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Potential of Chitinolytic *Bacillus amyloliquefaciens* SAHA 12.07 and *Serratia marcescens* KAHN 15.12 as Biocontrol Agents of *Ganoderma boninense*

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ABSTRACT

The objective of this research was to screen and identify of chitinolytic bacteria which have an ability to inhibit the growth of *G. boninense* and also to characterize its chitinase activity on degrading the chitin of *G. boninense*. Screening of chitinolytic bacteria against *G. boninense* by using dual culture method on Potato Dextrose Agar (PDA) showed that bacterial isolates SAHA 12.07 and KAHN 15.12 had the highest percentage inhibition of 68,19 and 40, 29%, respectively. Based on 16S rRNA identifications, SAHA 12.07 and KAHN 15.12 were identified as *Bacillus amyloliquefaciens* and *Serratia marcescens*, respectively. Isolate SAHA 12.07 produced optimum chitinase at 48 h and KAHN 15.12 at 54 h of incubation in chitin medium. Crude chitinase of SAHA 12.07 and KAHN 15.12 have precipitated by acetone at 60 and 20%, respectively. Chitinase activity had active at pH 4-10 and temperature about 20-80°C. *In vitro* lysis of *G. boninense* bioassay showed that the chitinase of SAHA 12.07 and KAHN 15.12 were capable to inhibit the growth of mycelium *G. boninense* and could release of N-acetylglucosamine by incubation of *G. boninense* with partially purified enzyme.

Key words: *Ganoderma boninense*, chitinolytic bacteria, chitinase activity

INTRODUCTION

Oil palm (*Elaeis guineensis jacq*.) is one of crop plantations that has known as a high market value of vegetable oil producer in Indonesia. However, one of problems in cultivation of oil palm is the diseases, such as the most important and detrimental disease on oil palm is Basal Stem Rot (BSR) that is caused by *Ganoderma boninense*. The disease was reported that caused losses about 50-80% ha^{-1} of 13 year old plantings (Singh, 1991). But currently, *Ganoderma* is also found in nursery and upper stem (Sanderson, 2005; Paterson, 2007). The control effort of BSR disease using technical culture and chemical fungicides still does not show the maximum results, even causing problems such as environmental pollution and resistance of pathogen to fungicides. Biological control using endophyte bacteria was conducted to suppress the growth of *G. boninense* (Bivi *et al*., 2010; Suryanto *et al*., 2012). However, applications in the field need consistency in eradicating of *G. boninense*, because of differences environmental. There are a lot of studies carried out on biocontrol development to degrade fungal cell wall, particularly its chitin (Ilyina *et al*., 2013).

Chitin is a β-1,4-homopolymer consist N-acetylglucosamine (NAG) which is the second polymer abundance in nature after cellulose (Patil *et al*., 2000). Chitin is naturally found in the fungal cell

walls, crustacean shells, nematodes and insect exoskeletons. In fungi, chitin is wide spread particularly on Basidiomycetes, Ascomycetes, Phycomycetes and has fungi on as constituentcell wallthat is contained in the mycelium, stalk and spores. The content of chitin in the mycelium of the phylum Basidiomycota approximately 26.4-64.5% (Di Mario *et al*., 2008), whereas in *Ganoderma lucidum* is 41% (Alvarez *et al*., 2014). Chitin can be degraded enzymatically by chitinase which function as biocontrol in fungal pathogens (Singh *et al*., 1999; Dai *et al*., 2011) and insect pathogens (Mubarik *et al*., 2010; Nurdebyandaru *et al*., 2010; Tu *et al*., 2010; Lee and Kim, 2015).

Chitinases are hydrolytic enzymes complex that work synergistically in degrade chitin. One of chitinase fungsion as an antifungal which effective and widely used as biocontrol agents of fungal pathogens (Singh *et al*., 1999; Wang *et al*., 2002; Zarei *et al*., 2011; Dai *et al*., 2011; Patil *et al*., 2013; Fadhil *et al*., 2014). Chitinase can be produced by bacteria, fungi, insects and plants. But the production and application of chitinases from bacteria has been usedwidely (Bhattacharya *et al*., 2007; Ilyina *et al*., 2013; Lee and Kim, 2015). Previously research reported that chitinolytic bacteria have been screened from soil of oil palm plantation in Jambi Province, Indonesia and the chitinase could inhibit the growth of *Curvularia affinis* and *Colletotrichum gloeosporioides* causing leaf blight disease on oil palm (Asril *et al*., 2014). However, there still no reports of the chitinase inhibition against *G. boninense.* This study aimed to select and identify chitinolytic bacteria from soil of oil palm plantation in Jambi Province in inhibiting the growth of *G. boninense* and to examine chitinase in degrading the cell wall of *G. boninense.*

MATERIALS AND METHODS

Microorganisms and their pre culture condition: A total of 40 chitinolityc bacterial were previously isolated from soil of oilin Jambi Province, Indonesia and collected in IPB Culture Collection, Department of Biology, Bogor Agricultural University, Indonesia (Haryanto, 2013; Purnamasari, 2013). Those isolates were cultured and maintained on solid mediachitin agar (0.3% colloidal chitin, 0.1% $MgSO_4 \cdot 7H_2O$, 0.02% K_2HPO_4, 0.1% yeast extract and 1.5% bacto agar) at 37°C for 2 days. *Gadonerma boninense* was isolated from fruit bodies of infected oil palm tree and obtained from oil palm plantation in Pontianak Province, West Kalimantan, Indonesia. This fungi was cultured on Potato Dextrose Agar (PDA) media at 30ºC for 7-10 days.

***In vitro* screening of chitinolytic bacteria against *Ganoderma boninense*:** Inhibition activity of chitinolitic bacteria against *G. boninense* based on the percentage inhibition of radial growth were conducted by the modified by the dual culture methods (Bivi *et al*., 2010). One centimeter diameter agar 7 age *G. boninense* plugged centrally on PDA media. Bacterial colonies were streaked 3 cm from both sides of *G. boninense* plug then incubated at 30°C for 7 days. The percentage inhibition of *G. boninense* was measured by using the formula:

$$\frac{r_1 - r_2}{r_1} \times 100$$

where, r_1 is radius of the mycelium growth in the plate control (3 cm) and r_2 is radius of the mycelium growth toward bacterial. Chitinolytic bacteria which the highest percentage inhibition used for further study.

Identification of selected chitinolytic bacterial DNA as *Ganoderma boninense* inhibitor: Selected bacterial genomic DNA was extracted using Presto™ Mini gDNA Bacteria Kit (Genaid). Amplification of 16S rRNA gene was performed by PCR using specific primers 63F (5'-CAGGCCCACATGTAACAAGTC-3') and 1387R (5'-GGGCGGGTAWGT CAAGGC-3') (Marchesi *et al*., 1998). The PCR reaction was performed in a total volume of 50 μL containing 25 μL GoTaq Green Master Mix, 0.5 μL of each primer, 1 μL DNA template and 23 μL nuclease free water. The PCR was performed under the following conditions: Initial denaturation at 94°C for 2 min, denaturation 92°C for 30 sec, annealing 55°C for 45 sec, extension at 72°C for 5 min with 30 cycles and final extension at 72°C for 10 min. The PCR products were purified and sequenced according to standard from sequencing services company. Sequences were analyzed using Bioedit program then aligned with the 16S rRNA gene data base using BLAST-N program. Phylogenetic analysis was performed using MEGA 6 software with 1000x bootstrap.

Determination of growth curve and chitinase production of selected bacteria: Inoculation of 2 loopsof selected bacteria were grown into 50 mL broth chitin media and incubated ona rotary shaker at 37°C and 120 rpm for 36 h. The 2.5 mL of bacterial (10^8 CFU mL^{-1}) as inoculum was inoculated into100 mL both chitin media. Cultures were collected every 6 until 78 h and their optical density were measured spectrophotometrically at 600 nm. The same cell cultures were centrifuged at 3820×g for 10 min at 4°C to obtain the crude enzyme of extracellular chitinase in supernatant.

Measurement of chitinase activity and protein concentration: Chitinase activity was measured by Spindler (1997). A volume 150 mL crude enzyme was added to 300 mL 0.3% colloidal chitin and 150 mL 0.1 M phosphate buffer pH 7 at 37°C for 30 min. Incubation was stopped at 100°C for 10 min and centrifuged at 8400×g for 5 min at 4°C. The 200 mL of filtrates was added to 500 mL distilled water and 1000 mL Schales reagent and the mixture was boiled at 100°C for 10 min. Enzyme activity was determined by measuring absorbance at 420 nm. One unit of enzyme activity is defined as the amount of enzyme that produced 1 mol of N-acetylglucosamine per min. Protein concentration was determined by Bradford (1976) using bovine albumin as a standard.

Partial purification of chitinase: Crude enzyme was precipitated with acetone concentration 0-70% (Scopes, 1987). Addition of acetone into crude enzyme was done with stirring. After stirring, the mixture were stored at 0±1°C for 4 h. The precipitate was collected by centrifuging the mixture at 10.000×g for 20 min at 0°C and dissolved in buffer (1:1) as partially purification enzyme. Chitinase activity and protein concentration were measured both of supernatant and precipitate.

Characterization of chitinase activity: Crude and partially purification enzyme of selected isolates were characterized based on the optimum activity pH and temperature. The optimum pH of enzyme activity was tested using 0.3% colloidal chitinase substrate at different pH values 4-10 (interval 1 unit). The buffers used were as follows: 0.1 M citrate buffer (pH 4-6), 0.1 M phosphate buffer (pH 7-8) and 0.1 M glycine-NaOH buffer (pH 9-10). The optimum temperature of enzyme activity was determined by incubating the enzyme at different temperature from range 20-60°C (10°C interval) from the optimum pH.

Inhibition of chitinase against *Ganoderma boninense*: Inhibition of *G. boninense* using crude and partially purification enzyme of selected isolates were conducted by the poisoning food methods

(Bivi *et al*., 2010). Crude and partially purification enzyme were filtered through a 0.25 μm membrane filter and the concentration were used 10 ppm in 10 mL liquid PDA media, poured into a sterile petridish and allowed the PDA to be solidified. One centimeter diameter agar 7 age *G. boninense* mycelium plug was inoculated in the central of PDA media. The diameter of mycelium growth was measured after 7 days incubation.

Lysis of *Ganoderma boninense* cell wall: The ability of chitinase in lysing cell wall of *G. boninense* was determined by measuring the concentrations of NAG that were released after mixed with partially purification enzyme according to the modified study of Singh *et al.* (1999). *Ganoderma boninense* mycelium was grown into broth Potato Dextrose Yeast (PDY) media at 30°C for 7 days on a rotary shaker at 150 rpm. The mycelium was harvested by centrifugated it at 3000×g for 10 min. Pellets were washed with sterile distilled water three times and then suspended into 0.1 M phosphate buffer pH 7. Mycelium suspension and partially purification enzyme were mixed with a ratio of 1:1 and incubated at 30°C for 12 h. The concentration of N-acetylglucosamine that was released from the cell wall of *G. boninense* measured by Spindler (1997) every 2 h for 12 h.

RESULTS

Screening of chitinolytic bacteria as *Ganoderma boninense* inhibitor: There are three chitinolytic bacteria had an antagonistic activity against *G. boninense* growth shown by the inhibition zone between bacteria and fungi (Table 1). The other chitinolytic bacteria from Jambi can not formed inhibition zone toward *G. boninense* growth. Isolates SAHA 12.07 and KAHN 15.12 had percentage inhibition and specific chitinase activity higher than KAHA 15.39, so isolates SAHA 12.07 and KAHN 15.12 were chosen for the further tests.

Identification of selected bacteria as *Ganoderma boninense* inhibitor: Selected bacteria which have an ability to inhibit the growth of *G. boninense* have been identified based on 16S rRNA gene as *Bacillus amyloliquefaciens* SAHA 12.07 and *Serratia marcescens* KAHN 15.12. Visualization of 16S rRNA gene amplification showed a band size ±1300 bp. Sequence analysis of gene encoding 16S rRNA in the Genbank (BLAST-N) showed that isolate SAHA 12.07 had 99% of similarity with *Bacillus amyloliquefaciens* strain MPA 1034. Philogenetic analysis showed that isolate SAHA 12.07 had also consistent closely with *Bacillus amyloliquefaciens* (Fig. 1). Isolate KAHN 15.12 had similarity with *Serratia marcescens* strain H3010 that had been identified previously by Astriani *et al.* (2015).

Growth curve and enzyme production of selected bacteria: Selected bacteria chitinolytic namely *Bacillus amyloliquefaciens* SAHA 12.07 and *Serratia marcescens* KAHN 15.12 had a growth and chitinase activity in colloidal chitin media (Fig. 2). Both of those bacteria growth entered the logarithmic phase in 0-6 h then the stationary phase up to 78 h. Chitinase production of *B. amyloliquefaciens* SAHA 12.07 at 36-60 h incubation with the optimum activity at 48th h up to 63.35 U mg^{-1}. Chitinase activity of *S. marcescens* KAHN 15.12 at 48-72 h incubation

Table 1: Percentage of inhibition of chitinolytic bacteria against *Ganoderma boninense*

Isolates code	Inhibition zone (%)	Chitinolytic index	Specific chitinase activity (U mg^{-1})
SAHA 12.07	68.19±0.78	0.35	41.96
KAHN 15.12	40.29±3.94	2.30	44.34
KAHN 15.39	37.50±6.31	0.14	0

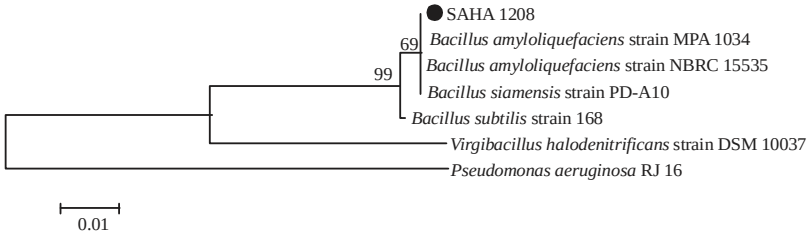

Fig. 1: Phylogenetic tree of isolate SAHA 12.07 16S rRNA using neighbor joining method with 1000x bootstrap value

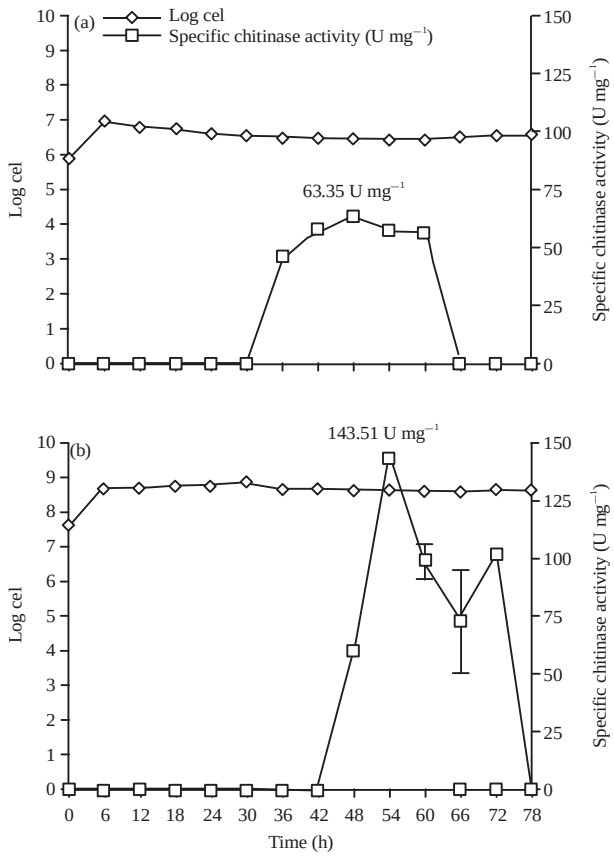

Fig. 2(a-b): Growth and chitinase activity of (a) *Bacillus amyloliquefaciens* SAHA 12.07 and (b) *Serratia marcescens* KAHN 15.12

with the optimum activity at 54th h up to 143.51 U mg^{-1}. *Serratia marcescens* KAHA 15.12 had capability of producing chitinase higher than *B. amyloliquefaciens* SAHA 12.07.

Partial purification of chitinase: The results of chitinase partial purification of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 using acetone were summarized in Table 2. The specific activity of *B. amyloliquefaciens* SAHA 12.07 improved up to 33.372 U mg^{-1} using acetone at the concentration of 50-60% and increased its purity up to 1.34 fold than the previous. Chitinase of *S. marcescens* KAHN 15.12 was able to improve its

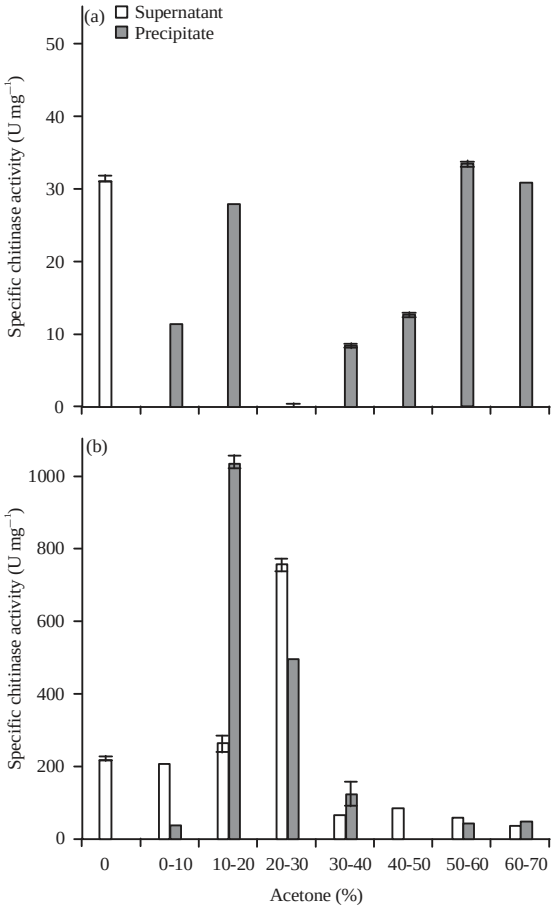

Fig. 3(a-b): Effect of acetone concentration on precipitation of (a) *Bacillus amyloliquefaciens* SAHA 12.07 and (b) *Serratia marcescens* KAHN 15.12

Table 2: Summary of chitinase partially purification

Steps	Volume (mL)	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Purification (fold)	Yield (%)
***B. amyloliquefaciens* SAHA 12.07**						
Crude enzyme	100	2.881	113.0	39.23	1	100
60% acetone precipitation	1	0.021	1.134	52.65	1.34	1.003
***S. marcescens* KAHA 15.12**						
Crude enzyme	100	0.750	109.1	145.5	1	100
20% acetone precipitation	1	0.002	1.135	446.5	3.06	1.04

specific activity up to 1029.2 U mg^{-1} using acetone at the concentration of 10-20% (Fig. 3) and increased its purity up to 3.06 (Table 2).

Characterization of chitinase activity: Crude and partially purification enzyme of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 had active at pH 4-10 and temperature about 20-80°C. Crude and partially purification enzyme of *B. amyloliquefaciens* SAHA 12.07 had optimum activity at pH 5 and 6 with the chitinase activity 1.158 and 1.130 U mL^{-1}, respectively. Crude and partially purification enzyme of *S. marcescens* KAHN 15.12 had the same optimum activity at pH 7 with chitinase activity 1.136 and 1.132 U mL^{-1}, respectively (Fig. 4). The optimum temperature of crude and partially purification enzyme of

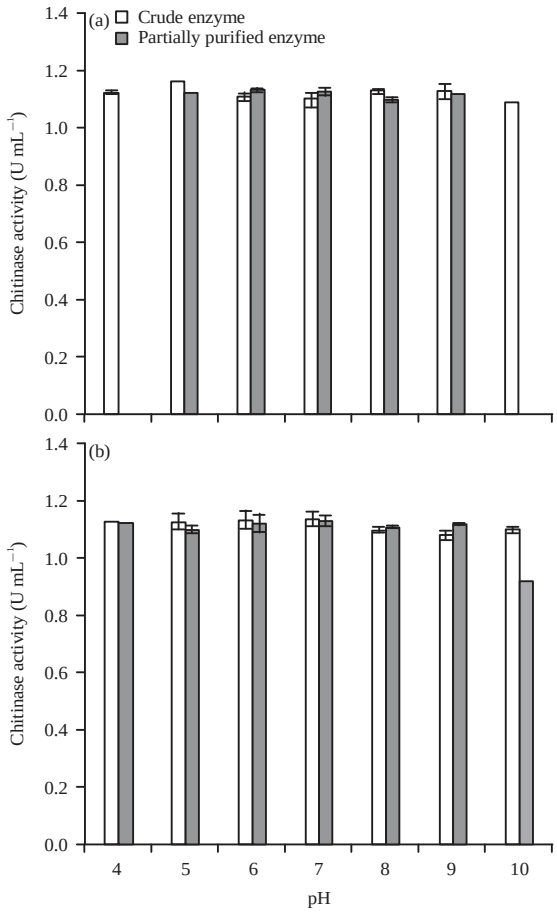

Fig. 4(a-b): Effect of pH value on chitinase activity of crude and partially purified enzyme of (a) *Bacillus amyloliquefaciens* SAHA 12.07 and (b) *Serratia marcescens* KAHN 15.12

B. amyloliquefaciens SAHA 12.07 were 50 and 60°C with chitinase activity 1.114 and 1.117 U mL^{-1}, respectively. While crude and partially purification enzyme of *S. marcescens* KAHN 15.12 had optimum chitinase activity at 60 and 70°C with chitinase activity 1.155 and 1.117 U mL^{-1}, respectively (Fig. 5).

Inhibition of chitinase against *Ganoderma boninense*: Crude and partially purification enzyme of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 were able to inhibit the growth of *G. boninense* compared to the control (Fig. 6). Both of partially purification enzyme of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 had a higher inhibition percentage against mycelial growth of *G. boninense* by an average 54.92 and 40.84%, compared to crude enzyme by an average 11.97 and 8.45%, respectively. Partially purification enzyme of *B. amyloliquefaciens* SAHA 12.07 had a high inhibition percentage against mycelial growth of *G. boninense* than *S. marcescens* KAHN 15.12.

Lysis of *Ganoderma boninense* cell wall: Partial purification enzyme of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 were able to lysis *G. boninense* cell wall that were measured by an increase of N-acetylglucosamine (Fig. 7). Chitinase of *B. amyloliquefaciens*

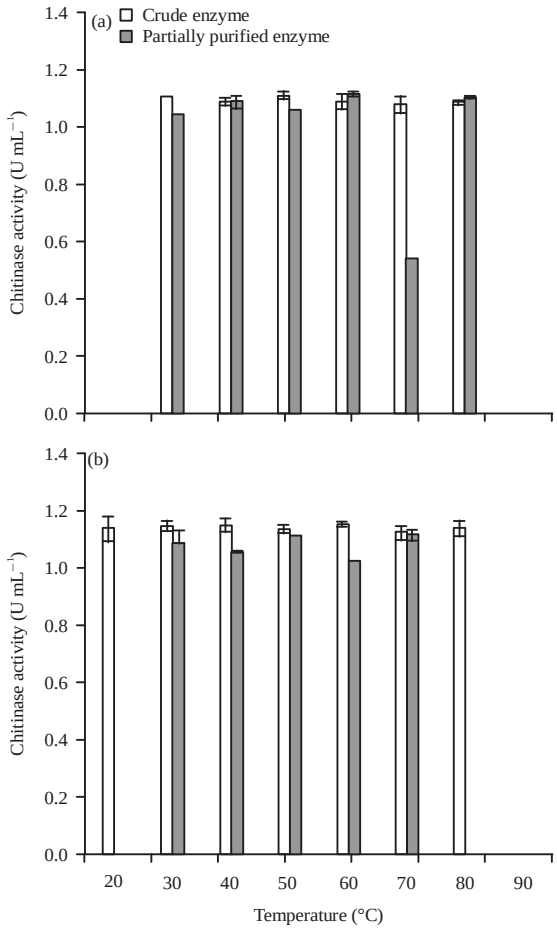

Fig. 5(a-b): Effect of temperature value on chitinase activity of crude and partially purified enzyme of (a) *Bacillus amyloliquefaciens* SAHA 12.07 and (b) *Serratia marcescens* KAHN 15.12

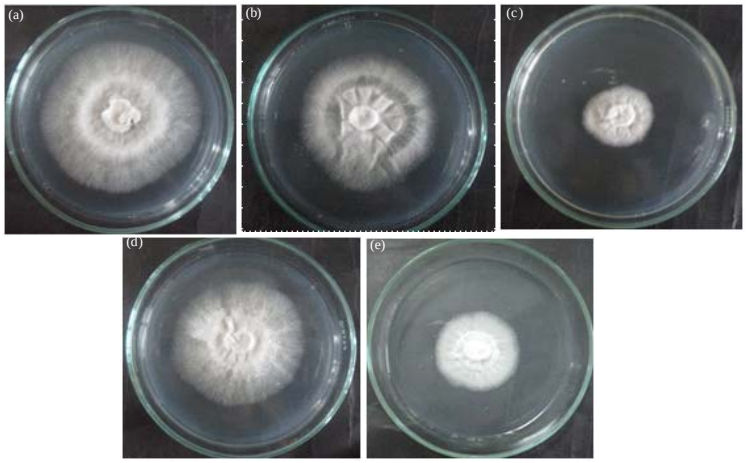

Fig. 6(a-e): Inhibition of *Ganoderma boninense* using chitinase after 7 days incubation. (a) Control, (b) Crude enzyme of SAHA 12.07, (c) Partially purified enzyme of SAHA 12.07, (d) Crude enzyme of KAHN 15.12 and (e) Partially purified enzyme of KAHN 15.12

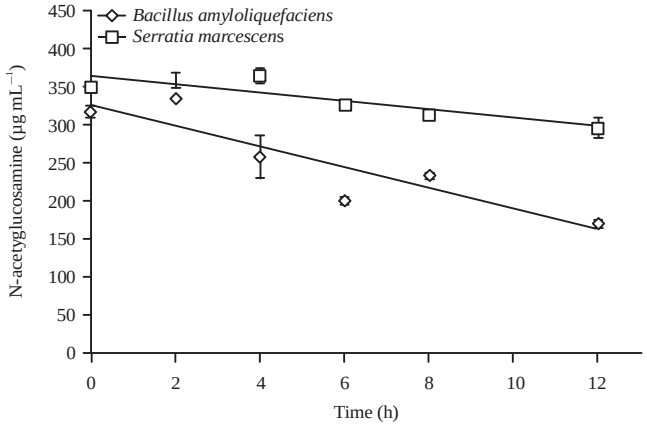

Fig. 7: Release of N-acetylglucosamine from a living cell wall preparation of *Ganoderma boninense* by partially purified enzyme of *Bacillus amyloliquefaciens* and *Serratia marcescens* KAHN 15.12

SAHA 12.07 released the highest NAG 334.89 U mg^{-1} at optimum incubation time 2nd h. Unlike the chitinase *S. marcescens* KAHN 15.12 released the highest NAG 364.63 U mg^{-1} at optimum incubation time 4th h. Partially purification enzyme of *S. marcescens* KAHN 15.12 was higher in releasing NAG on mycelial of *G. boninense* compared to *B. amyloliquefaciens* SAHA 12.07.

DISCUSSION

Chitinolytic bacteria SAHA 12.07 and KAHN 15.12 were able to inhibit the growth of *G. boninense* mycelium with a high inhibition percentage compared to other isolates. The percentage inhibition compliance with inhibition zone among bacteria against *G. boninense*. Inhibition zone around bacterial colonies were allegedly due to hydrolysis of chitin in the *G. boninense* cell wall by antifungal chitinase that was released by the bacteria. *Ganoderma boninense* mycelium could not grow to approach bacterial colonies and damaged visible. Chitinase and other hydrolytic enzymes such as β-glucanase is a key enzyme in lysing fungi cell wall that work synergistically (Bormann *et al*., 1999). Inhibition of fungi through chitinolytic system is able to produce antifungal compounds chitin-binding protein, which it will bind the formed new α chitin (nascent chitin) strongly on the tip of hyphae that will affect to exocytosis mechanism in the cell wall formation (Schnellmann *et al*., 1994).

Chitinolytic bacterial isolates *Enterobacter* sp. KR05, *Enterobacter cloacae* LK08, *Bacillus* sp. BK13, *Enterobacter* sp. BK15 and *Bacillus* sp. BK17 were also able to suppressed the growth of *G. boninense* in chitin media agar (Suryanto *et al*., 2012) but in our study using PDA as a good media for the growth of fungi. The result was indicated by the growth of *G. boninense* on chitin media during antagonistic test against bacteria which has lower fungal mycelium density (Suryanto *et al*., 2012), compared to the growth of *G. boninense* on PDA media (control) in this study. The efforts to get a potential biocontrol agent during antagonist test is needed to maintain all the conditions of well growth the pathogen. It aims to reduce the inhibitory effect of pathogens except by the bio control agent.

Isolate SAHA 12.07 was identified as *Bacillus amyloliquefaciens*. *Bacillus amyloliquefaciens* has been widely reported as a biocontrol agent on *Colletotrichum lagenarium*, *Sclerotinia sclerotiorum*, *Ralstonia solanacearum*, *Rhizopus stolonifer*, *Botritis cinerea* and *Penicillium*

expansum (Kim and Chung, 2004; Abdullah *et al*., 2008; Arrebola *et al*., 2010; Hu *et al*., 2010). *Serratia marcescens* was also reported as a potential biocontrol agent on *Pythium ultimum*, *Pyricularia oryzae* and *Varroa destructor* (Jaiganesh *et al*., 2007; Roberts *et al*., 2007; Tu *et al*., 2010). Microbial which have a broad spectrum of inhibition pathogenic are very well be used as biocontrol agents (Wang *et al*., 2014).

Bacillus amyloliquefaciens SAHA 12.07 and *Serratia marcescens* KAHN 15.12 had a similar growth pattern in the chitin media and were able to produce chitinase. Both of those bacteria had ability to grow chitin media, it showed that media could be able to supply nutrients for cell growth. Chitin would be hydrolysed by extracellular chitinase which is the primary metabolite of bacteria. Chitin hydrolysis produces N-acetylglucosamine that will be reused by both bacteria as a carbon source for their growth. The highest chitinase production by both of bacteria were in the stationary phase. The similar result also reported that chitinase is generally produced by bacteria in the stationary phase at 36-72 h of incubation time (Toharisman *et al*., 2005; Mubarik *et al*., 2010; Nurdebyandaru *et al*., 2010; Asril *et al*., 2014).

Crude enzyme was obtained from the highest production of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 were able to be improved its specific activity using acetone. The percentage of acetone was different in precipitating chitinase for various isolates. Chitinase of *B. amyloliquefaciens* SAHA 12.07 partially was able to be enhanced its purity by 1.34 using 60% acetone while *S. marcescens* KAHN 15.12 used 20% acetone were able to increase 3.06 of the purity. Generally, all of the proteins can be precipitated using acetone 20-50% (Scopes, 1987). Chitinase of *Stenotrophomonas maltophilia* and *Penicillium ochrochloronwere* successfully precipitated using 70% acetone (Patil *et al*., 2013; Jankiewicz and Brzezinska, 2015).

Crude and partially purification enzyme activity of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 had a broad pH range between 4-10. The optimum activity of crude and partially purification enzyme of *B. amyloliquefaciens* SAHA 12.07 at pH 5 and 6, while *S. marcescens* KAHN 15.12 at pH 7. The similar result also reported that chitinase of *B. amyloliquefaciens* V656 had optimum activity at pH 6 and 7 (Wang *et al*., 2002). Similarly, chitinase of *S. marcescens* GEI and *Serratia marcescens* MO-1 had optimum activity at pH 7 (Tu *et al*., 2010; Okay *et al*., 2013). Chitinase of bacteria generally have a pH range from acidic to alkaline. Antifungal protein which have a wide range of pH and temperature are excellent to be used as biocontrol (Bhattacharya *et al*., 2007; Wang *et al*., 2014). Therefore, chitinase can be used extensively under various conditions to address plant diseases (Ilyina *et al*., 2013).

Crude and partially purification enzyme activity of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 also had a wide range of temperature between 20-80°C. Generally, optimum chitinase activity of both bacteria was in termofilic temperature. The optimum activity of crude and partially purification enzyme *B. amyloliquefaciens* SAHA 12.07 at 50 and 60°C, respectively, while *S. marcescens* KAHN 15.12 at 60 and 70°C, respectively. Chitinase activity of crude and partially purification enzyme of both bacteria were not found at 90°C. Chitinase activity at temperature 20°C was only found on *S. marcescens* KAHN 15.12 although, it was lower than the optimum temperature. The same result also reported that chitinase had optimum activity between 35-75°C (Wang *et al*., 2002; Dai *et al*., 2011; Tu *et al*., 2010; Zarei *et al*., 2011; Asril *et al*., 2014; Laribi-Habchi *et al*., 2015).

Crude and partially purification enzyme of *B. amyloliquefaciens* SAHA 12.07 dan *S. marcescens* KAHN 15.12 were able to inhibit the growth of *G. boninense*, but the higher inhibition percentage was obtained using partially purification enzyme. Partially purification enzyme of

B. amyloliquefaciens SAHA 12.07 had a high percentage of inhibition compared to other. This occurred because of *B. amyloliquefaciens* SAHA 12.07 had a higher protein content after precipitation compared to *S. marcescens* KAHN 15.12, so that it was allegedly any synergysm presence of chitinase with the other antifungals. *Bacillus amyloliquefaciens* produces chitinase and other peptide anti fungal (Arrebola *et al*., 2010; Hu *et al*., 2010). *Bacillus* also produces antibiotic compounds, including volatile, lipopeptide and peptides. *Bacillus* has heat and drought resistant endospore and can colonizes in the plant micro environment to prevent infection of pathogen (Wang *et al*., 2014). *Serratia marcescens* KAHN 15.12 was also reported the ability to produce IAA, that has a role as growth promotionin roots oil palm seedlings (Astriani *et al*., 2015). Good rooting system in oil palm seedling indicates adequate plant nutrients and serves to prevent pathogen infection, particularly soil-borne diseases such as *G. boninense* which attacks roots (Paterson, 2007). Rhizo bacteria such as *Bacillus* and *Serratia* had potential as plant growth promoting rhizobacteria which play a vital role in growth promotion, pathogen suppression and improvement of soil health (Raaijmakers *et al*., 2009).

Partially purification chitinase of *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 were able to lyse chitin of *G. boninense* characterized by increase of N-acetylglucosamine (NAG) which was released from *G. boninense* mycelium. Chitinase of *S. marcescens* KAHN 15.12 was higher in releasing NAG compared to chitinase of *B. amyloliquefaciens* SAHA 12.07, although *B. amyloliquefaciens* SAHA 12.07 has a higher inhibitory consistency using cell, crude and partially purification enzyme. This occurred because of *S. marcescens* KAHN 15.12 had a chitinase specific activity that was higher than *B. amyloliquefaciens* SAHA 12.07, it was alleged the ability to lyse *G. boninense* cell wall will be higher and NAG will be produced in the higher number. This is supported that *Serratia marcescens* has also known as a potential chitinase producing bacteria that has been widely applied as biocontrol (Tu *et al*., 2010; Zarei *et al*., 2011; Okay *et al*., 2013; Fadhil *et al*., 2014). In the condition above optimum time, here concentration of NAG decreased. The same results also reported that precipitated chitinase of *Streptomyces* sp. 285 and *Paenibacillus* sp. The 300 were able to lyse the cell wall of *Fusarium oxysporum* which characterized by release of NAG at 24 h incubation (Singh *et al*., 1999).

These results suggest that *B. amyloliquefaciens* SAHA 12.07 and *S. marcescens* KAHN 15.12 isolates are highly potential as biocontrol agents of *G. boninense*. However, this should be supported by field study to confirm their potential. Bacterial consortia are proven to be useful for plant protection and enhancing plant growth, compared to single inoculation. However, for application of enzyme as bio control in field was needed enzyme immobilization using encapsulated which is fungi on to protect enzymes against environmental factors and keep their activity (Ilyina *et al*., 2013; Okay *et al*., 2013).

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