Isolation and Determination of Enzymatic Activity of Selected Fungi on Sugarcane Bagasse as Feed for Ruminant

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ABSTRACT

It was found that ester and covalent bond between lignin, polysaccharides, and protein could reduce the digestibility of cellulose and hemicelluloses of sugarcane bagasse. Objectives of this experiment were to identify and to isolate the fungi that capable of degrading the lignocellulosic materials in sugarcane bagasse and to determine the decomposition ability of enzymatic selected fungi. The method used to culture the fungi was enrichment and platting method, while the method used for selection of fungi was the enzymatic selection method. Isolated fungi in this experiment were: *Aspergillus* sp, *Penicillium citrinum*, *Penicillium* sp(2), *Penicillium* sp(3), *Penicillium* sp(4), *Penicillium* sp(5), *Penicillium* sp(6), *Memnoniella* sp(1), *Memnoniella* sp(2), dan *Helminthosporium* sp. Further test showed that these isolated fungi have cellulolytic activity.

Key words: fungi, cellulase, and sugarcane bagasse

INTRODUCTION

As a center of agro-based industry in Indonesia, Lampung Province has a very high fibrous agricultural residues and agro-industrial byproducts, including sugarcane bagasse. This resource could be used as a main feed for ruminants (goats, sheep, and cows) in the future. These animals have the ability to digest the cellulosic materials using microorganisms in the rumen to help in breaking down the feed and nutrients, so that the host animals can get the nutrients from it. However, lignocellulosic materials, such as sugarcane bagasse, have long been demonstrated to have high degree of resistance to ruminal degradation. Therefore, this abundant renewable biomass in fact still has a minimum benefit as a feed for ruminants.

Kirby (2006) explained that lignin has a highly complex and relatively random structure that provides this organic material with a high degree of resistance to degradation. Their wide varieties of chemical bonds make specific cleavage by the active site of an enzyme difficult, and would require many enzymes, each with a specific active site, for degradation. Moreover, Taherzadeh and Karimi (2008) stated that lignin is a complex molecule constructed of phenyl propane units linked in a three-dimensional structure, which is particularly difficult to biodegradation.

Numerous attempts have been made to improve the utilization of cellulosic materials as a feed for ruminant, include pretreatment of cellulosic materials and optimizing the bioprocess in the rumen. The whole digestion process in the digestive tract of the ruminants, especially in the rumen, could be accelerated by application of feed treatment (pretreatment), including chemical and biological treatments. Mosier *et al*., (2005) stated that pretreatment is an important tool for improving cellulose conversion or degradation processes. Pretreatment is required to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. The ultimate goal of pretreatment is to break the lignin seal and disrupt the crystalline structure of cellulose.

A number of preliminary studies have investigated the benefit effects of fungal cultures on improving the lignocelluloses decomposition. Culture of *Trichoderma viride* in sugarcane bagasse could improve the availability of structural carbohydrate (Prayuwidayati, 2006) and improve the crude protein content of fermentation product (Prayuwidayati and Muhtarudin, 2006). However, the exact mechanism or process of the effects is still not yet explored. Moreover, early enzymatic exploration of several fungal that could be

cultured in sugarcane bagasse (Prayuwidayati *et. al.*, 2008) revealed that *Trichoderma viride*, *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus oryzae* has cellulase activity of 0.034, 0.007, 0.007, 0.004 units/ml respectively.

The main objective of this study was to identify and to isolate the fungi that capable of degrading the lignocellulosic materials in sugarcane bagasse and to determine the decomposition ability of enzymatic selected fungi.

MATERIALS AND METHODS

This research was conducted at the Department of Animal Science, Faculty of Agriculture and Department of Biology, Faculty of Natural Science University of Lampung in April - October 2009.

Substrate. Substrate material used in this experiment is sugarcane bagasse obtained from the local sugarcane industry PT. Gunung Madu Plantation, in Center of Lampung District. In this experiment, this material was used as its original condition from the factory without any physical and chemical treatment.

Fungi Exploration. Exploration of the fungi that capable of degrading sugarcane bagasse was conducted through culturing the fungi using moist chamber method and then followed by direct plating method. Moist sugarcane bagasse as a substrate in petri dish was placed in incubator for several days until all potential fungi were grown. All grown fungi were then isolated and cultured with PDA medium in separated petri dish and then placed in incubator until the colony of fungi produced enough spore for further evaluation.

Cellulase Activity Test. After isolation and identification, enzymatic activities of all grown fungi were then qualitatively analyzed using Congo red indicator method. In this method, cultured fungi on CMC medium (on top side) and PDA (on below side) was dropped by 1 - 3 drop of Congo red. After at least 24 hour placed in refrigerator, cultured fungi were then washed with NaCl physiological solution. Cellulase activity produced by fungi was observed as halo or clear zone on the medium. Cellulase activity could also be observed as change in the color of medium from red to dark-blue.

Table 1. Result of identification of isolate from sugarcane bagasse.

Codes	Colony colors	Hyphae	Conidophore	Metula	Phialide	Spore/Conidia	Name of Isolate
F1	black	septate	upright, with vesicle	-	-	Ovale, globose → ovoid to globose	*Aspergillus sp*
F2	green	septate	smooth-wall, nearly green, apex swelled	-	flask-shaped, each metula contains 6 to 10 phialide	spheric, chained, densed	*Penicillium citrinum*
F3	green	septate	dark-colored, simple, not branched	each conidiophore contains 2 to 6 metula)	short phialides, conidiophore contains 3 to 6 phialide	catenulate, spheric	*Memnoniella sp*
F4	green, densed	septate	smooth-wall, not swelled	each conidiophore contains 3 to 5 metula	each metula contains 2 to 4 phialide	globose to spheric	*Penicillium sp* (2)
F5	purple	septate	smooth, short, simple	-	-	thin, each two side with sharp point	*Helminthosporium*
F6	white at center , green at side	septate	not branched	-	-	spheric, dark-colored	*Penicillium sp* (3)
F7	green, in layers	septate	branched		each metula contains 3 to 6 phialide	spheric, catenulate	*Penicillium sp* (4)
F8	nearly brown - green	septate	smooth-wall, apex swelled		each metula contains 2 to 4 phialide		*Penicillium sp* (5)
F9	dark green	septate	not branched, dark-colored		short phialides, conidiophore contains 2 to 4 phialide, chained	ovoid to spheric	*Memnoniella sp*
F10	green, densed	septate	smooth-wall apex not-swelled	each conidiophore contains 3 to 5 metula	each metula contains 2 to 4 phialide	globose to spheric	*Penicillium sp* (6)

RESULTS AND DISCUSSION

Isolation and Identification of Fungi

Isolated and indentified fungi that capable of degrading the sugarcane bagasse in this experiment were: *Aspergillus* sp, *Penicillium citrinum*, *Penicillium* sp(2), *Penicillium* sp(3), *Penicillium* sp(4), *Penicillium* sp(5), *Penicillium* sp(6), *Memnoniella* sp(1), *Memnoniella* sp(2), dan *Helminthosporium* sp. Picture of microscopic form of these isolated and identified fungi were presented in following figures:

Figure 1. Fungi 1 (F1): *Aspergillus* sp

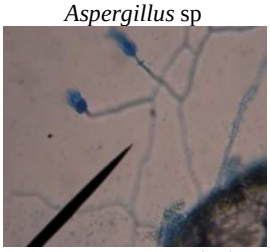


Figure 2. Fungi 2 (F2): *Memnoniella* sp(1)

Figure 3. Fungi 3 (F3): *Penicillium citrinum*

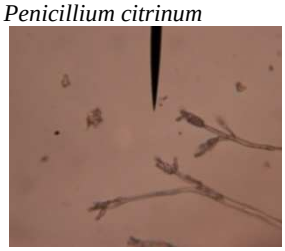


Figure 4. Fungi 4 (F4): *Penicillium* sp (2)

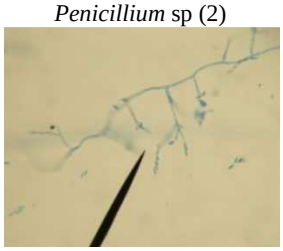


Figure 5. *Helminthosporium* sp.

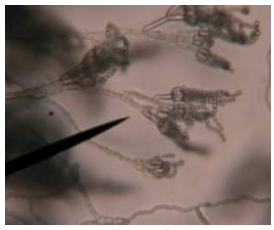


Figure 6. *Penicillium* sp (3)

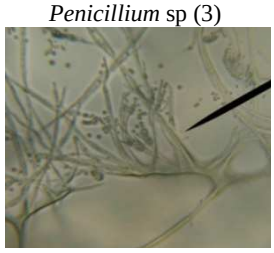


Figure 7. *Penicillium* sp (4)

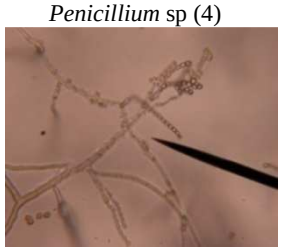


Figure 8. *Penicillium* sp (5)

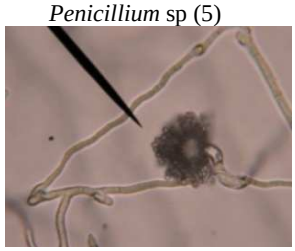


Figure 9. *Memmoniella* sp (2)

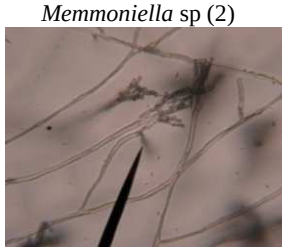
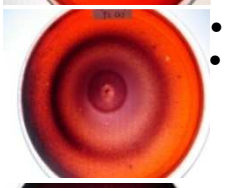
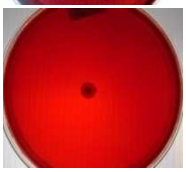
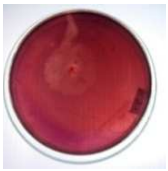


Figure 10. *Penicillium* sp (6).

Table 2. Activity of cellulolytic enzymes based on Congo red test

Fungi Code	Congo Red Test	Observation	+/-
F1		• Halo zone • Blue color	+
F2		• Halo zone • Blue color	+
F3		• Halo zone • Blue color	+
F4		• Halo zone • Blue color	+
F5		• Halo zone • Blue color	+
F6		• Halo zone • Blue color	+
F7		• Halo zone • Blue color	+
F8		• Halo zone • Blue color	+
F9		• Halo zone • Blue color	+
F10		• Halo zone not clear • Blue color	+

+ = fungi produce cellulolytic enzymes;- = fungi not produce cellulolytic enzymes

Activity of Cellulolytic Enzymes

All isolated fungi were tested qualitatively for measurement of cellulase activity. Based on the result of the test, all isolated fungi have the cellulase activity. Qualitative analysis was conducted at the age of culture of 3 - 4 days. It could be seen clearly on the medium that the color of the colony were not so much different among the tested isolated fungi. The color of colony of all tested isolated fungi are close to white, because at this stage all the isolated fungi just form the miselium, or no spora produced yet. Results of Congo red test to measure the cellulase activity of all isolated fungi were presented in Table 2.

Colony of isolated fungi had different response to Congo red indicator. Congo red indicator could associate with the glycoside bound in cellulosic matter in CMC medium. Cellulase produced by fungi could break the glycoside bound and this process would be an indicator as formation of the halo zone or clear area around its colony. The color of Congo red indicator will be red in base environment and blue in acid environment. Therefore, decomposition of CMC by cellulase that produced organic acids will lead to the formation of blue color in medium. In other words, the blue colors seen in the medium indicate that the isolated fungi could produce cellulase that enabling them to have decomposition ability.

CONCLUSIONS

It can be concluded that *Aspergillus* sp, *Penicillium citrinum*, *Penicillium* sp (2), *Penicillium* sp (3), *Penicillium* sp (4), *Penicillium* sp (5), *Penicillium* sp (6),*Memnoniella* sp (1), *Memnoniella* sp (2), dan *Helminthosporium* sp produce cellulase that enabling them to decompose the cellulosic materials.

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