Screening of Ligninolytic activity of some Basidiomycota from domestic Oil Palm Plantation in Bogor

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Abstract. Lignin is an essential bio-polymer which is required in the formation of plant cell wall. The degradation of lignin in the nature is a major challenge due to the complexity of its structure. It has been long known that white rot fungi produce ligninolytic enzyme that can degrade lignin efficiently. To identify white rot fungi, screening test was carried out by testing different strains of Basidiomycota using czapek dox agar supplemented with Guaiacol (0.02%). The results showed that two out of thirteen strains of Basidiomycota have ligninolytic activity. In this study, we successfully obtained two strains that have ligninolytic activity. In terms of ligninolytic activity, the potency index of phenol oxidation was higher in SP1 which accounts for 4.30, meanwhile, SP13 only reached potency index of 0.94. This study sheds light on further observation of these strains which potentially can be used for lignocellulose waste treatment especially oil palm empty fruit bunch (OPEFB) to produce valuable product.

1. Introduction

Palm oil is the biggest commodity in Indonesia which is extravagantly produced with annual market 31 million tons. The main reason causing palm oil largely scattered in Indonesia is the high demand for domestic purposes, for instance, cooking oil and soap. As such, tremendous waste has been produced through palm oil production. Generally, palm oil production produces many varieties of biomass waste including oil palm empty fruit bunch (OPEFB), palm kernel meal (PKM), palm oil mills effluent (POME), palm kernel shell (PKS), palm kernel meal (PKM) mesocarp fiber (MF) [1]. OFEB is one of the most abundant lignocellulosic waste and potentially could be used for possible bioconversion towards bioethanol production [2]. However, due to the high concentration of lignin causing OFEB is difficult to be fermented to further produce ethanol.

Due to its complexity, Lignin is highly recalcitrant towards biological degradation [3]. Ligninolytic microbes have been known for its ability to degrade lignin in nature. White rot fungi (WRF) are a group of fungus that has an enzymatic system which enables the lignin degradation. It is known that biodegradation of lignin is done through multienzymes process with the present of ligninolytic enzymes are Lignin Peroxide (LiP), manganese peroxide (MnP) and Laccase.

Basidiomycota have been linked to the study of lignin degradation due to its ability to produce ligninolytic enzyme [4]. Most of the studies of lignin degradation have been carried out with a well-
known Basidiomycota such as *Pleurotus ostreatus* sp. [5]. As a comparison little is known about the ligninolytic activity of another Basidiomycota isolated from palm oil plantation.

Identification of WRF for its ligninolytic ability through screening test plays a significant role in biotechnology fields [6]. This study was aimed to obtain ligninolytic fungi from oil palm plantation and examine the ligninolytic activity of selected isolates. The study conveyed a proper selection of fungus for pretreatment of OPEFB to produce valuable product such as bioethanol.

2. Research Methodology

2.1. Collection of fungal strain

All fungal strains which were grown on wood and soil surface were collected from domestic oil palm plantation, Bogor, Indonesia. Samples were collected in a sterilized plastic bag for further isolation and investigation. The different sample code was given to each collected fungus. All the collected fungus was a member of the family basidiomycetes.

2.2. Screening of Ligninolytic Enzyme Activity

Screening of ligninolytic enzyme activity was qualitatively performed by using Bavendamm test [7]. The fungal isolates were grown on czapek dox agar supplemented with Guaiacol (0.02%) and incubated at 29°C. When a reddish-brown colour is formed on the medium, it indicates the activity of phenol oxidation, which shows that fungi belong to the group of ligninolytic fungi.

2.3. Measurement of Potency index (PI) value for Phenol oxidation

The selected fungal isolates were grown in Malt Extract Agar (MEA) with 0.5 % tannic acid. Positive ligninolytic activity was shown by the formation of a brownish zone resulting from oxidation of phenol. Tannin acid was used as a chromogenic indicator for quantification of ligninolytic potential. The area of the colony was estimated in terms of square millimetres. Potency index indicates the efficiency of the ligninolytic activity [8]. PI was calculated by using the following equation:

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

According to Kaur et al., PI is obtained to find out the hyper and hypo secretors of ligninolytic enzymes. Measurement of PI was performed every two days for four days of incubation.

3. Result and discussion

3.1. Screening of Ligninolytic Enzyme Activity with Bavendamm Test

The qualitative screening studies showed that two out of thirteen isolates were positive of ligninolytic enzyme activity by using the Bavendamm test (Figure 1). Positive results were indicated by the presence of reddish-brown zone in the media.
According to Badalyan et al., Bavendamm test was used to detect the production of extracellular polyphenol oxidase activities and growth on the lignocellulosic substrate [9]. In this study, we successfully obtained two WRF isolates. Sepwin et al., also found two out of five isolates isolated from OPEFB produced brown deposit in Bavendamm test [10].

Moreover, in this particular study we used czapek dox medium supplemented with guaiacol to test the activity of ligninolytic. The capability of the fungus to oxidize and decolourise guaiacol also demonstrated that the fungus can produce phenol oxidases and peroxidises and hydrogen peroxide–producing oxidases [8].

3.2. Growth Zone and Potency index (PI) value for Phenol oxidation

To find the potency index of the isolates, a quantification of qualitative measurement of a brownish zone on medium was conducted by culturing the positive fungal isolates (SP1 and SP13) on MEA 0.5% tannic acid. The fungal isolates grew exponentially in the medium. Interestingly, the results showed a difference in growth and the efficiency ligninolytic activity (figure 3) when the two isolates were grown on MEA with 0.5 % tannic acid. Frequently, Malt agar medium supplemented with tannic acid has been used to detect the presence of extracellular oxidases which are generally characteristic of ligninolytic activity [11].

Table 1. Areas (mm²) of fungal colonies on agar plates supplemented with tannin acid.

| Fungal Strain | Day 0  | Day 2  | Day 4    |
|---------------|--------|--------|----------|
| SP1           | 0      | 47.7   | 218.05   |
| SP13          | 0      | 1690.07| 4763.7   |

In terms of growth, SP13 showed better growth compared to SP1. SP13 colonised the entire plate at the fourth day of incubation (figure 2) which has an area of the colony of 4763.7 mm². Meanwhile at the fourth day of incubation SP1 only reach 47.7 mm² area of the colony (Table 1). Ang et al., reported that *P. chrysosporium* was able to colonize the entire plate from the second day onwards of incubation [8].
Potency index of the area of halo zone to area of colony indicates the activity of ligninolytic enzyme as shown in figure 3. The potency index of phenol oxidation was higher in SP1 (4.30). Meanwhile, phenol oxidation in SP13 was four-time lower than the SP1. Based on the data of PI, SP13 showed a hypo ligninolytic potentials hence indicate low ligninolytic activity. While, SP1 was found to have a hyper ligninolytic potential. The enzymatic activities of two fungal isolates remained constants on the second and the fourth day of incubation.

Kaur et al., reported that strain Ganoderma lucidum (GL)-1 and Ganoderma lucidum-2 strains showed a hyper production potential (PIs ≥ 1.000), however GL-3 and GL-4 strains showed low potency index values (PIs < 1.000) due to hypo ligninolytic potentials.

On the Bavendam test, SP1 showed an intense reddish-brown colour which eventually leads to higher potency index value. In terms of growth, SP1 showed a lower growth compared to SP13. It is might due to the difference in terms of enzyme production [10].

Kaur et al., stated that the higher potency index value, the more intense the colour zone and the lower the growth of the fungus. Vasquez et al., also mentioned that fungi that produced low growth are responsible for the initial process to degrade wood [12].
4. Conclusion

Two out of thirteen fungal isolates showed positive result of the ligninolytic activity. On the potency index of phenol oxidation, SP1 was found producing higher ligninolytic activity. This study provides baseline information of lignin degrading fungi which can be optimized well for lignocellulose pretreatment to produce bioethanol.

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