33] S. Droby, and E. Chalutz. "Mode of action of biocontrol agents of postharvest disease, in Biological Control of Postharvest Diseases of Fruits and Vegetables-Theory and Practice," C.L. Wilson, M.E. Wisniewski, Ed. *Boca Raton*: CRC Press, 1994, pp. 63-75.

[34] S. Hartati, S. Wiyono, S. H. Hidayat, M. S. Sinaga. "Selection of Epiphytic Yeasts as Antagonist of Anthracnose on Chili." *Jurnal Hortikultura*, Vol. 24 (3), pp. 258-265, 2014. [Inpress].

[35] S.K. Bencheqroun, M. Bajji, S. Massart, M. Labhilili, S. Jaafari, and H. Jijakli. "In vitro and in situ study of postharvest apple blue mold biocontrol by *Aureobasidium pullulans*: evidence for the involvement of competition for nutrients." *Postharvest Biology and Technology*, Vol. 46, pp. 128–135, 2007.

[36] T. Hijwegen, H. Buchenauer. "Isolation and identification of hyperparasitic fungi associated with Erysiphaceae." *Netherlands Journal of Plant Pathology*, Vol. 90, pp. 79-83, 1984.