Cellulase Production from *Paecilomyces Lilacinus* ICP1 Using Coffee Pulp as Substrate

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Abstract. Cellulase can be produced by a microorganism, such as fungi. The cellulolytic microorganism used in this study was *Paecilomyces lilacinus* ICP1. *P. lilacinus* ICP1 is one of the indigenous fungi from coffee pulp that has been known to produce cellulose. Coffee pulp is expected to be able to replace the use of pure cellulose substrate because of its high cellulose content of 63% and other organic materials that can support the growth of cellulolytic microorganisms. One method that is often used for enzyme production by utilizing agricultural or plantation waste is Solid State Fermentation (SSF). Cellulase production by *Paecilomyces lilacinus* ICP1 using coffee pulp waste as a substrate has been investigated. Through Solid State Fermentation of coffee pulp substrate based the *P. lilacinus* ICP1 can produce cellulase up to 0.17 U/ml with 3 days of incubation at 30 °C. Cellulase activity was measured base on reducing sugar produced using the Somogyi-Nelson method against 0.5% carboxymethyl cellulose in 20 mM acetate buffer pH 5. The crude cellulase was then dialyzed through a cellulose membrane with 12–14 kDa pores in the same buffer. In this step, the activity was 0.47 U/ml, an increase about three times from the initial. These results proved that *P. lilacinus* ICP1 could secrete extracellular cellulase through Solid State Fermentation using coffee pulp waste without any nutrients added so that it may become a strategy industrial scale to produce cellulase cheaply.

Keywords: Cellulase · Coffee pulp · *Paecilomyces lilacinus* ICP1 · Solid state fermentation

1 Introduction

Cellulase is an enzyme that hydrolyses β-1,4 glycosidic bonds in linear polysaccharides such as cellulose into glucose monomers [1]. Cellulose is currently widely used in the industrial sector, such as textiles, animal food, ethanol production, detergents, paper, and pulp [2, 3]. This causes the production of cellulose on an industrial scale to be urgently needed. The very high cost is one of the obstacles to industrial-scale cellulose production. Therefore, many studies have focused on reducing production costs by exploring cellulolytic microorganisms [4].

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Cellulase can be produced by microorganisms, such as fungi, bacteria, protozoa, and *actinomycetes* [4]. The cellulolytic microorganism used in this study was *Paecilomyces lilacinus* ICP1. *P. lilacinus* ICP1 is an indigenous fungus of coffee pulp that has been known to produce cellulose. Based on the research that has been carried out, shows that *P. lilacinus* ICP1 can produce cellulose using a coffee pulp substrate with an activity of 0.18 U/ml after 3 days of incubation at 30 °C [5].

The use of cellulose substrates from agricultural and plantation waste can also be considered to reduce costs in cellulose production [4]. One of the plantation wastes used as s substrate for cellulose production in this study is coffee pulp. Coffee pulp is expected to be able to replace the use of pure cellulose substrate because of its high cellulose content of 63% and other organic materials that can support the growth of cellulolytic microorganisms [6]. Furthermore, the availability of coffee pulp waste in Indonesia is also very abundant. This is because every processing of coffee cherries will produce 60% of coffee pulp, while Indonesia in 2020 will be able to produce 685.980 tons of coffee [7, 8]. This abundance indicates that coffee pulp waste has a high potential to be used as a substrate for cellulose production in Indonesia.

The utilization of coffee pulp as a substrate for cellulase production by microorganisms has been done in several studies. *Aspergillus* sp. VT12 can produce cellulase using coffee pulp with cellulase activity reaching 1.8 U/ml for 96 h of incubation through solid-state fermentation [9]. The coffee pulp can also be used by *Aspergillus* sp. VTM1 produces cellulase under solid-state fermentation with the activity reaching up to 1.18 U/ml at 120 h of incubation [10]. The fungi isolate VT11 from [11] can also produce cellulase from the coffee pulp through solid-state-fermentation with the highest cellulase activity reaching 1.86 U/ml after 96 h of incubation. However, the thing that is different in some of the studies above with this research is that the fungi used are not indigenous fungi of coffee pulp.

One method that is often used for enzyme production by utilizing agricultural or plantation waste is Solid State Fermentation (SSF). The Solid-State Fermentation (SSF) method is the most suitable solid fermentation method for cellulase production by fungi because of the low humidity in the substrate so which is under natural environmental conditions for fungi growth [12]. This method has several advantages, including high fermented products simple technique used, and low possibility of contamination [13]. Therefore, it is necessary to conduct further research on the production of cellulase from *P. lilacinus* ICP1, as indigenous fungi of coffee pulp, through Solid State Fermentation (SSF) using coffee pulp waste as a substrate so that it may become a strategy industrial scale to produce cellulase cheaply.

2 Materials and Methods

2.1 Inoculum Preparation

Paecilomyce lilacinus ICP1 is one of the indigenous fungi of coffee pulp that has been isolated and identified by previous research [5]. Pre-cultured *P. lilacinus* ICP1 was carried out on slanted Potato Dextrose Agar (PDA) medium. *P. lilacinus* ICP1 from culture stock was inoculated on a slanted PDA medium using the scratch method and incubated at 30 °C for 6 days [14].

2.2 Solid State Fermentation (SSF)

Solid State Fermentation was made using 80 g coffee pulp as a substrate with 168 ml aquadest in a 2000 ml Erlenmeyer flask. Then, the SSF medium was sterilized at 121 °C for 25 min using an autoclave. The SSF medium was added to 8 ml inoculum *P. lilacinus* ICP1 which reached 10^7 spores/ml and it incubated at 30 °C for 3 days [9].

2.3 Extracting of Crude Enzyme

Extracting the crude enzyme was started by adding NaN_3 0,01% and NaCl 1% in 160 ml aquadest. Then, it was incubated at a rotary shaker (120 rpm) for 12 h. The crude enzyme was extracted with filtration and centrifuged at 8000 rpm for 5 min. The supernatant was taken as an extracellular enzyme and it was stored at 4 °C [14].

2.4 Cellulase Activity Assay (Somogyi-Nelson)

Cellulase activity assay was started with 500 µl CMC 0,5% in acetate buffer 20 mM pH 5 incubated at 37 °C for 20 min in the water bath. Then, added 50 µl crude enzyme and incubated at 37 °C for 2 h. The Somogyi reagent was added as much as 500 µl to the sample and then boiled for 15 min. After the sample was cold, 500 µl of Nelson reagent and 2.5 ml aquadest were added to it and then homogenized using a vortex. The sample was taken at 1.3 ml and centrifuged at 8000 rpm for 10 min. Then, the supernatant obtained was measured in its absorbance using a spectrophotometer with a wavelength of 500 nm. The absorbance value is converted into a linear equation of the glucose standard curve and then it was converted into an enzyme activity formula to know the activity of cellulase. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the conversion of 1 µmol of the substrate to the product under specified assay conditions. Measurement of protein content was carried out using a spectrophotometer with a wavelength of 280 nm [14, 15].

2.5 Dialysis with a Cellulose Membrane

Dialysis was carried out by inserting 25 ml of the crude enzyme into the cellulose membrane with 12–14 kDa pores. The crude enzyme in the cellulose membrane was soaked in acetate buffer of 20 mM pH 5 for 24 h and stirred using a magnetic stirrer. Acetate buffer is replaced every 12 h. The enzyme activity of the dialysis results was tested using the Somogyi-Nelson method [14].

3 Results and Discussion

3.1 Cellulase Production

Cellulase production was carried out by adding the isolate *Paecilomyces lilacinus* ICP1 with 75.2 x 10^7 spores/ml on 80 g of coffee pulp substrate in 168 ml of aquadest. The spore density of *P. lilacinus* ICP1 used in this research was optimal. This is because according to Naik [16] the inoculum size plays an important role in enzyme production.

Table 1. Cellulase activity from *Paecilomyces lilacinus* ICP1 at the enzyme purification stage

Purification stage	Total volume (ml)	Enzyme activity (U/ml)	Total activity (U)	Abs.280 nm	Total abs. 280 nm (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude enzyme	350	0.17	59.5	0.83	289.80	0.21	100.00	1.00
Dialysis 12–14 kDa cellulose membrane	413	0.47	194.11	0.32	133.81	1.45	326.24	7.07

The optimal spore density of fungi for enzyme production ranges from 10^6 to 10^8 spores/ml [17]. A lower level of inoculum may not be sufficient for initiating growth and enzyme production. An increase in inoculum size ensures a rapid proliferation of biomass and enzyme production. After a certain limit, enzyme production could decrease because of depletion of nutrition due to the enhanced biomass, which would result in a decrease in metabolic activity [18].

The incubation time obtains optimum enzyme activity in the crude enzyme production process refers to the previous study Ubaidillah [5], which is 3 days. Incubation time plays an important role in the production of an enzyme [19, 20]. The incubation time of this research was different from other studies. Evelyn [21] reported that *Eupenicillium javanicum* can produce cellulase using pineapple crown leaves waste with the highest activity after 4 days of incubation at 35 °C through solid-state fermentation. Maftukhah and Abdullah [22] also reported that *A. niger* can produce cellulase from rice straw under the SSF method with the optimum incubation time being 6 days at 30 °C. *Trichoderma viride* can produce cellulase using maize husk silage with the highest activity after 7 days of incubation at 30 °C through the SSF method [23]. The difference in incubation time is due to differences in substrates, types of microorganisms, and other factors in the fermentation process.

Cellulase production from *P. lilacinus* ICP1 in this study resulted in cellulase with an activity of 0.17 U/ml with 3 days of incubation at 30 °C (Table 1). The value of this activity is not much different when compared with the optimization results in the Ubaidillah study [5] of 0.18 U/ml with the same incubation. This is because all parameters in enzyme production were carried out under the same conditions, such as the ratio of substrate volume to Erlenmeyer flask, inoculum volume, water content, number of spores, and incubation time.

3.2 Dialysis

Crude cellulase produced needs to be purified because it still contains protein and other metabolites. This purification is carried out through a series of purification processes. One of the purification steps carried out in this research is dialysis using cellulose membrane 12–14 kDa. The purpose of dialysis is to separate the target protein in solution from

other impurity particles [24]. The success rate of the dialysis process can be determined by the enzyme activity assay of the dialysis results. Cellulase activity assay from the dialysis stage showed the results of 0.47 U/ml with a specific activity of 1.45 U/mg or 7.07 times purer than the crude enzyme produced (Table 1).

According to Hapsari et al. [25], the higher the specific activity value of the enzyme, the higher the purity level of the enzyme. This is because in the dialysis process there is a separation of other molecules that do not target proteins based on size through the pores of the cellulose membrane. Molecules larger than the membrane pores such as enzymes will be retained in the membrane, while small molecules such as salts, solvents, and other metabolites that do not target proteins will diffuse through the cellulose membrane [26, 27].

Through Solid State Fermentation of coffee pulp substrate based on the *P. lilacinus* ICP1 can produce cellulase up to 0.17 U/ml with 3 days of incubation at 30 °C and cellulase activity increased after dialysis to 0.48 U/ml or 7.07 times than initial. These results proved that *P. lilacinus* ICP1 could secrete extracellular cellulase through Solid State Fermentation using coffee pulp waste without any nutrient added so that it may become a strategy industrial scale to produce cellulase cheaply.

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Authors' Contributions. T carried out all the experiments, analyzed data, drafted, and wrote the manuscript. S, RW, and HTW participated in manuscript revision. KM participated in the design of the study, and manuscript revision, and become the corresponding author. All authors read and approved the final manuscript.

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