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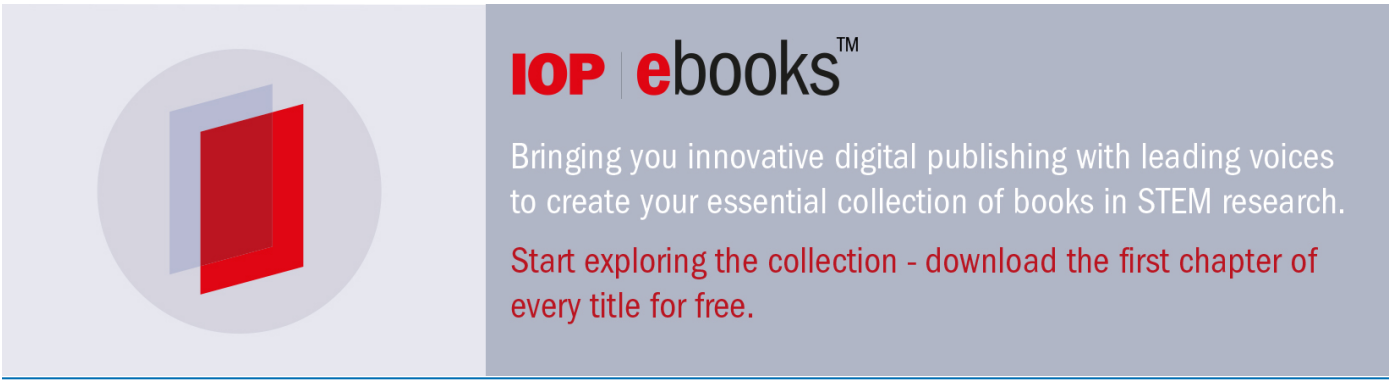

The Ability of Rot Fungi From Cocoa Plant In Producing Lignocellulosic Enzymes

I Rahim[1], Suherman[1], Hakzah[1], A Nasruddin[2]

[1] Faculty of Agriculture, Animal Husbandry, and Fisheries, Universitas Muhammadiyah Pare pare, South Sulawesi, Indonesia
[2] Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar; Indonesia

E-mail: iradhat76@gmail.com

Abstract. Rot fungi has the ability to degrade components of lignocellulosic which difficult to decompose. Rot fungi degrades components of cellulosic to get the nutrients and excrete the lignocellulosic enzyme. The study aims to examine the ability of rot fungi from decayed cocoa plant to grow on carbon compounds and produce the cellulosic enzyme. The capability of producing the enzyme performed by growing a piece of rot fungi isolates on Czapek Dox Agar media added Remazol dyes Briliant Blue 0.1%. The media was divided into 5 parts and each added one type of substrate lignocellulose: lignin, cellulose, pectin, amylose, and chitin as much as 0.1%. The formation of a clear zone around isolates indicative of enzyme activity. Scoring by measuring the magnitude of the clear zone formed in comparison to the area of petri dish. The results showed there were 9 kinds of rot fungi found in cocoa, namely *Mycena sp, Lycoperdon sp, Auricularia sp, Schizophyllum sp, Coprinus sp, Tremella sp, Crepidotus sp, Trametes sp, dan Pleurotus sp*. *Tremella sp* has the highest ability to produce enzyme ligninase, kitinase, and pectinase, than all other isolates. *Lycoperdon sp* produces the highest cellulose enzyme, while the highest amylase founded on *Pleurotus sp, Tremella sp, Schizophyllum sp,* and .*Crepidotus sp.*.

1. Introduction

Rot fungi many found in stems of decayed cocoa plants. The fungus degrades decayed cocoa organs into simpler compounds and then can be absorbed by plants to metabolic activities. There are three groups of fungi in nature that can decompose the components of wood (*lignocelluloses*), namely brown rot, white rot, and soft rot. The grouping of the rot fungi was based on the results of the degradation process. Brown rot fungi produce a brown residual result while white rot fungi produces white residual. The three types of rot fungi have different characteristics. White rot fungi have a high lignin degrading ability with less in cellulose loss [1,2].

Some clusters of fungi have the ability to produce lignocellulose enzymes. This enzyme were useful to decompose the compounds contained carbon source. The fungus *Panerochaete chrysosporium,* for example, has enzymes to decompose lignin and cellulose. It also has the ability to decompose toxic substances that are persistent, due to the presence of dehalogenase enzymes, lignin peroxidase, and manganese peroxidase[3]. Oyster mushroom (*Pleurotus spp*.) has long been known in Indonesia as a consumption fungi and for medicinal ingredients. *Pleurotus spp.* was also a major organic matter decomposer that can efficiently and selectively degrade lignocellulose without

chemical or biological treatment. *Pleurotus* spp. can utilize a wide range of lignocellulosic materials, such as rice straw, sawn remaining, cocoa pod husk, bagasse, coffee pulp, and cotton rods [4].

White rot fungi were grouped according to microscopic characteristics of white pockets, white spots, and white fibres. It is influenced by species of fungi, wood species, and ecological conditions. The decomposition process by white rot fungi, carbohydrate, and lignin fungi degrade at the same time and the same level during all stages of degradation. The decay of cell walls begins by producing micro hyphae holes in secondary walls, which flow together for larger wall openings by extending decay. The hyphae grow in the lumen close to near the tertiary wall. The hyphae was surrounded by a layer of mucus that secretes an active substance only in the immediate distance of the hyphae. Thus, the hyphae lysis zone develops below, and the hyphae produces a groove on the wall that gradually diminishes its thickness, as the river erodes the soil [5]

This research was to show the type of rot fungi that has a fruiting body were found in cocoa cultivation. In addition to looking at its ability to produce lignocellulosic enzymes.

2. Material and Methods

2.1. Isolation and Propagation of Fungi

Isolates of rot fungi were obtained from decayed stems of cocoa plants in cocoa plantation in Bila Village, Pitu Riase Subdistrict, Sidenreng Rappang District, South Sulawesi. The fruit bodies of the fungi were stored in paper bags. Pieces of fungal fruit bodies were surface sterilized with 70% alcohol, rinsed twice with sterile water and placed on to sterile filter paper. Each piece was grown aseptically on Potato Dextrose Agar (PDA) medium and incubated at room temperature. After sub culturing and purification, isolates were then identified and labelled with the species of fungi.

2.2. Test of Isolate Growth on Carbon Source Media

The test used a solid Czapek dox medium. Media added Remazol Brillant Blue 0.1% and divided into 4 parts. Each section added a substrate of carbon source, ie cellulose, lignin, chitin, and pectin. The media was homogenized on hot plate stirer for 15 minutes, then sterilized in autoclave for 2 hours. After the cold, the media was poured into a sterile petri dish in Laminar Air Flow. The fungal isolates were cut using a cork bohrer, grown on the media, and incubated in the dark for 7 days. Based on the methods of Wirth and Wolf 1990, two to three days after cultivation, a brightly colored zone was formed around the culture. Growing ability was obtained by measuring the diameter of the colony.

2.3. Measurement of clear zone were formed

The formed of clear zone on the media was measured in percentage. The clear zone area formed 0 - 25% of the petridish area was scored one plus (+), between 25% - 50% the score is two plus (++), greater than 50% - 75% of the score is three plus (+++), and when more than 75%, the score is four plus (++++). The assessment of the clear zone area was shown in Figure 1.

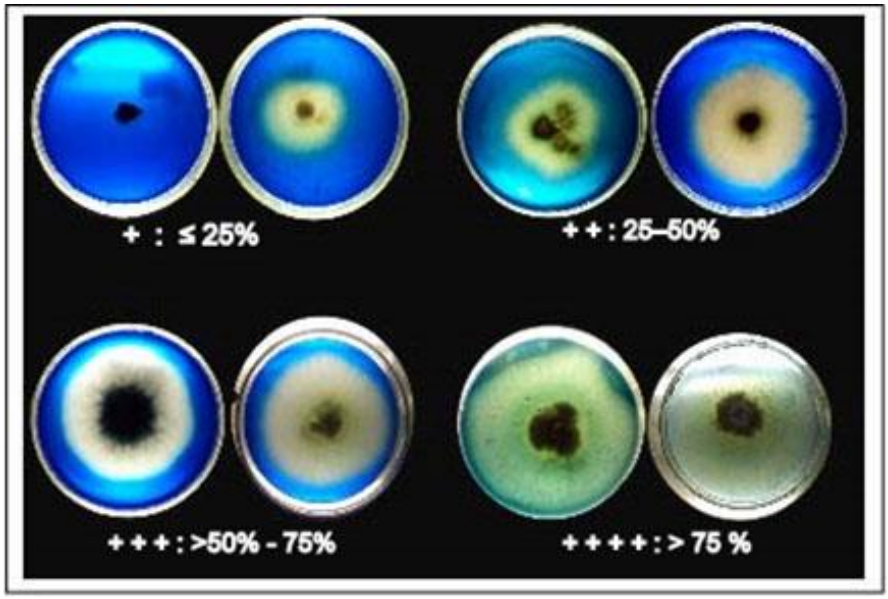

Figure 1. Skooring based on the clear zone formed on the media

3. Result and Discussion

3.1. Rot fungi found in cocoa cultivation

There were nine rot fungi that have a fruiting body found in cocoa cultivation. All of these fungi were included in Divisio Basidiomycota. The fungi belong to the class of Tremellomycetes, Agaricomycetes, Basidiomycetes, and Homobasidiomycetes (Table 1).

Table 1. Rot Fungi were founded in Cocoa Cultivation

No.	Rot Fungi	Class	Family
1.	*Tremella sp*	Tremellomycetes	Tremellaceae
2.	*Mycena sp*	Agaricomycetes	Mycenaceae
3.	*Lycoperdon sp*	Agaricomycetes	Agaricaceae
4.	*Auricularia sp*	Agaricomycetes	Auriculariaceae
5.	*Schizosphyllum sp*	Agaricomycetes	Schizophyllaceae
6.	*Coprinus sp*	Agaricomycetes	Agaricaceae
7.	*Trametes sp*	Agaricomycetes	Polyporaceae
8.	*Crepidopus sp*	Basidiomycetes	Crepidotaceae
9.	*Pleurotus sp*	Homobasidiomycetes	Tricholomateceae

3.2. Level of Lignosellulotic Enzymes in Rot Fungi Isolate

The level of lignocellulosic enzyme produced by the rot fungi were founded in the cocoa plantation was presented in Table 2.

Table 2. Lignosellulotic Enzyme Levels on Rot Fungi Isolate based on Clear Zone

Rot Fungi	Lignin	Chitin	Pectin	Cellulose	Amylose
Tremella sp	+ + + +	+ + + +	+ + + +	+ + +	+ + +
Mycena sp	+	+	+	+	+ +
Lycoperdon sp	+ + + +	+ + +	+	+ + + +	+ +
Auricularia sp	+	+ +	+ +	+	+ +
Schizosphyllum sp	+	+ +	+	+	+ + +
Coprinus sp	+ +	+	+	+ +	+ +
Trametes sp	+ +	+	+	+	+ +
Crepidotus sp	+	+ +	+ +	+	+ + +
Pleurotus sp	+	+ +	+	+ + +	+ + +

Description: Skooring based on clear zone. If the clear zone formed $\leq 25\ \% = +$; $25 - 50\% = + +$; $> 50\%\text{-}75\% = + + +$; and if $> 75\ \% = + + + +$).

Table 2 showed clearly that Tremella sp producing the highest lignin, chitin, and pectin enzymes compared to other fungi. The resulting enzyme level which shown by a clear zone that reaches more than 75% of the media area. The highest cellulose enzyme levels were produced by the Lycoperdon sp, while the highest amylose levels were produced by Tremella sp, Schizosphyllum sp, Crepidotus sp, and Pleurotus sp. The ability of Tremella sp to produce enzymes based on the clear zone formed on 4 types of lignocellulosic media shown in Figure 2.

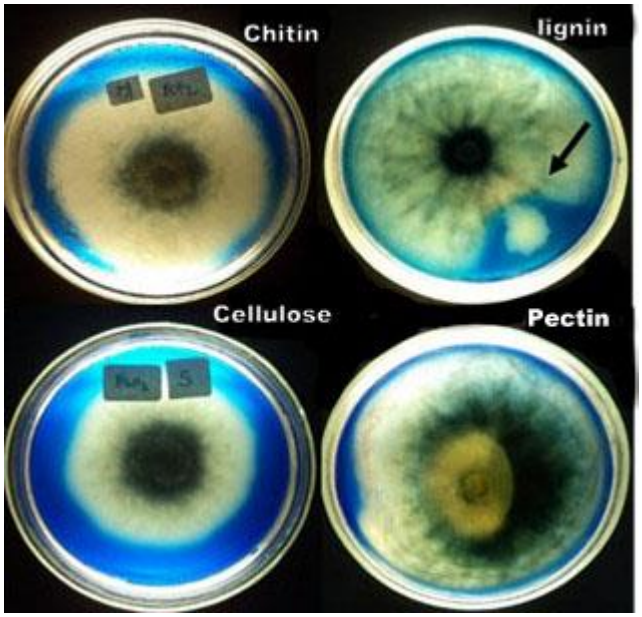

Figure 2. Isolates of *Tremella sp* produce clear zones (arrows) on various media. The clear zone was an indicator that isolates producing lignocellulosic enzymes.

All of the rot fungi isolated from the cocoa plantation was Basidiomycota Division. Basidiomycota has a large fruit body so easily observed. Fruit body consists of a hood (pileus), blades (lamella), and stalk (stipe). There are 9 rot fungi isolated from cacao plantations, namely *Mycena sp, Lycoperdon sp, Auricularia sp, Schizophyllum sp, Coprinus sp, Tremella sp, Crepidopus sp, Trametes sp*, and *Pleurotus sp*. All of these fungi have the ability to solubilize phosphates [7]. *Tremella sp* and *Pleurotus sp* can also produce Indole Acetic Acid (IAA) and Giberelic Acid (GA) hormones. *Tremella sp* produced 2.44 μgL-1 IAA and 4,117 μgL-1 GA, while *Pleurotus sp* also produced IAA and GA respectively 1,794 μgL-1 and 2,551 μgL-1 [8].

The other of the secondary metabolites produced by the rot fungi is a lignocellulosic enzyme. The presence of this enzyme was characterized by a clear zone formed around the fungal isolates grown on solid Czapek Dox Agar (CDA) medium, and each of medium were added to one of 4 substrate types, namely chitin, lignin, pectin and cellulose. Transfer of atoms by enzymes requires the acceptor which was compound not found in the cell naturally. One acceptor is methylene blue [9], which in this study used Remazol Brillian Blue (RBB). The ability of fungal isolates to produce lignocellulosic enzymes was measured by scoring based on the area of the clear zone formed. *Tremella sp* have the highest ability to produce ligninase, chitinase, and pectinase enzymes, compared to other isolates. The *Lycoperdon sp* produces the highest cellulase enzyme, while the highest amylase enzyme were found on *Tremella sp, Schizosphyllum sp, Crepidotus sp*, and *Pleurotus sp*. Although the fungi produce amylase enzymes were still less than 75% (Table 2).

The processes occurring in the bodies of organism both synthesis and degradation require substances that can speed up the reaction. The substance was an enzyme that the a protein group and serves as a catalyst. Catalyst is a compound that serves to speed up the reaction without taking reaction. Enzymes have 2 types, namely extracellular/exogenous enzymes that function outside the cell to made changes to surrounding nutrients that allow the nutrients to enter the cell. The function of intracellular/endoenzyme enzymes to synthesize cellular substances and synthesize nutrients for cell energy needs [9].

The lignocellulosic enzyme were an extracellular enzyme associated with the degradation of the lignocellulosic substrate. In this study, *Tremella sp* have the highest ability compared to other isolates. These fungi can formed clear zones of more than 75% for the three types of media added to the carbon source substrate, ie lignin, pectin, and chitin (Figure 2). Growing media environments may be optimum and suitable for *Tremella sp* strains, compared to other fungi. The other research reported that the qualitative measurements of lignocellulosic enzyme *Tremetes vesicolor* with Lignin Modifying Enzyme (LME) assay and CMC assay test show cellulolytic and ligninolytic activity at maximum level [10]

Research conducted by Hatakka [11] and Archibald [12] showed the production of enzymes in addition to environmental influences, also influenced by fungal strains. Optimal conditions of *P. chrysosporium* may not be appropriate for other white rot fungi strains, such as an oxygen-rich environment will not increase the lignin degradation enzyme in most white rot fungi strains, although the species are the same. It detected that the production of LiP and MnP on solid media without prior concentration or protein purification. The high level LiP and MnP in liquid medium would happen with the presence of air. Nitrogen and oxygen in various lignocellulosic activities and these conditions should be optimal for all organisms of the same species. Further [13], said that some whit rot fungi can produce MnP in the main phase of its growth.

4. Conclusion

Rot fungi derived from cocoa planting was in Divisio Basidiomycetes and on classes of Tremellomycetes, Agaricomycetes, Basidiomycetes, and Homobasidiomycetes. The rot fungi have the ability to produced lignocellulosic enzymes. *Tremella sp* produced the highest chitinase, ligninase, and pectinase enzymes compared to other fungi. While *Lycoperdon sp* produces the highest cellulase

enzyme. *Tremella sp* has the potential to be developed as an effective biodegradation of plantation waste.

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