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Characteristics of Various Moulds that Potentially as Decomposers Pineapple Industry By-Product

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ABSTRACT

The purpose of this study was to determine the potency of three mold isolates Bio GGP2, Bio GGP3 and Bio GGP5. The mold isolate was isolated from pineapple waste. Molds are selected have cellulolytic, xylanolytic, ligninolytic, amylolytic, annanolytic, and proteolytic. Enzyme detection on agar media with suitable substrate. Then, the selected fungi were tested for their decomposition ability in pineapple waste. The results of the decomposition test showed that the combination of the three isolates gave the best results of 45% decomposed material at 14 days of age. The three isolates have the potential to be used to decompose cellulose waste from pineapple.

INTRODUCTION

Pineapple plants are planted on dry land In Indonesia. The plant can be maintained in tropical and subtropical areas, recently. According Ali *et al*. (2020) pineapple can grow in various types of soil. This plant has a shallow root system, so it requires soil that has a good drainage and air system, such as sandy soil and contains organic matter. Pineapple production is very abundant in Indonesia (Manik *et al.* 2019). Therefore, the industry by-produced will increase. There are several pineapple processing industrial centers spread across various places in Indonesia. One of them is PT. Great Giant Pineapple (GGP) which is located in the Terbanggi Besar area, Central Lampung.

PT. GGP company is experiencing problems regarding wild pineapple plants. The wild plants are not wanted to grow on the plantation that was usually called volunteering. The volunteer plants can appear due to second post-harvest processing which is carried out using a chopping system. The system was hoped to cause decomposition so that the land has a source of organic material. However, a new problem emerged. The chopping productions have still leave parts of the plant that it's potent to grow to wild pineapples due to not decomposing completely. Decomposer microorganisms such as fungi break down litter or dead plant parts in the process. The fungi used materials and nutrients for life. Therefore, this research was conducted to determine the decomposition of pineapple by-product on a laboratory scale by the mold.

MATERIAL AND METHODS

Fungi

The fungi used are *Trichoderma* sp1 (BioGGP2), *Apergillus* sp1 (BioGGP5), and *Trichoderma* sp2 (BioGGP5). The three isolates collected from GGP agricultural land.

Determining Extracellular Enzymatic Activity

Fungal cultures were inoculated onto 5 g peptone, 5 g yeast extract, 0.2 g K2HPO4, 20 g agar, 10 g Carboxymethyl Cellulose (CMC) and 1000 mL distilled water media and incubated for 4 days at room temperature. Then the petri dish was soaked with 15 mL of 0.1% Congo red solution and let stand for 30 minutes, then rinsed twice using 15 mL of 1 N NaCl and let stand for 15 minutes. A positive reaction was indicated by the presence of a clear zone around the colony which was determination of cellulolytic activity. Fungal characters were also test for the activity of other enzymes degrading. The carbon source content the media is replaced with another substrate. Substrate analogues such, oat spelt xylan, guaiacol, amylum, locust bean gum (mannan), and skim milk were used to estimate the potential activity of xylanolytic, ligninolytic, amylolytic, mannanolytic, and proteolytic, respectively.

Mold Starter

The basic ingredient for making mold starter is corn. Corn that has been washed clean, cooked by steaming. The corn took and cool it on a tray. The corn is then put into a glass bottle as much as 60 g. Corn media was sterilized by autoclaving at 121ºC for 15 minutes. Then, the corn media inoculated with the mold. The bottle containing the corn media is then incubated at room temperature for 4-7 days. A sign that the propagation process has been successful is when the corn media changes color to green.

Pineapple Waste Decomposition

Wet pineapple waste was weighed 45 g. Then the pineapple waste was sterilized by autoclaving at 121ºC for 15 minutes. The pineapple waste was inoculated with starter at 5% of the total volume. After the incubation time is complete, the culture is harvested and weighed. Weight reduction due to fungal growth was observed. Observations of the decomposition rate are in the form of numbers in percentage form.

$$Decomposition\ percentage = \frac{initial\ weight - final\ weight}{initial\ weight} \text{ x } 100\%$$

Data Analysis

Fungal characterization data of qualitative enzyme tests and polysaccharide degradation are made in separate tables. Each table is analyzed descriptively. Polysaccharide degradation uses a comparison of decomposition percentages.

RESULTS AND DISCUSSIONS

Determining Extracellular Enzymatic Activity

Isolates showed differences in the abilities to produced extracellular enzyme. Isolate BioGGP2 has ligninolytic ability because the isolate produces lignocellulase enzymes. While, it’s not the ability to degrade other macromolecules. Isolate BioGGP2 does not have cellulolytic, xylanolytic, amylolytic, mannanolytic, and proteolytic abilities. After the fungi observed by a microscope, it was discovered that the mold was *Trichoderma* sp1 (Table 1).

Table 1. Comparison of the ability of fungal isolates to break down cellulose, xylan, lignin, starch, mannan and protein

No	Isolate	Cellulolytic	Xylanolytic	Ligninolytic	Amylolytic	Mannanolytic	Proteolytic
1	BioGGP2	-	-	+	-	-	-
2	BioGGP3	+	+	-	-	-	-
3	BioGGP5	-	-	+	-	-	+

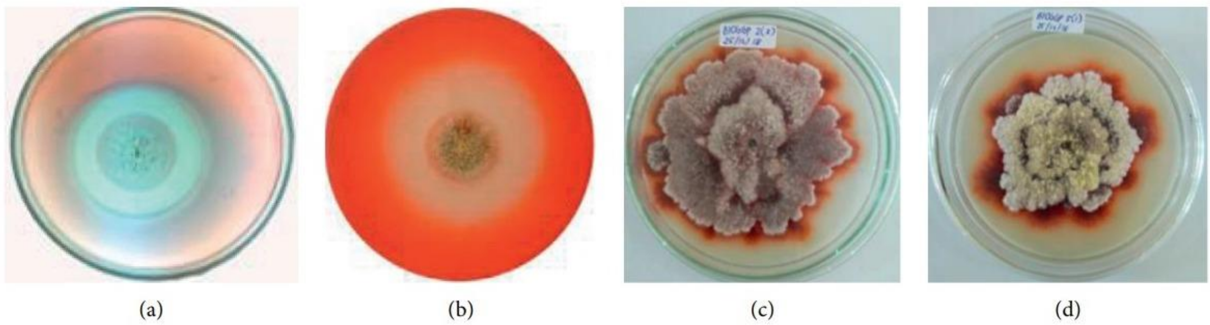

Figure 1. (a) Clear zone of cellulolytic (BioGGP 3); (b) xylanolytic (BioGGP 3) isolate; (c) oxidation of guaiacol of ligninolytic (BioGGP 2); and (d) (BioGGP 5) isolate on specific media (Irawan *et al.* 2022)

BioGGP3 isolate has cellulolytic and xylanolytic abilities. Meanwhile, the other enzyme did not produce, such as ligninolytic, amylolytic, mannanolytic and proteolytic. After being identified using a microscope, it was discovered that the mold was *Aspergillus* sp1 (Figure 1). BioGGP5 isolate has ligninolytic and proteolytic abilities. Meanwhile, the other enzyme did not produce such as cellulolytic, xylanolytic, amylolytic, mannanolytic and proteolytic. After being identified using a microscope, it was discovered that the mold was *Trichoderma* sp2.

Due to the differences in the characteristics of the three isolates, they are very good to combine to degrade pineapple waste.The three fungi have different characters. The differences in the characters of the three isolates provide complementary to degrade fiber polymers. The fiber polymer is in the form of lignicellulose. Pineapple leaves are the most waste produced from pineapple farming, which is around 90% each time they are harvested. Pineapple leaves contain 69.5-71.5% cellulose and 4.4-4.7% lignin (Haryani *et al.* 2015).

Pineapple fiber polymers contain lignin, cellulose, and hemicellulose (Nurfaizin and Hartati 2023). The lignocellulose is degraded into various phenylpropane units, namely guaiacyl units (G), syringyl units (S) and p-hydroxyphenyl units (H). The units can be degraded into coniferyl, sinapyl and p-coumaryl alcohols (Kumar and Chandra 2020). Furthermore, the simple units will be utilized for the growth of soil microbes. According to Sutarman (2019), soil microbes help plants in several important ways to decompose organic matter, protect against pathogenic microbes, symbiotically with roots, and produce bioactive compounds.

Pineapple Waste Decomposition

The ability of mold isolates to decompose pineapple waste can be done by calculating the dry weight of the waste, comparing the initial dry weight (before treatment) and after incubation treatment. Substrate decomposition at the end of the observation showed different results in each treatment (Table 2). Decomposition of all treatments for 7 days apparently resulted in pineapple waste degradation of between 19-35%. However, when the isolate was treated for 14 days, there was more significant degradation. BioGGP2, BioGGP3, and BioGGP5 isolates could degrade waste by 38%, 37%, and 35%, respectively. Meanwhile, for mixed isolates, the results were even higher, namely 45%.

Table 1. Percentage of pineapple waste degraded by mold isolates BioGGP2, BioGGP3, BioGGP5 and a mixture of the isolates

Isolate	The Percentage of Waste Degraded (%)	
	7 days	14 days
BioGGP2	32	38
BioGGP3	35	37
BioGGP5	19	35
Mix isolates	28	45

The decomposition process is characterized by changes, namely changes in particle size, changes in color and appearance, and odor. The decomposition waste also occurs regularly every week. The weight decreases little by little, because decomposition is the process of gradually destroying an organism so that its structure can no recognized anymore. In this destruction process, extracellular enzymes play a very important role, such as cellulase, xylanase, protease, amylase, and others. The characteristics of the types of extracellular enzymes produced were different by the BioGGP2, BioGGP3, and BioGGP5 isolates. In this way, the decomposition of pineapple waste makes a difference. However, the combination of the three types of isolates will produce the greatest decomposition (45% at 14 days).

The decomposition process is known by changes. The changes in particle size, changes in color and appearance, and odor (Hanum and Kuswytasari 2014). Waste decomposition also occurs routinely every week. Its weight decreases little by little. Decomposition is the process of gradually destroying an organism so that the structure can't be recognized anymore. Extracellular enzymes play a very important role, such as cellulase, xylanase, protease, amylase, and others in the destruction process. The more complex the material will result in slower the decomposition process. The decomposition process of complex compounds involves more enzymes that work together comprehensively and synergistically (Ratih *et al.* 2020). In this study, the characteristics of the types of extracellular enzymes produced differed in the isolates of BioGGP2, BioGGP3, and BioGGP5. In this way, the decomposition of pineapple waste can provide a significant-results. The combination of the three types of isolates will produce the greatest decomposition 45% in 14 days of incubations.

CONCLUSION

Fungi have different character to produce extracellular enzyme. BioGGP2 isolate has ligninolytic enzyme, BioGGP5 isolate has ligninolytic and proteolytic enzyme, and BioGGP3 isolate has cellulolytic and xylanolytic. The combination of the three types of isolates will produce the greatest decomposition for pineapple waste i.e:45% for 14 days.

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