Ligninase Profiling and Optimization of Laccase Production from Indigenous Wood Rot Fungus (WRF) KLUM₂ in Kirk Medium-Alkali Lignin Kayu Jati (MK-ALKJ)

Siti Mutmainah¹, Evi Susanti¹²*

¹Department of Chemistry, Faculty of Mathematic and Natural Science, Universitas Negeri Malang,
²Department of Biotechnology, Faculty of Mathematic and Natural Science, Universitas Negeri Malang

*Corresponding author: evi.susanti.fmipa@um.ac.id

Received: June 2021; Revision: June 2021; Accepted: July 2021; Available online: July 2021

Abstrak

The production of ligninase by wood rot fungus (WRF) is determined by carbon source and growth condition. The goal of this study is to determine the ligninase profile produced by WRF KLUM₂ in Kirk Medium using teak wood alkaline lignin as a carbon source known as Kirk Medium-Alkali lignin Kayu Jati (MK-ALKJ), optimization of dominant ligninase production in the MK-ALKJ compared to the one that is produced in the Kirk’s medium with glucose as a carbon source (MK-Glucose). This research was conducted in an experimental laboratory consisting of: (1) spore suspension preparation, (2) ligninase profiling at various growth times, (3) ligninase profiling at various temperature variations, (4) optimization of laccase production including pH and the amount of nitrogen source. Growth was identified based on the specific activity of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. The results showed that relatively the three types of ligninase, namely LiP, MnP, and laccase, were produced in the same amount by the wood rotting fungus isolates KLUM₂ in MK-ALKJ. All three were produced with the highest yield of respectively 55.65; 52.48; 57.64 U/mg. Laccase as the dominant ligninase can be optimized to reach 83.52 U/mg by inoculating 2.10⁻⁴°C, pH = 3.5, and a nitrogen source. Growth was identified based on the specific activity of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. The results showed that relatively the three types of ligninase, namely LiP, MnP, and laccase, were produced in the same amount by the wood rotting fungus isolates KLUM₂ in MK-ALKJ. All three were produced with the highest yield of respectively 55.65; 52.48; 57.64 U/mg. Laccase as the dominant ligninase can be optimized to reach 83.52 U/mg by inoculating 2.10⁻⁴°C, pH = 3.5, and a nitrogen source of 20mM (NH₄)₂SO₄ for 6 days. Therefore, it can be concluded that the ligninase activity of indigenous WRF KLUM₂ in MK-ALKJ medium is higher than in the MK-Glucose.

Keywords: Kirk-Medium, KLUM₂ isolate, ligninase, wood rot fungus.

DOI: 10.15408/jkv.v7i1.20895

1. INTRODUCTION

Ligninase enzyme is a typical enzyme produced by the wood rot fungi (WRF) group (Deshmukh & Sao, 2015). Ligninase is divided into two groups, heme peroxidase and phenol oxidase. Heme peroxidase consists of lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), and versatile peroxidase (EC 1.11.1.16). Phenol oxidase only consists of laccase (EC1.10.3.2) (Hamid, 2013). The medium that is commonly used for the growth and production of ligninase is Kirk’s medium (Urek et al., 2007; Acevedo et al., 2011; Susanti et al., 2016). The components in Kirk’s medium are carbon sources; ammonium sulfate (source of nitrogen); thiamine-HCl (precursor of B vitamins necessary for fungus growth); veratryl alcohol (ligninase inducer); KH₂PO₄ (buffers); MgSO₄.7H₂O, CaCl₂, NaCl, FeSO₄, CuSO₄.5H₂O, CoCl₂, and ZnSO₄ (micro minerals), nitirloacetate; and ALK(SO₄)₂ (trace minerals) (Susanti et al., 2016).

According to Dekker et al., (2001), the ligninase enzyme is a secondary metabolite produced in the iodophase or stationary phase by the WRF in a medium with a limited carbon and nitrogen sources. Therefore, the use of complex carbon sources is expected to accelerate the iodophase conditions, hence ligninase activity can be produced faster and higher. Complex carbon sources such as lignin extract are known to produce ligninase with higher activity than glucose and glycerol carbon sources (Hu et al., 2016; 14 Kenkebashvili et al., 2012). Genoderma applanatum in Indulin AT carbon source media (lignin extract) produced higher
ligninase (LiP 7.512 U/mg; MnP 13.133 U/mg; laccase 0.977 U/mg), compared to acacia sawdust carbon source (LiP 5.420 U/mg; MnP 13.705 U/mg; laccase 0.906 U/mg). Ball Milled Cellulose (LiP 7.512 U/mg; MnP 1.751 U/mg; laccase 0.356 U/mg) and glucose (LiP 4.512 U/mg; MnP 0.275 U/mg; laccase 0.064 U/mg) (Artiningsih, 2009).

Ligninase production by wood-rotting fungi is also influenced by growth conditions such as temperature and pH. WRF isolates classified as mesophyll fungi can live in a temperature range of 15-40 °C (Pham et al., 2019). The optimum pH for the production of heme peroxidase is at pH of 4.5-5.5 (Acevedo et al., 2011), and for the production of phenol oxidase (laccase) is between pH of 3-7 (Hamid et al., 2013).

Indigenous WRF KLUM2 isolated from the weathered cocoa husk of Sepawon plantation, Kediri, East Java has the potential as a source of ligninase since it can reduce methylene blue in selective media (Delila, 2016). Arinta (2017) showed that WRF KLUM2 in Kirk’s medium with a glucose carbon source (MK-Glucose) produced a LiP ligninase profile (60.67 U/mg) much higher than MnP (46.56 U/mg) and laccase (23.38 U/mg). In this study, we are going to observe the ligninase profile of WRF KLUM2 grown in Kirk medium using teak wood alkaline lignin as a carbon source known as Kirk Medium-Alkali lignin Kayu Jati (MK-ALKJ). Observations were made at various growth times and temperatures. The production optimization was carried out towards the dominant ligninase type which included the optimization at various pH and the amount of (NH₄)₂SO₄.

2. MATERIALS AND METHODS

Materials and tools

The materials used in this study were the indigenous WRF KLUM2 of a collection group of Dr. Evi Susanti at the Biochemistry Research Laboratory, State University of Malang, which is continuously subcultured in PDA (Potato Dextrose Agar) medium. Materials with a grade of p.a. are: Potato Dextrose Agar (PDA), hydrogen peroxide (H₂O₂), MnSO₄, H₂O, CaCl₂, NaCl, FeSO₄, CoCl₂, ZnSO₄, AlK(SO₄)₂, H₂SO₄, HCl, guaiacol, tartaric acid, citric acid, veratryl alcohol, Folin-Ciocalteu reagent, sodium citrate, citric acid, nitrosoacetate, Tween-80, and ammonium sulfate. The technical grade materials are: distilled water, lysorin, detergent, teak powder, rubbing alcohol and 70% alcohol. Wood rot fungi local isolate KLUM2 is a collection of the Biochemistry Laboratory, Department of Chemistry, State University of Malang.

The equipment used in this study were test tubes, Erlenmeyer flask, watch glass, spatula, stirring rod, beaker, volume pipette, dropper pipette, measuring cup, glass funnel, filter paper, sieve, milipore, ose wire, enten wire, plastic wrap, spirit lap, lighter, tissue, inoculum bottle, tip, micropipette, microtube, hot plate and stirrer, centrifuge (80-1 Table Top Low speed), oven (Memmert U10 Oven), incubator (Memmert BM400), autoclave (Tommy SX vertical chamber), vortex (Dragon Lab MX-5), refrigerator, analytical balance (Precisa XT 120 A), and Visible spectrophotometer (Spectrophotometer B-ONE, Vis 50 DA).

Spore Suspension Preparation

All stages of spore suspension preparation were conducted aseptically. A pure isolate of indigenous WRF KLUM2 was inoculated on Potato Dextrose Agar (PDA) media and incubated for 14 days. The spores formed were resuspended using a 0.02% Tween-80 solution with the help of an ose needle, and vortexed for 10 minutes, and then left idle for 30 minutes. The mixture was filtered using sterile cotton in a sieve to obtain a spore suspension. The number of initial spores in each production was set at 2.10 x 10⁷ cells. Determination of the volume of the inoculated suspension with an OD value of OD₆₆₀ = 0.41 is equivalent to a cell density of 22.6 x 10⁵ cells/mL, the volume of spore suspension required for each experiment is calculated based on equation (2) which is a derivative from equation (1):

\[ \Sigma \text{cell} = \frac{OD_{\text{measured}}}{OD_{\text{standard}}} \times \text{stand. cell density (cell/mL). vol}(mL) \]  

\[ \text{Vol.(mL)} = \frac{2.10 \times 10^7 \times OD_{660 \text{ standard}}}{\text{cell density} \times OD_{660 \text{ measured}}} \]  

Kirk Medium-Alkali lignin Kayu Jati (MK-ALKJ) Preparation

Teak wood powder was added with 1% sulfuric acid and heated at 80 °C for 20 minutes, until the solution becomes cloudy brown. 4% sodium hydroxide solution then added and boiled for 30 minutes until the
solution turns from brown to dark brown and smells pungent. The mixture was autoclaved at 121 °C with the pressure of 15 psi. The results of the autoclave were filtered using filter paper and the residue obtained was neutralized until it reached pH 7 with hot distilled water. The alkaline lignin residue further baked at 70 °C to obtain dry product.

The alkaline lignin medium was made using a modified method of Susanti et al. (2016). The composition of the production media per L consists of: 10 g Alkali lignin teak wood; 1 X basal medium; 1 X trace element solution; 0.5 g NH₄(SO₄)₂; 0.3 g Tween-80; 640 ml acetate buffer pH 4.5 0.2 M; 0.5 g veratryl alcohol; and 0.001 g thiamine-HCl. Trace element solution 1 X per L contains: 3 g MgSO₄·7H₂O; 5 g NaCl; 0.1 g FeSO₄·7H₂O; 0.1 g CuSO₄·5H₂O; 10 mg AlK(SO₄)₂·12H₂O and 1 g nitriloacetate, and 1 X per L basal medium contains: 0.2 g KH₂PO₄; 0.05 g MgSO₄·7H₂O; and 0.01 g CaCl₂.

**Ligninase Profiling of WRF KLUM₁ in MK-ALKJ**

Ligninase profiling was conducted in 2.10 x 10⁷ spore cells of WRF KLUM₂ inoculated into 20 mL of MK-ALKJ with pH=4.5, incubated for 12 days. The fermented products on days 0, 2, 4, 6, 8, 10, and 12 were centrifuged at 1000 rpm for 15 minutes. The supernatant obtained was crude extract of ligninase, tested for protein content and activity of ligninase enzymes including LiP, MnP, and Laccase activities. The second observation was carried out based on the time of ligninase production at various temperature of 27, 30, 37, and 45 °C. Each treatment was conducted in triplicate.

**Assay of Protein Concentration**

A total of 0.5 mL protein standard solution containing 0 (blank), 25, 50, 75, 100 g/mL of bovine serum albumin (BSA) protein and the sample was put in a dry and clean test tube with the addition of 2.5 mL of Biuret solution, briefly vortexed. It was left idle at room temperature for 10 minutes and 0.25 mL of Folin-Ciocalteu 1 N was added then vortexed and left incubated for 20 minutes. The absorbance was measured at a wavelength of 750 nm. The obtained absorbance data was used to create protein standard curve. Determination of sample protein concentration was determined by interpolating sample absorbance value into the protein standard curve.

**LiP Activity Test**

LiP activity test conducted by taking 0.8 mL of a 10 mM veratryl alcohol solution placed in a test tube with the addition of 1 mL of 0.2M tartaric acid solution, 1.5 mL of crude extract of the enzyme, and 0.5 mL of 50 mM H₂O₂ solution. The absorbance was measured at a wavelength of 310 nm, at minutes 0 and 1 after the addition of H₂O₂. Based on the absorbance value obtained, the activity of the LiP enzyme was determined using the equation (3), the value of the excitation coefficient molarity (εₘₐₓ) is 90300 M⁻¹cm⁻¹.

\[
\text{Enzyme Activity} \left( \frac{\text{U}}{\text{ml}} \right) = \frac{(\text{A}_t - \text{A}_0) \times V_{\text{total}} \times 10^6}{\varepsilon_{\text{max}} \times d \times V_{\text{enzyme}} \times t} \quad \text{(3)}
\]

- A₀ : Absorbance at minute 0
- A₀ : Absorbance at minute 0
- εₘₐₓ : substrate molar absorptivity
- d : thickness of cuvette (cm)
- t : time (minutes)
- V_{total} : total volume ingredients (ml)
- V_{enzyme} : enzyme filtrate volume (ml)

**MnP Activity Test**

A total of 0.5 mL acetate buffer solution pH 5.5 was added with 0.8 mL of 1 mM guaiacol solution and 1 mL of 20 mM citric acid solution. 0.5 mL of 50 mM H₂O₂ 1.5 mL of 0.1 mM MnSO₄ solution and 0.2 mL of crude extract of the enzyme then added. The mixture left idle for 15 minutes. The MnP activity test was determined based on the amount of guaiacol that was oxidized, which was then measured at a maximum wavelength of 465 nm at minute 0 and minute 1. The absorbance value obtained is to determine the activity of the MnP enzyme using equation (3), with a molar excitation coefficient value (εₘₐₓ) of 12100 M⁻¹cm⁻¹.

**Laccase Activity Test**

Laccase activity was measured based on its ability to oxidize guaiacol. Oxidized guaiacol was measured spectrophotometrically at a wavelength of 470 nm with a molar excitation coefficient of 6740 M⁻¹ cm⁻¹ (Praveen et al., 2010). The step of the laccase
activity test was 0.8 mL of 10 mM guaiacol solution which was added with 0.8 mL of pH 5 0.2 M acetate buffer and 0.2 mL of extra crude enzyme, then measured at a wavelength of 470 nm at minute 0 and 1. The absorbance value obtained is to determine the activity of the Laccase enzyme using equation (3). With a molar excitation coefficient ($\varepsilon_{\text{max}}$) of 6740 M$^{-1}$cm$^{-1}$.

**Optimization of the Production of the Dominant Ligninase Enzyme KLUM2 Isolate**

Optimization of the production of the dominant ligninase enzyme KLUM2 isolate includes pH and the amount of nitrogen source. The variation of pH used refers to the work by Susanti et al., (2016), which are 3; 3.5; 4/5; 5; and 5.5 using 0.2 M acetate buffer. The nitrogen source used was (NH$_4$)$_2$SO$_4$ with 5; 10; 20; and 40 mM variations. Each independent variable is observed by referring to the previous subchapter, by adjusting the observed independent variables. The dependent variable is the protein content and the activity of the dominant ligninase enzyme.

### 3. RESULTS AND DISCUSSIONS

#### Inoculum Preparation and Spore Suspension of WRF KLUM$_2$

The WRF KLUM$_2$ is a collection of the research group of Dr. Evi Susanti at the Biochemistry Research Laboratory, State University of Malang, which is continuously subcultured in potato dextrose agar (PDA) medium. At the spore preparation stage, the cultures were incubated at 37 °C for 14 days and the inoculum was obtained in the form of a brownish pore suspension (Figure 1a). This result is similar with the results of Delila’s work (2017). The spore suspension on first until third repetitions was cloudy brown, while on the fourth repetition, it was lighter (Figure 1b). This is comparable to the OD value shown in Table 1, which indicated that the lighter the brown color, the lower the OD value.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (a) Culture of WRF KLUM$_2$ in PDA slant which was incubated at 37 °C on the 14th day and (b) WFH KLUM$_2$ Spore Suspension Experiment 1 (A), Experiment 2 (B), Experiment 3 (C), Experiment 4 (D).

#### Growth of WRF KLUM$_2$

The color and turbidity of MK-ALKJ which had been inoculated with WRF KLUM$_2$ spore suspension for 12 days had changed. On day 0, MK-ALKJ was clear. Entering day 2 and 3, it became slightly cloudy and became cloudier with increasing growth time. This is in line with Arinta (2017) that stated the growth of KLUM$_2$ wood rot fungi isolate spores in PDB (Potato Dextrose Broth) medium becoming reddish brown after 6 days. The opinion was confirmed by the results of measurements of protein levels carried out on days 0, 2, 4, 6, and 8. Protein levels on day 0 were 67.22 g/mL, increased to 77.08 g/mL on day 2. A gradual increase occurred until day 8 to 81.11 g/mL (Figure 2).

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Growth curve of WRF KLUM$_2$ in MK-ALKJ at 37 °C, pH 4.5 and (NH$_4$)$_2$SO$_4$ 20 mM

The increase in protein levels showed that the spore suspension of the KLUM$_2$ wood rot fungi isolate experienced growth as mentioned from Susanti et al., (2016) which stated that an increase in protein content indicated an increase in cell components,

#### Table 1. OD$_{660}$ WFH KLUM$_2$ Spore Suspension

| Experiment | OD$_{660}$ |
|------------|------------|
| 1          | 1.252      |
| 2          | 1.242      |
| 3          | 1.305      |
| 4          | 1.167      |
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which was directly proportional to the increase in the mass of a microorganism and was a feature of microbial growth. Susanti et al., (2016) also showed that the increase in extracellular protein levels of P. chrysosporium ITB isolate was proportional to the increase in dry weight of the mycelium.

The growth of WRF KLUM2 in MK-ALKJ from day 2 to 8 tends to be gentle. Contrasting growth in the Kirk’s medium with a glucose carbon source (Arinta, 2017), which shows a sharp increase (Figure 3.). This condition is similar with Susanti et al., (2016). The growth of P. chrysosporium ITB isolate using sawdust carbon sources is gentler than in glucose carbon sources medium. This is because glucose can immediately become energy source for the formation of primary metabolite components such as cell biomass constituents so that the increase in protein levels is higher and faster, whilst the carbon source in MK-ALKJ is harder to digest, forcing the wood rot fungi to produce ligninase to degrade the carbon source as an energy source for its growth. As a result, the increase in protein during growth is relatively sloping (Artiningsih, 2006; and Susanti et al., 2016).

![Figure 3. Comparison of extracellular protein concentration of WRF KLUM2 increment cultured in different carbon sources.](image)

**Ligninase Profile of WRF KLUM2 in MK-ALKJ at Various Growth Times**

The results of the study in Figure 4 show that on day 0 the specific activity value was measured. Whereas on day 0 there should have been no activity at all, this is presumably due to the presence of interfering compounds in the growth medium components, which also absorb during the measurement of enzyme activity. This opinion is supported by a decrease in the value of specific activities on the 2nd day. The decrease occurs because the components of the medium are changed and used to produce energy and cell growth. Furthermore, there was an increase from the eighth to the twelfth day. These results were similar with the ligninase profile Kirk’s medium with glucose carbon source which showed ligninase activity by the KLUM2. Isolate began to increase and showed the highest activity on day 6 (Arinta, 2017). The lower ligninase until the sixth day was assumed because the cells are adapting to the medium, then after the nutrients in the medium are reduced, the production of ligninase will also decrease (Subagyo et al., 2014).

The ligninase profile on the sixth day shows that the three enzymes of LiP, MnP, and Laccase that were produced were relatively equal. This result is different from Arinta (2017) which showed that LiP was more dominant than MnP and Laccase in MK-glucose. This proves that the type of carbon source affects the ligninase profile of the WRF KLUM2. Cells do not need to immediately produce ligninase in MK-glucose. On the other hand, although the growth of the WRF in MK-ALKJ is slow, lignin alkaline as the only carbon sources in the medium will trigger ligninase production to convert alkaline lignin into a carbon source that can be converted into energy for growth.

![Figure 4. Specific activity of ligninase produced by WRF KLUM2 at pH 4.5, 37 °C, (NH₄)₂SO₄ 20 mM at various growth times.](image)

**Ligninase Enzyme Profile of WRF KLUM2 at Various Growth Temperatures**

The production of ligninase at various temperatures was conducted at the optimum time of ligninase production which is shown in Figure 5 for the sixth day. In general, the temperature gave the same effect on the production of LiP, MnP and Laccase from the KLUM2 isolate, which increased from 27 to the optimum temperature at 37 °C, then decreased at 45 °C and showed consistency.
that laccase was the dominant ligninase produced in MK-ALKJ. This fits with Susanti’s (2016) statement that stated mesophyll fungus generally grow at temperatures between 25-40 °C which have enzymes in the form of thermolabile proteins that have been denatured at temperatures above 40 °C.

Other studies such as Manimozhi et al. (2012) showed that the pH value of 5.5 is the optimum value for Laccase production from *Agaricus heterocystis*. Kumar, (2016) reported that the optimum pH for Laccase production from *Aspergillus Flavus* was at pH 7. This proves that the optimum pH for Laccase production varied depending on the fungus strain.

**Optimization of (NH₄)₂SO₄ levels for Dominant Ligninase Production by the indigenous Wood Rot Fungi KLUM₂ Isolate in Kirk-Alkali Lignin Medium**

The optimization of (NH₄)₂SO₄ levels was conducted after knowing the optimum pH level for the production of dominant ligninase (laccase) KLUM₂ isolate. The results showed that the levels of (NH₄)₂SO₄ affected the production of laccase. (NH₄)₂SO₄ has a role as a nitrogen source for the growth of WRF KLUM₂ and induces ligninase production. Figure 7 shows low laccase activity at 5 mM (NH₄)₂SO₄ levels, because at that amount the nitrogen source is still low. Thus, the formation of ligninase is also low. Laccase activity began to increase at 10 mM levels, then experienced a maximum increase at 20 mM levels of 83.52 U/mg, and decreased drastically at 40 mM levels. The production of ligninase requires an adequate nitrogen source to induce and supply ligninase precursors. The excess number of nitrogen sources resulted in insufficient or even excessive energy supply, thus triggering the KLUM₂ isolate to be slow and not induced to produce ligninase enzymes. Mantovani *et al.*, (2007) argue that a lack or excess of nitrogen content can inhibit the growth of a fungi. However, Mikiashvili *et al* (2006) stated that in secondary metabolism such as laccase production, nutritional requirements differ depending on the culture and strain conditions of the fungus, laccase activity can increase in medium with limited nitrogen or vice versa. Hence, it can be concluded that the optimum condition of laccase production is 83.52 U/mg by wood rot fungi isolate KLUM₂ in MK-ALKJ is at pH 3.5 and nitrogen content of 20 mM, at 37 °C for 6 days.

Laccase produced by the KLUM₂ isolate in MK-ALJK on the sixth day with an incubation temperature of 37 °C, pH 4.5, and levels of (NH₄)₂SO₄ at 20 mM, showed a higher specific activity value (83.52 U/mg),
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compared by Arinta’s work (2017) on the production of laccase by the same fungi in MK-glucose which is 23.28 U/mg. These results prove that the carbon source affects the amount of ligninase produced. Glucose carbon source is a simple carbon source that can directly enter cells and is used for cell growth so that cells do not need to immediately produce ligninase. On the other hand, alkaline lignin is a complex carbon source similar to the natural substrate of wood rot fungi. This causes slow growth but triggers the production of ligninase to convert alkaline lignin into a carbon source that can be converted into energy for growth.

Gambar 7. Specific Activity of Laccase Enzyme Produced by WRF KLUM2 in MK-ALJK on Day 6, with temperature at 37 °C, pH 3.5 at various concentration of (NH4)2SO4.

4. CONCLUSIONS
Relatively, the three types of ligninase, which are LiP, MnP, and laccase, were produced in the same amount by the wood rot fungi isolates KLUM2 in MK-ALKJ. All three enzymes were produced with the highest yield of 55.65, 52.48 and 57.64 U/mg, respectively. Laccase as the dominant ligninase can be optimized to reach 83.52 U/mg by means of 2.107 spore cells inoculated in MK-ALKJ at 37 °C, pH 3.5, and a nitrogen source of 20mM (NH4)2SO4 for 6 days. Therefore, it can be concluded that the ligninase activity of indigenous WRF KLUM2 in MK-ALJK medium is higher than in the MK-Glucose.

ACKNOWLEDGMENTS
The writer thanked KEMENRISTEK DIKTI for funding the study through a PDUPAT 2019 scheme, and LP2M UM that financed this publication through Underthesis publications grant.

REFERENCES
Acevedo F, Pizzul L, Castillo MP, Rubilar O, Lienqueo E, Tortella G, Diez MC. 2011. A Practical culture technique for enchaned production of manganese peroxidase by Antrophillum discolor Sp-f. Brazilian Archives od Biology and technology. 54(6):1175-1186.

Arinta A. 2018. Penentuan Profil Ligninase dari Kapang Pelapuk Kayu Isolat Indigenus KLUM1, KLUM2, dan Phut/M. Skripsi. Malang (ID): Universitas Negeri Malang.

Artiningsih T. 2006. Aktivitas ligninolitik jenis genoderma pada berbagai sumber karbon. Biodiversitas. 7(4):307-311

Dekker, Robert FH, Vasconzales AFD, Barbosa AM, Giese EC, Mairesles LP. 2001. A new role veratile alkolohol: regulation of synthesis of lignosellulose-degrading enzyme in the lignilitic Ascomiteeous fungus, Botryosphaeria sp.: influence of carbon Source. Biotechnology letters. 23: 1987-1993

Delila L, Susanti E, Sanjaya EH. 2016. Isolation and screening of indigenous fungus producing lignin peroxidase from the cocoa plantation in sepawon kediri regency Indonesia. KnE Life Sciences, International Conference on Natural Resources and Life Sciences (NRLS-2016). doi 10.18502/kls. v3i5.985

Deshmukh Y, Sao S. 2015. Degradation of Lignin through Carbon Utilization. Microbial Ligninolytic Enzymes for Environmental Management. 5: 27–31

Hamid, A. 2013. Insights into lignin degradation and its potential industrial applications. Advances in Applied Microbiology. Volume 82 ISSN. 0065-2164

Hu J, Yuan B, Zhang Y, Guo M. 2015. Immobilization of laccase on magnetic silica nanoparticles and its application in the oxidation of guaiacol a phenolic lignin model compound. RSC advances. 5(120): 99439-99447

Kenkebashvili N, Elisashvili V, Wasser P. 2012. Effect of carbon, nitrogen sources, and copper concentration on the lignolytic enzyme production by coriolopsis gallica. Journal of Waste Conversion, Bioproduct and Biotechnology. 1(2): 22-27

Kumar R, Kaur J, Kumar A. 2016. Optimization of laccase production from Aspergillus flavus by
design of experiment technique: Partial purification and characterization. *Journal of Genetic Engineering and Biotechnology*. doi.org/10.1016/jgeb

Manimozhi M, Kaviarasan V. 2012. Effect of nutritional parameters on biomass and laccase production in submerged medium by litter decomposing basidiomycetes agaricus heterocytis. *International Journal of Pharmacy and Pharmaceutical*. 4(3): 592-599

Mikiashvili N, Wasser SP, Nevo E, Eliazhivili V. 2006. Effects of carbon and nitrogen sources on pleurotus ostreatus lignolitic enzyme activity. *World Journal of Microbiology and Biotechnology*. 22(9): 999-1002

Neto MSL, Matheus R, Machado G. 2009. Influence of pH on the growth, laccase activity and RBBR decolorization by Tropical Basidiomycetes. *Brazilian Archives of Biology and Technology*. 1516-893

Nurhayati T. 1988. Analisis kimia 75 jenis kayu dari beberapa lokasi di Indonesia. *Jurnal Penelitian Hasil Hutan*. 5(10): 6-11

Pham MT, Huang, Kirschner R. 2019. The plant growth-promoting potential of the mesophilic wood-rot mushroom Pleurotus pulmonarius. *Journal of Applied Microbiology*. 127: 1157-1171

Subagyo T, Susanti E, Suharti. 2014. Uji potensi dan optimasi konsentrasi lignoselulosa beberapa limbah pertanian sebagai sumber karbon produksi enzim lignin peroksidase oleh *Phanerochaete chrysosporium*. *Prosiding Seminar Nasional Kimia dan Pembelajarannya (SNKP) 2014*: 275-281

Suryana Y. 2001. Budidaya Jati. Bogor (ID): Swadaya

Susanti E, Ardyati T, Suharjono, Aulani’am. 2016. Optimizing of lignin peroxidase production by the suspected novel strain of *Phanerochaete chrysosporium* ITB Isolate. *International Journal of ChemTech Research*. 9(11): 24-33.

Urek RO, Pazarlioglu NK. 2007. Enchanced production of manganese peroxidase by *Phanerochaete chrysosporium*. *Brazilian Archives of Biology and Technology*. 50(6): 913-922.