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Effect Fermentation of Sugarcane Shoots With Phanerochaetechrysosoporiumon the Activity of Lacase Enzymes, Lignin Peroxidase and Manganese Peroxidase

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Abstract. Sugarcane shoots are very potential for make feed because there are quite a lot of them. Efforts to use sugarcane shoots by products have been carried out but it is still not optimal. One strategy that can be done is to use it as fermented feed. Main constraints on use of sugarcane shoots for animal feed are nutritional value, low digestibility and high lignin. Lignin physically and chemically is a major factor in the inability of livestock to digest feed ingredients. Fungi that have the ability to degrade high lignin are fungi which are included in the white rot fungi. One of them is Phanerochaetechrysosporium which is known to produce enzyme lignin peroxidase (LiP), manganese peroxidase (MnP) and lacase. Phanerochaetechrysosporium is one of the fungi could break down the bonds and degradate lignin with the help of lignin degrading enzymes. In this study sugarcane shoots were fermented using Phanerochaetechrysosporium. This method study used Completely Randomized Design (CRD) with 4 treatments inoculum dose A (0%), B (5%), C (10%), D (15%) with 5 replications. Data processing by analysis of variance (ANOVA), if it was significantly different in further testing with DMRT. Results showed that treatment C (10%) was the best dose that could be used with the production of lacase enzyme activity 2.016 U / ml, Lignin Peroxidase (LiP) 1.677 U / ml and Manganese peroxidase (MnP) 0.328 U / ml.

1. Introduction

Indonesia is agriculture country has most variety of agricultural side products. Sugarcane is one of strategic agricultural commodities to provide many side products from both on farm and off farm. Largest area of sugarcane plantation in 2010 was 418,259 ha with national sugarcane production 34,218,549 tons [1]. Sugarcane waste produced from this area will reach 17,793,645 tons [2].

One of the side products of sugarcane is sugarcane shoots, very potential used for feed cause the amount is quite large. Efforts to use sugarcan by products have been carried out but it is still not optimal. This requires an appropriate technology approach to further increase added value of sugarcane shoots. One strategy that can be done is to use it as fermented feed. Main constraints on the use of sugarcane shoots for animal feed are nutritional value, low digestibility and high lignin. Lignin physically and chemically is a major factor in the inability of livestock has digest feed ingredients. Lignin is chemically bound to structural carbohydrate components and physically acts as a barrier to the process of reforming cell walls by rumen microbes[2].

Some microbes could act as decomposers in fermented feed are fungi, yeast and bacteria. One of fungi type has the ability degradation high lignin which as fungi included in white rot fungi. These

fungi call Phanerochaetechrysosporium, known to produce the enzyme lignin peroxidase (LiP), manganese peroxidase (MnP) and lacase. Phanerochaetechrysosporium is one of the fungi can break down the bonds and degradate lignin with help of lignin degrading enzymes. This fungi also degrades cellulose, hemicellulose and lignin polymers with the help of extracellular enzymes. Biodegradation is the process of changing substrate by microorganisms involving a number of reactions into simpler products. Activities of overhauling the substrate component requires nutrients obtained from the results of reshuffle.[3]

Lignin peroxidase (LiP) and manganese peroxidase (MnP) are extracellular peroxidase enzymes that use H2O2 to degrade lignin, whereas lacase is a copper-containing enzyme using oxygen molecules to degrade lignin [4]. According to Ref. [5], enzyme work is influenced by several factors, namely substrate, temperature, pH, cofactor and inhibitor. Use of sugarcane shoots as microbial substrate will increase the nutritional value of sugarcane shoots. In this study sugarcane shoots were fermented using Phanerochaetechrysosporium with different doses and the same incubation period. Based on the analysis of the dose of the inoculum it affected the activity of the lacase enzyme, lignin peroxidase (Lip) and manganese peroxidase (MnP).

2. Material and Methods

2.1. Material

This research was conducted at the Feed Industry Technology Laboratory of the Faculty of Animal Husbandry, Andalas University. The materials used in this study were Phanerochaetechrysosporiumand sugarcane shoots.The equipment used in this study was 1700 Shimadzu UV-Vis Spectrophotometer, autoclave, measuring tube, test tube, electric stove, colony counter, micro pipette, electric heater, and a set of glass tools.

2.1.1. Preparation of Phanerochaetechrysosporium Inoculum

Phanerochaetechrysosporium isolate was rejuvenated on PDA sloping media, incubated at 30 oC for 7 days. Sterilize 100 grams of sugar cane which has been added to distilled water up to 60%. Autoclave for 30 minutes at 121oC, after being warm mixed with 1 test tube, isolates of Phanerochaetechrysosoporium. Incubation for seven days.

2.1.2. Fermentation of sugarcane shoots with Phanerochaetechrysosporium

As much 100 grams of sugarcane mashed, distilled water to 60% moisture content, sterilize in an autoclave for 30 minutes. Added inoculum Phanerochaetechrysosporium as much as 0%, 5%, 10% and 15% then incubated for 21 days.

2.1.3. Test Activity of Lignin Peroxidase (LiP) Enzymes (Tien & Kirk, 1984)[6]

0.2 ml of enzyme filtrate, 0.05 ml of H2O2 5 mM; 0.1 ml of veratril alcohol 8 mM; 0.2 ml of acetate buffer 0.05 M pH 3 and 0.45 ml of distilled water are added to cuvette and then shaken. Solution was read the absorbance at a wavelength of 310 nm at 0 and 30 minute intervals. One unit of enzyme activity in LiP is defined as amount of enzyme that causes conversion of 1 micromol (1µmol = 10-6 mol) veratril alcohol per minute.

2.1.4. Test the Activity of Manganese Peroxidase (MnP) Enzymes (Wariishi, Valli& Gold, 1962) [7]

As much as 0.1 ml of 50 mM Na-lactate buffer pH 5 is added with 0.1 ml of 4 mMguaiacol, 0.2 ml of MnSO4 1 mM, 0.1 ml of H2O2 1 mM and distilled water 0.3 ml then 0.2 filtrate is added enzyme. Solution was then checked and read at a wavelength of 465 nm in the 0 and 30 minutes.

2.1.5. Test the Activity of Lakase Enzymes (Leonowicz&Grzywnowicz, 1981) [8]

As much as 0.4 ml of enzyme filtrate plus 0.5 ml pH 5 acetate buffer of 0.5 ml and 0.1 ml of 1 mM ABTS. then it was checked and measurements were read 420 nm wavelength. Reading is done in the 0 and 30 minutes.

2.2. Research Methode

2.2.1. Analysis of Statistic (Steel and Torrie , 1960) [9]
This experiment by completely randomized design (CRD) with 4 treatments, namely inoculum doses A (0%), B (5%), C (10%), D (15%) with 5 replications. Data processing used analysis of variance (ANOVA). If got significantly different in further testing with DMRT.

3. Result and Discussions
This below results of the analysis of the activity of the lacase enzyme, lignin peroxidase (LiP) and Manganese peroxidase (MnP) are presented in Table 1.

Table 1. Results of measurement of activity of Lacase enzyme, Lignin peroxidase (LiP) and Manganese peroxidase (MnP) fermented sugarcane shoots

Treatment	Enzym Activity (U/ml)		
	Lacase	LiP	MnP
A (0 %)	0.505d ± 0.09	1.153 c± 0.03	0.030d ± 0.01
B (5 %)	1.213c ± 0.17	1.464 b± 0.04	0.107b ± 0.01
C (10%)	2.016a ± 0.10	1.677 a± 0.06	0.328a ± 0.04
D (15 %)	1.663b ± 0.06	1.413 b± 0.04	0.129b ± 0.04

Note: Different letters in the same column show a significant difference based on the DMRT test at the 5% real level

According Table 1 results of the lacase enzyme activity test showed that at a dose of 10% (C) the highest value was 1,213 U/ml. ANOVA observations for lacase activity showed that different inoculum doses showed a significant difference with the same incubation period of 21 days. At doses of 0%, 5% and 10% the activity has increased by 0.505 U/ml, 1.213 U/ml and 2.016 U / ml, respectively. At a dose of 15% (D) activity decreases, this occurs because the number of fungi is more than the availability of substrate nutrients. According to Supriyanto (2009) [5],

Enzyme work is influenced by several factors, namely substrate, temperature, pH, cofactor and inhibitor.mThe highest test results for Lignin peroxidase (LiP) enzyme activity were found at 10% (C) inocolum dose of 1.677 U / ml. Overall the activity of lignin peroxidase is higher than that of lacase activity. From the data produced with higher doses it does not always provide higher activities results. In treatment D (15%) with same period of incubation Lignin peroxidase (LiP) activity decreased to 1,663 U / ml. Lignin peroxidase (LiP) is produced by Phanerochaetechrysosporium to break C-C or C-O-C bonds in aromatic non-phenolic chains of substrates with fast The degraded substrate will be more easily utilized by the Phanerochaetechrysosporium for its growth (Wong, 2009; Pogni et al, 2007) [9] [10]

Manganese peroxidase (MnP) activity test results showed the lowest value compared to lacase and Lignin peroxidase (LiP). The largest manganese peroxidase (MnP) activity in treatment D (10%) is 0.328 U / ml, higher than doses A (0%) and B (5%), which are 0.030 U / ml and 0.107 U / ml respectively and decrease in treatment D (15%) which is 0.129 U / ml. Lignolytic enzyme activity is influenced by pH, substrate concentration, temperature, amount of nitrogen, and supporting minerals (Patel et al., 2009).[11]. Ergun et al (2015) [12] showed that MnP activity value would be relatively stable at the incubation period of 50 days. The enzyme has an optimum activity at a certain pH which is also called the optimum pH. The role of the enzyme in catalyzing a reaction depends on the type of specific substrate.

4. Conclusion
Based the results of the study could concluded that sugarcane shoot fermentation with mold Phanerochaetechrysosporium produces the highest enzyme activity at a dose of 10% with a 21-day incubation period. Lacase activity was 2.016 U / ml, Lignin Peroxidase (LiP) 1.677 U / ml and Manganese peroxidase (MnP) 0.328 U

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