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2nd IBOC 2014

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Biodiversity and Biotechnology for Human Welfare

November 8th, 2014

Volume II: Biotechnology, Agriculture and Food Technology

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Faculty of Mathematics and Natural Science
Institut Teknologi Sepuluh Nopember
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Preface

We would like to thank God for the blessing since the 2nd International Biology Conference (IBOC) could be held in November 2014 by The Biology Department. This conference is purposed to build a promising international networking between our department and international institutions which have a similar biological interest for saving the environment, as the theme of conference is Biodiversity and Biotechnology for Human Welfare.

It is well recognized that our environment faces many problems due to many natural and anthropogenic causes that bring many impacts for human being itself. Global warming, losing biological biodiversity, losing environmental water, land and air quality are the examples that has a significant effect for the human welfare.

Through the conference we would like to achieve many personal contacts, ideas, biological-environmental problem solve sharing and fruitful discussions in order to save the earth together. Therefore we are really grateful and thankful that the participants who are interested to joint with are from Japan, Thailand, Norway, Australia, Malaysia, Germany and Indonesia.

We would like also to thank to the Mathematics and Natural Sciences Faculty and the Institut Teknologi Sepuluh Nopember (ITS), Surabaya-Indonesia for supporting the conference. The big remarkable applause is also going to our students who are giving their excellent hands for keeping the conference running on schedule.

Last but not least, we have a big hope that a real excellent networking in the future may arise from this event.

Thank you and best regards.

Surabaya, November 8th 2014
Head of Biology Department

Dr.rer.nat. Maya SHOVITRI, M.Si

Content

Cellulases Production By Cellulolytic Mold Isolated From Soil Teak Forest Kresek, Madiun

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ABSTRACT

The research is conducted to determine the productivity of cellulases from cellulolytic mold isolated from soil of teak forest, Madiun. Results of previous studies showing that the cellulolytic mold were identified are Aspergillus, Fusarium, Penicillium and Rhizopus. The mold that exhibited higher cellulase activity is *Aspergillus* sp. with 21,17 ± 1,53 mm of clear zone diametre and 0,68 of clear zone ratio. This research used a completely randomized factorial design consisted of two factors to know the activity and productivity of the best cellulolytic mold. The first factor are three levels of incubation time (5, 7, and 9 days). The second factor are three levels of inoculum concentration (10%, 15%, and 20%). The parameters that used in this research are the value of glucose reduction and soluble proteins. The results showed that the incubation time and inoculum concentration influential to activity and productivity of cellulases at *Aspergillus* sp. The best cellulases activity occur on day 5 and 20% inoculum concentration with 0.072% db glucose reduction and the best protein content occur on day 9 and 10% of inoculum concentration with have an average value of 0.065% wb.

Keyword : Cellulases, Cellulolytic mold, Cellulases activity , Soil of Teak Forest

Introduction

Forest ecosystem produces a lot of organic matter in the form of leaves, twigs, branches, fruits and reproductive parts, such as fl owers, seeds, spores (Tandel et al. 2009). Plant residues added to the soil are transformed into CO2, microbial material and relatively stable humus components (Shields et al. 1973). Cellulose is considered as one of the most important sources of carbon on this planet and its annual biosynthesis by both land plants and marine algae occurs at a rate of 0.85×10 11 tonnes per annum (Nowak *et al*., 2005). Cellulose degradation and its subsequent utilisations are important for global carbon sources. The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest (Bhat, 2000). Over the years, a number of organisms, in particular fungi, possessing cellulose degrading enzymes have been isolated and studied extensively (Nowak *et al*., 2005). Cellulose may be hydrolyzed using enzymes to produce glucose, which can be used for the production of ethanol (Olsson and Hahn Hagerdahl, 1996), organic acids (Luo *et al*., 1997) and other chemicals (Cao *et al*., 1997).

Enzymatic components act sequentially in a synergistic system to facilitate the break down of cellulose and the subsequent biological conversion to an utilizable energy source, glucose (Beguin and Aube rt, 1994). Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen *et al*., 2005). Therefore, there has been much research aimed at obtaining new microorganisms producing cellulase enzymes with higher specific activities and greater efficiency (Subramaniyan and Prema, 2000).

Cellulases have attracted much interest because of the diversity of their applications, and also for facilitating the understanding of mechanism of enzymic hydrolysis of plant carbohydrate polymers (Bhat, 1997). The major industrial applications of cellulases are in textile industry for 'biopolishing' of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness. Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juices, in baking etc. Utilisation in deinking of paper is yet another emerging application. The cellulases that are used so far for the above mentioned industrial applications are those from fungal sources (Tolan and Foody, 1999).

Cellulolytic mold are produced in large amounts, which include all the components of a multi enzyme system with different specificities and mode of action, i.e. endoglucanases, cellobiohydrolases (exoglucanases) and b-glucosidase, acting in synergism for complete hydrolysis of cellulose. The present work is aimed at screening cellulolytic fungi from the soil of teak forest samples collected from different areas of Hyderabad and producing the crude enzyme from the best cellulolytic mold. Further, efforts were also made to optimize the cultural and environmental conditions for maximizing of yield of the enzyme. The present study was undertaken with the following objectives:

- To isolate mold from teak forest soil samples that produce cellulase enzyme using a selective medium after enrichment.
- To determine the ability from the best Cellulolytic mold to degrade cellulose

Materials and methods

Screening cellulolytic molds start with collected the soil from teak forest in Kresek, Madiun with depth of 5-10 inches from the top and sieved through a 2 mm sieve constituted the soil sample. The samples were dispensed into bags and were brought to the laboratory and soil enrichment was done by adding 1 g of cellulose. Enrichment broth with cellulose as carbon source and peptone as nitrogen source was used for isolation of cellulolytic fungi. The selective medium i.e., Mandel's enriched medium with pH 5 was employed to get desired fungi (Mandels, 1985). The plate screening medium was used which contains Mandels mineral salts solution along with cellulose thus enabling the growth of many cellulase secreting fungi. The growth obtained on Mendel's enriched agar medium was isolated and inoculated into petri plates containing CMC (Carboxy methyl cellulose) agar medium. The strains isolated were then inoculated into the production medium to identify the ability of the strain for cellulase production under optimized conditions. The obtained pure cultures of the fungi were maintained at 29°C and then transfered to Potato dextrose agar (PDA) slants. The isolated fungi were identified based on the morphological, staining and molecular techniques.

Production of cellulases from fungal isolates. The isolated mold culture, *Aspergillus* sp. were used to know the potential for cellulases production. A volume of 100 ml of Czapek-Dox broth medium amended with 1% cellulose was distributed into separate 250 ml Erlenmeyer conical flasks. pH of the medium was adjusted to 5. After autoclaving at 121 °C. pressure, the flasks were inoculated with the fungal spore suspensions. The flasks were incubated at 32 °C on a rotary shaker at 120 rpm for 9 days. After incubation, the contents of the flasks were passed through Whatman filter paper No.1 to separate mycelial from culture filtrate. The filtrate thus obtained was used for the estimation of biomass, extracellular protein content (Biuret method), total glucose reduction (Nelson Somogyi method).

Results

Kresek is one of centra teak forest in Madiun. The location of this research is about 07° 41'53,07' S and 111° 37' 24,64'' E. Generally, the physico-chemical characteristic of the soil showed with 30% of moisturity, 6,7 pH and 21 of C/N ratio. The condition of the soil is the general characteristic of forest. The mold that can be isolated from teak forest in Kresek Madiun are Aspergillus, Fusarium, Penicillium and Rhizopus. The genus that can be isolated is showed in figure 1. The soil sample contained considerable population of the cellulolytic mold. The mold grown on the selective media supported the growth of the mold by using cellulose as the carbon source (Khalid *et al*., 2006). Efficient cellulolytic mold isolates were finally selected based on the zone of the clearing around t he mold on Carboxy Methyl Cellulose agar (CMC agar) plates (Immanuel *et al*., 2006). The appearance of the clear zone around the colony when the Congo red solution was added (Wood *and* Bhat, 1998), was a strong evidence that the cellulolytic mold inorder to degrade cellulose (figure 2). The clear zone diametre of each mold are shown in Table 1. After we know the best cellulolytic mold based on their clear zone (Table 1). After best fungus is known (*Aspergillus* sp), then we produced a crude enzyme from that mold. the cellulases activity and productivity analyzed from the best mold (Table 1; Table 2).

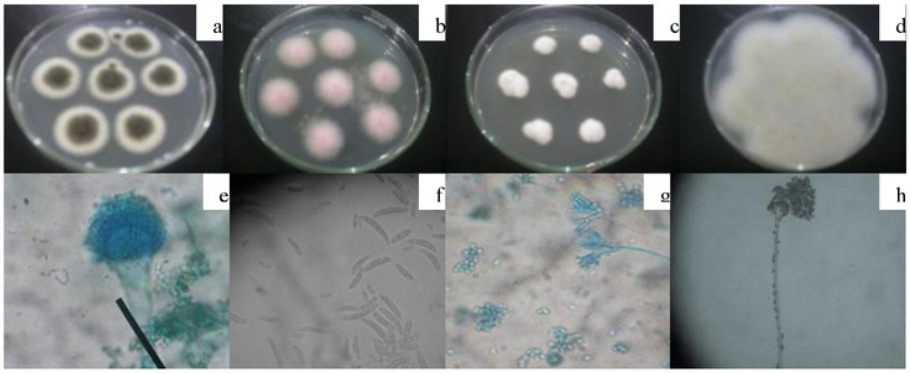

Figure 1. Morphological characteristic of cellulolytic mold in PDA medium a) *Aspergillus* sp., b) *Fusarium* sp., c) *Penicillium* sp., d) *Rhizopus* sp., e) *Aspergillus* sp. spore, f) *Fusarium* sp. spore, g) *Penicillium* sp. spore, d) *Rhizopus* sp. spore

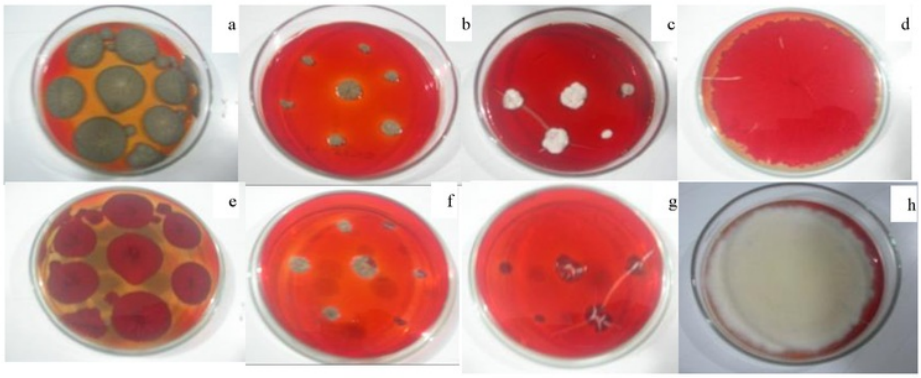

Figure 2. Cellulolytic Mold in CMC agar medium with Congo red solution a) *Aspergillus* sp looks foward, b) *Fusarium* sp. looks foward, c) *Penicillium* sp. looks foward, d) *Rhizopus* sp. looks foward looks foward, e) *Aspergillus* sp looks back, f) *Fusarium* sp. looks back, g) *Penicillium* sp. looks back, h) *Rhizopus* sp. looks back

Table 1. The diametre of clear zone CMC agar with Congo Red solution

No	Genus	Clear zone (mm)	Colony Diametre (mm)	Ratio of clear zone
1	*Aspergillus* sp.	21,17 ± 1,53	31	0,68
2	*Fusarium* sp.	4,67 ± 2,08	9	0,52
3	*Penicillium* sp.	0,63 ± 0,35	11	0,06
4	*Rhizopus* sp.	8,00 ± 1,73	89	0,09

Table 2. Protein content on crude enzyme from *Aspergillus* sp. (% wb)

No	Incubation time/Concentration (%)	10%	15%	20%
1	5 day	0,063 ± 0,004	0,060 ± 0,005	0,049 ± 0,000
2	7 day	0,055 ± 0,001	0,052 ± 0,001	0,042 ± 0,005
3	9 day	0,065 ± 0,001	0,057 ± 0,001	0,047 ± 0,001

Table 3. Glucose reduction on crude enzyme from *Aspergillus* sp. (% db)

No	Incubation time/Concentration (%)	10%	15%	20%
1	5 day	0,055 ± 0,003	0,054 ± 0,001	0,072 ± 0,004
2	7 day	0,017 ± 0,003	0,018 ± 0,001	0,016 ± 0,000
3	9 day	0,029 ± 0,003	0,027 ± 0,000	0,026± 0,001

Discussion

a. Isolation of the celulolytic mold

The teak forest soil sample contained population of the cellulolytic mold. The mold grown on the selective media supported the growth of the mold by using cellulose as the carbon source (Khalid et al., 2006). Efficient cellulase producing mold isolates were finally selected based on the clearing zone around the mold on carboxy methyl cellulase agar (CMC agar) plates (Immanuel et al., 2006). The appearance of the clear zone around the colony when the Congo red solution was added (Wood and Bhat, 1998), was a strong evidence that the fungi produced cellulase inorder to degrade cellulose. Based on the research showed that the biggest diametre of clear zone is aspergillus which then Rhizopus.

b. Morphological identification

The isolated mold was purified by repeated sub-culturing on the Potato Dextrose Agar medium at regular intervals and incubating at 29°C. The isolate was identified based on the colony morphology and microscopic observation like colony shape, color of colony, spore shape, hyphae, conidia, conidiofor etc.

The colonies size of *Aspergillus* sp. are medium, brown hyphae, hyphae's texture are smooth, rapid growth, yellowish brown hyphae in under surface. Conidiophores ± 11 µm in length, conidiophores head diametre ± 63 µm conidiophores, brown vesicles and brown conidia, septat conidiophores. *Fusarium* sp. has a white hyphae initially. At the age of 2-3 days later the white spores appear yellow interspersed. Under the surface of the hyphae interspersed yellowish. The conidiophores in 45-50 µm in length, macroconidia ± 2,6-5, 2 µm in length. The colonies of *Penicillium* sp. are white with dense hyphae at the first. At the age of 3-4 days the colonies changed to a dark green interspersed with white and form a vortex in the middle, sometimes showing a clear zone. Under the surface of the hyphae interspersed brown yellowish. The conidiophores ± 36 µm in length, the head of conidiophores ± 7 µm in length, has a black translucent vesicles and black conidia. The Colonies of *Rhizopus* sp. like a thread with white color; the sections of sporangium and sporangiofora looks certain form of black dots as pin, has a aseptat hyphae, multinucleated and have a stolon and dark color rhizoid if it is old.

c. Cellulases activity

The crude enzyme that produced then analyzed the cellulases activity with measured the glucose reduction (Nelson Somogyi method). In this study shown that the best cellulolytic mold Aspergillus sp have maximum glucose reduction on day 5, 20% of concentration with 0,072 ± 0,004 % db for the average. Beside that we also measured the protein content from the crude enzyme. The result shown that the best protein content on day 9, 10% of concentration with 0,065 ± 0,001 % wb for the average.

Conclusion

In the present study, it could be concluded that the fungal cultures isolated from forest litter soil possess cellulolytic activity. Among these fungal cultures, *Aspergillus* sp. was noticed to show maximum zone of hydrolysis (21,17 ± 1,53 mm) of carboxy - methyl cellulose, the best cellulases activity that measured from glucose reduction is 0,072 ± 0,004 % db (day 5, 20% of concentration), the best protein content is 0,065 ± 0,001 % wb (day 9, 10% of concentration). The fungal cultures isolated in the present investigation need to be further studied in depth for their cellulolytic potential for conversion of cellulosic waste material into useful products.

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We wish to thank to the Faculty of Mathematic and Natural Science Education IKIP PGRI Madiun especially Department of Biology Education, Dr. Parji M.Pd to great support and cooperation that enabled us to carry out this study. Authors would also like to thank to Mrs. Nurul Kusuma Dewi S.Si, M.Sc and Mrs. W. Lienda Wiguno for their suggestions and help.

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CERTIFICATE

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