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The Activity Of Cellulose Enzyme From Indigenous Bacteria "*Bacillus Sp YLB1*" As Bioactivator

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Abstract. This study aims to test the activity of cellulase enzyme (CMCase) from indigenous bacteria "*Bacillus Sp YLB1*" as bioactivator of palm oil based feed. The treatment consists of various combinations of substart, namely: P1 = 80% palm leaves + 10% palm kernel cake + 10% sludge; P2 = 60% palm leaves + 20% palm kernel cake + 20% sludge and P3 = 60% palm leaves + 30% palm kernel cake
+ 30% sludge. The parameters observed were cellulase enzyme activity (CMCase) on various combinations of palm oil waste and fermentation time. The results showed that cellulase enzyme (CMCase) production of *Bacillus sp YLB*1 bacteria during fermentation fluctuated ie 0.143 Units / ml; 0.372 Units / ml; 0.588 Units / ml; 1.013 Units / ml; 0.906 Units / ml; 1,065 Units / ml and 1,198 Units / ml.The activity of cellulase enzyme (CMCase) on substrate P1 is lower than P2 and P3. As conclutions that cellulase enzyme activity of *Bacillus sp Y*LB1 influenced by the combination of substrate and fermentation time. The P3 fermented with Bacillus sp YLB1 for 7 days gave a more optimal result than the other treatments.

Keywords: cellulase enzyme, indigenous bacteria, substrate

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1 Introduction

Indigenous bacteria are bacteria that are exploited from their own substrate which have the optimal ability to degrade the substrate. Processing of feed ingredients based on oil palm plantations by utilizing indigenous cellulolytic bacteria from the waste itself is expected to optimize the ability of rumen microorganisms in digesting high-fiber feed. [1] said that

indigenous bacteria (*Bacillus sp YLB1*) have the ability to produce cellulase (CMCase) enzymes in addition to xylanases and mannanases.

Cellulase enzymes are conceptually enzymes that can degrade cellulose into simple sugars so that they can pass through microbial cell walls. Cellulose degradation is a complex process and is a synergistic action by several enzymes [2]. Cellulase enzyme is actually an enzyme complex consisting of several enzymes that work gradually to break down cellulose into glucose [3]. Cellulase enzymes include extracellular enzymes that have a great ability to degrade organic waste, especially agricultural waste and industrial waste [4]. The biological function of extracellular enzymes is hydrolyzing macromolecules that are too large such as cellulose to be carried into cells [5].

Palm frond biomass, solid, BIS, squeezed fibers, and empty fruit bunches are byproducts from oil palm plantations and industry, potentially being used as animal feed. However, its utilization is not optimal because of the limiting factors in the form of high fiber content, especially lignin which is difficult to digest. Lignin forms a strong bond with cellulose in the form of lignocellulose.

Cellulose is a linear polymer of glucose which is bound by β-1,4-glycosidic bonds. The length of a cellulose chain is between 2,000 and 14,000 residues. Intra and intermolecular hydrogen bonds between chains in cellulose fibers result in cellulose having a dense and tight crystalline structure [6]. Cellulose and hemicellulose are constituents of plant tissue composed of different sugars. Cellulose is a linear polymer composed of D-glucose which is bound by β-1,4 glycosides with the molecular formula (C6H10O5) to form cellobiose [7].

Bacteria remodel cellulose extracellularly because cellulose is not water-soluble. Microbes have two types of extracellular enzyme work systems: (1) Hydrolytic system, namely by producing hydrolase enzymes that work to remodel cellulose and hemicellulose, and (2) Oxidative system and extracellular ligninase secretion by lignin depolymerization [8].

Cellulolytic indigenous bacteria are bacteria that come from the substrate itself which has the ability to overhaul polysaccharides (lignocellulose). Bacterial isolation from the waste itself will be obtained by bacteria that have the optimal ability to remodel/degrade lignocellulose originating from the waste. The results of isolation of indigenous bacteria from the waste of plantation and oil palm industry obtained 10 cellulolytic isolates and 5 of them showed better growth than others [1]. The result of isolation, one of the bacteria which has the highest enzyme activity compared to the other qualitatively is Bacillus sp YLB1. The optimal ability of these bacteria to degrade cellulose can be done through quantitative enzyme activity testing.

2. Methods

The method used in this study is an experimental method with a completely randomized design (CRD) 3 treatments and 6 replications. The steps taken are the making of indigenous bacterial inoculums. Fermentation using solid substrate media [9], the measurement of enzyme activity using the Dinitrosalicylic Acid (DNS) method.

Materials used include indigenous Bacillus Sp YLB1 isolates isolated from plantation and palm oil industry waste [1], palm fronds and leaves, palm mud, palm kernel cake (Figure 1), Acetic acid, Sodium acetate, dinitrosalicylic acid (DNS), sodium potassium tartrate, sodium sulfite, NaOH, veratryl alcohol, H2O2, sodium tartrate, Mn2SO2, sodium malonate buffer.

The tools used are autoclaves, incubators, pH meters, Erlenmeyer, spectrophotometers, stirrers, test tubes, micropipets, penagas, centrifuge, filter paper, gauze, 1 kg plastic, incase.

Figure 1. Substrate based on oil palm plantation and industrial waste (palm leaf, palm sludge and palm kernel cake)

Research procedure

Making general agar medium (nutrient agar)

Preparation of nutrient agar (NA) can be by heating 14 grams of NA in 1 liter of aquadest until boiling then cooled and then put into a 500 ml Erlenmeyer tube covered with cotton then coated with aluminum foil, then sterilized in an autoclave for 15 minutes at 121^0C.

Rejuvenation of indigenous bacteria

The isolation of indigenous bacteria was first rejuvenated so that the bacteria used were pure indigenous bacteria aged + 24 hours. Sterilized NA medium is poured into sterile Petri dish, after it has cooled down, then scratch indigenous bacteria and incubated in an incubator at 37^0C for 24 hours.

Solid Fermentation

Fermentation carried out is complete feed fermentation based on the plantation and industrial oil palm wastes (palm fronds and leaves, palm kernel cake and mud) refers to the method of solid substrate fermentation according to [9].

Samples of oil palm fronds and leaves, palm kernel cake and palm mud are put into plastic bags then add distilled water (Aquades) as the final subtract with a moisture content of + 70%. The substrate was sterilized using an autoclave, cooled at room temperature, then inoculated with

5% (w / w) Bacillus Sp YLB1 inoculum and incubated for 7 days and all samples were repeated 3 times. Samples are harvested every day and the crude enzymes are extracted immediately. Substrate formula consists of: Substrate P1 = 80% palm leaves + 10% palm kernel cake + 10% sludge; P2 = 60% palm leaves + 20% palm kernel cake + 20% sludge and P3 = 60% palm leaves + 30% palm kernel cake + 30% sludge.

Measurement of enzyme activity

The measurement of specific activities is done by counting the number of products produced from the enzyme reaction. The product measured is reducing sugar (glucose from CMC). Glucose solution is used as a standard. One unit of enzyme is the number of enzymes that produce 1 μmol glucose in one minute.

Crude enzyme extract. Fermentation results of 10 grams were immersed in 90 ml acetate buffer 0.05 M pH 5 for 2 hours (Figure 2). The enzyme extract was then filtered, then centrifuged with a refrigerated centrifuge at a speed of 5000 rpm for 1 hour. The centrifuge results are filtered using No. 1 filter paper watmant to separate the supernatant (crude enzyme extract) with microbial biomass. The supernatant is stored at 4^0C (crude enzyme) to measure its enzyme activity.

Figure 2. Extracts of crude enzymes fermented using palm oil *Bacillus sp YLB1*

Glucose standard solution. The standard solution is prepared by dissolving 1 gram of glucose in 10 ml of sterile aquades as follows: (1) The solution is diluted to obtain a standard stock with a concentration of 1 μg / ml. The dilution series is made from 0 ppm, 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm, 0.5 ppm, 0.6 ppm, 0.7 ppm and 0.7 ppm, (2) One ml of standard stock solution plus 1

ml of DNS, then mix the solution was incubated at a temperature of 100^0C for 15 minutes, (3) The solution was cooled and then the absorbance was measured at a wavelength of 540 nm, (4) The measurement results were made linear regression equations for glucose standards to be used in the measurement of enzyme levels.

Testing enzyme activity.

Sample. Crude enzyme extract 0.5 ml plus 0.5 ml CMC substrate then vortex, then incubate temperature 400C for 30 minutes, after incubation add 1 ml DNS then vortex then heatst 100^0C for 15 minutes, cool and measure absorbance at 540 nm wavelength (Figure 3) as following: (1) Blank. Extracts of crude enzymes 0.5 ml plus 1 ml of DNS in a test tube and then added 0.5 ml of sterile distilled water then vortex, then heated at a temperature of 100^0C for 15 minutes, cool and measure the absorbance at a wavelength of 540 nm, (2) Control. A crude enzyme extract of 1 ml plus 1 ml of DNS in a tube then vortex. Then heated at a temperature of 100^0C for 15 minutes, cool and measure the absorbance at a wavelength of 540 nm.

$$\text{Enzyme activity (U / ml)} = \frac{(C \times 2 \times P \times 1000)}{(t \times BM)}$$

C = Concentration of examples

P = Dilution

t = time

BM = molecular weight

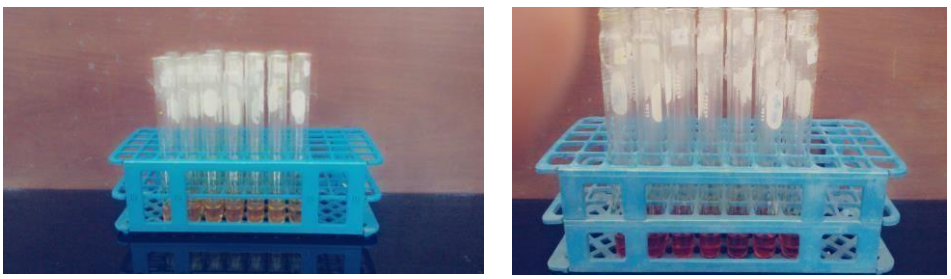

Figure 3. enzyme extract sample for cellulase enzyme activity testing

3. Results and Analysis

The cellulolytic ability of Bacillus sp YLB1 can be measured qualitatively by measuring the clear zone formed on the substrate and quantitatively from the activity of cellulase enzymes (U / ml). The production of cellulase enzymes by bacteria shows that the bacteria are able to hydrolyze cellulose contained in the substrate into glucose (simple sugar) which is soluble and can be used as a source of carbon for its growth.

The fermentation time for cellulase enzyme activity

Enzyme activity is expressed as the amount of reducing sugars released by the action of the enzyme per unit time. Determination of the amount of reducing sugar was carried out on crude extracts of enzymes from fermentation by the bacterium Bacillus sp YLB1 incubated for 30 minutes at + 40^0C.

The results of the average activity of cellulase enzymes (CMCase) from the complete fermentation of palm oil plant waste (palm fronds and leaves, BIS, palm mud) with Bacillus sp YLB1 for 7 days can be seen in Table 1.

Table 1. Average activity of the enzyme cellulase (CMCase) of palm oil substrate fermented with Bacillus Sp (Unit/ml)

Duration of fermentation (7 days)	cellulase (CMCase) enzyme activity			Average
	P1	P2	P3	
1	0.149	0.083	0.196	0.143^{A}
2	0.161	0.503	0.452	0.372^{AB}
3	0.375	0.735	0.655	0.588^{B}
4	0.321	1.576	1.142	1.013^{CD}
5	0.740	1.002	0.975	0.906^{C}
6	0.375	1.367	1.453	1.065^{D}
7	1.099	1.015	1.479	1.198^{D}
Average	0.460^{A}	0.897^{B}	0.908^{B}	

The fermentation time had a very significant effect (P <0.01) on cellulase enzyme activity. Cellulase enzyme activity in the fermented palm waste substrate for 1 day was significantly different from 3, 5 days and was significantly different from 4, 6, and 7 days of fermentation. The difference in the activity of the enzyme Bacillus sp YLB1 during fermentation is due to the bacteria used to undergo several phases in degrading the substrate.

From the activity of cellulase enzymes (CMCase) for 7 days of fermentation illustrates that on the first day of fermentation showed a lag phase marked by obtaining a low average cellulase enzyme activity compared to other days that is equal to 0.143 U / ml. In the lag phase, Bacillus sp YLB1 adapts itself to the existing substrate so that the enzyme activity produced is still low.

Enzyme activity continues to increase with increasing fermentation time, and cellulase enzyme activity reaches the first peak of day 4 (1.013 U / ml), after that, it decreases and reaches the second peak on day 7 (1,198 U / ml). This illustrates that there are several peaks of the

exponential phase of cellulase enzyme activity due to the repeated attack of the bacterium Bacillus sp YLB1 on the substrate.

Effect of various substrate combinations on cellulase enzyme activity

The fermentation results showed that the combination of the substrate had a very significant effect (P <0.01) on cellulase enzyme activity, and Duncan's multiple range tests showed that the activity of cellulase enzyme P1 substrate was very significantly different from P2 and P3. P2 substrate is not significantly different from P3. This can be due to cellulose content from different substrates.

Enzyme activity is influenced by cellulose content contained in the substrate in the form of amorphous and crystalline. It is suspected that during the first exponential phase, the amorphous cellulose portion was hydrolyzed by the bacterium *Bacillus sp YLB1*, while in the second exponential phase the crystalline portion was hydrolyzed.

The mean CMCase enzyme activity of complete palm oil waste substrate fermented with Bacillus sp was 0.4600 U / ml (P1 substrate), 0.8973 U / ml (P2 substrate) and 0.9075 U / ml (P3 substrate) (Figure 4). The results of cellulase enzyme activity using Bacillus sp YLB1 were higher than the cellulase enzyme activity of Bacillus subtilis of 0.00346 U / ml and 0.00566 U / ml [10]. This result is higher than the cellulase enzyme activity of Bacillus circulans in corncob fermentation at 0.07 U / ml [11]. Different cellulolytic bacteria will produce different cellulase enzyme complexes. This can be due to the genes they have and the carbon source used. The results of this study prove that the isolate derived from the substrate has a high ability to degrade the substrate, according to the statement of [1].

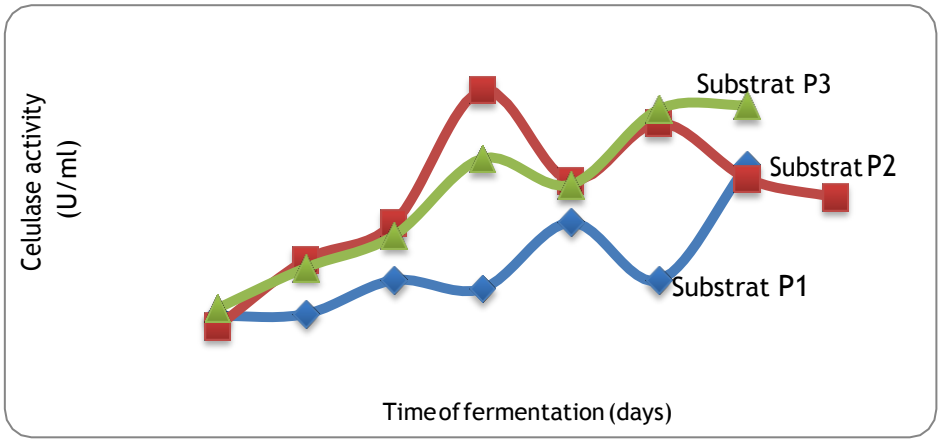

Figure 4. Cellulase (CMCase) enzyme activity of Bacillus Sp YLB1 (Unit/ml)

The results of the study above there are interactions between the use of various combinations of substrates with fermentation time (P <0.05) on the activity of cellulase enzymes. Enzyme

activity on the substrate P1 (0.460 U / ml) was significantly different from P2 (0.879 U / ml) and P3 (0.908 U / ml) but P2 was not significantly different from P3. This shows the existence of different enzymatic reactions due to contact cellulase enzymes produced by the bacterium Bacillus sp YLB1 with the combination of the substrate used. Different enzymatic reactions between substrates can be caused by the composition of the different substrates used.

[12]) said that enzyme activity depends on the type and concentration of substrate, temperature, pH, and the composition and amount of other ingredients/liquids added. The substrate concentration is small, there is also a small enzyme activity. The substrate is in excess, then the reaction depends on the amount/concentration of enzymes that exist. Enzyme reaction speed does not depend on the concentration of the existing substrate.

4. Conclusions

Cellulase enzyme (CMCase) activity of indigenous cellulolytic bacteria (Bacillus sp YLB1) is affected not only by substrate combination but also by fermentation time. The bacterial enzyme activity of Bacillus sp YLB1 is influenced by the cellulose content contained in the palm oil substrate in the form of amorphous and crystalline. P3 substrate fermented with Bacillus sp YLB1 for 7 days gave more optimal results than other treatments.

References

[1]. Yunilas, L. Warly, Y. Marlida, and I. Riyanto. 2013. Potency of indigenous bacteria from oil palm waste in degrades lignocellulose as a sources of inoculum fermented to high fibre feed. Pakistan J. Nutr. 12: 851-853.

[2]. Pandey, A., C. R. Soccol, P. Nigam, and V. T. Soccol. 2000. Biotecnological potential of agro-industrial residues. I: sugarcane bagasse. Bioresource Technol. 74: 69-80.

[3]. Cruz, S. P. B., J. Freer, M. Siika-Aho, and A. Ferraz. 2004. Extraction and determination of enzyme produced by Ceriporiopsis subvermispora during biopulpung of pinus taeda wood chips. Enzyme Microb. Technol. 34: 228-234.

[4]. Ferraz, A., A. M. Cordova, and A. Machuca. 2003. Wood biodegradation and enzim production by Ceriporiopsis subvermispora guring solid state fermentation of Eucalyptus grandis. Enzyme Microb. Technol. 32: 59-65.

[5]. Machuca, A. and A. Ferraz. 2001. Hydrolytic and oxidative enzymes produced by white- and brown rot fungi during Eucalyptus grandis decay in solid state medium. Enzyme Microb. Technol. 29: 386-391.

[6]. Chaplin, M. 2007. Water Structure and Science: Cellulose. London South Bank University. http://www.Isbu.ac.uk/water/, Accessed 10 Januari 2008.

[7]. Sanchez, C. 2009. Lignocellulosic residues: biodegradation and bioconvertion by fungi. J. Biotech. Adv. 27: 185-194.

[8]. Peres, J., J. Munoz-Dorado, T. de la Rubia, and J. Martinez. 2002. Biodegradation and biological treatment of cellulose, hemicellulose and lignin: an overview. Int. Microbiol. 5: 53-56.

[9]. Andayani, P., A. K. Wardanie, dan S. Murtini. 2008. Isolasi dan identifikasi mikrob dari tempe Sorgum coklat (Sorghum bicolor) serta potensinya dalam mendegradasi pati dan protein. Jurnal Teknologi Pertanian 9: 95-105.

[10]. Sholihati, A. M., M. Baharuddin, and Santi. 2015. Produksi dan uji aktivitas enzim selulase dari bakteri Bacillus subtilis. J. Al Kimia 3: 78-90.

[11]. Septiningrum, K. dan C. Apriana. 2011. Produksi xilanase dari tongkol jagung dengan sistem bioproses menggunakan Bacillus circulans untuk pra-pemutihan pulp production of xylanase from corn cob by bioprocess system using Bacillus circulans for pre-bleaching pulp. Jurnal Riset Industri 5: 87-97.

[12]. Susanti, R. dan F. Fibriana. 2017. Teknologi Enzim. Penerbit Andi, Yogyakarta.