Microbial Cellulolytic Isolation And Identification From Durian Leather Waste

Hapsoh[1)], Gusmawartati[1)] dan Ujang Al Husnah[2)]*

[1)]Lecturer Department of Agrotechnology Faculty of Agriculture, University of Riau
[2)]Student Department of Agrotechnology Faculty of Agriculture, University of Riau
author correspondence:email: **hapsohdin@yahoo.co.id**

Abstract

Isolation of microbial cellulolytic (cellulose decomposer) can grow naturally or intentionally given to accelerate the recast of organic materials containing cellulose. This microbe has an important role in biogeochemical cycles and very responsive to the recycling of organic compounds. Durian's peel is the household waste that being disposed as waste and has no economic value so that the recast of durian waste peel still occur naturally. Durian's peel is one of the sources of cellulose which has not been utilized optimally. The research was aimed to obtain potentially cellulolytic microbial isolates derived from durian waste peel so that it can be utilized in recasting the cellulose either from the durian waste peel itselfs or other agricultural wastes. Cellulolytic microbes were isolated from durian shell waste that has naturally half decaying. The Microbes are grown in Carboxymethil microbial cellulose media (CMC) with some level of dilution. The microbes' isolation uses the scratch methods and pour-jelly methods. Cellulolytic microbial isolates was observed microscopically by gram staining. Microbes that can be isolated as much as seven isolates that four isolates of bacteria and three isolates of fungal. Based on the results of the bacterial gram staining, two Gram negative bacteria and two Gram positive bacteria were obtained. The ability test of cellulolytic bacteria was tested qualitatively that seen from the resulting index cellulolytic bacterial isolates respectively of 3.9, 2.6, 2.8, 3.5 while the index cellulolytic generated by each of the fungi is 2.2, 1.8, 1.5. The larger the index cellulolytic generated, the greater the ability of microbes to degrade cellulose there.

Keywords : *Isolation, cellulolytic microbes, durian peel waste, CMC .*

I. INTRODUCTION

Durian is known as favorite fruit in some Indonesian people. At the harvest time, durian peel waste that was produced is very much and has not been utilized optimally. Durian skin proportionally contains high cellulose elements (50-60%) and lignin content (5%) and low starch content (Fadli, 2010). Because it has a high content of cellulose allegedly contained various cellulolytic microbial (bacteria and fungi) that can produce the enzyme cellulase so as to overhaul waste durian skin naturally by the microbe itself in a long time.

According to Milala et al. (2005) the composition of the cellulose in plants in general can reach 40-50% of the mass of the plant that cellulase is the most abundant renewable biopolymer in nature. Waste can be derived from agricultural waste products of which may result in environmental pollution management if not done properly.

Microbial utilization in various fields has been done today, but exploration and exposure to the microbe remains to be done in order to maximize the potential of microbial biodiversity in Indonesia. Microbial diversity has important value which catalyzes the transformation of microbial unique and cheap in biogeochemical cycles in the biosphere, producing critical components in the Earth's atmosphere, and represent a large part of the genetic diversity of organisms (Whitman et al. 1998 in Suryanto, 2009). Use of microbial in agriculture as a biological fertilizer currently being developed to support environmentally friendly farming (Sutanto, 2002).

Potential cellulolytic microbes can grow naturally or intentionally given to accelerate the recast of organic matter. Microbial isolation can be done in different natural habitats as places containing organic compounds derived from the remains of dead plants or from waste or degraded already started to decay naturally.

Beside from durian skin, the other agricultural waste management is still not optimal, so the microbial cellulolytic obtained may be an opportunity in the development of bioenergy from organic material that adds value and supports sustainable farming practices. This study aims to isolate microbial cellulolytic that derived from durian skin so that it can be potentially in outline agricultural waste containing cellulose from waste

leather durian either itself or other agricultural wastes.

II. MATERIALS AND METHODS

Cellulolytic microbes Isolation from durian peel waste. Durian peel waste obtained from Muara Fajar landfill, Pekanbaru. A total of 20 g sample durian peel put in 180 mL of sterile distilled water and then being shaken in the shaker so that it becomes homogeneous. After that as many as 1 mL suspension put in 9 mL of sterile distilled water (10-1) dilution and so on (10-6). Cellulolytic microbes isolation from the durian peel grown using the pour jelly and scratch methods (Hadioetomo, 1993). Cellulolytic bacterial growth media CMC of 0.5 g, 1 g NaNO3, 1.2 g Na2HPO4, 0.5 g KCl, 0.5 g MgSO4.7H2O, 0.9 g KH2PO4, 0.5 g of yeast extract, 0.5 g casein hydrolyzate, 20 g of agar bakto (in 1 L distilled water), whereas fungi grown on medium potato dextrose agar (PDA).

Morphology of the isolates was observed by microscopic way through Gram staining that is seen with a microscope. Gram staining was done by fixing the bacteria on the glass with a glass object using a solution of distilled water over a Bunsen flame. Smear preparations of bacteria that have been heat fixed flooded purple dye crystal violet for 1 min, rinsed with water, and drained. Smear flooded iodized salt for 1 min and washed with 95% 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. Smear flooded back with safranin solution for 1 min and rinsed with distilled water, drained it dry. Bacteria that have been observed with colored bright field microscope at a magnification of x 1000-2000 (Cappucino and Sherman 1983).

Cellulolytic activity test conducted qualitatively. Qualitative test was conducted using 0.1% Congo red staining. Isolates were spotted on the jelly medium CMC. Bacteria were incubated for 3 days at a temperature of 280C. Then tested the activity of the bacteria by adding 0.1% congo red as much as 15 mL and allowed to stand for 30-60 minutes. After it is rinsed 2-3 times with 15 mL of 1 M NaCl and allowed to stand for 15 minutes. Diameter of the clear zone and colony diameter were formed was measured. Cellulase activity test is seen from cellulolytic index which is the ratio between the diameters of the clear zone to colony diameter. The greater the cellulolytic index, the greater the resulting enzyme produced by the bacterial isolates. Cellulolytic index or cellulase activity index (IAS) is the ratio between the diameters of the clear zone to colony diameter.

III. RESULTS AND DISCUSSION

Microorganisms are defined as organisms that are so small (typically less than 1 millimeter) so, in order to observe the necessary help like microscope or loupe is needed. Microorganisms can be a single cell or group of cells that have the ability to regulate the cell independently of other life. Microorganisms consist of bacteria, viruses, and fungi (fungi), each of which have different morphological characteristics, ecology, and physiology. Bacteria are prokaryotic cells with bacterial rRNA were linked by an ester bond and membrane lipid diacyl glycerol which is a dieter (Madigan et al. 2000).

From the research that has been conducted, it was found that seven potential cellulolytic isolates consist of four isolates of bacterial and three isolates of fungi. Based on the microscopic observations obtained each of the two isolates of Gram positive bacteria and two Gram negative. Some forms of bacteria observed that the form of rods and cocci (Figure 1)

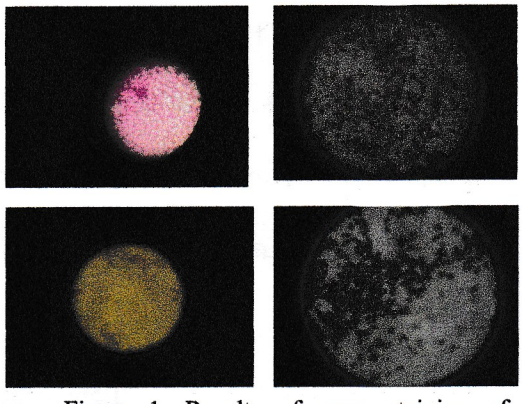
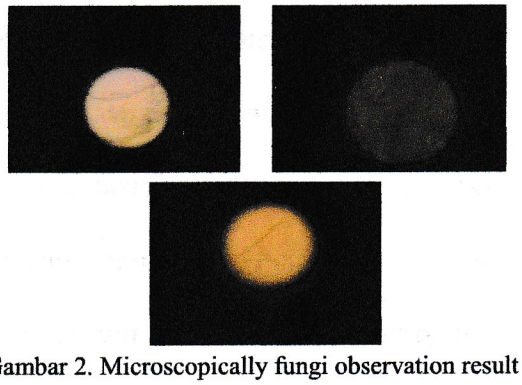


Figure 1. Results of gram staining of bacteria (Gram positive (above), Gram negative (bottom)

In addition to bacterial isolates, obtained three fungal isolates were isolated microscopically observed under a microscope. Based on observations that these bacteria have a different color hyphae. There is a brown, white and yellowish red (Figure 2).



Gambar 2. Microscopically fungi observation result

Qualitative test cellulase produced by microbial cellulolytic characterized by the formation of a clear zone around the colony zone

on agar medium containing cellulose. Teather and Wood (1982), conduct ' rapid screening of cellulolytic microbial index measurement by clear zone. Wide clear zone produced depends on the concentration of CMC and gelatins are used. The more CMC and gelatin given it will lead to smaller pores so that the cellulase enzymes secreted more difficult to pass through the pores and lead to inhibition of the degradation process (Hankin & Anagnostakis 1997). Zverlova et al. (2003) stated that the diameter of the clear zone is generally larger than the diameter of the colony, because the cellulase enzymes are secreted into the surrounding environment by cellulose degrading bacteria.

Qualitative test cellulolytic microbial isolates obtained from seven diverse produce cellulolytic index. The resulting index cellulolytic bacterial isolates respectively of 3.9, 2.6, 2.8, 3.5 while the index cellulolytic generated by each of the fungi are 2.2, 1.8, 1.5. Cellulolytic bacterial isolates produce an index greater than the index cellulolytic produced by fungi. This is due to the fungal colony diameter is much larger than bacteria. As the result, the resulting fungal cellulolytic index is smaller than the index cellulolytic bacteria.

Clear zone resulting from the treatment given the congo red that has the ability to bind the reducing sugar formed by the hydrolysis of cellulose by cellulase enzymes. According to Srinivasan (1973), a reducing sugar is glucose or carbohydrate is a monosaccharide group containing aldehydes and ketones that can reduce metal ions such as Cu and Ag in an alkaline solution. Cellulase enzyme activity can be determined by two methods: by reducing sugar increased enzymatic activity on substrate soluble or insoluble and decreased thickness at 0.5 or 1.0% CMC solution.

In the ecosystem, decomposer organisms of organic matter plays an important role because the organic matter derived from waste can be decomposed into elements that are returned to the soil and the atmosphere as a nutrient that can be reused by plants so that the nutrient cycle and the process runs as it should in the face of life the earth can take.

Beside as a source of microbes that can potentially to produce bioenergy. Cellulolytic microbial utilization can be used as inoculants to speed up the composting of agricultural waste both large and small scale industries. The microbes will hydrolyze cellulose into glucose which can then be converted into ethanol, organic acids, single cell proteins or compounds are useful. The microbial decomposition of cellulose by cellulase enzymes help to sugar the microbial reduction required as a source of carbon and nutrients (Stevenson, 1986).

Cellulase enzyme or enzymes known as systematic β-1, 4-glucan 4-glukano hydrolase is an enzyme that can hydrolyze cellulose to break the glycosidic β-1, 4 in cellulose, selodektrin, cellobiose, and other cellulose derivatives into simple sugars or glucose. The breakdown system of cellulose to glucose system consists of three types of cellulase enzymes are endo-β-1,4-glucanase, exo-β-1,4-glucanase and β-glucosidase. Endo-β-1,4-glucanase attack the middle of the chain at random, exo-β-1,4-glucanase (selobiohidrolase) break disaccharide units (cellobiose) from the end of the chain, and β-glucosidase break down cellobiose to glucose (Da silva et al. 2005).

There are several kinds of cellulolytic enzymes, including aviselase, CMCase and cellulase and produced by cellulolytic microbes that live in the nature, either freely or in the animal body. Some publications indicate that the cellulose decomposers microbes can decompose cellulose derivatives, whereas, decomposing cellulose derivatives are not necessarily able to decipher the cellulose.

Some examples of bacteria genus that are known to have cellulolytic activity is Acetobacter, Bacillus, Clostridium, Cellulomonas, Pseudomonas, Cytophaga, Sarcina, and Vibrio, while examples of fungi genus that have cellulolytic activity is Bulgaria, Chaetomium, Helotium, Coriolus, Phanerochaete, Poria, Schizophyllum, Serpula, Aspergillus, Cladosporium, Fusarium, Geotrichum, Myrothecium, Paecilomyces, Penicillium, and Trichoderma (Rao 1994). Some types of organisms may also produce cellulase enzymes such as termites (Watanabe and Tokuda 2001), mussels (Xu et al. 2000), and Arabidopsis.

CMC media are commonly used in isolating cellulolytic microbes. Cellulolytic enzymes produced by the microbial species are encoded by different genes, both in terms of the number of base pairs and nucleotide sequences. In addition, expression of genes encoding cellulolytic enzymes is influenced by the availability of cellulosic material in the growth medium.

The microbes' growth medium should contain all the necessary substances such as organic compounds (proteins, carbohydrates, and fats), minerals and vitamins. According to media usefulness consists of general media (be overgrown by microbes in general), selective media (certain microbes that can live), differential media (to distinguish microbial species from one another) and enrichment media (Gandjar et al. 1992). Grow the cellulolytic microbes using selective media that is media CMC which is one of the carbon sources that can be used for the microbes' growth medium.

Research on cellulolytic bacteria or cellulose decomposer microbes among which (Cellulomonas sp, Planococcus sp, Moraxella sp) isolated from soil degradation in Bangladesh to encourage household waste and agricultural waste

through the increased release of CO2 (54.3 and 37.62 mg) , reduction of fiber (46.86% and 45.11%), reduction of sugar (72.52% and 74.27%), reduction of fat (65.20% and 61.22%), endoglucanase activity (0.097 mg / hour / mL) and selobiose (0.82 mg / h / mL) (Barman et al. 2011). Subsequently Kim et al. (2011) found the Bacillus subtilis that is isolated from agricultural land has great potential as a cellulolytic microbes seen from CMCase enzyme production, Avicelase, β-glucosidase and xylanase that produced.

However, qualitative testing remains to be done to further quantitative test, and characterize the enzyme cellulase produced so that it can be known with certainty the ability of cellulolytic microbes to produce cellulase enzymes that can be used to degrade agricultural waste especially waste durian skin is the source of these isolates . Thus, the reform process of organic matter takes place rapidly and the management of agricultural waste into energy source can be realized.

IV. CONCLUSION

Durian skin is agricultural waste that has a potential as a source of cellulolytic isolates were able to degrade the durian peel waste and other agricultural wastes that can be bioenergy. Cellolytic bacteria and fungi was known from the cellulolytic. Bacterial cellulolytic index was greater than the index of the cellulolytic that produced by fungi. However, the ability of fungi to degrade cellulose was greater than that seen from the large bacterial clear zone formed around the colony. This indicates that the seven isolates were isolated from durian peel waste can be utilized as a potential isolates to degrade waste durian peel itself and other agricultural wastes that are currently very abundant.

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