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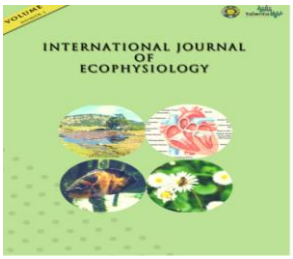

The Effectivity of Bacteria Isolated From of Liquid Waste Palm Oil Plantation on *Ganoderma boninense*

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Abstract. *Ganoderma boninense* is one of the main pathogenic fungus in oil palm plantations. Generally, these pathogen cause root rot (basal stem rot). Biological control that has been widely used reduce the infection is using bacteria. Liquid waste palm oil has potential to produce bacteria that is able to degrade *Ganoderma boninense* that causes root rot in oil palm. Liquid waste were obtained from Muaro Sabak Regency Jambi Province. Bacteri were isolated and cultivated in nutrient agar medium, characterized and identified for antagonistic test against *G. boninense*. Results showed that 16 bacterial isolates were identified, among of them are able to inhibit *Ganoderma boninense*

Keywords: *Ganoderma boninense*, palm oil, liquid, stem, waste

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1. Introduction

Palm oil (*Elaeis guineensis*) is one of the growing plantations in the Sumatra and Kalimantan regions. Palm oil is a superior commodity that can increase foreign exchange. This has caused the area of oil palm plantations to continue to expand and develop quite rapidly. Jambi is one of the provinces that make oil palm the main commodity that needs to be developed. Data from the Agriculture Office of Jambi Province shows that, outside the oil palm plantation area, it reached 465,265 ha. (Directorate General of Plantation, 2015). This figure shows that oil palm plantations are more dominant compared to other plantation products such as rubber, coffee, tea, etc.

In a few decades, there has been a decline in palm oil production. Some research shows that one of the main enemies in oil palm plantations is *Ganoderma boninense*. *Ganoderma*

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boninense is a group of fungi that causes infection at the base of the palm oil stem (basal stem rot). In some oil palm plantations in Indonesia, this disease has caused the death of up to 50% or more of the total oil palm plant population, resulting in a decrease in oil palm per unit area (Turner 1981). Oil palm plants that are attacked by a stem rot (basal stem rot) can be known from the tree crown. The sick tree has a janur (leaves that have not opened, spear leaves) more than usual. Pale green leaves. Old leaves wither, break on the midrib, and hang around the stem (Semangun 2000).

One form of biological control that has been widely used is to use various microorganisms (Duffy 1995) such as bacteria. Bacteria are often used as biological control agents based on the ability of microorganisms to produce chitinase and β 1,3-glucanase which can lyse fungal cells (El-Katatny *et al*, 2000). Based on how the hydrolysis works according to Brurberg *et a,*. (1996), chitinase is grouped into: (1) endocitinase, which randomly cuts chitin polymer internally resulting in a short oligomer, (2) exokitinase (1,4-β-ketobiosidase), which cuts the ketobiose trimer unit at the end of the polymer terminal chitin, and (3) N-acetylglucosamidase, which cuts the monomer unit at the terminal end of the chitin polymer.

PT Wirakarya Sakti is a paper industry company producing 100 tons of waste per day (Sunarti, 2004). It is known that paper industry wastewater contains high organic pollutants such as lignin, cellulose, hemicellulose and small amounts of pectin and lignin which are one of the bacterial habitats. Based on the content, it is possible that bacteria from the waste have the ability to degrade fungi, one of which is *Ganoderma boninense* which causes stem rot in oil palm. Thus, it is necessary to conduct research on the effectiveness of the origin of WKS wastewater in inhibiting the growth of pathogenic fungi, *Ganoderma boninense*, which causes root rot of oil palm stems.

2. Materials and Methods

This research was carried out in the laboratory of Biotechnology and Engineering, Faculty of Science and Technology, University of Jambi from April to November 2017. Location of wastewater extraction for HTI Wira Karya Sakti in Muaro Sabak Regency, Jambi Province, while Ganoderma boninense is isolated from oil palm which experiences stem rot in the oil palm plantation of RT 15, Sungai Duren Village. The tools used in this study were analytic scales, autoclaves, ovens, incubators, light microscopes, refrigerators, scalpels, tweezers, glassware, pH meters, erlenmeyers, test tubes, petri dishes, measuring pipettes, oases, Pasteur pipettes, bunsen lights, spatulas, shovels, and digital cameras. While the ingredients used are PDA medium (Potato Dextrosa Agar), aquades, 70% alcohol, 5% chlorine, NA medium (Nutrient Agar), Violet Crystal, iodine, safranin solution, immersion oil, Sulfide Indole Motility (SIM) media, media Triple Sugar Iron Agar (TSIA).

Research design

Isolation of Potential Bacteria from Liquid Waste Wira Sakti Works Potential bacteria which are biological control agents of pathogenic fungi *Ganoderma boninense* are isolated from liquid waste taken from HTI Wira Karya Sakti Muara Sabak Regency, Jambi Province. Isolation was carried out by taking as much as 1 ml of liquid waste sample which had previously been measured by pH and the electrical conductivity into the NA media was modified. The sample was diluted to 10-2 and then planted in modified NA media using the pour plate method. Next it was incubated for 24-48 hours at 30ºC.

Potential Isolation

Screening of Liquid Waste Wira Karya Sakti Isolates that had previously been grown on NA media were purified by streak plate technique, incubated for 24-48 hours at 30 ºC until a single colony grew at the end of the scratch. Separate growing bacterial colonies were taken and moved into maintenance medium (NA-sloping) as pure culture for further characterization and identification stages.

Characterization of Potential

Isolates from Liquid Waste Wira Karya Sakti Characterization of isolates was carried out on isolates grown on modified NA media, including morphological and biochemical characters in the form of: macroscopic colonies by observing the shape, color, elevation, and edges of bacterial colonies, while the microscopic morphology of bacterial cells was carried out by Gram staining, SIM test (Sulfide Indol Motility), TSIA (Triple Sugar Iron Agar) test, and catalase test.

Gram Painting Potential Isolates

The glass to be used is cleaned with alcohol, then baked in a bunsen flame. Bacterial culture of 24 hours of age is taken with ose, then flattened over glass of objects and dried. Fixation is carried out on the flame of a spirit lamp. After cold and stains are formed, the drops are painted with gram A (crystal violet) paint on thin preparations until they are all covered and left for 1 minute, then washed with running water and dried. After that, it is dripped with gram gram B (Lugol iodine) allowed to stand for 2 minutes, then washed again with running water and dried. Then decolorization using gram C diluent solution (70% alcohol) for 30 seconds and made again using running water and dried. The last paint is gram D (safranin) for 1-2 minutes, then washed and dried. After that, it was observed under a microscope with 1000x magnification.

SIM Test (Sulfide Indol Motility)

Motility test is done by inoculating bacterial isolates by inserting an ose needle perpendicular to half the height of Sulfide Indol Motility media in a test tube. The tube was incubated for 48 hours at 30 ° C, after which trace trace of bacteria was observed.

TSIA (Triple Sugar Iron Agar) Test

TSIA test is a biochemical test to determine the ability of isolates in fermenting glucose, sucrose and lactose contained in the medium. This test is done by making a scratch on the media so that it sticks and thrusts it into the bottom media. Then incubated at room temperature for 48 hours. Positive reactions are indicated by changes in the medium color to black.

Catalase test

The bacterial isolate is taken one ounce and placed in a glass of sterile object. Penetrated 30% H2O2 solution. Positive results are indicated by the formation of air bubbles. Identification of Potential Isolates from Wira Liquid Waste Sakti Works Identification of isolates that showed positive was done using the Bergey Manual of Derminative of Bacteriology (Hot el al., 1994) and the Bergey Manual of Systematic Bacteriology Vol 2A Benner al.,1923).

Isolation of Pathogenic Fungi, Ganoderma boninense

Aseptically G. boninens cause of base rot the palm oil stem is taken and put into a plastic bag.

3. Result and Discussion

The results of gram staining can be seen that the forms of isolate cells are diverse, in the form of bacillus, coccus basil and streptococcus which are negative and positive. The TSIA media aims to distinguish the type of bacteria based on the ability to break down glucose, lactose, sucrose. Positive results are marked by changes in color to yellow which shows the ability of bacteria to ferment the three types of sugar. From the test results, only 3 isolates namely Sp2, Sp6, and Sp7 were able to ferment the three types of sugar, while others were categorized as not having the ability to break down glucose, lactose, and sucrose so that the implications were negative. SIM (Sulfate, Indol Motility) test aims to see the movement of bacteria and produce sulfite, this is indicated by a pattern in the bacterial puncture which shows that generally isolates are motile, while 2 other isolates such as Sp5 and Sp8 do not flatten. In the sulfite test, it was seen that all isolates were negative, this was due to the absence of black deposits on the medium which indicated that all isolates did not produce H2S. The indol test aims to look at the ability of bacteria to contain the enzyme tryptophanase which is a catalyst that decomposes indole groups contained in the amino acid tryptophan.

Positive results are indicated by the formation of a red ring on the dividing line, while the formation of the red ring between the media and reagent does not show a negative result. From these tests it can be seen that all isolates are negative indole, meaning that isolates do not have tryptophanase enzyme in catalyzing the amino acid tryptophan. The catalase test shows that all isolates are catalase negative, meaning that the isolates do not have the ability to produce the enzyme catalase or peroxidase which can dehydrate hydrogen peroxide (H202) to be catalyzed to H2O and O2. WKS Liquid Waste is known to be one of them accumulated in a flooded area which is located alongside the Industrial Plantation Forest of PT Wirakaryasakti Jambi. Based on physical measurements, this waste has a temperature of 28 - 31 ºC with a degree of acidity ranging from 3 - 3.4. Periodic decomposition of solids from organic and inorganic materials such as litter and chemical fertilizers that settle does not determine the isolates obtained can reduce sulfur to sulfide, this is evident from all the isolates that are negative for catalase test and sulfide test. However, most of these isolates are able to ferment glucose and are motile, meaning that the possibility of selected isolates is the *enterobacteriacee* group.

Saraswathi and Saseetharan [9] states that *Pseudomonas alkaligenes, Bacillus pumilus, Bacillus subtilis* are bacteria that can degrade paper. However, some characters did not show that the isolates had similarities with these isolates, thus isolates isolated from WKS Liquid Waste are certain species of enterobacteriace group that need to be identified more specifically. After all bacterial isolates from WKS wastewater were characterized, antagonistic tests were carried out between bacterial isolates and fungi that cause the base rot of oil palm stem, namely *G. boninense*

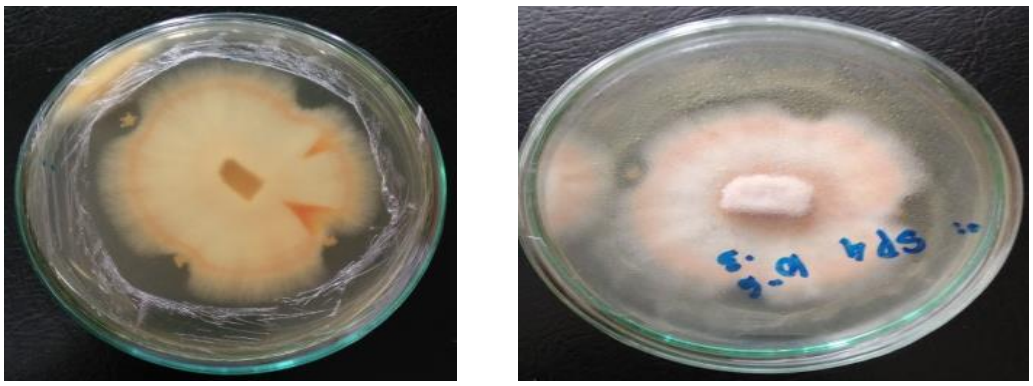

Figure 3. Antagonism Bakteri Sp4 and *G. boninense* A.upper side B. down side

From the results of the identification, it was found that bacterial isolates which have the potential to inhibit the growth of *G. boninense* are gram-negative bacteria from the enterobacter group. The clear zone formed on day 5 after incubation was 2.04 mm. Inhibition with a clear 2.04 mm zone is included in the low criteria, as said by Martin *et al* (2015) that if a small clear zone of 16,315 mm is included in the low criteria. While the criteria is high if the clear zone is

large from 25,807 mm and the criteria is moderate if the clear zone is 16,315 to 25,807 mm. The results of the study Martin *et al* [5] stated that as many as 3 bacterial isolates tested for antifungal activity against target fungi *Colletotrichum capsici* and *G.boninense* found no bacterial isolates that had antifungal activity against the target fungi which were characterized by no clear zones around test fungi isolates.

The clear zone formed indicates the growth inhibition of the test fungus namely G. boninense. Bacterial isolates from WKS liquid waste that can inhibit the growth of G. boninense are likely to have antifungal compounds that can inhibit the growth of pathogenic fungi, as stated by Propagdee et al. (2008) and Augustine et al [1] that some of the antifungal compounds produced by Streptomyces include chitinase, β-1,3-glucanase, nystatin and natamycin enzymes have been reported to inhibit the growth of pathogenic fungi in plants and can damage fungal cell walls consisting of chitin and other polysaccharides [4;6]

4. Conclussion

Results showed that 16 bacterial isolates were identified, among of them are able to inhibit *Ganoderma boninense*.

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