Isolation And Characteristics Molding Fungus Of Stalks And Empty Fruit Bunches Of Oil Palms

Sukriming Sapareng, Ambo Ala, Tutik Kuswinanti, Burhanuddin Rasyid

Abstract: Fungus has the ability to degrade the stem and EFB because it produces an enzyme that can decompose cellulose, hemicellulose, and lignin. Fungal isolates were obtained from decayed palm oil and EFB. Isolates were observed macroscopically and microscopically. Macroscopic observations include growth rate, colony color, elevation and shape of the colony's edge. Microscopic observations include spores and conidiofor fungi. The result of the research was found that there were 32 isolate mushrooms consisting of 17 isolates from crushed palm oil and 15 isolates from EFB. Trichoderma sp3 isolates had the highest colony diameter, and the lowest isolates of Absidia sp grown on PDA media.

Keywords: isolation, isolates, characteristics, fungi

1. Introduction

The palm stem has a productive period in general for approximately 25 years, after which oil palm has to be rejuvenated. From rejuvenation will be generated a number of biomass. Returning biomass to the plantation area takes a long time. Biomass that remains in the plantation area after rejuvenation can be a source of nutrients for new plants. In order for nutrients to be available for the crops, the cutting of the palm tree that has been felled needs to be decomposed first. Similarly, with empty fruit bunches (EFB), EFB production is estimated at 30 million tons per year [1]. However, this waste has not been well utilized by most palm oil mills in Indonesia. In a relatively long time, the presence of this waste brings problems of pollution. Utilization of this waste is expected to reduce the problem and bring profits if managed to be valuable goods using fungus. The fungus has the ability to lower the stem and EFB because it produces enzymes that can decompose cellulose, hemicellulose, and lignin. The palm stem is a lignocellulosic material such as wood, the chemical content of the palm stem is cellulose 54.38%, lignin 23.95%, 2.02% ash, and other elements. With the approach that the coconut tree trunk is lignocellulose then the decomposition of the palm is not much different from the decomposition of wood [2].

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Based on this, the utilization of identified wood fungi allows for use in accelerating the process of degradation of stems and EFB of oil palm. The ability of fungi to degrade lignin is due to the presence of extracellular enzymes secreted by fungal hyphae [3], such as speeding up the composting process by Trichoderma [4]. Eaton and Hale (1993) [5] mentioned various enzymes that play a role in lignin degradation processes secreted by white fungus including lignin peroxidase (LiP), manganese peroxidase (MnP), lacase, demetoxylase, H_2O_2-generating enzyme, and monomer degradation enzymes such as selobiose dehydrogenase , vanilic acid hydrolase, and trihydroxy benzendioxigenase. However, the major ligninolytic enzymes produced by fungi are lignin peroxidase (LiP), manganese peroxidase (MnP), and lacase.

2. Materials and methods

2.1. Isolation of Endophytic Fungus.

Trunk and EFB oil palm washed with distilled water, cut 1-2 cm. The surface was sterilized with three submersions, first in ethanol 70% for 1 minute, the second in a 5.25% NaOCl solution for 5 minutes and last, in a 70% ethanol solution for 0.5 minutes. The composition of the medium is 15 g of agar, 15 g of dried powder from oil palm rod, 0.2 g chloramphenicol, and up to 1 L of distilled water. Incubation is done at room temperature (28 ^{o}C) for 1-3 weeks depending on the growth rate of the fungus. The mushroom colon was then transferred to a new medium with a composition of 15 g of agar, 15 g of Malt Extract, and up to 1 L of distilled water, and adjusted to a pH of 7.4 to 7.8.

2.2. Rejuvenation Isolate.

One isolate of the fungus from the culture of the collection was transferred into a new PDA (potatoes dextrose agar) medium. This rejuvenation is done in a sterile cabinet laminar air flow. The isolated isolates were then allowed to grow for 3-7 days at room temperature.

2.3. Mushroom Morphology Observation.

Morphological fungi are observed macroscopically and microscopically. The macroscopic observations refer to Shahid et al. (2013) [6] which includes the growth rate, colony color, elevation and shape of the colony's edge. Microscopic observation begins by dripping KOH solution over glass preparations and then the isolates are mixed in one ose [7]. Preparations are covered with a glass cover

and then observed the shape of spores and conidiofornya using a light microscope.

3. Results and Discussion

3.1. Morphology of Fungi

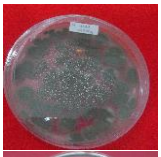
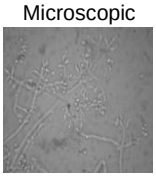
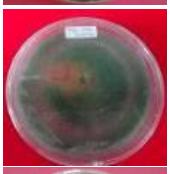
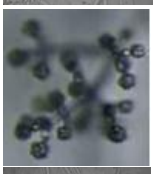
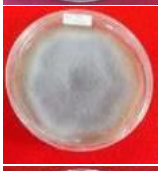
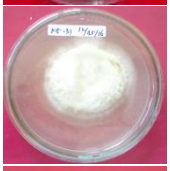
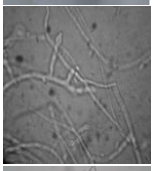
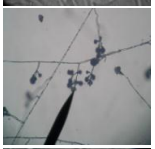
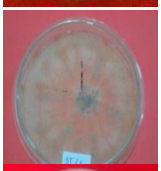
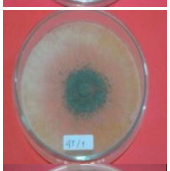
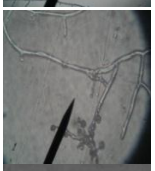
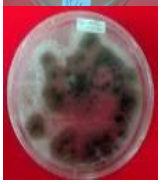
The result of isolation and purification of fungal isolates from two locations were 32 isolates of fungi consisting of 15 isolates isolated from oil palm rods that had been decomposed on smallholder plantation in Pattimang Village, Malangke District, North Luwu Regency, South Sulawesi Province, and the remaining 17 isolates isolation results from EFB at Luwu 1 Palm Plantation Plant, Lagego Village, Burau Subdistrict, East Luwu Regency South Sulawesi Province (Table 1).

Table 1. *Results of isolation and purification of fungal isolates from palm oil stems and EFB*

Isolates	Source	Location	Amount
BT	The stem of the Palm Oil is decaying	Oil Palm Plantation Pattimang Village	15
TK	Empty fruit bunch of Palm Oil	Palm Oil Factory in Lagego Village	17
Total			32

BT = The stem of the Palm Oil is decaying,
TK = Empty fruit bunch (EFB) of Palm Oil

The purified isolate (32 isolates) measured the growth velocity on PDA media and selected 15 best molding fungi isolates. Observations using a light microscope showed that the most isolates in EFB were Trichoderma genus isolates (isolate TK9, TK10, TK14), rapidly growing, had aerial mycelium, initially white, then turned greenish or greenish yellow (Trichoderma sp. 1; Trichoderma sp 3), and dark green and clot (Trichoderma sp. 2). The hypha structure, chained, branched konidiofor and there is a fialid in each branch. The branches of the fialid are densely packed. A set of conidia is attached to the fialid end, round or oval conidia and hyaline (Fig. 1). The number of spores in three-day-old isolates indicates relatively rapid growth rates of isolates. The number of spores between each isolate approximately the same shows a uniform growth rate.

Isolates	Fungi Colony	Microscopic	Genus	Isolates	Fungi Colony	Microscopic	Genus
BT1			Penicillium sp1	TK9			Trichoderma sp1
BT2			Pleurotus sp	TK10			Trichoderma sp2
BT3			Fusarium sp.	TK11			Phytophthora sp
BT4			Chrysosporium sp	TK12			Ulocladium sp
BT5			Penicillium sp.2	TK13			Chaetomium sp
BT6			Aspergillus sp1	TK14			Trichoderma sp3

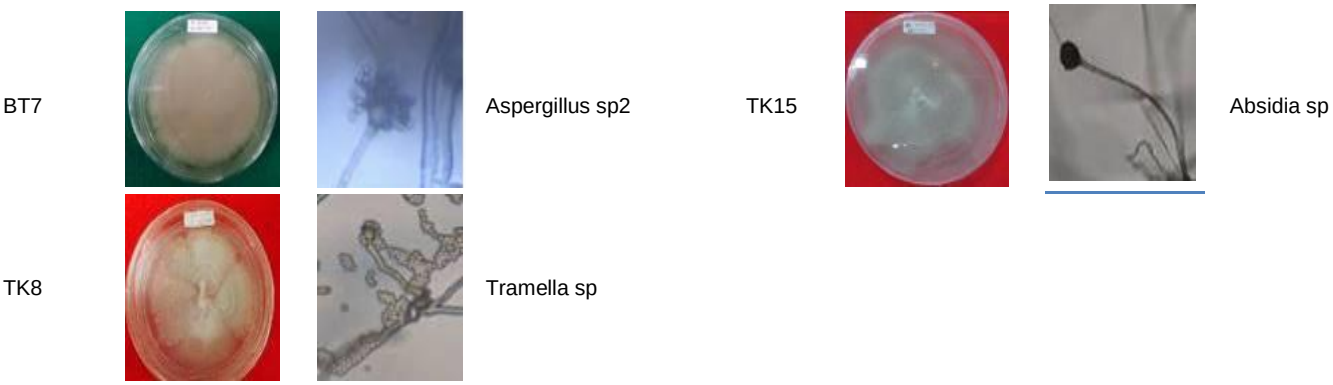


Fig 1. *Results of fungi isolation from trunk and EFB of oil palm*

All high growth isolates showed a fairly high, pyramid-shaped branching of conidiofor. This is in accordance with the first morphological results classified by Rifai (1969) [8] that the branching conidiofor Trichoderma sp. very high with a pyramid or conical pattern. The observed spores are green to gray in spheres and spread around the conidiofor. Athul and Jisha (2013) [9] also state that conidia Trichoderma sp. round to elliptical, unicellular, and produced from branched conidiofors such as pyramidal arrangements. Previous research results (Khang et al., 2013) [10] show Trichoderma sp. has a flat surface with soft structures such as wool, spreading green spores, and yellow-green pigments that diffuse into the growing medium. This is in accordance with a study previously conducted by Druzhinina et al. (2006) [11] that Trichoderma asperellum is the most common isolate found in tropical regions. Hoyos-Carvajal et al. (2009) [12] also states that the common species found in the neotropical region are Trichoderma asperellum followed by Trichoderma harzianum.

3.2. Fungi Characteristics

The growth of fungi colony isolate on the 7th day of the incubation period reached 7-8 cm in PDA media. The highest growth was achieved by 10 isolates, namely BT1, BT2, BT3, BT4, BT5, BT7, TK8, TK9, TK10 and TK4, while the lowest growth was found in 5 isolates, namely BT6, TK11, TK12, TK13 and TK15. Kecepatam growing hyphae is closely related to the characteristic of each isolate. In addition, it is also influenced by genetic, growing media content, growing environment, temperature and pH of the media (Table 2).

Table 2. *Characterization of morphology of isolates of fungi isolated from stems and EFB of oil palm*

Isolates	Colony color		Form	Texture
	On	Under		
BT1	Green	Green	Spherical small	Rather rough
BT2	Clean white	White	Spread	Smooth
BT3	White	White	Thick outer circle	Smooth
BT4	White	Brownish white	It expands like cotton in the middle	Rather rough
BT5	White	Whitish green	Thicker edges spread	Smooth
BT6	White black	Black	Spreads unevenly	Rather rough
BT7	White brown	Light brown	Spread, round compact	Rude
TK8	White gray	Gray	Spread	Rather rough
TK9	Whitish green	Green	Spreads unevenly	Rude
TK10	Green	Green	Spherical, uneven	Rude
TK11	White	White	Spherical, jagged edges	Smooth
TK12	Green	Green	Round	Smooth
TK13	Whitish green	Green	Bark, rounded edges	Smooth
TK14	White	White	Spread	Smooth cotton
TK15	White	Brownish white	Round	Smooth

The macroscopic morphology of 15 isolates generally has flat surfaces resembling wool with regular shaped edges. Almost all isolates are white, and only two isolates are green. An increasingly green color indicates an increasing number of spores. The diversity of the fungus is influenced by the growing medium. The content of organic matter greatly affects the microbial population because organic matter is used as the body's compiler and energy source for microbes. The quality and quantity of organic matter has a direct influence on the abundance of the fungus as most fungi are heterotrophic [13].

3.3. Growth Testing

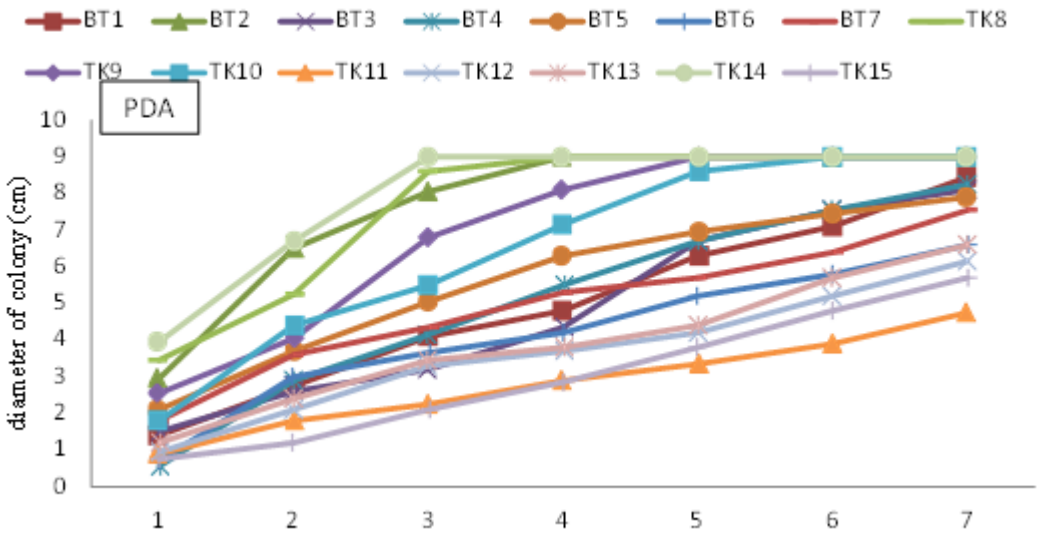
The purified filtrate fungus tested the ability and speed of growth in three types of solid media. Each solid medium contains different material compositions. PDA contains potato extract, peptone on MPA, and malt extract at MEA. Incubated at room temperature and performed until age 7 days (Table 3).

Table 3. *Mean growth of colonies (cm) of fungal isolates from stems and EFB of palm oil on various growing media*

Isolates	Media		
	PDA	MEA	MPA
BT1	8.45	8.60	8.00
BT2	9.00	9.00	9.00
BT3	8.10	6.70	5.05
BT4	8.25	7.35	5.65
BT5	7.90	7.80	4.15
BT6	6.60	9.00	8.70
BT7	7.55	9.00	8.40
TK8	9.00	9.00	9.00
TK9	9.00	9.00	9.00
TK10	9.00	9.00	9.00
TK11	4.75	4.10	3.25
TK12	6.15	3.60	2.65
TK13	6.60	3.15	1.95
TK14	9.00	9.00	9.00
TK15	5.70	5.00	4.20

Potato Dextrose Agar (PDA),
Malt Peptone Agar (MPA),
Malt Extract Agar (MEA).

All fungal isolates can grow on three types of solid media. In general, the isolates can fill the petri dish starting on the 4th day after it is grown. The average growth rate of fungus on the 7th day after incubation was 7-8 cm on PDA media, 6-7 cm on MEA medium, and 5-6 cm on MPA medium (Table 3). Although all isolates were grown on the same medium, ie agar medium containing potato extract. But according to Baon et al. (2012) [14] in addition to genetic influences, colonic variation may be due to environmental conditions in the sample area and growth media, including carbon sources, pH and temperature. Samples were taken in different areas ie oil palm and EFB cultivation around Palm Oil Factory. Colony color differences can be influenced by temperature in laboratory tests and the availability of nutrients in the medium.



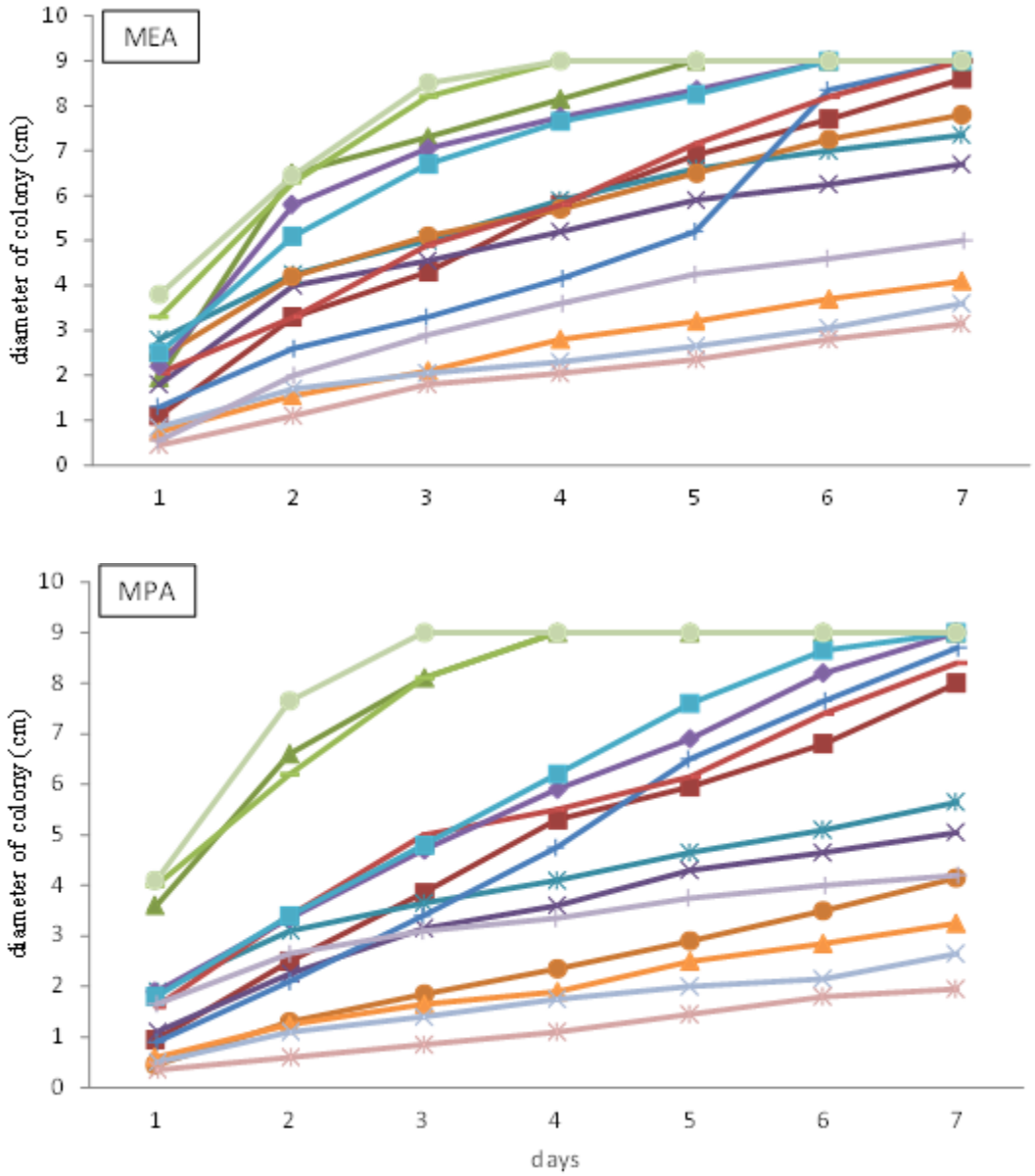


Fig 2. *Fungal isolation growth rates in three media types (PDA, MEA and MPA) for 7 days incubation*

Figure 2 also shows on PDA media isolates TK14 has the highest colony diameter, and the lowest in isolate TK15. There are 7 isolates that grow maximally on all media after 7 days incubation, ie isolate BT2, BT6, BT7, TK8, TK9, TK10 and TK14. PDA media have nutritional content of carbohydrates, water, and proteins derived from potato, glucose, and gelatin substrate. The MEA medium has nitrogen, carbohydrate, sodium chloride, and gelatin composition, while MPA media contain nutrients of nitrogen, carbohydrate, sodium chloride, peptone, and agar. The carbon content of the media has two functions, first for metabolism for heterotrophic organisms such as fungi. Carbon content in the form of element C is required for the synthesis process in cell survival. Such elements are carbon, nucleic acids, cell wall materials, and as food. The second function as the main energy source derived from the oxidation process of carbon elements [15].

4. Conclusions

The isolation result was 32 isolates consisting of 17 isolates from crushed oil palm and 15 isolates from EFB. Fungi from decayed palm oil rods are Penicillium sp, Pleurotus sp, Fusarium sp, Chrysosporium sp, Aspergillus sp, and from EFB ie Tremella sp, Trichoderma sp, Phytophthora sp, Ulocladium sp, Chaetomium sp and Absidia sp.

Acknowledgement

The authors are grateful to the Ministry of Research and Higher Education of Indonesia for the financial support through Research Doctoral Dissertation (RDD) research funding 2018.

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