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Research Paper

The Effect of the Addition of Palm Kernel Cake in Making *Lactobacillus sp*Inoculum on Enzyme Activity

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ABSTRACT
This study aims to determine the effect of adding palm kernel cake (PKC) in the manufacture of Lactobacillus sp inoculum on enzyme activity. The material in this study used 1) PKC from PT. Incasi Raya Padang, West Sumatra, 2) Lactobacillus sp. isolated from decomposed PKC, 3) rice bran, 4) Nutrient Agar (NA) media produced from Difoo Becton Dickinson, 5) aquades and standard minerals. This study used an experimental method completely randomized design (CRD) with 5 treatments and 5 replications. The treatments in this study were A = 100% rice bran, B = 100% PKC, C = 80% PKC + 20% rice bran, D = 75% PKC + 25% rice bran, E = 50% PKC + 50% rice bran. The variables observed were cellulase, mannanase and protease enzyme activity. The results showed that the treatment had a very significant effect (P <0.01) on the activity of cellulase, mannanase and protease enzymes. From the results of the study it can be concluded that the addition of 80% PKC in the manufacture of Lactobacillus sp inoculum can increase the activity of cellulase, mannanase and protease enzymes. In this condition, cellulase activity was obtained 18.84 U/ml, mannanase activity was 24.86 U/ml and protease activity was 10.45 U/ml.

Keywords:*Enzyme activity, PKC, Inoculum, Lactobacillus sp.*

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I. INTRODUCTION

Oil palm is a crop that produces palm oil. Palm oil production in Indonesia continues to increase every year. This is because Indonesia is the largest country in producing palm oil. In 2018, the total area of Indonesian oil palm plantations reached 14.326.350 hectares (Ditjenbun, 2019). Especially in West Sumatra, oil palm production in 2018 was 1.248.269 tonnes and increased in 2019 to 1.298.038 tonnes (Ditjenbun, 2019). He further explained that Indonesia's CPO production increased from 31 million tonnes in 2015 to 42.9 million tonnes in 2018 or an increase of 11.8 million in the last 4 years. The high production of CPO is also accompanied by an increase in processing waste or palm oil by-products, one of which is palm kernel cake (PKC). Judging from the nutritional content of PKC is quite high, namely 17.31% crude protein, 27.62% crude fiber, 7.14% crude fat, 0.27% Ca, 0.94% P and 48.4 ppm Cu (Mirnawati *et al*., 2018). Although the crude protein content of PKC is quite high, its use in broiler rations is still limited, namely 10% (Sinurat, 2003).

The low use of PKC in broiler rations is due to its low palatability and high content of crude fiber in the form of β-manan. β-manan is a polysaccharide component that is difficult to digest and utilize by livestock, so to increase its utilization it is necessary to degrade it into monosaccharides. Biochemical studies on the composition of PKC revealed that 57.8% of the hemicellulose content of PKC consisted of β-manan (Azman *et al*., 2016; Cerveró *et al*., 2010). β-manan is a limiting factor for poultry because the digestive tract of poultry does not produce enzymes to digest food. Efforts that can be made to increase the use of PKC in poultry rations are by processing by fermentation.

Fermentation is a process of changing complex food substances into simple ones with the help of enzymes produced by microbes to produce products that are easier to digest and use by livestock. Walugembe *et al*. (2014) stated that fermented food substances usually have better nutritional value than before fermentation due to the presence of catabolic microorganisms that break down complex components into simpler ones, making them easier to digest.

Mirnawati *et al*. (2017) carried out fermentation of PKC using mananolytic fungi such as *Sclerotium rolfsii* and *Eupenicillium javanicum*. Of the two fungi, *Sclerotium rolfsii* mold has a higher ability than *Eupenicillium javanicum*, seen from its mannanase activity, namely 24.58 U/ml and cellulase activity of 21.89 U/ml. Furthermore, Mirnawati *et al*. (2019) carried out fermentation of PKC with mananolytic bacteria, namely *Bacillus subtilis* with a fermentation time of 6 days which was able to provide the best results in increasing mannanase activity of 24.27 U/ml, cellulase 17.13 U/ml and protease 10.27 U/ml.

Lactobacillus sp. is one type of bacteria that can degrade fiber by producing several types of fiber-breaking enzymes. Krabi *et al*. (2015) stated that *Lactobacillus sp*. able to produce extracellular enzymes, namely 2.67% pectinase enzymes, 8% β-glucosidase enzymes and 5.33% cellulase enzymes. According to Walter and Kohler (1978) *Lactobacillus sp*. able to produce cellulase enzymes which break down the fiber in the digestive tract so as to increase the digestibility of feed. To increase the productivity of *Lactobacillus sp.*in order to increase enzyme activity, it is necessary to make an inoculum from *Lactbacillus sp.* with a mixture of bran and PKC as an inducer. The addition of PKC in the manufacture of the inoculum serves as an inducer to accelerate the adaptation phase so that it can improve the quality of the inoculum which can later be used in processing fermented palm kernel cake.

For this reason, it is necessary to conduct a study to determine the effect of adding palm kernel cake in making *Lactobacillus sp*. thus producing an inoculum that produces maximum enzyme activity.

II. MATERIALS AND METHODS

Research Materials

The materials used in this study were 1) Palm kernel cake from PT. Incasi Raya Padang, West Sumatra, 2) *Lactobacillus sp*. isolated from decomposed PKC, 3) Agar Nutrient Media, 4) Rice bran, 5) Aquades and standard minerals, 6) Buffer solutions and chemicals for analysis of cellulase, manhanase and protease activity. The tools used are autoclave, analytical scale, spectrophotometer, centrifuge, incubator, waterbath shaker, erlenmeyer, eppendorf and test tubes.

Experimental design

This research was conducted with an experimental method using a completely randomized design (CRD) with 5 treatments and 5 replications. The treatments given were A = 100% rice bran, B = 100% PKC, C = 80% PKC + 20% rice bran, D = 75% PKC + 25% rice bran, E = 50% PKC + 50% rice bran. The parameters measured were cellulase, mannanase and protease activity.

Making *Lactobacillus sp.* Inoculum

Preparation of inoculum consisting of treatments A, B, C and D, each treatment was added with 70 ml of distilled water then sterilized by autoclave for 15 minutes at a temperature of 121ºC. Then cool at room temperature. Then dilute the bacteria in the testube with mineral solution and inoculate it in each treatment and then incubate it for 3 days.

Enzyme Extract

Put 10 grams of each treatment sample into the Erlenmeyer and add 90 ml of 0.05 M pH buffer phosphate. Then put it in a shaker incubator at 100 rpm for 30 minutes. After that, filter it with filter paper and take the filtrate. The filtrate obtained was centrifuged at 10,000 rpm, temperature -4oC for 15 minutes. Take the supernatant and the enzyme activity can be analyzed.

Measurement of Enzyme Activity

- Cellulase and Mannanase with the Somogyi-Nelson Method (1944)

One ml of crude enzyme extract is added to one ml of the manan substrate (0.5 g / ml of manan plus 10 ml of buffer phosphate). Incubation for 30 minutes at temperature (40 cellulases and 60 mannanases) in a water bath shaker. Take one ml of the enzyme extract that has been incubated, then add Nelson AB's solution. Heat in boiling water for 20 minutes, then cool and add one ml of phosphomolybdate solution and seven ml of distilled water, the absorbance is measured by a Uv-Vis spectrophotometer at a wavelength of 575 nm.

- Protease

The proteolytic activity of the crude enzyme extract was determined based on Cupp and Enyard (2008). One ml of crude enzyme extract was added to the 0.65% casein substrate (0.65 g casein in 100 ml of 0.05 M phosphate buffer pH 7.5). The reaction mixture was incubated at 37°C for 10 minutes. Termination of the reaction was carried out by adding five ml of 110 ml TCA reagent and reincubating at 37°C for 30 minutes. Two ml of the filtrate were separated by centrifugation at 10 000 rpm for 10 minutes. Five ml of Na_2CO_3 and one ml of Folin Ciocalteau reagent were added to the filtrate and incubated at 37°C for 30 minutes. The absorbance was measured with a UV-vis spectrophotometer at a wavelength of 660 nm.

Data analysis

Data were processed statistically by analysis of diversity according to Steel and Torie (1991). The differences between treatments were tested by the Duncan Multiple Range Test (DMRT).

III. RESULTS AND DISCUSSION

Effect of Treatment on Cellulase Activity

Mean inoculum cellulase activity of *Lactobacillus sp.* can be seen in Table 1.

Table 1. Mean inoculum cellulase activity of *Lactobacillus sp.*

Replication	Treatment				
	A	B	C	D	E
1	9.73	16,73	19,82	14,84	10,76
2	11.58	16,87	19,14	15,02	12,28
3	10.80	16,76	18,03	14,96	14,23
4	8.60	16,42	17,24	14,92	13,74
5	9.71	16,90	19,95	15,05	11,49
Mean	10.08^{e}	16,74^{b}	18,84^{a}	14,96^{c}	12,50^{d}

Note: Different lowercase letters on the same line have a very significant effect (P <0.01). Mean cellulase activity in U/ml.

The effect of adding palm kernel cake in making the inoculum of *Lactobacillus sp.* on cellulase activity was statistically significant (P<0.01). The highest cellulase activity was found in treatment C (18.84 U/ml) with the addition of 80% palm kernel cake. Based on the data in table 1, it can be seen that the more palm kernel meal given, the increase in cellulase activity. The increase in cellulase activity is caused by the bacteria *Lactobacillus sp.* work according to the inducer. This is related to the bacteria used in fermentation derived from decomposed palm kernel cake. The increase in inducer concentration is directly proportional to the high activity of the resulting cellulases, this continues until the optimal point of inducer concentration is reached. The production of cellulase enzymes by microbes requires an inducer in the fermentation medium. The inducer will induce the formation of cellulase enzymes in microbial cells. The number of enzymes present in the cell does not remain dependent on the inducer, the number will increase several times if the medium contains the inducing substrate. Adri *et al*. (2013) stated that the inducer compound needed is generally in the form of the enzyme substrate. According to Purkan *et al*. (2015) stated that the type of inducer and the optimum conditions greatly influence the activity of the cellulase enzyme.

This result is higher than the results obtained by Mirnawati *et al*. (2019) where fermentation of palm kernel cake with *Bacillus subtilis* gave cellulase enzyme activity of 17.13 U/ml. However, this result is lower than the results obtained by Mirnawati *et al*. (2017) where fermentation of palm kernel cake with *Sclerotium rolfsii* mold gave cellulase enzyme activity of 21.89 U/ml. Based on these data, it turns out that the ability of molds to degrade cellulose is more effective than bacteria. This is consistent with the statement of Purwadaria (2003) that the ability of molds as microbes to degrade cellulose is more effective than bacteria because the enzyme components that break down cellulose (endogluconase, exogluconase and glucosidase) produce higher enzyme activity than bacteria, especially glucosidase.

Low cellulase activity was found in treatment A (10.08 U/ml) without the addition of palm kernel cake. The low activity of cellulases is due to the low or nonexistent inducer concentrations. In accordance with the opinion of Purkan *et al*. (2015) stated that the small inducer concentration causes low inducer binding affinity to the repressor. Likewise, too large an inducer concentration causes saturation in the production of cellulase enzymes because too large an inducer concentration can inhibit the formation of the substrate enzyme complex so that enzyme production does not run optimally.

Effect of Treatment on Mannanase Activity

Mean inoculum mannanase activity of *Lactobacillus sp.* can be seen in Table 2.

Table 2. Mean inoculum mannanase activity of *Lactobacillus sp.*

Replication	Treatment				
	A	B	C	D	E
1	13,55	18,08	24,58	21,95	14,63
2	12,20	17,64	24,02	21,01	14,26
3	14,52	17,47	27,16	20,64	16,16
4	12,87	19,88	22,39	22,07	16,23
5	13,65	17,14	26,16	21,50	16,60
Mean	13,36^{e}	18,04^{c}	24,86^{a}	21,43^{b}	15,58^{d}

Note: Different lowercase letters on the same line have a very significant effect (P <0.01). Mean mannanase activity in U/ml.

The effect of the addition of palm kernel cake in the manufacture of *Lactobacillus sp* inoculum on mannanase activity was statistically significant (P<0.01). The highest mananase activity was found in treatment C (24.86 U/ml) with the addition of 80% palm kernel cake. Based on the data in table 2, it can be seen that the more the addition of palm kernel cake, the higher the resulting mannanase activity. The increase in mannanase activity is due to the optimal availability of the inducer in the inoculum. The increase in the availability of the inducer is directly proportional to the activity of the mannanase enzyme. This is in accordance with the opinion of Kurnia (2010) that the increase in enzyme activity is optimal based on the content of the substrate inducer in the inoculum. The increased activity of this enzyme also shows that the more the substrate is hydrolyzed.

Palm kernel cake contains a lot of manan, where it functions as an inducer in the fermentation process. According to Purkan *et al*. (2016) the inducer will bind to the repressor protein so that the repressor protein undergoes allosetric changes which can change its shape and cause the repressor to no longer bind the operator. As a result, RNA polymerase can transcribe the genes needed for food degradation and the bacteria are able to synthesize the enzymes needed for their metabolism. In this fermentation, palm kernel meal is used as a source of food because the manan content in palm kernel meal is very high. This result is higher than the results obtained by Mirnawati *et al*. (2019) where fermentation of palm kernel cake with *Bacillus subtilis* gave 24.27 U/ml of mannanase enzyme activity.

Low mannanase activity was found in treatment A (13.36 U/ml) without the addition of palm kernel cake. This is due to the absence of an inducer in the fermentation process. The inducer functions as an inducer for the formation of the enzyme mannanase in microbial cells. In accordance with the opinion of Purkan *et al*. (2015) stated that the availability of a small inducer causes the affinity of the repressor binding by the inducer to be low, so that the bacteria are unable to synthesize the enzymes needed for their metabolism.

Effect of Treatment on Protease Activity

Mean inoculum protease activity of *Lactobacillus sp*. can be seen in Table 3.

Table 3. Mean inoculum protease activity *with Lactobacillus sp.*

Replication	Treatment				
	A	B	C	D	E
1	3,05	8,47	10,35	6,66	4,89
2	4,61	7,70	10,38	6,34	5,02
3	3,84	8,34	10,30	5,49	5,17
4	4,47	8,56	10,61	7,34	5,03
5	3,73	7,98	10,59	7,45	5,30
Mean	3,94^{e}	8,21^{b}	10,45^{a}	6,66^{c}	5,08^{d}

Note: Different lowercase letters on the same line have a very significant effect (P <0.01). Mean protease activity in U/ml.

The effect of adding palm kernel cake in making the inoculum of *Lactobacillus sp*. on protease activity was statistically significant (P<0.01). The highest protease activity was found in treatment C (10.45 U/ml) with the addition of 80% palm kernel cake. Based on the data in table 3, it can be seen that the more palm kernel cake given the higher the protease activity produced. The increase in protease activity is due to the availability of large amounts of nutrients needed by bacteria to carry out cell metabolism. Where the protein content of palm kernel cake is quite high, namely 17.31% (Mirnawati *et al*., 2018) which can be used as a metabolic process. This result is higher than the results obtained by Mirnawati *et al*. (2019) where fermentation of palm kernel cake with *Bacillus subtilis* gave protease enzyme activity of 10.27 U/ml.

In addition, the high protease activity is also due to optimal substrate availability in the inoculum. In accordance with Yunita's opinion (2012), if the amount of substrate in the growth medium is optimal, it will produce a high enzyme catalytic rate. However, too large a substrate concentration can also cause saturation in the production of enzymes because too large a substrate concentration can inhibit the formation of the substrate enzyme complex so that enzyme production does not run optimally.

Low protease activity was found in treatment A (3.94 U/ml) without the addition of palm kernel cake. This is due to the absence of palm kernel cake substrate added in the treatment. In accordance with the opinion of Yuniati *et al*. (2015) stated that a decrease in proteolytic activity can occur due to a reduced number of substrates which will inhibit the formation of the substrate enzyme complex and changes in the enzyme structure which will cause a decrease in the catalytic rate. Due to changes in the structure of the enzyme, the active side of the enzyme changes shape so that it cannot be used properly in binding to the substrate (Yunita, 2012).

IV. CONCLUSION

The best composition of rice bran and PKC is the best in making the inoculum of *Lactobacillus sp.* based on enzyme activity was treatment C (80% PKC + 20% rice bran). In this condition, cellulase activity (18.84 U/ml), mannanase activity (24.86 U/ml) and protease activity (10.45 U/ml) were obtained.

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