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# Isolation and Characterization of Chitinolytic Bacteria Used as Biology Control of Pathogenic Fungi on Oil Palm Leaves

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***Abstract:*** *Oil palm (Elaeis guineensis Jacq.) is one of the main plantation commodities in Indonesia. Production of oil palm is influenced by several environmental condition such as rainfall, soil, climate, and pathogen outbreak. Pathogen outbreak such as anthracnose and leaf blight cause rotting on leaves of seedling oil palm which in the end will kill the plant. Prevention on pathogenic fungi on oil palm can be done by using chitinolytic bacteria which can produce chitinase enzyme as biology control. Chitin is one of the elements in cell wall, mycelia, stalks, and spore of the fungi. The aims of this experiments were to isolate chitinolytic bacteria and their potential as biology control of pathogenic fungi causing anthracnose and leaf blight on oil palm. Among isolated 28 chitinolytic bacteria, 10 chitinolytic isolates with highest chitinolytic index were tested against pathogenic fungi. Results showed strain SAHA12.08, SAHA12.10, and SAHA12.13 had antagonistic activity to the growth of Curvularia pathogenic and Botryodiplodia pathogenic whereas strain KAHN15.12 only had antagonistic activity to Curvularia pathogenic. Chitinolytic index of strain SAHA12.08, SAHA12.10, SAHA12.13 and KAHN15.12 respectively are 3.40, 0.40, 0.71, and 0.76. Isolate SAHA 12.08 which is identified as Bacillus thuringiensis showed inhibition activity against Curvularia sp. using leaf of oil palm in vitro test. Leaves treated only with Curvularia sp. as negative control resulted in larger surface area of leaf spot compared to leaf treated with SAHA 12.08. The oil palm leaf which infected by Curvularia sp. was damaged in the epidermal tissue and mesophyll tissue.*

***Keywords:*** *antagonistic activity, anthracnose, Bacillus thuringiensis, Curvularia sp.*

## 1. Introduction

Oil palm is one of the main plantation commodities which contributes in national incomes significantly. It generates profitable export earnings for the country especially country like Indonesia which is one of the world's largest producer and exporter of oil palm. Production of oil palm products increased approximately from 167.000 tones in 1967 to 18 million tones in 2009, or almost 107 times within 42 years. Areas of oil palms have increased from 105.000 hectares in 1967 to 7,8 million hectares (Ditjenbun 2009). Some of disease that attack oil palm such anthracnose (*Botryodiplodia palmarum*, *Melanconium* sp., and *Glomerella cingulata*) and leaf blight (*Curvularia eragrostidis*) which are commonly found at southeast Asia greatly damaged oil palm seedling (Aderungboye 1977).

Biological control using microorganism has been studied intensively since not many environmental friendly alternavites to control are available. Introduction of chitinolytic bacteria as antagonist agents is to control pathogenic fungi which causes anthracnose and leaf blight. As stated in many previous reports, the production of chitinase enzyme was related to fungal growth inhibition and the biological control of fungal pathogen was possible because of the ability of the chitinolytic bacteria to degrade fungal cell walls (Kamil *et al.* 2007; Suryanto *et al.* 2010; Gomaa 2012). Chitinolytic bacteria is capable to inhibit fungal activity because it can produce chitinase which is one of the element in cell wall, mycelia, stalks, and spore in the fungi (Peter 2005). Introduction chitinolytic bacteria is one of environmental friendly alternatives which is safer to control

pathogenic fungi than using fungicide. The aims of this experiments were to isolate chitinolytic bacteria and their potential as biology control of pathogenic fungi causing anthracnose and leaf blight on oil palm.

## 2. Methods

### 2.1. Isolation and Identification of Chitinolytic Bacteria

In amount of 10 soil sample was collected from Hutan Nasional Bukit Dua Belas, Jambi. Soil samples were taken from soil around oil palms and rubber trees. Every 3.0 g of soil sample is diluted in 30 mL of nutrient broth (NB) with 1 % chitin colloidal and it was incubated at 25 $^{o}$C for 24 hours. Then, all cultures were done with serial dilution from $10^{-6}$ to $10^{-8}$ in NaCl 0.85 %. Suspension was spreaded on chitin agar (1% chitin colloidal, 0.1% $MgSO_4 \cdot 7H_2O$, 0.02% $K_2HPO_4$, 0.1% yeast extract, and 1.5% agar) which was incubated at 37$^{o}$C for 48 hours. Every colony of different bacteria is streaked on the new agar chitin until single colony of bacteria was found. Cultures were incubated at 37$^{o}$C for 48 hours. Isolates were observed based on chitinolytic index (CI) which was hinted by clear zone. Every chitinolytic was identified by using Gram staining.

### 2.2. Isolation and Identification of Pathogenic Fungi

Isolation was began with preparation of infected leaves which were collected from Indonesia Biotechnology Research Institute for Estate Crops. Every three brown spot on infected leaves is cut into 1x1 cm covering half healthy leaf and half sick leaf. The leaves were rinsed with flowing water and then it were soaked into sodium hypochlorite (NaClO) 1% for a minute. The leaves then were rinsed with sterilized water and it was dried with sterilized tissue. Finally, the leaves were put on potato dextrose agar (PDA) containing chloramphenicol 0.5 %. Fungal cultures were incubated at 25oC for a week. Every different hyphae were reinoculated into new PDA to get pure cultures. Hyphae were identified by its morphology characteristic. Molecular identification was also done by using internal transcribed spacers (ITS) of the rDNA regions.

### 2.3. *In vitro* Screening of Chitinolytic Bacteria against Pathogenic Fungi

The bacterial isolates were screened for their antagonistic activity against pathogenic fungi *in vitro* based on percentage inhibition of radial growth (Fokema 1973). To evaluate the antagonistic activity of the chitinolytic bacteria isolates, a loopful of the bacterial isolates was streaked 2 cm from the margin of PDA plates (9 cm diameter) and 4 cm long. Opposite the bacterial isolates, at a distance of at least 3 cm, pathogenic fungi was placed. After incubation for 6 days at 25$^{o}$C, inhibition of the pathogen's development was assessed by two parameters, the percentage of inhibition of radial growth [100% x $(r_1-r_2)/r_1$) and the width of the zone of inhibition measured at the smallest distance between both colonies (d). R1 is length of radial growth towards plate margin (4 cm) and R2 is length of radial growth towards antagonistic (length of inhibited hyphea)

### 2.4. Identification of Chitinolytic Bacteria which has Antagonistic Activity

Isolates which had antagonistic activity to all pathogenic fungi was identified by using kit bioMérieux, USA. Isolates with antagonistic activity were streaked on chitin agar. Cultures were incubated at 37$^{o}$C for 24 hours. Cultures then were stained with Gram staining for verification. Three loopful of isolates were diluted into kit API 50 CHB/E medium. In amount of 200 µl kit API 50 CHB/E medium was inoculated into the tube. In addition, the first tube is filled by kit API 50 CHB/E medium before it was inoculated by isolates (sterile). Result which shown was interpretated by using the *apiweb identification software with the database (V4.0).*

### 2.5. Modeling Bacterial Growth Curve from Selected Isolates and Antagonistic Activity.

Inoculation of 2-4 loopful of selected isolates into 50 ml NB with 1% chitin colloidal which was incubated at 37$^{o}$C for 15 hours. In amount of 1 ml culture was inoculated into 100 ml NB with 1% chitin colloidal as enzyme production medium. Enzyme production medium was incubated on incubator shaker at 120 rpm at 37$^{o}$C. Optical density (OD) and antagonistic activity of the culture were measured every 12 hours. Antagonistic activity was tested by using cell and supernant of the strain with agar well diffusion method. Supernatant which contains extracellular metabolites was obtained by centrifugation on 12.000 rpm.

### 2.6. Biocontrol Assay Against Curvularia sp. using In vitro Test

Inhibition test of SAHA 12.08 against *Curvularia* sp. was done by using in vitro test Detached-Leaf Assays method (Parke *et al.* 2005). Oil palm leaf was 3 months washed using sterile aquadest. For positive control oil palm leaves were inoculated with fungi pathogens only using *Curvularia* sp. The whole treatments was conducted four times. Each The leaves will be treated cut up to size of 4 cm x 3 cm. Thereafter the leaf was dipped into cell culture 60 hours, crude enzyme, partial purification enzymes enzyme for 30 min, then air dried. The leaves put and placed into a petri dish containing a wet filter paper to maintain the humidity, as well as a buffer so that the leaves do not directly touch the surface of petri dish. After 1-2 h, 50 µl of fungal of pathogens at a concentration of 4 x $10^5$ spores $ml^{-1}$ to the one end of the surface of the leaves with micropipette and then incubated for 7 days in the dark conditions at room temperature. Percentage of disease incidence and severity of disease as indicated by increased wound diameter (lesion diameter) were recorded 3 days until 7 days after inoculation. The ability to reduce disease incidence of the treatments was compared. Observation of anatomical structures was performed by cross-cutting the leaves using a frozen microtome performed at the LIPI Cibinong Botanical Laboratory. The result of preparations was dyed by using safranin and observed on a microscope with 400x magnification. The part observed is the epidermal tissue, mesophyll tissue, and vascular bundles.

## 3. Results

### 3.1. Isolation of Chitinolytic Bacteria

Among 10 soil samples, there were 28 isolates which had been successfully isolated and identified. There were 14 isolates which were isolated from soil around oil palms whereas the other 14 isolates were isolated from soil around rubber tree. Isolates were identified with Gram staining. Results showed that chitinolytic index was ranged from 0.04 to 3.40 (Table 1). The strain SAHA12.08 had the highest chitinolytic index (CI) while strain KAHN10.05 had the lowest chitinolytic index.

### 3.2. Isolation and Identification of Pathogenic Fungi

Two pathogenic fungi were obtained from sick leaves of oil palm at Indonesia Biotechnology Research Institute for Estate Crops. Identification results through morphology characterics and molecular showed the isolates are most likely belonging to *Curvularia* sp. and *Botryodiplodia* sp. (Figure 1).

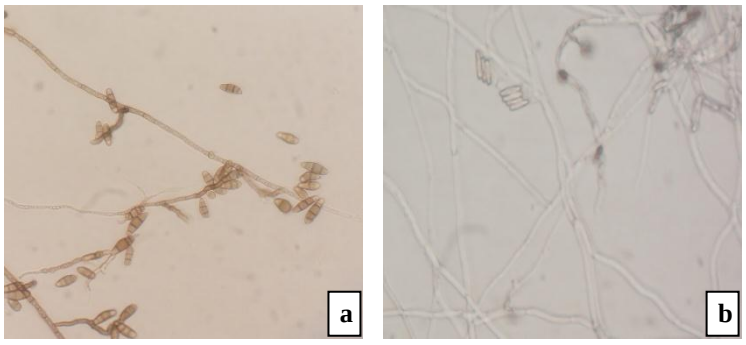


Fig 1 Morphology of pathogenic fungi from oil palm. (a) *Curvularia* sp. (b) *Botryodiplodia* sp

Table I Identification results of chitinolytic isolates

| Soil Origin | Total Isolate | Isolates Code | Gram | Chitinolytic Index |
|---|---|---|---|---|
| Oil Palms | 14 | SAHA3.01 | + | 0.33 |
| | | SAHA3.02 | + | 0.15 |
| | | SAHA12.04 | + | 0.53 |
| | | SAHA12.05 | + | 0.25 |
| | | SAHA12.06 | + | 1.27 |
| | | SAHA12.07 | + | 0.29 |
| | | SAHA12.08 | + | 3.40 |
| | | SAHA12.09 | + | 0.25 |
| | | SAHA12.10 | + | 0.40 |
| | | SAHA12.11 | + | 1 |
| | | SAHA12.12 | + | 0.29 |
| | | SAHA12.13 | + | 0.71 |
| | | SAHA12.14 | - | 0.20 |
| | | SAHA12.15 | + | 0.50 |
| Rubber Tree | 14 | KAHA7.01 | + | 0.33 |
| | | KAHA7.02 | + | 0.21 |
| | | KAHA7.03 | + | 0.22 |
| | | KAHN10.01 | - | 0.08 |
| | | KAHN10.02 | + | 0.16 |
| | | KAHN10.03 | + | 0.20 |
| | | KAHN10.04 | + | 0.11 |
| | | KAHN10.05 | + | 0.04 |
| | | KAHN10.06 | + | 0.07 |
| | | KAHN10.07 | + | 0.25 |
| | | KAHN10.08 | + | 0.18 |
| | | KAHN13.09 | + | 0.13 |
| | | KAHN13.10 | + | 1.67 |
| | | KAHN15.12 | + | 0.76 |

### 3.3. *In vitro* Screening of Chitinolytic Bacteria against Pathogenic Fungi

Ten chitinolytic bacteria with highest chitinolytic index were antagonistic tested against two isolates pathogenic fungi (Table 2). The test showed strain SAHA12.08, SAHA12.10, and SAHA12.13 were able to inhibit the growth of two pathogenic fungus at once (Botryodiplodia sp. and Curvularia sp.) whereas KAHN15.12 was only able to inhibit the growth of Curvularia sp. pathogenic (Table 3).

### 3.4. Identification of Chitinolytic Bacteria which has Antagonistic Activity

Two isolate of higher chitinolytic index which had antagonistic activity towards two pathogenic fungus were identified by using kit bioMérieux (API 50 CH). Strain SAHA12.08 and SAHA12.13 which had higher chitinolytic index showed antagonistic activity to two pathogenic fungus. Therefore, strain SAHA12.08 and SAHA12.13 were identified with kit BioMérieux (API 50 CH) which identification was based on biochemical characteristics (Table 4). Results showed that strain SAHA12.13 had similarities to *Bacillus cereus* in amount of 82.7% while strain SAHA12.08 had similarities to *Bacillus thuringiensis* in amount of 99.9% (Figure 2).

Table II Antagonistic activity of ten highest chitinolytic index (CI) isolates

| Isolates Code | CI | Pathogenic Fungi | Antagonistic Activity |
|---|---|---|---|
| SAHA12.08 | 3.40 | *Curvularia* sp. | + |
| | | *Botryodiplodia* sp. | + |
| KAHN13.10 | 1.67 | *Curvularia* sp. | - |
| | | *Botryodiplodia* sp. | - |
| SAHA12.06 | 1.27 | *Curvularia* sp. | - |
| | | *Botryodiplodia* sp. | - |
| SAHA12.11 | 1.00 | *Curvularia* sp. | - |
| | | *Botryodiplodia* sp. | - |
| KAHN15.12 | 0.76 | *Curvularia* sp. | + |
| | | *Botryodiplodia* sp. | - |
| SAHA12.13 | 0.71 | *Curvularia* sp. | + |
| | | *Botryodiplodia* sp. | + |
| SAHA12.04 | 0.53 | *Curvularia* sp. | - |
| | | *Botryodiplodia* sp. | - |
| SAHA12.15 | 0.50 | *Curvularia* sp. | - |
| | | *Botryodiplodia* sp. | - |
| SAHA12.10 | 0.40 | *Curvularia* sp. | + |
| | | *Botryodiplodia* sp. | + |
| SAHA3.01 | 0.33 | *Curvularia* sp. | - |
| | | *Botryodiplodia* sp. | - |

+ antagonistic activity - no antagnonistic activity

Table III Antagonistic properties on pathogenic fungi

| Pathogenic Fungi | Isolates Code | Zone of Inhibition (mm) | Inhibition on Radial Growth (%) |
|---|---|---|---|
| *Curvularia* sp. | SAHA12.08 | 1.5 | 36.25 |
| | SAHA12.13 | 8.5 | 58.75 |
| | SAHA12.10 | 9 | 57.50 |
| | KAHN15.12 | 2 | 41.25 |
| *Botryodiplodia* | SAHA12.08 | 1 | 16.82 |
| | SAHA12.10 | 6.5 | 46.97 |
| | SAHA12.13 | 8.5 | 52.50 |

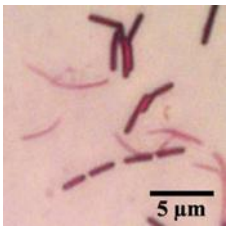


Fig 2 Gram staining of isolate SAHA 12.08

Table IV Biochemical characteristics of isolates SAHA12.13 using API 50 CH

| Test | SAHA12.08 | SAHA12.13 |
|---|---|---|
| Glycerol | - | - |
| Erythritol | - | - |
| D-Arabinose | + | - |
| L-Arabinose | + | - |
| Ribose | - | + |
| D-Xylose | + | - |
| L-Xylose | - | - |
| Adonitol | - | - |
| B-Methyl – D-Xyloside | - | - |
| Galactose | + | - |
| Glucose | + | + |
| Fructose | - | + |
| Mannose | + | + |
| Sorbose | - | - |
| Rhamnose | + | - |
| Dulcitol | - | - |
| Inositol | - | - |
| Mannitol | - | - |
| Sorbitol | - | - |
| a-Methyl-D-Mannoside | - | - |
| a-Methyl-D-Glucoside | - | - |
| N-Acetyl Glucosamine | - | - |
| Amygdalin | - | - |
| Arbutin | - | - |
| Esculin | + | + |
| Salicin | - | - |
| Cellobiose | - | - |
| Maltose | + | - |
| Lactose | - | - |
| Melibiose | - | - |
| Sucrose | + | - |
| Trehalose | + | + |
| Inulin | - | - |
| Melezitose | - | - |
| Raffinose | - | - |
| Starch | - | + |
| Glycogen | - | + |
| Xylitol | - | - |
| Gentiobiose | - | - |
| Turanose | + | - |
| D-Lyxose | - | - |
| D-Tagatose | - | - |
| D-Fucose | - | - |
| L-Fucosel | - | - |
| D-Arabitol | - | - |
| L-Arabitol | - | - |
| Gluconate | - | - |
| 2-Keto Gluconate | - | - |
| 5-Keto Gluconate | - | - |

### 3.5. Modeling Bacterial Growth Curve from Selected Isolates and Antagonistic Activity

Two isolates namely SAHA12.08 and SAHA12.13 which higher chitinolytic index and showed antagonistic activity to pathogenic fungi were measured its growth as well as antagonistic activity. Strain SAHA12.08 reached exponential phase at 0 hour to 24 hours. Stationary phase occurred within 24 hours to 60 hours while death phase was reached at 60 hours to 72 hours. Antagonistic activity also showed that optimum inhibition occurred at 0 hour to 24 hours while decreasing occurred after 24 hours (Figure 1). Strain SAHA12.13 was also observed its growth and antagonistic activity, however this strain showed no activity at all to inhibit pathogenic fungi.

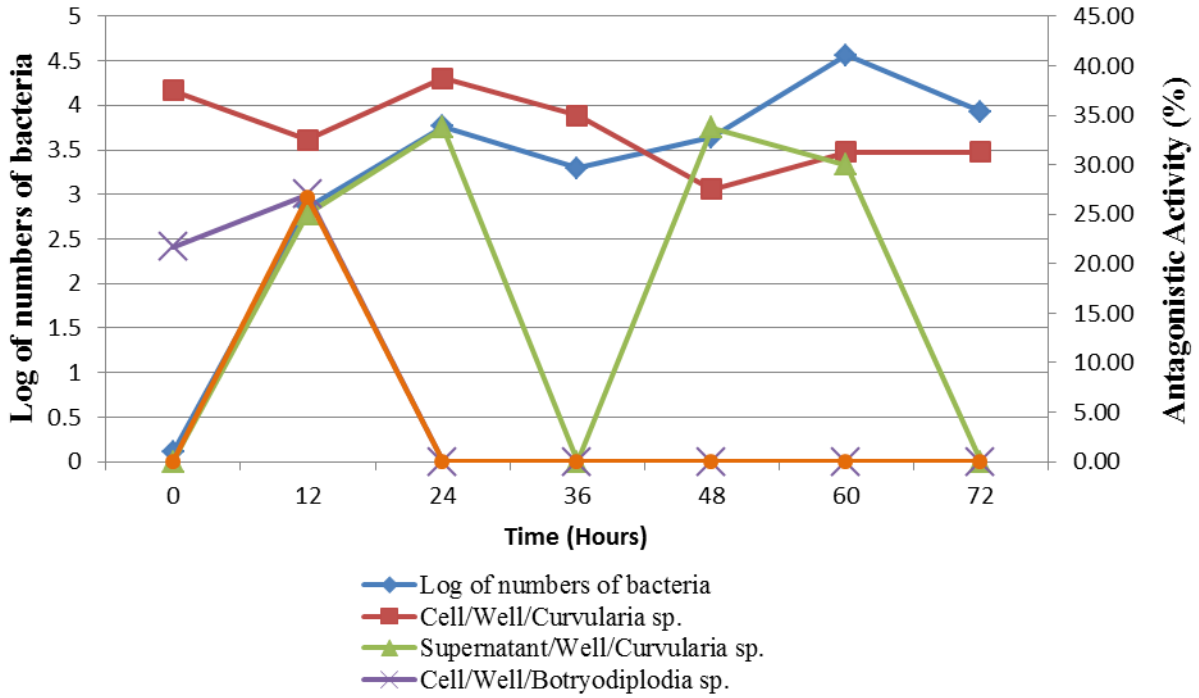


Fig 3 Growth curve of strain SAHA 12.08 and its antagonistic activity toward *Curvularia* sp. and *Botryodiplodia* sp.

### 3.6. Test for Inhibition of Fungal Attack on Oil Palm Leaf

A total of 1.5x106 conidium / µL suspension of the conidium of Curvularia sp. fungus was inoculated as much as 100 µL on the oil palm leaf. Filtrate culture of 24-hour SAHA 12.08 are inoculated on leaf treatment using Curvularia sp. Leaves treated with Curvularia sp. as control resulted in a large surface area of 244 mm2 leaf spot, compared with leaf treated with SAHA 12.08 leaf surface area of 51 mm2 and 21 mm2 (Table 5). While observing anatomical structures, leaves infected by Curvularia sp. was damaged in the epidermal tissue and mesophyll tissue (Figure 4).

Table V Surface area of oil palm leaf spot that attacked by Curvularia sp. after being treated with Bacillus thuringiensis SAHA 12.08.

| Treatment | Average of leaf surface area ($mm^2$)* |
|---|---|
| *Curvularia* sp. | 244 c |
| *Curvularia* sp. (3rd day) + SAHA 12.08 | 51 ab |
| *Curvularia* sp. (7th day) + *SAHA 12.08* | 21 a |

*The number was followed by the same letter was not significantly different based on Duncan's multiple range test (DMRT) at 5% level.

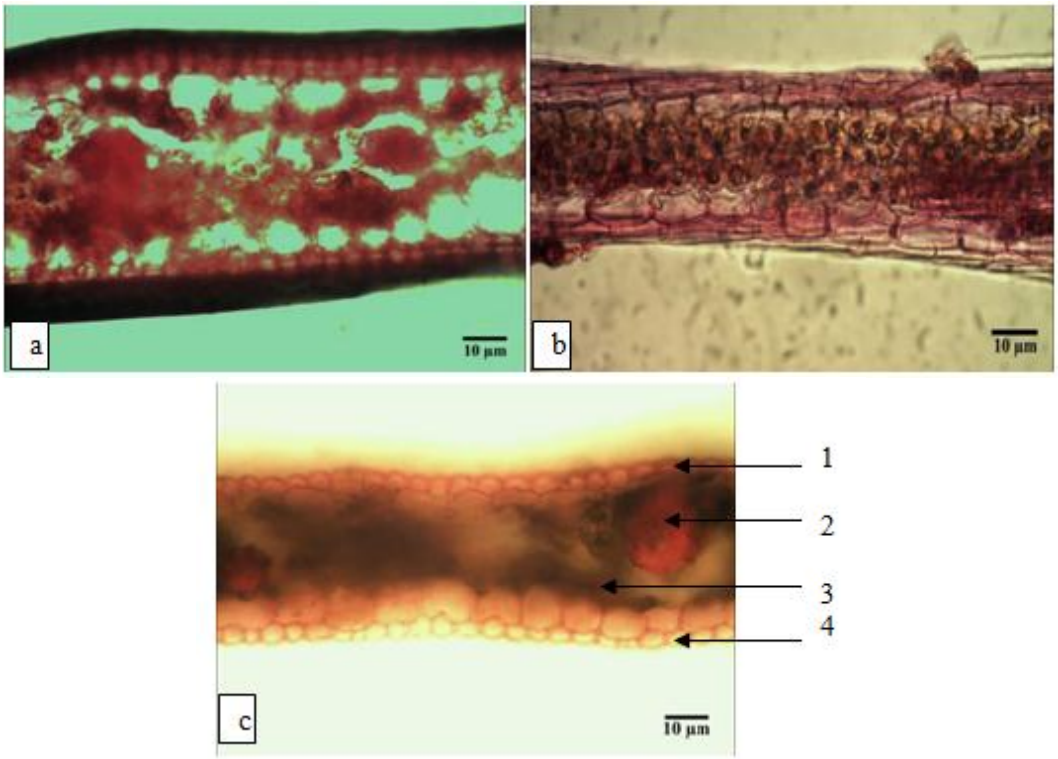


Fig 4 Anatomical structure of oil palm leaves after being treated with Curvularia sp. and Bacillus thuringiensis SA 12.08. (a) Curvularia sp. (b) Curvularia sp. + Bacillus thuringiensis, (c) negative control (-). 1) upper epidermis, 2) vascular files, 3) mesophyll, 4) lower epidermis.

## 4. Discussion

An amount of 28 isolates of chitinolytic bacteria were successfully isolated by using chitin agar (1% chitin colloidal, 0.1% $MgSO_4 \cdot 7H_2O$, 0.02% $K_2HPO_4$, 0.1% yeast extract, and 1.5% agar). Colloidal chitin was common substrate which was used as chitinase enzyme inductor (Haran & Chet 1995). Chitinolytic isolates can be detected through clear zone around bacterial colony. Isolation resulted 28 chitinolytic isolates which had chitinolytic index ranged from 0.04 to 3.40. Ten of the highest chitinolytic index isolates were selected for antagonistic testing. Those isolates were SAHA3.01, SAHA12.04, SAHA12.06, SAHA12.08, SAHA12.10, SAHA12.11, SAHA12.13, SAHA12.15, KAHN13.10, and KAHN15.12. Strain SAHA12.08 showed highest chitinolytic index in amount of 3.40. Chitinolytic index of strain SAHA12.08 (3.40) was higher than the average of chitinolytic index of isolates from chili plant roots which was 1.00 (Nurdebyandaru *et al*. 2008). Strain SAHA12.08 also showed higher chitinolytic index than the highest chitinolytic index of isolates from rubber tree which was 0.52 (Muharni & Widjajanti 2011). Q11

There were two pathogenic fungus which were successfully isolated from infected leaves of oil palms. *Curvularia* sp. is classified into phylum Ascomycota. It's morphological characteristics include brown hypae, brown conidiophores which are simple or branched, and white to pinkish gray initial colony which turns to olive brown or black as the colony matures (Larone 1995). Another pathogenic fungi which was isolated was *Botryodiplodia* sp. *Botryodiplodia* was first used as a subdivision of *Diplodia*. *Botryodiplodia* sp. belong to the class of Ascomycetes and live in a saprophytic way. They are molds which normally need injured tissue to parasite the plant. Characteristically for this fungus is the generation of pycnidia in which the spores of the fungus are formed. Pycnidia develop on artificial media only rarely and after a long time. The spores are

elliptical and compared to other spores relatively big. Young and immature spore are colourless and unicellular whereas mature spores are brown coloured, distichous, and thick-walled (Kunz 2007).

From 10 of higher chitinolytic index isolates, only 4 isolates showed antagonistic activity. Those isolates were SAHA12.08, SAHA12.10, SAHA12.13 and KAHN15.12. Strain SAHA12.13 showed strongest inhibition towards pathogenic fungus which was indicated by 58.75% of inhibition of radial growth to *Curvularia* sp. and 52.50% of inhibition of radial growth to *Botryodiplodia* sp. Strain SAHA12.08 and SAHA12.10 were also able to inhibit th growth of *Curvularia* sp. and *Botryodiplodia* sp. however its antagonistic activity were not as strong as strain SAHA12.13. Strain SAHA12.10 showed inhibition of radial growth to *Cuvularia* sp. in amount of 57.50% and 46.97% of inhibition to the growth of *Botryodiplodia* sp. Inhibition of radial growth to *Curvularia* sp. and *Botryodiplodia* sp. by strain SAHA12.10 was slightly lower than strain SAHA12.13, however SAHA12.10 showed low chitinolytic index and therefore this strain was not tested any further. Strain KAHN15.12 were only able to inhibit the growth of *Curvularia* sp. Variation in inhibition maybe was caused by specification enzyme to the species, differences chitinase activity, and chitin composition in fungi cell wall, also existence of antifungal metabolites. Fungi cell wall generally consist of not only chitin but also another type of sugars. For example, β-1,3 glucan which was bound to chitin. In that case, there are more than one enzyme which are responsible in degradation of cell wall (Anand & Reddy 2009).

Strain KAHN13.10 showed the second highest chitinolytic index but surprisingly strain KAHN13.10 was not able to inhibit pathogenic fungi. On the other hand strain SAHA12.10 and SAHA12.13 showed lower chitinolytic index than strain KAHN13.10, but they were able to inhibit the growth of pathogenic fungi especially strain SAHA12.13 which had strongest inhibition to pathogenic fungi. This results showed that compatibility of enzyme to the substrate is very essential in playing antagonistic activity as well as antifungal properties (Gohel *et al.* 2006).

Strain SAHA12.08 was selected for modeling growth curve and antagonistic activity for its higher chitinolytic index than SAHA12.10 while being able to inhibit all pathogenic fungi at once. Result showed that strain SAHA12.08 was able to inhibit *Curvularia* sp. and *Botryodiplodia* sp. optimally at 0 hour to 24 hours. Its ability to inhibit pathogenic fungi decreased after 24 hours. The growth curve indicated that exponential phase occurred at 0 hour to 24 hours, stationary phase occurred at 24 hours to 60 hours, and finally death phase was reached at 60 hours to 72 hours. On the other hand, strain SAHA12.13 showed no antagonistic activity at all, despite of stronger inhibition to all pathogenic fungi which it had at the early antagonistic testing. This might be due decreasing of viability of the strain in the culture for its had been stored for long time.

Optimum inhibition of strain SAHA12.08 at 0 hour to 24 hours probably was caused by high level of chitinase extracellular production on exponential phase. It has been reported that extracellular chitinase production was sharply increased during the exponential phase and dramatically declined when the cells entered the the stationary phase (Prapagdee *et al*. 2008). Strain SAHA12.08 was still able to inhibit *Curvularia* sp. on stationary phase probably due to secondary metabolites which were produce on stationary phase, although concentration of hydrolytic enzyme had decreased. Somehow, strain SAHA12.08 was not able to inhibit *Botryodiplodia* sp. past 24 hours. This might be caused by the decreasing production of hydrolytic enzymes and incapability of secondary metabolites which were produced on stationary phase to inhibit *Botryodiplodia* sp.

The antagonistic testing by using supernatant (cell-free-filtrate) which contains extracellular metabolites was meant to observe whether hydrolytic enzyme such as chitinase in the extracellular metabolites was responsible in inhibiting pathogenic fungal growth. Result showed that cell-free-filtrate was able to inhibit the growth of pathogenic fungi, albeit the inhibition was lower than inhibition by cell. Differences in ability to inhibit might be caused by differences in concentration of hydrolytic enzyme or even secondary metabolites on the filtrate (Prapagdee *et al*. 2008).

Identification using kit API 50 CH (bioMérieux, USA) showed isolate SAHA12.08 was most likely *Bacillus thuringiensis*, whereas strain SAHA12.13 was most likely *Bacillus cereus*. Identification using API 50 CHB

system turned out to be inapplicable for strain SAHA12.08 because discrepancies in the reaction patterns resulted in differing identifications. Isolate SAHA 12.08 showed inhibition activity against *Curvularia* sp. using leaf of oil palm in vitro test. Leaves treated only with *Curvularia* sp. as negative control resulted in larger surface area of leaf spot compared to leaf treated with SAHA 12.08. The results indicated the synergism of action of bio-control mechanisms of cell culture such as antibiotic production and induction of plant resistance with genes activation such as chitinase, β 1,3- glucanase, peroxydases, and phenylalanine ammonia lyase (Chang *et al*. 2007 ).

## 5. Conclusion

In conclusion, an amount of 28 chitinolytic isolates were successfully isolated. Strain SAHA12.08, SAHA12.10, and SAHA12.13 were able to inhibit the growth of Curvularia sp. and Botryodiplodia sp. Whereas, strain KAHN15.12 was only able to inhibit Curvularia sp. Strain SAHA12.08 showed optimally antagonistic activity to all pathogenic fungi at exponential phase. Isolate SAHA 12.08 which is identified as Bacillus thuringiensis showed inhibition activity against Curvularia sp. using leaf of oil palm in vitro test. Leaves treated only with Curvularia sp. as negative control resulted in larger surface area of leaf spot compared to leaf treated with SAHA 12.08.The oil palm leave which infected by Curvularia sp. was damaged in the epidermal tissue and mesophyll tissue.

## 6. Acknowledgement

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