academicJournals

Vol. 13(15), pp. 1631-1637, 9 April, 2014
DOI: 10.5897/AJB11.3687
Article Number: 68411E343915
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Utilization of chitinolytic bacterial isolates to control anthracnose of cocoa leaf caused by *Colletotrichum gloeosporioides*

Dwi Suryanto[1]*, Sri Wahyuni[2], Edy Batara Mulya Siregar[3] and Erman Munir[2]

[1]Research Center for Environment and Population Issues, Universitas Sumatera Utara, Jln. Perpustakaan No. 5, Medan 20155.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia 20155.
[3]Department of Forestry, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia 20155.

Received 18 November, 2011; Accepted 25 July, 2013

***Colletotrichum* spp. are causal agents of anthracnose in many plant species. Biological control of *Colletotrichum* spp. utilizing bacterial isolates and fungi has been reported. However, chitinolytic bacterial isolate utilization to control anthracnose of cocoa leaf has not seemingly been studied yet. In this study, we used chitinolytic bacterial isolates to reduce anthracnose severity and incidence caused by *Colletotrichum gloeosporioides*. Identification of the chitinolytic isolates was conducted for their morphological and biochemical traits, and the sequencing of 16S rRNA was to know their related species. Assay of antagonistic bacterial chitinolytic to *C. gloeosporioides* was conducted in minimum salt medium agar with 2% colloidal chitin as sole carbon source. To examine ability of the chitinolytic isolates in reducing anthracnose severity and incidence, cocoa leaves were treated with the isolates prior infestation of the conidia. Identification of 16S rRNA showed that KR05, LK08, BK13, BK15 and BK17 isolates were *Enterobacter* sp., *Enterobacter cloacae*, *Bacillus* sp., *Enterobacter* sp., and *Bacillus* sp., respectively. All chitinolytic isolates inhibited growth of *C. gloeosporioides in vitro* to some extent. Microscopic studies showed morphological abnormalities of *C. gloeosporioides* hyphae that is, broken, lytic, rolled, twisted, curled and abnormal branching of hyphae as a result of antagonistic mechanism caused by the chitinolytic isolates. All chitinolytic isolates were able to reduce anthracnose severity and incidence on cocoa leaves from 0.8 to 3.2% and 4 to 12%, respectively.**

Key words: Anthracnose, biological control, chitinolytic bacteria, *C. gloeosporioides*, cocoa.

INTRODUCTION

The genus *Colletotrichum* represents an economically important group of fungal plant pathogens and is recorded from approximately 2.200 plant host species (Farr and Rossman, 2009). As plant pathogens *Colletotrichum* spp. are the principal cause of anthracnose as well as pre- and postharvest fruit rots, damping-off and blossom and seedling blight diseases (Bailey and Jeger, 1992). One species, *C. gloeosporioides*, causes

*Corresponding author. E-mail: d.suryanto@lycos.com.

leaf spots in plantation plant such as cacao (Rojas et al., 2010; Yee and Sariah, 1993) and para rubber tree (Adekunle and Ogbebor, 2009), and also attacks other economic plants/fruits such as yellow passion fruit (Anaruma et al., 2010), mango (Nelson, 2008), avocado, and almond (Freeman et al., 1996).

Control of this disease has been largely dependent on chemical fungicides inhibiting vegetative pathogen growth. However, the chemical practices to overcome plant disease problem have adverse environmental effects affecting non-target organisms and causing health hazards to humans, besides demanding high costs (Ningthoujam et al., 2009). Biological control of plant pathogens has been shown to have potential to control over many diseases in plant. Biological control may operate through antibiosis, competition, predation or parasitism (Alabouvette et al., 2006; Ozbay and Newman, 2004). As a general method, biological control using antagonistic microorganisms has been successfully demonstrated in a number of plant species. Antagonistic microorganisms, by their interactions with various plant pathogens, play a major role in microbial equilibrium and serve as powerful agents for the biological control of diseases (Ozbay and Newman, 2004; Alabouvette et al., 2006). The lytic activity of bacteria is one of a number of mechanisms that has been implicated in biocontrol (Alabouvette et al., 2006). Recently, the use of naturally occurring bacteria (Sangeetha et al., 2010; Akinbode and Ikotun, 2008; Mahadtanapuk et al., 2007) and antagonistic fungi (Adekunle and Ogbebor, 2009; Akinbode and Ikotun, 2008; Shovan et al., 2008) for biocontrol of *Colletotrichum* spp. has been proposed.

A number of fungi are particularly susceptible to be degraded by microorganisms (Kim et al., 2008). Mycolytic enzymes producing by microorganisms such as chitinase have great potential in solving such problems (Anitha and Rabeeth, 2010; Patel et al., 2007; Gohel et al., 2006). For this purpose of employing such chitinolytic bacterial isolates for biological control of anthracnose in cocoa leaves caused by *C. gloeosporioides*, assay on their ability to inhibit the fungal growth on agar and on cocoa leaves were conducted. Abnormal hyphae of *C. gloeosporioides* as a result of antagonistic assay were also observed. To our knowledge, possible utilization of chitinolytic bacteria in controlling *C. gloeosporioides* causal agent of anthracnose in cocoa leaves has not been reported.

MATERIALS AND METHODS

Chitinolytic bacterial isolates

Bacterial isolates KR05, LK08, BK13, BK15 and BK17 used in this study were collection from Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. These isolates were not morphologically and biochemically characterized except that of KR05 and LK08 (Suryanto et al., 2011). The isolates were kept at 30°C in a modified salt medium (0.7 g K_2HPO_4, 0.3 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 0.001 g $ZnSO_4$, and 0.001 g $MnCl_2$ in 1.000 ml) containing 2% (w/v) chitin colloidal (MSMC) agar, with a pH of 6.8.

Examination of cell morphology and biochemical properties

Cell shape and Gram staining were evaluated using a microscope, while colony shape was observed directly. Motility was observed using semi-solid medium sulfide indole motility. Biochemical properties were examined including gelatin test using gelatin nutrient medium, citrate test using Simons citrate agar, catalase test using 3% H_2O_2 solution, and starch metabolism using starch agar.

16S rRNA gene of chitinolytic bacterial isolate identification

Bacterial DNA was prepared following the manufacturer's protocol of Wizard® Genomic DNA Purification Kit (Promega). The 16S-rRNA genes were polymerase chain reaction (PCR)-amplified using specific primers of 63f and 1387r (Marchesi et al., 1998) from the bacterial DNA. PCR reactions were performed in thermal cycler using the following conditions: initial denaturation for 1 min at 95°C; annealing (1 min at 50°C), and extension (1.5 min at 72°C); prolong the extension (5 min at 72°C). PCR product was purified using QIAquick PCR Purification Kit Protocol (Qiagen). Part of the genes of 16S-rRNA was pre-sequenced using thermo cycler. Pre-sequencing reaction products was precipitated using manufacturer's protocol of Pellet Paint® NF Co-Precipitant (Novagen). The sequencing reaction was run on ABI PRISM® 310 Genetic Analyzer (Applied Biosystem). The sequence was analyzed using Blast (http://blast.ncbi.nlm.nih.gov.).

Isolation and reisolation of *C. gloeosporioides* of cocoa leaves

Cocoa leaves showing symptoms of leaf spot disease were collected from mature cocoa trees. For re-isolation after conidia infestation, leaves with symptom were taken. Pieces of the infected leaf, 3 x 3 mm were surface disinfected for few seconds in 70% alcohol and in 1% aqueous sodium hypochlorite for 5 min, and then rinsed thoroughly with sterile distilled water (SDW). Surface-disinfected leaves were grown on potato dextrose agar (PDA) for 72 h at ±25 to 30°C. Growing fungal colonies were then purified. Culture of the isolate was sub-cultured onto and maintained on PDA throughout this study. Identification morphological characteristics such as colony, mycelia as well as shape and size of conidia under light microscopy observation were carried out.

Assay of bacterial-fungal antagonism

The ability of the chitinolytic bacterial isolates to inhibit *C. gloeosporioides* growth was conducted *in vitro*. Fungal culture was grown at the center of MSMC agar. Two pieces of paper discs immersed with ≈ 10^8 cells/ml of bacterial suspension were placed in the opposite direction about 3.5 cm from the fungal culture. Culture was incubated at ±25 to 30°C. Inhibitory activity was determined based on the inhibition zone formed around bacterial colonies on medium MSMC. Inhibition zone was measured from three to seven days of incubation as the radius of the normal fungal growth subtracted the radius of the inhibited fungal growth. The assay was replicated four times.

Observation of hyphal abnormality

Inhibited hyphae of *C. gloeosporioides* of antagonistic assay were cut by 1 cm^2. The hyphae were examined under light microscope and compared with normal ones.

Table 1. Identification and characterization of the chitinolytic bacterial isolates.

Character	Bacterial isolate				
	BK13	BK15	BK17	KR05*	LK08*
Colony shape	Entire, flat	Irregular, flat	Entire, flat		
Colony color	White	White	Yellowish		
Cell shape	Rod	Rod	Rod		
Biochemical traits					
Gram	+	-	+		
Motility	+	+	+		
Gelatine	-	-	-		
Citrate	+	+	+		
Catalase	+	+	+		
Starch	+	-	+		
16S rRNA gene partial	*Bacillus* sp.	*Enterobacter* sp.	*Bacillus* sp.	*Enterobacter* sp.	*Enterobacter cloacae*

*Morphological and biochemical characterization was previously described (Suryanto et al., 2011).

Assay of chitinolytic bacterial isolates to *C. gloeosporioides* on cocoa leaves

Chitinolytic bacterial isolates were sub-cultured in nutrient agar for two days. Bacterial suspension was prepared in 0.09% NaCl with cell density of ≈ 10^8 cells/ml. The isolated *C. gloeosporioides* was sub-cultured on PDA. Fungal colony was removed and put into 10 ml of 0.09% NaCl. Conidia were harvested by filtering them through muslin cloth to remove mycelia. Conidia concentration was then determined using a hemocytometer and adjusted to ≈ $2x10^5$ conidia/ml. All microbial cultures were incubated at ±25 to 30°C.

10 ml of chitinolytic bacterial suspension was thoroughly spray-inoculated into cocoa leaf surface of three-month old trees. Conidia suspension was applied after bacterial overnight- application. The leaf was then wrapped with transparent wrapping plastic. (+) Control was treated similarly but without chitinolytic bacterial inoculation, while (-) control had no conidia and chitinolytic bacterial inoculation. The work was replicated five times.

Observation on disease incidence and disease severity

Observation was conducted after 1 week of application every week for 1 month on 5 upper leaves below 2 tip-leaves. The Disease Incidence (DI) was measured using this following equation (Cooke, 2006):

$$DI = \frac{\text{Number of infected plant units}}{\text{Total number of plant units assessed}} \times 100\%$$

Meanwhile, the disease severity (DS) was determined according to alternative rating scale proposed by Bowen (2007) in which scale 0 = no symptom, scale 1 = 0 to 20% disease severity range, scale 2 = 20 to 40%, scale 3 = 40 to 60%, scale 4 = 60 to 80% and scale 5 = 80 to 100%, respectively. DS was measured using the equation proposed by Kranz (1988) as follows:

$$DS = \frac{\Sigma (axb)}{NZ} \times 100\%$$

Σ (axb) = Sum of the symptomatic plant and their corresponding score scale, N = total number of sampled plant and Z = highest score scale.

RESULTS

Characterization and identification of chitinolytic bacterial isolates

Identification of chitinolytic bacteria showed that KR05, LK08 and BK15 and except that of colony color BK13 and BK17 possessed similar characteristics. This indicated different species of bacteria (Table 1). Two isolates were Gram-positive, while three was Gram-negative. Chitinolytic bacteria spread among Gram-negative and Gram -positive (Anitha and Rabeeth, 2010; Singh et al., 2008; Metcalfe et al., 2002; Folders et al., 2001).

Identification of part of 16S rRNA gene revealed that KR05, LK08, BK13, BK15 and BK17 were closed to *Enterobacter* sp., *Enterobacter cloacae*, *Bacillus* sp., *Enterobacter* sp., and *Bacillus* sp. with similarity (forward-reverse) of 95 to 99, 94 to 98, 95 to 86, 96 to 99 and 97 to 98%, respectively.

Isolation and reisolation of *C. gloeosporioides* of cocoa leaf

Manifestation of anthracnose caused by *C. gloeosporioides* on cocoa leaf was shown as brownish lesions and chlorotic haloes symptoms (Figure 1a). These lesions had brown centers and then coalesced to rot. Identification of the isolate showed that the fungus had whitish colony (Figure 1b), with septate hyphae, and capsule-like conidia containing one cell (Figure 1c.). Reisolation of infected cocoa leaf showing anthracnose symptom revealed the same fungus.

Assay of bacterial-fungal antagonism

To know chitinolytic isolate ability to inhibit growth of *C.*

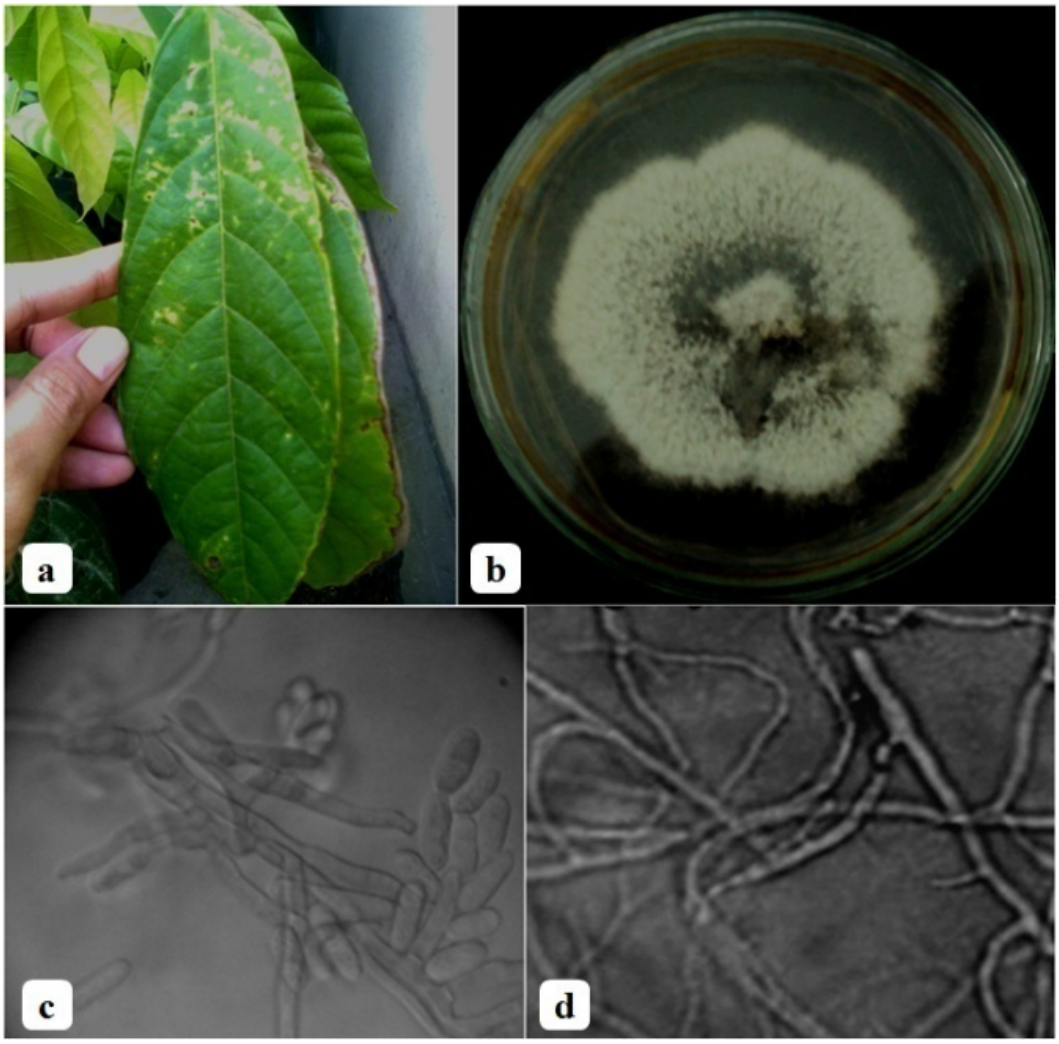

Figure 1. (a) Disease symptom on treated leaf. **(b)** Colony on PDA. **(c)** Conidia and hyphae. **(d)** Abnormal hyphae.

gloeosporioides hyphae, examination was conducted by growing chitinolytic isolates next to the fungi in chitin containing media. All isolates showed ability to inhibit growth of *C. gloeosporioides* hyphae to some extent (Table 2). Inhibition zone increased during incubation time, and was observed on 3-days of incubation and continued to 7-days of incubation, or more (Kim et al., 2008). This indicated that chitinase was still produced and diffused to the media to degrade fungal hyphae. The lytic activity of bacteria is one of a number of mechanisms that has been implicated in biocontrol (Anitha and Rabeeth et al., 2010; Gohel et al., 2006).

Observation of hyphal abnormality

Microscopic observation of fungal hyphae after the antagonistic assay showed the occurrence of abnormality hyphae. Abnormal hyphae were marked with broken, lytic, rolled, twisted, curled and abnormal branching of hyphae (Figure 2). Getha and Vikineswary (2002) reported that hyphal distortion like lytic of hyphal ends, swollen hyphae, abnormal branching of hyphae and the formation of hyphal protuberances is observed after contacted *Fusarium oxysporum* f.sp. *cubense* hyphae with *Streptomyces violaceusniger* strain G10.

Control of *C. gloeosporioides* on cocoa leaves

Efficacy of chitinolytic isolates to reduce infestation of *C. gloeosporioides* was conducted on cocoa leaves. All chitinolytic isolates showed reduce disease severity (Table 3) and disease incidence (Table 4) compared to that of (+) control. Unsterilized leaves of (-) control of cocoa leaves might result in contamination o f the leaves

Table 2. Inhibition zone of antagonistic assay of chitinolytic bacteria to *C. gloeosporioides.*

Isolate	Inhibition zone (mm) of days				
	3	4	5	6	7
KR05	6.0	6.2	8.7	17.1	28.9
LK08	10.0	9.9	12.4	18.3	30.5
BK13	6.1	6.5	9.6	18.8	31.8
BK15	6.1	6.4	8.4	17.7	28.7
BK17	6.1	6.1	9.5	18.5	29.9

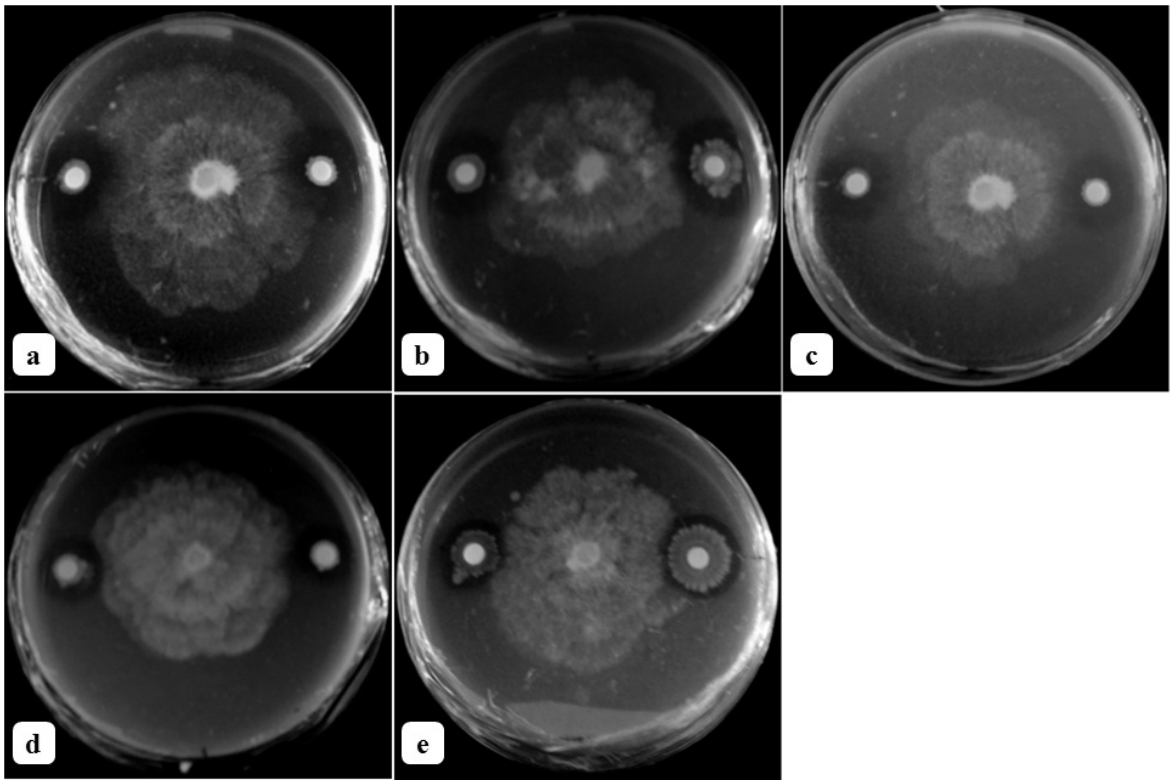

Figure 2. Antagonistic assay of **(a)** KR05, **(b)** LK08, **(c)** BK13, **(d)** BK15 and **(e)** BK17 on *C. gloeosporioides in vitro.*

to *C. gloeosporioides*. The fungus may spread by strong wind currents that dislodge spores from agitated leaves, by nursery workers handling diseased plants, and by movement of slugs or other pests (Uchida and Kadooka, 1997). Guyot et al. (2005) reported that the conidia of *C. gloeosporioides* disperse for several tens of meters in rubber tree. However, disease severity and disease incidence were still weak rather than of (+) control, in which fungal infestation was purposely inoculated. The maximum reduction of disease severity and disease incidence was shown by LK08 and BK17 by 0.8%, followed by BK13 and BK15 by 0.8 to 1.6 and 2.4 to 3.2%, respectively after eight weeks of the conidia infestation. The highest disease severity and disease incidence increased to 16 and 20%, respectively in (+) control, in which the conidia were infested without chitinolytic bacterial inoculation.

DISCUSSION

Biological control using microorganism has been studied intensively since not many alternatives for control are available. Various microbial antagonists have been investigated as potential antifungal biocontrol agents of plant diseases. Certain strain of microorganism has been reported to succesfully suppress the growth of plant pathogen (Adekunle and Ogbebor, 2009; Kim et al., 2008; Mahadtanapuk et al., 2007; Soytong et al., 2005).

Our study on utilization of chitinolytic bacterial isolates

Table 3. Disease severity on cocoa leaf caused by *C. gloeosporioides.*

Treatment	Disease severity (%)/week			
	1	2	3	4
KR05	1.6	1.6	1.6	2.4
LK08	0.8	0.8	0.8	0.8
BK13	0.8	0.8	0.8	1.6
BK15	2.4	2.4	3.2	3.2
BK17	0.8	0.8	0.8	0.8
(+) Control	15.2	15.2	15.2	16
(-) Control	3.2	3.2	3.2	3.2

Table 4. Disease incidence on cocoa leaf caused by *C. gloeosporioides.*

Treatment	Disease incidence (%)/week			
	1	2	3	4
KR05	8	8	8	12
LK08	4	4	4	4
BK13	4	4	4	8
BK15	8	8	8	8
BK17	4	4	4	8
(+) control	20	20	20	20
(-) control	8	8	8	8

as potential biological control agent to reduce disease severity and disease incidence caused by *C. gloeosporioides* on cocoa leaves showed that the isolates were capable of declining infestation of the fungi. Species in the genus *Colletotrichum* are common in the environment and frequently parasitize higher plants, causing anthracnose disease (Rojas et al., 2010; Nelson, 2008; Freeman et al., 1996; Yee and Sariah, 1993; Bailey and Jeger, 1992). The disease can occur on leaves, stems, and both preharvest and postharvest fruit (Ivey et al., 2004).

In vitro assay of antagonism showed that chitinolytic isolates inhibited fungal growth to some extent. Chitinase, antifungal protein, might play an important role to lytic fungal cell wall. Clear zone around the bacterial isolate colonies demonstrated ability of their chitinases to degrade chitin as C-source. Different ability of the chitinolytic bacterial isolates to inhibit fungal growth might be due to different chitinase produced by the isolates. Molecular and biochemical characterizations have revealed that chitinases, similar to other glycosyl hydro-lases, are molecular in nature and can differ according to their structural organization. Enzymes can vary both within and between microbes (Kobayashi et al., 2002; Metcalfe et al., 2002). Bacterial genera *Achromobacter, Bacillus, Chromobacterium, Pseudomonas* and *Vibrio,* along with bacteria from the *Flavobacterium-Cytophaga* group and the *Enterobacterioceae* family (Donderski and Brzezińska (2001), and *Streptomyces* (Anitha and Rabeeth, 2010) are the common chitinase producing bacteria. As shown by 16S rRNA gene identification, our isolates were *Enterobacter* spp., *Enterobacter cloacae*, and *Bacillus* spp.

Recently, the use of naturally occurring bacteria (Sangeetha et al., 2010; Akinbode and Ikotun, 2008; Mahadtanapuk et al., 2007) and antagonistic fungi (Adekunle and Ogbebor, 2009; Akinbode and Ikotun, 2008; Shovan et al., 2008) for biocontrol of *Colletotrichum* spp. has been recognized. The efficacy of chitinolytic bacterial isolates against different pathogens has been proven earlier (Maisuria et al., 2008; Gohel et al., 2006).

Our previous study have shown that several local chitinolytic bacterial isolates inhibited the growth of pathogenic fungi *Ganoderma boninense*, *Penicillium citrinum* and *F. oxysporum in vitro* (Suryanto et al., 2011). In this study, we observed that all tested chitinolytic bacterial isolates effectively suppressed the growth, and decreased anthracnose severity and intensity caused by *C. gloeosporioides*.

Application of chitinolytic bacteria on cocoa leaves before *C. gloeosporioides* conidia infestation reduced disease severity and disease incidence. This could be due in part to chitinase produced by the isolates that inhibited the fungal growth. Chitinolytic bacteria often show antago-nistic association with fungi (Gohel et al., 2006). Microbial antagonism implies direct interaction between two microorganisms sharing the same ecological niche (Alabouvette, 2006). Antagonistic effects responsible for disease suppression in biological control

results either from microbial interactions directed against the pathogen, mainly during its saprophytic phase, or from an indirect action through induced resistance of the host plant (Alabouvette, 2006).

ACKNOWLEDGEMENTS

We would like to thank DP2M of the Directorate General of Higher Education, Indonesia Ministry of National Education for supporting this research. Deep appreciation and thank are also addressed to the Research Laboratory of Fakultas Teknobiologi of UNIKA Atmajaya, Jakarta for helping us in the DNA sequencing.

REFERENCES

Adekunle AT, Ogbebor NO (2009). *In vitro* control of *Colletotrichum gloeosporioides* (Penz.) Sacc. and *Rigidoporus lignosus* using biological agents. Biosci. Res. Commun. 21:199-202.

Akinbode OA, Ikotun T (2008). Evaluation of some bioagents and botanicals in *in vitro* control of *Colletotrichum destructivum*. Afr. J. Biotechnol. 7:868-872.

Alabouvette C, Olivain C, Steinberg C (2006). Biological control of plant diseases: the European situation. Review. Eur. J. Plant Pathol. 114:329-341. DOI 10.1007/s10658-005-0233-0.

Anaruma AD, Schmidt FL, Duarte MCT, Figueira GM, Delarmelina C, Benato EA, Sartoratto A (2010). Control of *Colletotrichum gloeosporioides* (Penz.) Sacc. in yellow passion fruit using *Cymbopogon citratus* essential oil. Braz. J. Microbiol. 41:66-73.

Anitha A, Rabeeth M (2010). Degradation of fungal cell walls of phytopathogenic fungi by lytic enzyme of *Streptomyces griseus.* Afr. J. Plant. Sci. 4:61-66.

Bailey JA, Jeger MJ (1992). *Colletotrichum*: Biology, pathology and control. CAB International, Wallingford, UK, 388 pp.

Bowen KL (2007). Plant disease epidemiology. In: Trigiano RN et al. (eds.) Plant pathology, concepts and laboratory exercises. 2nd Ed. CRC Press, New York. pp. 281-294.

Cooke BM (2006). Disease assessment and yield loss. In: Cooke BM et al. (eds.) The epidemiology of plant diseases. 2nd Ed. Springer Netherlands. pp. 43-80.

Donderski W, Brzezińska MS (2001). Occurrence of chitinolytic bacteria in water and bottom sediment of eutrophic lakes in Iławskie Lake District. Polish J. Environ. Studies 10:331-336.

Farr DF, Rossman AY (2009). Fungal databases. Systematic Mycology and Microbiology Laboratory. ARS. USDA. http://nt.ars-grin.gov/fungaldatabases/. (downloaded 15 Sep 2009).

Folders J, Algra J, Roelofs MS, van Loon LC, Tommassen J, Bitter W (2001). Characterization of *Pseudomonas aeruginosa* chitinase, a gradually secreted protein. J. Bacteriol. 183:7044-7052.

Freeman S, Katan T, Shabi E (1996). Characterization of *Colletotrichum gloeosporioides* isolates from avocado and almond fruits with molecular and pathogenicity tests. Appl. Environ. Microbiol. 62:1014-1020.

Getha K, Vikineswary S (2002). Antagonistic effects of Streptomyces *violaceusniger* strain G10 on *Fusarium oxysporum* f.sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process. J. Ind. Microbiol. Biotechnol. 28:303-310, DOI: 10.1038/sj/jim/7000247.

Gohel V, Singh A, Vimal M, Ashwini P, Chhatpar HS (2006). Bioprospecting and antifungal potential of chitinolytic microorganisms. Review. Afr. J. Biotechnol. 5:54-72.

Guyot J, Omandab EN, Pinard F (2005). Some epidemiological investigations on *Colletotrichum* leaf disease on rubber tree. Crop Protect. 24:65-77.

Ivey MLL, Nava-Diaz C, Miller SA (2004). Identification and management of *Colletotrichum acutatum* on immature bell pepper. Plant Dis. 88:1198-1204.

Kim YC, Jung H, Kim KY, Park SK (2008). An effective biocontrol bioformulation against Phytophthora blight of pepper using growth mixtures of combined chitinolytic bacteria under different field conditions. Eur. J. Plant Pathol. 120:373-382. DOI 10.1007/s10658-007-9227-4.

Kobayashi DY, Reedy RM, Bick JA, Oudemans PV (2002). Characterization of a chitinase gene from *Stenotrophomonas maltophilia* strain 34S1 and its involvement in biological control. Appl. Environ. Microbiol. 68:1047-1054.

Kranz J (1988). Measuring plant disease. In: Kranz, J. and Rotem, J. (eds.) Experimental techniques in plant disease epidemiology. Springer, Berlin, pp. 35-50.

Mahadtanapuk S, Sanguansermsri M, Cutler RW, Sardsud V, Anuntalabhochai S (2007). Control of anthracnose caused by *Colletotrichum musae* on *Curcuma alismatifolia* Gagnep. using antagonistic *Bacillus* spp. Am. J. Agril. Biol. Sci. 2:54-68.

Maisuria VB, Gohel V, Mehta AN, Patel RR, Chhatpar HS (2008). Biological control of *Fusarium* wilt of pigeon pea by *Pantoea dispersa,* a field assessment. Ann. Microbiol. 58:411-419.

Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Wade WG (1998). Design and evaluation of useful bacterial specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64:795-799.

Metcalfe AC, Krsek M, Gooday GW, Prosser JI, Wellington EMH (2002). Molecular analysis of a bacterial chitinolytic community in an upland pasture. Appl. Environ. Microbiol. 68:5042-5050. DOI: 10.1128/AEM.68.10.5042–5050.2002.

Nelson SC (2008). Mango anthracnose (*Colletotrichum gloeosporioides*). Plant Disease. Department of Plant and Environmental Protection Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa, pp. 1-9.

Ningthoujam DS, Sanasam S, Tamreihao K, Nimaichand S (2009). Antagonistic activities of local actinomycete isolates against rice fungal pathogens. Afr. J. Microbiol. Research 3:737-742.

Ozbay N, Newman SE (2004). Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. Pakistan J. Biol. Sci. 7: 478-484.

Patel B, Gohel V, Raol B (2007). Statistical optimization of medium components for chitinase production by *Paenibacillus sabina* strain JD2. Ann. Microbiol. 57:589-597.

Rojas EI, Rehner SA, Samuels GJ, Van Bael SA, Herre EA, Cannon P, Chen R, Pang J, Wang R, Zhang Y, Peng Y-Q, Sha T (2010). *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102:1318-1338. DOI: 10.3852/09-244.

Sangeetha G, Thangavelu R, Rani SU, Muthukumar A, Udayakumar R. (2010) Induction of systemic resistance by mixtures of antagonist bacteria for the management of crown rot complex on banana. Acta Physiol. Plant 32:1177-1187. DOI 10.1007/s11738-010-0513-y.

Shovan LR, Bhuiyan MKA, Begum JA, Pervez Z (2008). *In vitro* control of *Colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum*. Int. J. Sustain. Crop Prod. 3:10-17.

Singh G, Sharma JR, Hoondal GS (2008). Chitinase production by *Serratia marcescens* GG5. Turk. J. Biol. 32:231-236.

Soytong K, Srinon W, Rattanacherdchai K, Kanokmedhakul S, Kanokmedhakul K (2005). Application of antagonistic fungi to control anthracnose disease of grape. J. Agric. Biotechnol. 1:33-41.

Suryanto D, Irawati N, Munir E (2011). Isolation and characterization of chitinolytic bacteria and their potential to inhibit plant pathogenic fungi. Microbiol. Indones. (accepted)

Uchida JY, Kadooka CY (1997). Colletotrichum leaf spot of red sealing wax palm. Plant Disease. Department of Plant Pathology, College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa. pp. 1-4.

Yee MF, Sariah M (1993). Comparative morphology and characterization of *Colletotrichum* isolates occurring on cocoa in Malaysia. Pertanika Trop. Agric. Sci. 16:45-51.