The Interaction between *Marasmius pulcheripes* J8 and Soil Fungi on Laccase Activity for POME Degradation

Yohanes Bernard Subowo, Arwan Sugiharto

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Department of Microbiology, Research Centre of Biology, Lembaga Ilmu Pengetahuan Indonesia

**Abstract**

A study on the effect of *Marasmius pulcheripes* J8 and soil fungi interaction on the activity and ability of laccase to degrade palm oil mill effluent (POME) was previously conducted, and some Basidiomycetes fungi were identified capable. Therefore, the aim of this study was to determine the ability of *Marasmius pulcheripes* J8 to degrade POME in the presence of inducers, and interactions with soil fungi. Furthermore, 3 types of inducers were applied to elevate its laccase activity, which include CuSO4, sucrose and Ammonium tartrate. In addition, *M. pulcheripes* J8 was grown together with soil fungi, encompassing *Aspergillus niger* NK and *Penicillium* sp R 75, in order to boost the action. The results showed the highest laccase activity was in *M. pulcheripes* J8 pure culture on a PDB medium of 3566.04 U / mL. Moreover, the POME decolorization was up to 74.25% after 20 days of incubation, and reduced COD level was 81%. Meanwhile, the addition of an inducer has never been performed before, and the outcome of this investigation showed the ability of *M. pulcheripes* J8 to degrade POME, and decrease environmental pollution. POME waste treatment using fungi is more affordable than other methods.

**History Article**

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**Keywords**

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Correspondence Author:  
Jl. Raya Bogor Km 46 Cibinong 16911 Bogor  
E-mail: yosubowo@yahoo.com
INTRODUCTION

Some industries that use plants as raw materials, including for textile and paper, require the use of laccase to degrade their lignin content, in order to obtain a better yield. This enzyme is able to break down wood, plastic, paint and jet fuel into nutrients (Majeau et al., 2010). Similarly, in the palm oil industry, it is also needed in the decomposition of lignin contained in Palm Oil Mill Effluent (POME), which consists of colloidal slurry of water (95 – 96%), oil (0.6 – 0.7%), total solid (4 – 5%), suspended solid (2 – 5%), temperature (80-90 °C), acidic (pH 3.84.5) (Onyia et al., 2001).

Lignin contained in POME is the byproduct of palm oil. Lignin content in palm fruit was found 25 – 28 % in mesocarp (Rizal et al., 2018) and 44 % in endocarp (Zainal et al., 2017). In addition, it spreads on the entire plant tissues in order to strengthen and maintain stability of the cell walls, creating a rigid pipe-like structure, which is necessary for vascular plants to transport water and nutrition. Simultaneously, tissues that consist of cellulose, hemicellulose and lignin form a biocomposite that supports the tree (Janusz et al., 2017).

Basidiomycetes are a group of fungi that are able to decompose lignin, especially the white rot type. These microorganisms secrete at least one of three extracellular enzymes to enhance the degradation, which include Lignin Peroxidase (LiP), Manganese Peroxidase (MnP) and Laccase (Lac). Laccase oxidizes organic and inorganic compounds, including phenol (catechol, hydroquinone, 2,6-dimethoxyphenol, and syringaldazine). Marasmius pulcheripes J8 is a member of the white rot group, small in size and is often found on the surface of litterfall.

Most of this group of fungi secretes laccase with low enzyme activity, which is possibly to be enhanced by the addition of inducers, including aromatic or phenolic compounds, metals, alcohols, and detergents (Leonowicz et al., 2001). Furthermore, elevated functionality results in superior and faster modification in substrate degradation, and also better efficiency in enzyme catalysis (Rao et al., 2014). Moreover, the addition of 200 μM CuSO_{4} into a submerged Volvariella volvacea culture was able to produce the highest laccase activity (Chen et al., 2003), which was also obtained through the application of 15 g / L sucrose to submerged Pleurotus ostreatus culture (Subowo, 2015). In addition, laccase activity has also been reported to be influenced by carbon and nitrogen concentrations in the media, D’Agostini et al. (2011) reported that, higher ratio of C/N led to better mycelium growth. However, it lowered laccase production.

The interaction between Basidiomycetes and soil fungi has been reported to ultimately elevate the laccase functionality. Baldrian (2004) stated an over 40 times increase in activity, by virtue of the addition of *Trichoderma harzianum* to *Trametes versicolor*. Furthermore, the addition of soil fungi or bacteria causes an upsurge in laccase activity as well, by up to 2–25 times, and the addition of soil or soil extract causes up to 10-15 times intensifications. Meanwhile, the usage of *Marasmius pulcheripes* J8 to decompose POME has never been performed, which was the reason for conducting this investigation. In addition, the objective of the study was to obtain data on the ability of *Marasmius pulcheripes* J8 to degrade palm oil mill effluent with the presence of inducers, as well as its interactions with soil fungi. Waste treatment using fungi will lower the cost needed compared to physical and chemical methods. Fungi are easier and cheaper to grow.

METHODS

*Marasmius pulcheripes* J8 isolates were obtained from the Microbiology department collection, which were stored on Potato Dextrose Agar (PDA) media at -20°C, together with *Aspergillus niger* NK and *Penicillium sp* R 7.5.

The media used were (1) PDA, composed of: 4.0 g Potato starch; 20.0 g Dextrose; 15.0 g Agar. (2) PDB (Potato Dextrose Broth), with the following composition: 400.0 g potatoes; 20.0 g Dextrose. (3) Poly R-478 media composition: 0.60 g KH_{2}PO_{4}; 0.50 g MgSO_{4} \cdot 7H_{2}O; 0.40 g K_{2}HPO_{4}; 0.22 g (NH_{4}) tartrate; 40.0 g Sorbose; 0.20 g Poly R-478 (Sigma); 15.0 g Agar (Oxoid No. 3); 10.0 ml Mineral solution; added with distilled water to 1L. Mineral solution: 7.4 g CaCl_{2} \cdot 2H_{2}O; 1.2 g Ferric citrate; 0.7 g ZnSO_{4} \cdot 7H_{2}O; 0.5 g MnSO_{4} \cdot 4H_{2}O; 0.1 g CoCl_{2} \cdot 6H_{2}O; 10.0 mg Thiamine HCl; with distilled water to 1L.

Up to 5 ml of *M. pulcheripes* mycelium was poured into 45 ml of PDB media, which was termed treatment A. Meanwhile, treatment AB encompassed 5 ml mixture of *M. pulcheripes* and *Penicillium sp* R75 mycelium, which was placed into 45 ml of PDB. In addition, treatment AC included a 5 ml mixture of *M. pulcheripes* and *A. niger* NK mycelium, added into 45 ml of PDB, and treatment ABC comprises of *M. pulcheripes*, *Penicillium sp* R 7.5 and *A. niger* NK myceliums, which were mixed together, and 5 ml of the mixture was poured into 45 ml of PDB. Further-
more, all cultures were incubated on shakers at 115 rpm, at room temperature. Subsequently, samples were taken on day 4, which were then centrifuged at 9000 rpm, and the supernatant was used to measure laccase, MnP and LiP activities.

Laccase activity was determined by the amount of 2,2’-azino-bis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) oxidized (Papinuti et al., 2003). The reaction mixture consisted of 0.5 mL citrate buffer of pH 6, 0.1 mL ABTS 1 mM, and 0.4 mL supernatants. In addition, ABTS oxidation was monitored through an increase in absorbance at 420 nm.

Manganese Peroxidase activity was measured based on the amount of guaiacol oxidized, using a spectrophotometer (Yoshida et al., 1996). The reaction mixture consisted of 0.1 mL guaiacol 4 mM, 0.1 mL lactate buffer 50 M at a pH of 4.5, 0.2 mL MnSO₄ 1 mM, 0.3 mL distilled water, 0.1 mL H₂O₂, and 0.2 mL supernatants. Furthermore, guaiacol oxidation was measured based on the increase in absorbance at 465 nm.

Lignin Peroxidase activity (LiP) was measured using Tien & Kirk (1983) method, based on the oxidation of veratral alcohol into veratral aldehyde, in the presence of H₂O₂. Moreover, the reaction mixture consisted of 0.1 mL veratral alcohol 8 mM, 0.2 mL acetate buffer 50 mM of pH 3, 0.45 mL distilled water, 0.05 H₂O₂ 5 mM, and 0.2 mL supernatants. Therefore, the increase in absorbance was observed at 310 nm, using three replications.

A 5 ml measurement of M. pulcheripes J8 mycelium suspension was inoculated into the Poly R-478 liquid media in an Erlenmeyer flask, up to the point where the final volume reached 50 ml (treatment A). In addition, Penicillium sp R 7.5 was integrated, and 5 ml of this admixture was poured into the Poly R-478 media (treatment of AB). Furthermore, Mycelium of M. pulcheripes J8 and A. niger NK were mixed and placed in 45 ml of Poly R-478 media (treatment AC). Subsequently, the myceliums of all three isolates were mixed, and 5 ml was obtained and added to the Poly R-478 media + 200 μM CuSO₄ (treatment ABC). In addition, all cultures were incubated for 20 days on a shaker, at 115 rpm and room temperature, the color reduction in the media was evaluated using a spectrophotometer after incubation, and absorbance was assessed at a wavelength of 520 nm.

A total of 5 mL of Marasmius pulcheripes J8 mycelium was poured into 45 mL of POME + 200 μM CuSO₄ (A). Penicillium sp R 7.5 was mixed with the fungi, and 5 mL of the combination was poured into the POME + 200 μM CuSO₄ (treatment of AB). Furthermore, A. niger NK was mixed with the fungi, and then integrated with 45 mL of POME + 200 μM CuSO₄ (treatment AC). Therefore, the myceliums of all three isolates were mixed, and 5 mL was taken to the POME + 200 μM CuSO₄ (treatment ABC). Subsequently, all cultures were incubated for 20 days on a shaker, at 115 rpm and room temperature, and therefore, samples were then taken and centrifuged at 9000 rpm, and room temperature. In addition, POME decolorization was evaluated by observing the absorbance of supernatant at 600 nm, using a spectrophotometer, and similarly, COD level was measured with the same procedure.

RESULTS AND DISCUSSIONS

Marasmius pulcheripes J8 produces three ligninolytic enzymes, including Laccase, Lignin Peroxidase and Manganese Peroxidase, in Potato Dextrose Broth (PDB) media during the experiment. A method to increase the laccase activity of the Basidiomycetes was performed by creating an interaction with soil fungi (Baldrian, 2004). However, the interaction between M. pulcheripes J8 and soil fungi (Penicillium sp R75 and Aspergil-
The yield of mycelium is directly correlated to fungal growth, and this study showed the highest to be produced was by AB mixed culture (mixture of \(M.\) \(pulcheripes\) and \(P\). \(enichillium\) sp R7.5), followed by the pure culture of \(M.\) \(pulcheripes\) J8. However, the results of both treatments were not significantly different, thus, it is assumed that fungal growth in pure cultures is generally better than that mixed cultures. In addition, competition for growth and nutrients occurred in mixed cultures resulted in inhibited growth of examined fungi.

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### Table 1. Ligninolytic enzymes activity of \(M.\) \(pulcheripes\) J8 and its mixed cultures

<table>
<thead>
<tr>
<th></th>
<th>A (U/mL)</th>
<th>AB (U/mL)</th>
<th>AC (U/mL)</th>
<th>ABC (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laccase</td>
<td>3566.04±524.71</td>
<td>1222.37±201.30</td>
<td>1453.08±129.32</td>
<td>1090.12±205.49</td>
</tr>
<tr>
<td>LiP</td>
<td>24764.63±2939.19</td>
<td>17745.51±2201.46</td>
<td>16064.51±762.46</td>
<td>13062.12±212.05</td>
</tr>
<tr>
<td>MnP</td>
<td>14336.08±1401.35</td>
<td>10785.11±1516.94</td>
<td>8677.68±453.67</td>
<td>6831.95±750.54</td>
</tr>
</tbody>
</table>

Notes: A= \(M.\) \(pulcheripes\) J8; B= \(P\). \(enichillium\) sp R75; C= \(A.\) \(niger\) NK.

### Table 2. \(M.\) \(pulcheripes\) J8 laccase activity with the addition of inducer and interaction with soil fungi

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A (U/mL)</th>
<th>AB (U/mL)</th>
<th>AC (U/mL)</th>
<th>ABC (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuSO(_4)</td>
<td>590.73±52.01</td>
<td>589.35±73.03</td>
<td>589.19±98.80</td>
<td>607.71±69.24</td>
</tr>
<tr>
<td>Sucrose</td>
<td>460.79±43.83</td>
<td>452.00±54.06</td>
<td>535.18±46.61</td>
<td>489.96±33.89</td>
</tr>
<tr>
<td>Ammonium tartrate</td>
<td>222.68±19.25</td>
<td>233.94±11.94</td>
<td>234.71±43.97</td>
<td>211.26±41.30</td>
</tr>
<tr>
<td>Control</td>
<td>313.26±16.57</td>
<td>312.65±18.55</td>
<td>294.13±4.66</td>
<td>253.54±29.43</td>
</tr>
</tbody>
</table>

Notes: A= \(M.\) \(pulcheripes\) J8; B= \(P\). \(enichillium\) sp R75; C= \(A.\) \(niger\) NK.
fungi. This result was in accordance with the study by Chatterjee et al. (2016), where Aspergillus niger and Fusarium verticillioides were grown on a minimal media with asparagine as the N source. Moreover, the biomass of both fungi increased in pure culture during the growing period and decreased in mixed cultures from the 10th to the 30th day of incubation.

The addition of inducer to the Marasmius pulcheripes J8 increased the activity of laccase, and the most significant occurred on the addition of 200 µM CuSO₄ followed by 15 g / L sucrose, while the addition of 1.6 g / L Ammonium tartrate resulted in a lower laccase activity than the control. Furthermore, the interaction with soil fungi was not capable of elevating this effect, and the value of the four treatments (A, AB, AC, ABC) were not significantly different (Table 2).

The additions of inducer increased the laccase activity of M. pulcheripes J8, e.g., 200µM CuSO₄ on Poly R-478 media contributed an 88.57% increase; 15 g / L of sucrose increased it by 47.09%, while the addition of Ammonium tartrate resulted in a decline in activity, which was observed to be lower than the control. Meanwhile, the interaction treatment with soil fungi had no impact on laccase activity. CuSO₄ is often used as inducer to grow some white rot fungi types. According to Baldrian and Gabriel (2002), some compounds are responsible for causing a positive response in the production of laccase, and they are known to consist of metal, copper and cadmium ions. Cu²⁺ has been reported to possess inductive capabilities by forming an inseparable prosthetic group (Soden & Dobson, 2001). Meanwhile, the addition of sucrose increases activity by many factors, one of which is the presence of carbon, nitrogen and inducing compounds (Majeau et al., 2010). Furthermore, carbon concentration in nutrient media and lignocellulose substrates have also been investigated to play an important role in enzyme activity (Elisashvili et al, 2002), which does not always occur with the addition of ammonium tartrate as a source of nitrogen.

The degradative ability of M. pulcheripes J8 is observed on Poly R-478 media, by measuring the color change that occurred. After incubation for 20 days, the highest color degradation occurred in the treatment of M. pulcheripes J8 pure culture at 68.89%, then mixed AC cultures (M. pulcheripes and A. niger NK), ABC treatment (M. pulcheripes, Penicillium sp R 75 and A. niger NK) and the last treatment AB (M. pulcheripes J8 and Penicillium sp R 75) at 61.63% (Figure 2).

**Figure 2.** M. pulcheripes J8 ability to decolorize Poly R-478 after 20 days incubation. A= M. pulcheripes J8; B= Penicillium sp R 75; C= A. niger NK

Poly R-478 is an anthraquinone-based polymeric dye with high molecular weight that is used to observe ligninolytic ability of an enzyme produced by microbes. In addition, the reduction of color indicates the fungus ability of decomposition, and the result showed a positive effect, which was different from one treatment to another. Furthermore, the most significant influence was observed in the treatment of pure M. pulcheripes J8 culture, which was reduced in mixed cultures. This happened because the single sample tends to produce more MnP as seen in Table 1, which is known according to Moreira et al. (2001), as the main factor initiating color modification in Poly R-478. Furthermore, the addition of H₂O₂ with a semicontinuous technique is able to boost the decolorization process.

*M. pulcheripes J8* was applied in the degradation of Palm Oil Mill Effluent (POME) pigment by adding 200 µM CuSO₄ to the liquid waste. Therefore, after a 20 days of incubation period, the maximum color reduction was observed in treatment A by 74.25%, which was followed by AC, then ABC, and the lowest was in treatment AB, by 24.97% (Table 3).

The degradation of POME is indicated by a reduction in color, where the maximum decline occurred in the pure culture by up to 74.25%, while the mixed cultures resulted in lower extent. This result was consistent with the most significant ligninolytic enzyme activity produced by the pure culture of *M. pulcheripes J8*. The color of POME is usually generated by compounds in plant tissues, including lignin, which is degraded by laccase, into simpler compound, and phenolic compound (Chanida & Poonsuk, 2011). Moreover, a more significant effect is observed when compared to *Coprinus cinereus*, which initiated a 75.26% reduction after 27 days of incubation (Subowo, 2017).

The treatment of *M. pulcheripes J8* was observed to also reduce the level of COD (Chemical Oxygen Demand) in POME, where the most sig-
A significant decline occurred in treatment A by 81% after 20 days of incubation period, followed by treatment AC, ABC and the lowest was AB by 77.79% (Figure 3).

![Figure 3](image-url)

**Table 3.** The ability of *Marasmius pulcheripes* J8 to degrade POME after 20 days of incubation

<table>
<thead>
<tr>
<th></th>
<th>A (%)</th>
<th>AB (%)</th>
<th>AC (%)</th>
<th>ABC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>73.99</td>
<td>17.55</td>
<td>67.51</td>
<td>55.37</td>
<td></td>
</tr>
<tr>
<td>74.44</td>
<td>32.77</td>
<td>62.35</td>
<td>56.83</td>
<td></td>
</tr>
<tr>
<td>74.34</td>
<td>24.61</td>
<td>66.06</td>
<td>56.65</td>
<td></td>
</tr>
<tr>
<td>74.25±0.23</td>
<td>24.97±7.61</td>
<td>65.30±2.66</td>
<td>56.28±0.79</td>
<td></td>
</tr>
</tbody>
</table>

Notes: A = *M. pulcheripes* J8; B = *M. pulcheripes* J8; C = *A. niger* NK

**CONCLUSION**

Pure culture of *Marasmius pulcheripes* J8 on PDB media showed the highest laccase activity (3566.04 U / mL) whereas it was lower in mixed cultures. The addition of CuSO4 on Poly R-478 media increased laccase activity by 88.57%. This fungus was able to reduce color in POME by 74.25% and reduce POME COD level by 81% after incubation for 20 days.

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