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Abstract. *Ganoderma boninense* is a serious fungal disease called basal stem rot (BSR) in oil palm plantation where the microbial ecosystem has been destroyed it could thrive and occupy the space that was created by sterilizing the soil. Infections by Ganoderma spp. begin their attack in the palm roots and gradually spread to the bole of the stem where they cause dry rot, which prevents absorption and transport of nutrients. Chitinoloytic microbes when in contact with chitin secrete an enzyme called chitinase to mainly degrade the chitin and utilize it as an energy source. The enzyme chitinase inhibits fungal growth by hydrolyzing the chitin present in the fungal cell wall resulting in inhibition of further progress of the fungi. There has been a lot of interest in this biological process because of its potential to be an agent for the biological control of plant disease and for engineering plants for resistance to phytopathogenic fungi by inducing the systemic resistance. Nine potential chitinase isolated from soil were tested for inhibit *Ganoderma boninense*. Based on the observations, it is the one isolate can inhibit the growth of *Ganoderma boninense* with activity of 0.02 U/mL.

1. Introduction

Using fungicides intensively has been accumulated toxic compounds potentially hazardous to humans and environment and also in the buildup of resistance of the pathogens [1]. In order to solve these problems, alternatives to chemical control are being investigated and the use of antagonistic microbes seems to be one of the promising approaches. Antagonism may be accomplished by competition, parasitism, antibiotics, or by a combination of these modes of action Parasitism involves the production of several hydrolytic enzymes that degrade cell walls of pathogenic fungi [2]. The importance of β-1,3-glucanase and chitinase as key enzymes responsible for fungal cell and sclerotial wall lysis and degradation has been reported [3]. These enzymes have been shown to be produced by several fungi and bacteria and may be an important factor in biological control [4]. Fungal cell walls contain chitin composed of N-acetyl-D-glucosamine molecules cross linked with other by β (1-4) glycosidic linkages which attains a highly insoluble crystalline structure organized into micro fibrils [5]. Most of the fungal cell wall hydrolyses have chitinolytic activity. Potential application of chitinases in bio control of unwanted fungi is promising.

Enzymes of chitin and glucan biosynthesis manufacture long linear chains of β1,4-linked N-acetylglucosamine and β1,3-linked glucose. However, the fungal cell wall contains abundant quantities of branched 1,3-β-, 1,6-β-glucan and there is evidence of extensive cross-linking between

chitin, glucan and other wall components. Furthermore, the wall is a highly dynamic structure subject to constant change, for example, during cell expansion and division in yeasts, and during spore germination, hyphal branching and septum formation in filamentous fungi. Cell wall polymer branching and cross-linking, and the maintenance of wall plasticity during morphogenesis, may depend upon the activities of a range of hydrolytic enzymes found intimately associated with the fungal cell wall. Most of the fungal cell wall hydrolases characterized to have chitinase or glucanase activity and a number of these enzymes also exhibit transglycosylase activity.

Fortunately, chitin is not a constituent biological part or metabolite of vertebrates. Thus bacteria producing chitinases have a great potential in controlling fungi and insects under select conditions [6].

Ganoderma boninense is a serious fungal disease called basal stem rot (BSR) in oil palm plantation where the microbial ecosystem has been destroyed it could thrive and occupy the space that was created by sterilizing the soil. [7]. Infection by *Ganoderma* spp. begin their attack in the palm roots and gradually spread to the bole of the stem where they cause dry rot, which prevents absorption and transport of nutrients. Infected oil palms gradually lose their ability to produce fruits and eventually collapse. By the time Ganoderma fruiting bodies are detectable on the oil palm, about 50% of the internal tissues would have already rotted [7]. *Ganoderma boninense* also has many forms of resting stages, including resistant mycelium, basidiospores, chlamydospores, and pseudosclerotia, and these are difficult to control

Many chitinolytic bacteria can play important role in biocontrol of fungi. One of the best-studied responses to chitin addition is the effect on the microbial species that act as antagonists of crop pathogens. Antagonistic microbes employ a number of methods to attack plant pests and pathogens. This includes, but is not limited to, the production of chitinases.

The beneficial effect of chitin-based treatments to antagonistic bacteria is not restricted to *Bacillus subtilis*, with both chitin and chitosan improving the control of Fusarium wilt in both tomato [8] and cucumber [9] when applied to the soil with a range of different species of chitinolytic microbes.

Chitinases also plays role in many areas such as the production of mosquito control, a biocontrol agent of fungal pathogens, and isolation of fungal protoplasts [10]. Microbial chitinase production has increased, and two purposes are reduce environmental hazards and increasing production for added value products. This research was screened microbe producing chitinase for inhibiting *Ganoderma boninense* from soil samples collected from Tangerang area, Banten, Indonesia.

2. Materials and Methods

2.1. Chemicals

The materials, media, reagents used for this study were chemicals for pro-analysis. Chitin was purchased from chitin-chitosan industry in Indonesia.

2.2. Methods

2.2.1. Collection soils samples

Lake and soil in near palm oil plantation of Tangerang areas in Banten, Indonesia were selected for the soil collection. Soil at the depth of approximately 9–12 cm was collected in a sterile zip-lock cover with the help of a sterile spatula and placed in an ice pack for transportation to the laboratory and it was processed.

2.2.2. Preparation of colloidal chitin [11]

To 10 g of chitin powder, added 120 ml of concentrate HCl, incubated at 37 °C, 180 rpm for 1 h. The mixture was transferred through glass wool to 50% ethanol and thoroughly mixed to obtain a homogenous suspension. This was further transferred through filter paper and washed with distilled water until the colloidal chitin reaches pH 7. Colloidal chitin was collected and stored at 4 °C.

2.2.3. Isolation of chitinase
Chitin utilizing bacteria from the collected soil sample was isolated by serial dilution and spread plate technique. 1 ml of each dilution was plated in triplicates on nutrient agar medium supplemented with 1% colloidal chitin and incubated at room temperature (37 °C) for 3 d, and isolation of bacteria was carried out from the third day. The chitinase producers were selected based on the morphology, color, and growth in the colloidal chitin medium.

2.2.4. Microorganisms and cultivation
Ganoderma boninense were obtained from Biotechnology Laboratory, Centre for Assessment of Biotechnology, Serpong, Banten, Indonesia. Potato dextrose agar was used to maintain cultures of pathogenic and antagonistic isolates. Media culture (w/v) for chitinase producing bacteria were K_2HPO_4 0.1%; $MgSO_4.7H_2O$ 0.01%; NaCl 3%; $(NH_4)_2SO_4$ 0.7%; yeastextract 0.05%; and colloidal chitin 3%.The medium was adjusted to 7.0 and autoclaved.

2.2.5. Screening of chitinase-producing bacteria
Quadrant streak of all the isolates was carried out in nutrient agar plate supplemented with colloidal chitin to isolate the potential organism based on the chitinase produced.Single streak inoculation measuring 2 cm length was performed for all the bacterial isolates on nutrient agar medium supplemented with colloidal chitin and incubated at room temperature for 2 d. The plates were stained with 0.1% congo red and distained with 1% NaCl, and the bacterial isolates producing a clear zone of more than 10 mm were selected. The screened pure isolates were stored in nutrient agar slants added with 1% colloidal chitin at 4 °C to maintain the viability of chitinase producers. Preliminary screening abetted the selection of isolates for further study. Screening technique of the enriched colonies involved selection of isolates which possessed the ability to produce chitinase which resulted in degradation of the substrate chitin. Potato Dextrose Agar (PDA) containing colloidal chitin was employed to select the isolates which were able to utilize chitin as a sole source of carbon and energy. The isolates which possessed the ability to degrade chitin produced a distinct zone of clearance around it. These colonies were sub-cultured onto sterile Luria Bertani (LB) slants and maintained for further studies

2.2.6. Chitinase activity
Enzyme activity is calculated based on the amount of reducing sugar produced using the NAG standard curve. One unit of chitinase activity is the amount of enzyme that produces 1mol of NAG per minute [11].

2.2.7. Antifungal activity of Ganoderma boninense
Agar plates (PDA; 10 % volume fraction) were prepared with *Ganoderma boninense* culture filtrates (sterilized by filtration) or with water (control). Chitinase producing bacteria was inoculated in the centre of agar plates using 5-mm mycelial discs and incubated at 30 °C for 3 days. The radial diameter of the colonies was measured at right angles every day, for threereplicate plates per treatment, measuring growth rate, and percent of inhibition was calculated.

3. Results and Discussion
Media LB is a good media for growing microorganisms because it is very efficient in the process of stimulation of growth and the media is suitable for many organisms. Fermentation was done by using a rotary shaker at a temperature of 37°C and 150 rpm for C14, C15, D6, WS4F, and WS7B isolates. At a temperature of 30°C and 200 rpm for CY1 and CY3isolates and temperature of 55°C, 200 rpm for KP 7.7 and 7.4 isolates. Incubation time was carried out during 24 hours to CY1, CY3, WS4F, WS7B, KP 7.4, and KP 7.7isolate. For C14, C15, and D6 isolates were incubated for 3 days (Figure 1).

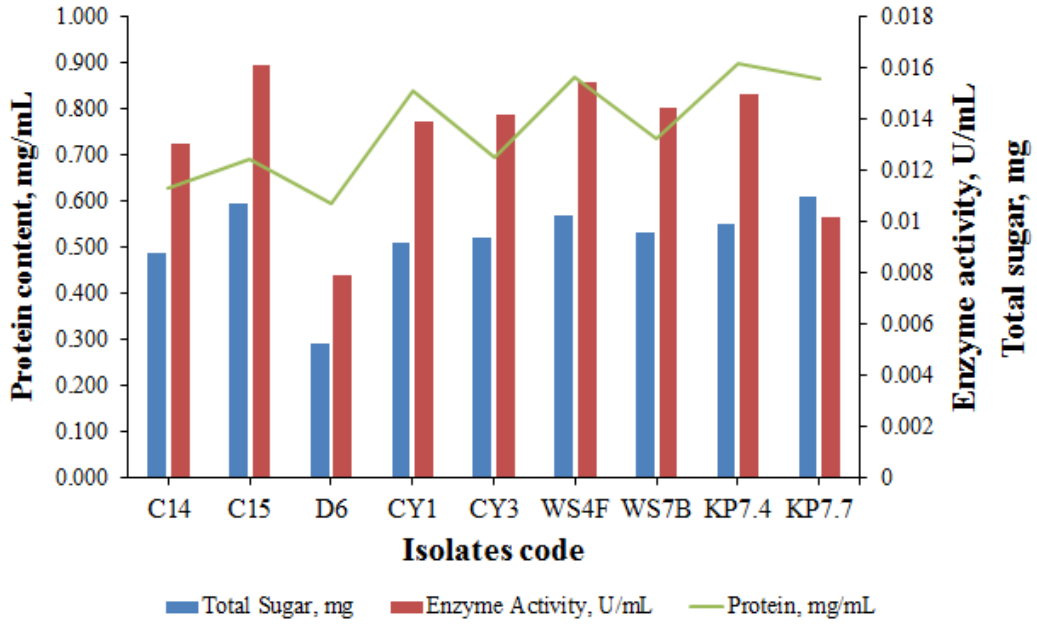

Figure 1. Nine isolates of chitinase were isolated from soil. Isolates C14, C15 and D6 were determined after 3 days incubation.

Based on the observations, the activity of the chitinase from C14, C15, and D6 isolates was increased from the first day until the third day. Isolate C14, C15 and D6were 300%, 300% and 225% increased. However, extracellular chitinase hydrolysis time to produce reducing sugars was 24 hours but on a reducing sugar C14, C15 and D6 isolates was 72 hours. In addition, the fungus can grow for 72-96 hours and the hydrolysis was carried out for 3 days. Park et al. [12] reported that the activity of the enzyme hydrolysis of chitin to chitinase can calculated from the reducing sugar because one unit of enzyme activity chitinase is the amount of enzyme required to produce one µmole of sugar reducing/minute which is equivalent to the N-acetyl D-glucosamine. All the chitinolytic activity bacteria isolates were examined and isolate KP7.7 was highest activity. KP 7.4 and KP 7.7 isolates is a bacteria.

Bacteria has been widely known to synthesize enzymes that has the ability to degrade the polymer chitin, which contain linear β-1.4 N-acetylglucosamine (GLcNAc) that cannot be broken down the main structure of components the cell walls of fungi [13].The presence of the enzyme chitinolytic on the growth medium containing colloidal chitin was produce GLcNAc. Testing chitinolytic bacteria conducted by growing *Ganoderma boninense* on PDA medium for 6 days. After *Ganoderma boninense* has been growing approximately 1.0 cm in PDA media containing 3% of colloidal chitin, 9 isolates inoculated in petri dishes at 30°C. 4 isolates were selected that have high *Ganoderma boninense* growth inhibition. Four isolates were CY3, WS4F, WS7B, and KP 7.7.

Based on the results of the observation clear zone area of chitinolytic bacteria and inhibition area of *Ganodermaboninense*, WS4F isolate has best ability to inhibit *Ganoderma boninense* (Figure 2).

Figure 2. Clear zone area of chitinolitic WS4F isolate.

The ability of WS4F in inhibiting the growth of *Ganoderma boninense* can be seen from the clear zones. Clear zone width resulting from the process of degradation of chitin found in *Ganoderma boninense* after inoculated by culture isolates WS4F using a paper disk is as follows:

Table 1. WS4F isolate inhibit *Ganoderma boninense* in PDA mediacontaining colloidal chitin 3 %

Days	*Ganoderma boninense* growth, cm
4	0.96
5	1.24
6	1.68
9	2.88
12	3.62
15	3.72

According to Chong et al. [14], chitinase was screened from tissue of the body the fruit and inoculated on selective medium can inhibit *Ganoderma* sp. at room temperature.WS4F selected isolate was tested its activities to produce the activity on the environmental conditions (pH 7 and 28°C).

From the results, WS4F producing chitinase have an activity 0.021 U/mL at optimum condition (28°C) and higher than at 30°C (0.014 U/mL).

4. Conclusion

Nine isolates (C14, C15, D6, WS4F, WS7B, KP 7.4, KP7, CY1, and CY3) of chitinolytic bacteria were tested to inhibit *Ganoderma boninense.* Based on the observations, isolates chitinase KP 7.7 on the optimum conditions have the activity of 0.016 U/mL. Chitinase from CY3 isolate on environment condition have the activity 0.021 U/mL similar with WS4F isolate, but only WS4F isolate can inhibit *Ganoderma boninense*. Compared to control, WS4F isolate can inhibit *Ganoderma boninense* 150%. WS4F isolates was inhibited *Ganoderma boninense* on PDA media of 0.4 cm and on PDA media containing colloidal chitin 0.3% of 0.5 cm.

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