Detection of ligninolytic capability of isolated fungi from decayed root and stem of oil palm tree

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Abstract. Lignin is difficult to be decomposed due to its extensive polymerization carbon chain. Previous studies have discovered that ligninolytic enzymes effectively degrade lignin waste. Numerous microorganism such as white rot fungi which belong to basidiomycetes have been taken into account to accelerate the decomposition process of lignin. The aim of this study is to detect the capability of the fungi which were isolated from decayed root and stem of oil palm tree. Qualitative test using guaiacol 0.02 % and quantitative test using agar with supplemented tannic acid test were carried out to observe the ligninolytic capability and potency index of fungal isolates. Out of ten isolates, AKS 5 and PLP 3 were found to be positive strains and grouped to basidiomycetes division. The potency index revealed was higher in AKS 5. Successful of this study could be used as baseline information of potential fungi which are able to degrade lignin waste in the environment.

1. Introduction

Indonesia is one of the world’s leading producers of palm oil. According to the Indonesian Palm Oil Association, total oil production reached 5.7 tons in early 2019 [1]. In line with the substantial number of productions, empty fruit bunches of palm oil (EFB) is abundantly discharged by the palm oil industry. Generally speaking, EFB consist of three major units i.e. cellulose, hemicellulose, and lignin [2]. The last one is a complex terrestrial aromatic compound containing extensive polymerization which forms an impenetrable physical seal as a barrier [3]. Therefore, it is highly insusceptible towards natural degradation. As the number of productions goes, the lignin over time will be tremendously accumulated in the environment. The abundance of lignin wastes produced by the EFB consequently becomes a critical challenge to be solved.

The scientific key to unlocking this problem is through the acceleration process of lignin’s decomposition. For years, it is already known that white rot fungi (WRF), belong to basidiomycetes, are considered as the most promising organism which able to mineralize lignin effectively [4,5]. WRF possess ligninolytic capability which utilizes the presence of the extracellular enzyme. The oxidative ligninolytic enzymes produced by WRF are laccase (Lac, E.C. 1.10.3.2) [6] and different peroxidases including manganese peroxidase (MnP, E.C. 1.11.1.13) and lignin peroxidase (LiP, E.C. 1.11.1.14) [7].

In recent years, screening works have been carried out in the finding and detection of potential fungi [8]. Primary screening test usually performed to check the ligninolytic capability by investigating the
change of visual appearance such as specific color reaction on agar medium. The specific color reaction of laccases acts on various types of substrates. The use of guaiacol as commonly used indicator pointer gives an advantage over the earliest study to detect the capability of enzyme production as it undergoes decolorization [9,10]. The appearance of the reddish dark zone on the solid plate is a virtuous proof of the ligninolytic activity produced by the fungi [11].

A qualitative determination also needs to carry to validate the screening test results. The use of agar with supplemented tannic acid (Bavendamm test) has been widely used to observe the brown zone around the fungal colony growth [12]. The quantification can be determined through the oxidation zone as Kameshwar and Qin stated that the appearance of the brown zone around the fungal colony represents the overall laccases oxidation of the fungi [13].

In this study, fungi from the decayed root and stem of the oil palm tree were investigated through a screening test. The aim of this study was to disclose qualitatively and quantitatively the alternative fungi which able to produce ligninolytic enzyme from the selected isolates to be used to accelerate the decomposition process of lignin waste.

2. Research methodology

2.1. Fungi collection
The fungi used in this study were collected from decayed root and stem of oil palm tree from domestic oil palm plantation in Bogor, West Java, Indonesia. There were 10 fungi collected which then labelled as ASK for root fungi and PLP for stem fungi.

2.2. Fungi isolation
The collected fungi taken from root and stem of the oil palm tree were sterilized using alcohol 70% and planted into the solid plate medium of Potato Dextrose Agar (PDA). The fungi then were isolated in the incubator for three days under the constant temperature of 29°C.

2.3. Primary screening test for ligninolytic activity
The Primary screening test was done using PDA with guaiacol 0.02% concentration to analyze qualitatively the ligninolytic capability through the assay for laccase activity [14]. The fungi were incubated and the reddish dark color was observed.

2.4. Enzyme potency index
The positive fungi obtained from the primary screening test were then tested again using agar with supplemented tannic acid medium to quantitatively obtain the potency index through the appearance of brown oxidation zone around the colonies [15]. However, the medium was autoclaved prior to tannic acid addition.

The Potency Index of the fungal can be determined using the following equation [16]:

\[
\text{Potency Index (PI)} = \frac{\text{Area of Color Zone (mm}^2\text{)}}{\text{Area of Colony (mm}^2\text{)}}
\]

3. Result and discussion

3.1. Screening of AKS and PLP decolorizing fungal strains on solid medium
Pure cultures of AKS and PLP were screened for guaiacol oxidation on solid plates. The result revealed that among 5 strains of AKS and 5 strains of PLP tested on 0.02% guaiacol medium, only 2 exhibited changes of colour reaction becoming reddish brown colour after 7-10 days of incubation (Figure 1). Those two strains were AKS 5 and PLP 3.
As shown in figure 1, the PLP 3 produced bigger color zone which occupied the entire plate with more concentrated colour than AKS 5. The reddish-brown colour resulted due to the oxidation of polymer enhanced with the guaiacol which were started to appear on the medium at the same time with the fungal mycelial growth on the solid plates as it is incubated [17].

3.2. Microscopic Examination of Strains
The morphological condition of positive strains AKS 5 and PLP 3 were examined. It was found that both strains belong to basidiomycetes due to the presence of clamp connector as shown in Figure 2 [18].

3.3. Color zone and potency index value
During the quantification studies using agar supplemented with tannic acid, all the two strains showed a positive reaction when incubated for 4 days with maintained 29°C temperature where the fungal isolates could successfully grow and produced brown color zone. The result shown that AKS 5 and PLP 3 have different growth and color zone (Figure 3).

![Figure 1. Screening result of (a) AKS and (b) PLP 3.](image)

![Figure 2. Clamp connector of (a) AKS 5 and (b) PLP 3](image)

![Figure 3. Color zone produced from three days of each strain (a) AKS 5 (b) PLP 3.](image)
The area of colony growth and color zone were measured 3 times performed on day 1, day 2, and day 4 incubation to calculate the potency index of each strain.

### Table 1. Area of fungal colony growth on agar supplemented with tannic acid.

| Fungal Strain | Area (mm²) | Day 1 | Day 2 | Day 4 |
|---------------|------------|-------|-------|-------|
| AKS 5         | 16.34      | 58.83 | 273.53|
| PLP 3         | 59.42      | 157.92| 432.28|

### Table 2. Area of color zone on agar supplemented with tannic acid.

| Fungal Strain | Area (mm²) | Day 1 | Day 2 | Day 4 |
|---------------|------------|-------|-------|-------|
| AKS 5         | 46.34      | 103.22| 332.04|
| PLP 3         | 27.32      | 189.00| 587.91|

Potency index indicates the activity of ligninolytic enzyme as shown in Figure 4.

![Figure 4. Potency index of AKS 5 and PLP 3.](image)

As shown in Figure 4, AKS 5 produced the highest potency index while PLP 3 produced the lowest potency index in which both are on the day 1 observation. According to Kaur et al., (2018) only PLP 3 in day 1 is grouped into the hypo-ligninolytic strains due to its low potency index (PI < 1.000) while the others are grouped into the hyper-ligninolytic strains due to its high potency index (PI > 1.000) [16]. Kaal et al. and Keyser et al. hypothesized that lower potency index fungi required longer adaptation to produce the enzyme to be oxidized [19,20]. Therefore, from day 1 to day 4 observation the potency index of PLP 3 increases. Higher potency index values signify higher ligninolytic enzyme activities and vice versa [21]. For the three days of observation, AKS 5 has better potency index than PLP 3 which means also more intense in producing ligninolytic activity.

In case of the growth, based on Table 1, PLP 3 is faster to grow than AKS 5 as it produces a bigger area of fungal colony growth on agar supplemented with tannic acid. The mycelia formation of PLP 3 is more rapid than AKS 5. The fungal colony growth of AKS 5 was also found to be difficult to observe than PLP 3 because the increment of the area was not significantly shown in the artificial medium used. Consequently, the day 3 observation cannot be done and the day 4 observation was preferably chosen to observe the increment which turned to be used for potency index determination. Generally, each fungi species has its own suitable substrate to support them to grow optimally [22]. As stated by Smith & Onions, in certain conditions some fungi can grow in poor nutrient medium but the other might need a rich nutrient medium [23].
4. Conclusion
From the study, two out of ten fungi (AKS 5 and PLP 3) isolated from decayed root and stem of oil palm tree are positively able to produce ligninolytic activity and both of the fungi belong to basidiomycetes. From the potency index, AKS 5 was found to be more intense and producing higher ligninolytic activity than PLP 3.

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