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Research Article

Production of Cellulase by *A. Niger* and *T. Reesei* Under Solid State Fermentation Using Bagasse as Substrate

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Abstract: Cellulase is a very important enzyme for ethanol production from lignocellulose and others. Bagasse is very good to be chosen as lignocellulosic material for cellulase production because it is cheap and already available in a huge amount. Solid-state fermentation has advantages than submerged fermentation because of its higher yield and activity. Cellulase production using solid-state fermentation has been researched by many people, but for different substrate, different pretreatment and different fungi producer, it is likely to have a different condition. It is very important to perform specific study to optimize cellulase production with specific substrate and specific fungi which available in Indonesia. Because large amount of fungi strains available so it is wise full if they are screened using effective and efficient methods. This research is started by initial screening of three strain of *Trichoderma reesei* and five strain of *Aspergillus niger* using Congo red dye staining on CMC agar plate. It is shown that a strain of *Trichoderma reesei* and two strains of *Aspergillus niger* has better activity than the others. Further screening is performed by conducting solid-state fermentation using bagasse by three best strains for five days. The activity of the cellulase is measured every 24-hour using Ghose method by dinitrosalicylic acid as reagent. The highest activity is shown by *A.niger* ITBCC L74 on third day at 0.525 Unit/gram.

Keywords: *A. niger*, *T.reesei* ,bagasse, cellulase, solid state fermentation

1. Introduction

Commercial enzymes or industrial importance are obtained from three main sources namely plants, animals and microorganisms. In the past, plants and animals served as main source of enzymes but today microbial sources of enzyme are becoming more popular for obvious reasons (Abubakar and Oloyede, 2013).

The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria. Large quantities of lignocellulosic wastes are generated through forestry, agricultural practices and industrial processes, particularly from agro-allied industries such as sugar cane, breweries, paper pulp, textile and timber industries. These wastes generally accumulate in the environment thereby causing pollution problem (Bhradwaj et al 2021). Lignocellulose is a major renewable natural resource of the world and represents a major source of renewable organic matter (Maeda et al 2013). The plant biomass regarded as "wastes" are biodegradable and can be converted into valuable products such as enzymes, biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients (Acharya *et al* 2008).

Industrially important enzymes have traditionally been obtained from submerged fermentation (SF) because of the ease of handling and greater control of environmental factors such as temperature and pH. However, solid state fermentation (SSF) technique can improve the yield and reduces the cost of enzyme production. Filamentous fungi are the most commonly used microorganisms in SSF because they are able to grow on solid materials with low water contents (Lynd et al 2017). The other advantages of SSF include superior productivity, simple technique; low capital investment, low energy requirement and less water (Mrudula and Murugammal 2011). The aim of this study is to find the best strains in producing cellulase enzyme under solid state fermentation using bagasse as substrate media among eight strains of *Aspergillus niger* and *Trichoderma reesei*.

2. Method

2.1. Chemical

All chemicals used were of analytical grade. Media and chemicals used in this study were purchased from Merck, Indonesia.

2.2. Bagasse materials

Bagasse was obtained from Sugar Factory, Mojo Panggung, Tulungagung, Indonesia and characterized using Chesson-Datta method (Chesson, 1981) to determine the content of cellulose, hemicellulose, lignin, and ash.

2.3. Microorganism

The micro-organism used in this study were *Aspergillus niger* ITBCC L51, L74, L76, and L161 obtained from Bandung Institute of Technology, and *Aspergillus niger* FNCC 6114, *Trichoderma reesei* FNCC 6012, 6013 and 6131 obtained from of Gajah Mada University and maintained at 4^{o}C on Potato Dextrose Agar (PDA) slants.

2.4. Cellulase Production with Congo Red Staining

Eight strains were inoculated aseptically on a PDA plate for 2 hours, then PDA cut with a diameter of approximately 1.5 cm and removed to a petri dish that contains agar (20 g/l), sodium CMC and Mandels nutrients and stored for 4 days. The samples were then treated with a solution of 10 ml congo red (2.5 g/l) for 10 minutes. Samples were washed with NaCl solution (10 g/l). Orange color of colony diameter than measured (Florencio et al 2012).

2.5 Solid state fermentation (SSF)

Ten grams of bagasse with water content of 80% was incubated with 2 mL inoculum in a 250 ml Erlenmeyer flask. Nutrients are added in accordance with nutrients Mandels. After reaching the specified time the enzyme was extracted with a solution of sodium citrate buffer pH 4.5 in the ratio of dry bagasse against buffer 1:10 w / v. The enzyme activity was tested by the Ghose method (Ghose, 1987)

3. Result and Discussion

3.1. The characteristic of bagasse

The bagasse was obtained from sugar industry, the characteristic of this waste is important in order to know the actual composition which definitely affects the results of the fermentation process. The result was shown in Table 1.

Table 1 The characteristic of bagasse

Element	Content (%)
Cellulose	57,76
Hemicellulose	12,94
Lignin	21,34
Others	7,96

3.2. Selection of strains

Eight strains were tested by congo red staining to find enzymatic index and are presented in Table 2. The result shows that three strains of *A. niger* ITBCC L74, *A. niger* ITBCC L161 and *T. reesei* UGM 6131 were higher than the four other strains with enzymatic index of 1.945, 1.608, and 1.875, respectively.

Table 2 Enzymatic index for eight strains

Strains	Colony diameter (D_k)	Effect of enzyme cellulase (D_p)	Enzymatic indexs (IE) = D_p/D_k
A. niger FNCCC 6114	2,23	-	-
A. niger ITBCC L51	2,21	3,36	1,520
A. niger ITBCC L74	1,63	3,17	1,945
A. niger ITBCC L76	1,91	2,17	1,136
A. niger ITBCC L161	1,94	3,12	1,608
T. reesei FNCC 6131	1,84	3,45	1,875
T. reesei FNCC 6012	1,88	2,23	1,186
T. reesei FNCC 6013	1,85	2,47	1,335

3.3. Time course of cellulase production

SSF was carried on bagasse with initial moisture content of 80 % at 30°C. Time course of cellulase production were given in Table 3. As shown in Table 3, after 72 hours incubation the enzyme activity of CMCase for *A. niger* ITBCC L74, *A. niger* ITBCC L161 and *T. reesei* UGM 6131 reached the maximum of 0.5251, 0.3927 and 0.3264 U/g, respectively. Incubation beyond 72 hours resulted in decreased enzyme activity, this might be done to the denaturation of the enzyme caused by interaction with other compounds in the medium. Similar trend also reported by Sun *et al* (2011) and Ma et al (2011).

Table 3. Enzyme activity (Unit/gram) vs time

Time (day)	*A. niger* ITBCC L74	*A. niger* ITBCC L161	*T. reesei* UGM 6131
1	0,0311	0,0264	0,0208
2	0,1868	0,1585	0,1246
3	0,5251	0,3927	0,3264
4	0,2602	0,2129	0,3122
5	0,1892	0,1608	0,1986

4. Conclusion

Bagasse have been used in production of cellulase by *Aspergillus niger*. The results highlight the potentials of the substrates as possible raw materials for cellulase production using *Aspergilius niger* ITBCC L74. Incubation beyond 72 hours resulted in decreased enzyme activity which may be caused the denaturation of the enzyme caused by interaction with other compounds in the medium.

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