ISOLATION CELLULOLYTIC BACTERIA DEGRADATION OF EMPTY FRUIT BUNCH OF OIL PALM IN PEATLAND

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ABSTRACT

The using of a peatland as the food crops cultivation have been motivate the part of an agriculture to more developing the agricultural innovations, such as the making of an organic fertilizer that can be used by the food crops in the peatland. An organic material that can be used as the raw material to make an organic fertilizer, which one it is the empty fruit bunch of oil palm (EFBP). It is something like the compost heap or the waste of oil palm trees, which is contain of cellulose with the result that the substrate for the growth of the cellulolytic bacteria. The election of EFBP as the source of the isolate is because there is many oil palm trees which is cultivated on the peat land and it is naturally degraded around the palm trees. The natural degradation of EFBP will require a long time enough. Therefore, this study is purposed to isolating the cellulolytic bacteria from EFBP and to obtain bacteria that having a high cellulolytic index and also acid-resistant bacteria, and later could be used to remodel the EFBP itself as well as the other organic matter in the peatland. Based on the isolation result and morphology observation according to gram coloring, it is obtained 12 isolates which there is 4 isolates that negative Gram cocci form , 1 isolate that positive Gram cocci form, 2 negative Gram basil, 2 gram negative diplobasil, 2 positive Gram basil, and 1 negative Gram basil. Furthermore to do the measuring of cellulolytic is to know the ability of the bacteria of producing an cellulase enzyme that pass through the clear zone which is formed around the colony. Based on the observation, the highest amount of cellulolytic index is 7 which are obtained from the diplobasil shaped positive gram. The best time to apply the making of organic fertilizer which one is should be done on the third day of incubation.

***Keywords**: peatland, empty fruit bunches of oil palm, cellulolytic bacteria, cellulolytic index*

INTRODUCTION

Peatlands area in Indonesia is estimated of 20.6 million ha, which is spread over five islands, which is : Sumatra (7.2 million ha), Kalimantan (4.4 million ha), Sulawesi (44,000 ha), Maluku (48,000 ha), and Papua (8.9 million ha) (Wahyunto, 2007). While the area of peatland in Riau Province is reaching 4.1 million ha, an the 817.593 ha area have been utilized became a plantations (Suwondo *et al*., 2012). A few commodity of crop plantations in peatland are oil palm and rubber. This is because they are supported by the majority of peatland in Riau Province which is thick enough (about 2-3 m even thicker). That according to the Ministry of Agriculture stated that peat with a thickness of 2-3 m is very suitable for the plantation plant (Sabiham *et al*., 2008).

As a means of agricultural cultivation, the shallow peatlands with a depth of less than 100 cm can be used for planting crops such as rice, soybean, maize, cassava, and various other vegetables plant (Agus and Subiksa, 2008). The development of the cultivation of food crops in peatlands have a constraints such as high soil acidity and the nutrient levels and low base saturation (Ratmini, 2012). Agus and Subiksa (2008) said that the peat has a low fertility rate because of the diverse of organic elements that most are toxic to plants, and micro elements which is contained in peatlands is very low and tightly tied with the result that it can not be used by the plants.

Subsidence and low nutrient content of the peat can be improved by adding an organic matter. The organic material can be described by using bio-activator that containing cellulolytic bacteria as a strategy to accelerate the decomposition process. Cellulolytic bacteria are bacteria that can produce cellulase enzymes for the decomposition of cellulose into its compounds such as glucose, maltose and so forth (Saraswati *et al*., 2008).

The resources of organic matter is containing a cellulose that can be come from agricultural waste, called oil palm empty bunches or EFBP. The quantity of EFBP waste in Riau Province in 2010 have reached 5, 050,367.6 tons and and increase in 2011 become 5,176,842.53 tons (Andriyati 2007 in Tarkono and Ali, 2015). This waste of EFBP can be converted into a value-added products such as an organic fertilizer that could support a sustainable farming practices.

The utilization EFBP as the raw material in the production of organic fertilizer in the peatlands have constraints like the difficulty of the organic materials are decomposed by bacteria from the soil minerals. Therefore, it is necessary to discover cellulolytic acid-resistant bacteria that can come from EFBP which is undergoing decomposition process around the palm trees that planted on the peatland.

The EFBP will continue to be degraded naturally, but the time it takes to become compost takes quite a long time. Therefore, the use of cellulolytic microbes that have been isolated are expected to provide some of the advantages of which is can accelerate the decomposition of that organic waste materials and TKKS also can be used as organic materials are widely available for the composting production.

An important role in degrading the cellulose inside the cellulolytic bacteria from EFBP, in result that this study is proposed to isolate and characterize the bacterial EFBP cellulolytic which is origin of the peatland in order to obtain a low pH-resistant bacteria as a cellulose degrading organic matter.

MATERIALS AND METHOD

This research was conducted at the Laboratory of Soil, Faculty of Agriculture, University of Riau, Campus Bina Widya KM 12.5 Simpang Baru Village, Tampan District, Pekanbaru.

Materials and tools. The materials that used in this study is rice straw. Some of the chemicals that used in this study are Carboxymethyl Cellulose (CMC), Nutrient Agar (NA), medium sugar (sucrose, glucose, mannitol, maltose), MgSO4.7H2O, K2HPO4, FeSO4.7H2O, CaCl2.2H2O, yeast extract, NH4NO3, KH2PO4, Kovac's reagent, reagent Barrit A, reagent Barrit B, spritus, alcohol 70%, kit Gram staining, Congo red, NaCl, distilled water and oil immersion.

Research method. The method which is used in this research is the method of explorative consists of seven phases: first phase is the land survey and determining the location, the second phase is taking a sample, the third phase is isolation and purification, the fourth phase is morphological characterization, the fifth phase is characterization of physiology, the sixth phase is biochemical characterization, and the seventh phase is potential testing of cellulolytic bacteria. The data which is obtained from the results of bacterial identification and test of resistance to low pH test is a qualitative data which later analyzed descriptively and presented in the form of tables and figures.

Implementation Research (Land survey and determination of the location). Land survey was conducted to determine the condition of the location where sample is taken is in the sei mandau village, siak regency. These activities include the measurement of pH, moisture and peat depth at EFBP sampling sites. The determining of the sampling location are done by purposive sampling, where these locations there are an expected samples in the form of half decay EFBP.

Isolation of Bacteria. A total of 1 g of EFBP are dissolved in 9 ml of sterile saline and homogenized using a vortex for 2 minutes. With the same activity, 1 ml of the suspension was moved into 9 ml of normal saline second (10-1). Furthermore, the dilution to 10-7. Dilution 10-5-10-7, inoculated as much as 100 mL of media to the NA. Isolates were incubated for ± 3 days at the same as the room temperature. The grown Isolates will be purified by separating the single isolates from colonies that formed into the new NA media.

Gram staining bacteria. As much as 1 isolate were fixed on a glass slide that has been poured with 3 drops of KH2PO4. Mixture spread of bacteria that has fixed with the heat and flooded with crystal violet and let stand for 1 minute. Furthermore flowing rinsed with distilled water and drained. Then flooded with an iodine Gram and let stand for 1 minute, rinsed with distilled water flows and drained. Dispense 95% of ethanol (decoloration solution) for 30 seconds until the crystal violet dye preparations not rinsed again and washed with distilled water until the color spreads into the lymph. The spreadable flooded back with safranin solution for 1 minute, rinsed with distilled water and drained to dry. Bacteria that have been colored will be observed under a bright field microscope with a magnification of 1000-2000 x (Cappuccino and Sherman 1983). Positive gram bacteria will be purple, while negative gram bacteria will be red.

Qualitative Cellulolytic Test. Qualitative test performed with the 0,1% Congo red staining method. The isolate was spotted on the CMC agar medium. The bacteria were incubated for 5 days with the 37°C/

room temperature. Then test the activity of bacteria by adding 0.1% congo red as much as 15 ml and allowed to stand for 30-60 minutes. After that, rinsed it for 2-3 times with 15 mL of 1 M NaCl and allowed to stand for 15 minutes (Chasanah *et al.*, 2013). Furthermore, the diameter of clear zone and the colonies formed was measured using a caliper. The test of cellulase activity could be seen from the index formed. Cellulolytic index is the ratio between the diameter of the clear zone diameter colony. The larger the index cellulolytic generated, the bigger enzyme will be produced by the bacterial isolates. Cellulolytic index or index cellulase activity (IAS) are obtained using the following formula (Kader and Omar, 1998):

$$\text{Index cellulolytic} = \frac{\text{Clear Zone Diameter(mm)} - \text{Colonies Diameter (mm)}}{\text{Colonies Diameter (mm)}}$$

Once known the cellulolytic index which is generated from the each isolates that obtained, we then have as many as three top isolates to be continued on the biochemical characterization of the test so that later can be obtained the type of bacteria that could potentially be used in the manufacture of organic fertilizer.

Biochemical characterization of bacteria. Biochemical characterization can be done in a test like the test of fermentable carbohydrates (glucose, sucrose, maltose and mannitol). Carbohydrate fermentation test conducted by growing bacteria cultures that have lived for 24 hours in test tubes, each containing carbohydrate fermentation medium in between glucose, sucrose, maltose, and manitol. After it was incubated at 37° C for 24 hours.

Anaerobic test. The testing of anaerobic bacteria that are carried out by means of cellulolytic bacteria were 24 hours old inoculated on NA medium in a test tube up into the media and incubated for 24-48 hours at a temperature of 37°C.

Turbidity measurement bacteria (spectrophotometer). Bacterial turbidity measurement is to know the turbidity of bacteria which is related with the testing of sugar reduction amount of the resulting sediment on the reduction sugar test. Measurements were made every day during the time of incubation cultures at CMC liquid media by means of 1 ml were taken using a micro pipette, then measured using a spectrophotometer with a wave length of 600 nm.

RESULT AND DISCUSSION

Cellulolytic bacteria is a bacteria that can produce cellulase enzymes for the decomposition of cellulose into its compounds such as glucose, maltose and so forth (Saraswati *et al.*, 2008). That bacteria can degraded the cellulose because it produces cellulase enzyme. Cellulase enzymes or enzyme which is known as systematic β-1,4-glucan 4-glukano hydrolase is an enzyme that can hydrolyze cellulose to break the β-1,4 glycosidic bonds in cellulose, selodektrin, cellobiose (Nur *et al.*, 2007).

Based on the results of isolation and morphological observation microscopically through Gram staining are obtained the total of 12 isolates among all which 4 isolates shaped Gram-negative cocci, 1 isolate shaped Gram-positive cocci, 2 Gram-negative bacilli, 2 Gram-positive diplobasil, 2 Gram-positive bacillus, and 1gram negative basil. 12 isolates of the bacteria are grown on agar medium containing 1% CMC which then form the clear zone on qualitative tests were performed (Figure 1). The resulting clear zone indicate the presence of extracellular cellulase enzymes released by isolates known as cellulolytic index (Table 1).

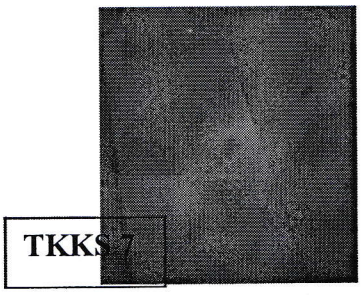


Figure 1.Clear zone isolate TKKS 7

Table 1. Index data of EFBP cellulolytic bacteria

Isolates	Diameter colony (mm)	Diameter of clear zone (mm)	The index value cellulolytic
TKKS 1	2	10	4
TKKS 2	2	3	0.5
TKKS 3	1	4	3
TKKS 4	44	48	0.04
TKKS 5	2	7	2.5
TKKS 6	5	6	0.2
TKKS 7	3	24	7
TKKS 8	3	10	2.33
TKKS 9	2	3	0.5
TKKS 10	4	7	2.33
TKKS 11	15	20	0.33
TKKS 12	2	3	0.5

In Table 1 we can see that the 12 isolates produce cellulolytic diversified index. The highest cellulolytic index isolates was produced by isolates with isolates code EFBP 7 on the fifth day of incubationand the temperature is 37° C is equal to 7. This means that the bacteria had a cellulase enzyme activity which is large enough to degrade cellulose around the colony. As stated by Zverlova *et al.* (2003), the diameter of clear zone is generally larger than the diameter of the colony, because of cellulase enzymes are secreted into the surrounding environment by cellulose degrading bacteria. However, the wide clear zone produced depends on the concentration of CMC and gelatin are used. The more CMC and gelatin are given then it will lead to smaller pores so that the cellulase enzymes secreted more difficult to pass through the pores and lead to delays in the process of degradation (Hankin and Anagnostakis, 1997).

Saraswati *et al.*, (2012) said that the EFBP contains lignocellulose which is difficult to degraded with the main organic matter cellulose that reached 45.95%. It also supports the high cellulolytic index produced by the bacteria. The ability of bacteria to degrade the cellulose to make the activity of cellulase enzyme complex produced by the bacterium is also high.

As stated Ahmed *et al.*, (2001), the rate of degradation of cellulose by microbial cellulolytic influenced by environmental conditions during the degradation process. The factors that affecting among other substances that are required by microorganisms especially essential is used both during the growth of microorganisms or the formation of an enzyme. Another factor that affects the pH and the optimum temperature is affecting the growth of microorganism and enzyme activity of cellulase. Their products either primary or secondary metabolites that can affect the working of enzymes to degrade cellulose. The presence oflarge amounts of cellobiose also affect the action of the enzyme. This is because cellobiose is the strongest inhibitor in the degradation process.

Each bacterium has a different strategy to degrade cellulose is influenced by the characteristics of the bacteria (Jeschu, 1995). Biochemical characteristics which is produced by cellulolytic bacteria can be seen in carbohydrate fermentation test (Table 2).

Table 2. Test results of carbohydrate fermentation TKKS

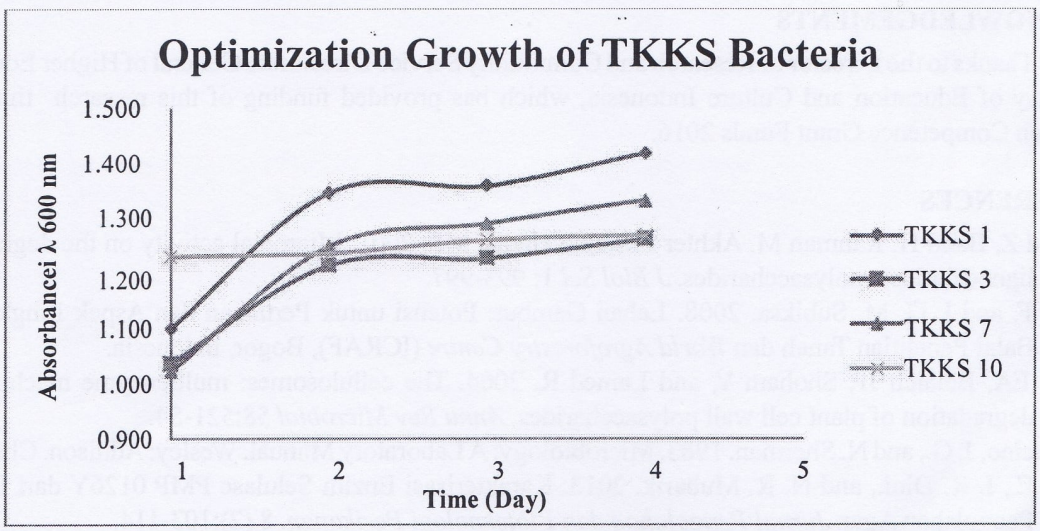
Isolates	Glucose test	Maltose test	Sucrose test	Mannitol test
TKKS 1	+	+	+	+
TKKS 2	+	+	+	+
TKKS 3	+	+	+	+
TKKS 4	+	+	+	+
TKKS 5	+	+	+	+

TKKS 6	+	+	+	+
TKKS 7	+	+	+	+
TKKS 8	+	+	+	+
TKKS 9	+	+	+	+
TKKS 10	+	+	+	+
TKKS 11	+	+	+	+
TKKS 12	+	+	+	+

Based on Table 2 can be seen that all of isolates can ferment sugars are glucose, maltose, sucrose, and mannitol. This suggests that the isolated cellulolytic bacteria can degrade all kinds of sugar because the content of cellulase enzymes or other enzyme in that bacteria such as amylase.

Next, on the needs oxygen to the bacteria test, most of the aerobic isolates (10 isolates) and 2 isolates are facultative anaerobes. This is in line with the statement Doi *et al.* (2003) and Bayer *et al.* (2004) that the degradation of cellulose in nature mostly done by aerobic microorganisms. Aerobic microorganisms produce cellulase enzymes noncomplex comprising endoglucanase, exoglucanase, and glucosidase which work synergistically to hydrolyze cellulose. Anaerobic microorganisms produce cellulase enzyme complex called selulosom. Zhang *et al.* (2006) stated that although anaerobic microorganisms accounted for only about 5-10% of the total biodegradation of cellulose in nature, but the role is very important because it is responsible for the degradation of anoxic areas on lakes, oceans, and the digestive tract ruminants and termites, which can not be performed by aerobic microorganisms.

Based on that, then at the time of overhaul of organic matter in aerobic conditions that should be expected in the degradation process is rapid and maximum. The speed of revamp organic materials into organic fertilizers are largely influenced bacterial initial conditions that will be applied. To support this, it is need to know the optimum time when the growth of the cellulolytic bacteria will be applied to organic materials. Especially in isolates EFBP 1,3,7, and 10 that have the highest index of the optimum time cellulolytic bacterial growth can be seen in Figure 2.



Based on Figure 2, it can be seen that the application all of bacteria to begin the revamp of organic material can started between day 2 to day 4. This is done so that the isolates did not take long time for the media adaptation phase in composting so that the expected production of cellulase enzymes in media production faster. On the second day, the bacteria already in the phase logarithmic the stage at which the

maximum bacterial growth. Fourth isolates decreased growth on the third day but rose back on the fourth day. This is because the first and second day, there are still a glucose that provide nutrients that are quickly and easily utilized by bacteria for growth. However, on the third day of glucose begins to run out and the bacteria begin to utilize cellulose as a source of glucose. As stated Fikrinda *et al.* (2001), glucose is one of the nutrients in the growth of bacteria as a carbon source. The use of glucose in small quantities to produce cellulase enzyme to function as an energy source for isolates to support its growth so that it can move better in hydrolyzing the cellulose amorphous or crystalline.

However, the decrease in the number of bacterial cells are not in line with the increased activity of cellulose that produced. On the third day, its suspected of cellulase enzymes produced are quite high so it is good if the day began to be applied to bacterial decomposition of organic material in composting. As the research conducted by Chasanah *et al.* (2013), the highest activity of cellulase enzyme is produced on the third day of incubation. This is because the bacteria in cellulose media will excrete cellulases with quick and its high activity in order to get the nutrients back to growth. This can be seen on the fourth day there was an increase of regrowth (Figure 2). However, the magnitude of the resulting enzyme activity should be known to be able to ensure the best time on the revamp of composting organic matter which can run up to a relatively quick time.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Based on research that has been done, it can be concluded that the EFBP contains about 12 cellulolytic microbial isolates. From the qualitative test that has been carried out on selective media cellulose (1% CMC) it is seen that all isolates producing the index cellulolytic diverse and isolates EFBP 7 produce the index cellulolytic highest is 7 in which biochemically these bacteria can ferment carbohydrates (glucose, mannitol, sucrose and maltose). The isolates are known as isolates were able to produce extracellular cellulase enzymes and can later be used as agricultural waste decomposers EFBP decomposers in the manufacture of organic fertilizers. Best time of application of bacteria to start the revamp of organic matters that able to do the third day of incubation time.

Recommendations

It is necessary to test the activity of cellulase enzyme to see the amount of activity generated by bacteria that have the highest index and to determine the cellulolytic bacterial species through the molecular identification.

ACKNOWLEDGEMENTS

Thanks to the Director of Research and Community Service Directorate General of Higher Education Ministry of Education and Culture Indonesia, which has provided funding of this research through a research Competence Grant Funds 2016.

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