Malaysian Journal of Microbiology, Vol 20(2) 2024, pp. 140-147
DOI: http://dx.doi.org/10.21161/mjm.230175

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

# Characterization of laccase from *Trichoderma* sp. UBDFT12 isolated from a Bornean tropical forest

**Abdul Zul'Adly Mohaimin, Pooja Shivanand* and Hussein Taha**

Environmental Life and Sciences Programme, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link BE1410, Brunei Darussalam.
Email: pooja.shivanand@ubd.edu.bn

Received 9 January 2023; Received in revised form 2 January 2024; Accepted 3 January 2024

## ABSTRACT

**Aims:** This study was aimed to characterize laccase from a selected fungal strain and examine the enzyme's ability to remove lignin from paper pulp.
**Methodology and results:** Twelve fungal strains were screened for laccase production, resulting in the selection of *Trichoderma* sp. UBDFT12. The highest laccase activity (103 U/L) was observed from the culture filtrate on the fourth day of incubation. The optimum temperature and pH for the enzyme were 40 °C and pH 4, respectively. However, the enzyme stability was found to be reduced with time after 1 h incubation. At 1 mM, it was found that $AgNO_3$, $CaCO_3$, $CuSO_4$, KCl, $MgSO_4$, $MnSO_4$ and $ZnSO_4$ increased the laccase activity to 107, 107, 111, 112, 106, 105 and 107%, respectively, whereas $FeSO_4$ and $NH_4Cl$ reduced the activity to 84 and 99%, respectively. The addition of 1% $H_2O_2$, 1% NaCl, 1% sodium dodecyl sulphate (SDS), 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM EDTA, 1 mM phenanthroline and 10 mM phenanthroline reduced the activity to 95, 73, 0, 79, 79, 73 and 37%, respectively. The culture filtrate was partially purified via ammonium sulphate precipitation and the recovered enzyme had a specific activity of 0.176 U/mg. Using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the molecular weight of the enzyme was approximately 65 kDa and its activity was confirmed by zymography. The culture filtrate was also found to be able to remove lignin from different types of paper pulp.
**Conclusion, significance and impact of study:** Laccase produced by *Trichoderma* sp. UBDFT12 was found to have the ability to remove lignin from paper pulp.

*Keywords:* Biodegradation, delignification, enzyme, laccase, *Trichoderma*

## INTRODUCTION

Laccase (EC 1.10.3.2; benzenediol: oxygen oxidoreductase) has generated much interest due to its ability to degrade phenolic and non-phenolic lignin-related compounds through the reduction of molecular oxygen to water (Khan *et al.*, 2016). Due to its attractive properties, such as being eco-friendly, low substrate specificity, high stability and high catalytic efficiency at a wide range of temperatures and pH conditions, laccase is considered an important enzyme. It has been widely used for biotechnological and environmental applications such as the decolourisation of dyes, degradation of xenobiotics compounds, waste effluent treatments, biosensors, biofuel cells, pulp and paper industry, food and beverage industry, bioethanol production, analytical applications, personal and medical care applications, and organic synthesis (Vantamuri and Kaliwal, 2016). Laccase can be found in insects, bacterial strains, plants and fungi (Madhavi and Lele, 2009). It was reported that laccases have been produced and purified from different groups of fungi, such as basidiomycetes, deuteromycetes and ascomycetes (Assavanig *et al.*, 1992).

Laccase can be used for the delignification of lignocellulose materials, which is considered more environmentally friendly than bleaching. This enzyme can be helpful in the paper and pulp industries that depend on the recycling of paper, tissue and wood pulp (Sridevi *et al.*, 2017). Generally, these industries utilize chlorine-based chemicals that are not only toxic but also costly (Aslam *et al.*, 2016). Organochlorine compounds are released using chlorine dioxide during the bleaching process of pulp, which causes harmful effects on the environment (Sharma, 2019). An alternative method is to use nonchlorine chemical reagents such as oxygen, hydrogen peroxide or ozone, which have been reported to be less efficient. Thus, laccase excreted from *Trichoderma* could reduce the usage of chlorine

*Corresponding author


ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 140-147
DOI: http://dx.doi.org/10.21161/mjm.230175

components for bleaching processes since it is easy to extract, environmentally friendly and cost-effective (Virk *et al*., 2012).

*Trichoderma* species, which belong to the family Hypocreaceae (Asomycota), are some of the most common fungi in forest soils (Yakop *et al*., 2019). The fungi naturally produce laccase for survival, which breaks the lignin component of dead leaves and trees. It has been reported that several *Trichoderma* species, including *T. harzianum*, *T. viride*, *T. pseudooningii*, *T. cremeum*, *T. longipile*, *T. atroviride*, *T. citrinoviride*, *T. beinartii*, *T. asperellum* and *T. virens* excreted decent amount of the enzyme (Umar, 2021). In this study, extracellular laccase from *Trichoderma* sp. UBDFT12, previously isolated from a Bornean tropical forest (Taha *et al*., 2020), was characterized and tested for its ability to remove lignin from paper pulp. As the fungal strain is novel, the characterization of its laccase is important, which could potentially benefit the biotechnology industry.

## MATERIALS AND METHODS

### Fungal samples

A total of twelve fungal isolates that were previously isolated from Brunei Darussalam, Borneo Island, were used in this study. Briefly, eight of the fungal isolates were from a mixed dipterocarp forest (4°38′33.1″N, 114°30′34.1″E) and four isolates were from a mangrove forest (4°53′23.3″N, 115°07′58.5″E), which were all identified by deoxyribonucleic acid (DNA) barcoding (Taha *et al*., 2020). For maintenance, the isolated fungal strains were subcultured on potato dextrose agar at 25 °C for 7 days and stored at 4 °C until the next subculturing.

### Qualitative screening of laccase

Potato dextrose agar was supplemented with 2 mM of 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). A mycelium-covered potato dextrose agar disc (5 mm diameter) from a week-old fungal culture was inoculated onto the ABTS-supplemented agar medium and incubated for 3 days at 25 °C. The formation of a dark green colour would indicate the presence of laccase. A fungal strain (*Trichoderma* sp. UBDFT12; GenBank accession no. MK116422) that showed the most prominent and darkest formation of green colour was selected for further study.

### Quantitative analysis of laccase

Using a four-day-old fungal culture, mycelium-covered agar discs (5 mm diameter) were inoculated into a number of 250 mL Erlenmeyer flasks containing 100 mL of yeast extract peptone dextrose-copper sulphate (YPD-Cu) medium (Vantamuri and Kaliwal, 2015). The flasks were incubated for 1 to 7 days at 30 °C and 150 rpm. Whatman filter paper (grade 1, 110 mm diameter, 11 µm pore size, cat. no. 1001-110) was used to filter the culture filtrate. The mycelial residue was dried at 60 °C before its dry weight (biomass) was measured. Laccase activity was measured by mixing 1 mL of the filtrate with 1 mL of 2.5 mM ABTS and 1 mL of 100 mM sodium acetate buffer (pH 4.5). The change of absorbance observed over a period of 5 min was recorded at 420 nm and the laccase activity (U/L) was calculated using the formula $(\Delta A)(V_t)(D_f)(10^6)/(t)(\varepsilon)(d)(V_s)$ (Baltierra-Trejo *et al*., 2015). One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 µM of ABTS per min.

### Characterization of laccase

The optimum temperature for laccase activity was determined to be between 20 °C and 80 °C. After a 1 h incubation at the selected temperature, an enzyme assay was carried out using ABTS as described above. After determining the optimum temperature, the optimum pH was also determined at pH 2.0 to 8.0. The culture filtrate was incubated in a buffer at the selected pH for 1 h at the optimum temperature. For pH ranging from 2.0 to 5.0, sodium acetate buffer was used, while sodium phosphate buffer was used for pH ranging from 6.0 to 8.0. After incubation, the culture filtrate was assayed using ABTS. The stability of the laccase with time was determined by incubating the culture filtrate at the optimum temperature and pH for 1 h. Afterward, laccase activity was measured at 30-min intervals for a period of 3 h.

For determining the effects of metal ions, additives, and inhibitors on laccase activity, an enzyme assay was similarly carried out as above but with 100 mM sodium acetate (pH 4.0) and at room temperature. A total of nine metal salts at a concentration of 1 mM were used: $AgNO_3$, $CaCO_3$, $CuSO_4$, $FeSO_4$, KCl, $MgSO_4$, $MnSO_4$, $NH_4Cl$ and $ZnSO_4$. Three different additives were used: 1% NaCl, 1% $H_2O_2$ and 1% SDS, and two different inhibitors at different concentrations were used: 1 mM EDTA, 10 mM EDTA, 1 mM 1,10-phenanthroline and 10 mM 1,10-phenanthroline. For control, no metal ions, additives or inhibitors were added.

### Partial purification of laccase

Partial purification was carried out using ammonium sulphate precipitation. The culture filtrate was mixed with 80% ammonium sulphate at a cold temperature and agitated for 30 min. The sample was centrifuged for 15 min at 10,000 ×*g*. The supernatant was removed, and the protein pellet was dissolved using 50 mM sodium acetate buffer (pH 4.5). Determination of protein concentration was done using Bradford assay with bovine serum albumin as standard. Enzyme assay using ABTS was similarly carried out using the partially purified sample.

### SDS-PAGE and zymography

SDS-PAGE was carried out using 12% resolving gel to determine the molecular weight of laccase. The partially purified sample was mixed with a sample buffer (Laemmli buffer containing freshly added β-mercaptoethanol) and heated at 90 °C for 5 min. After loading 10 to 15 µL of the

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

mixture into the well, the gel was run at 120 V for 2 h. For protein band observation, the gel was stained with silver nitrate staining.

Zymography was carried out according to El-Fakharany *et al.* (2016). The sample was prepared without any addition of reducing agent or heating. SDS-PAGE was similarly carried out using 12% resolving gel. After loading 20 µL of the mixture into the well, the gel was run at 120 V for 2 h. The gel was washed with distilled water twice for 15 min each and then soaked with 2.5% triton X-100 twice for 15 min each to remove SDS and renature the enzyme. To visualize laccase activity, the gel was incubated with 0.1 M sodium acetate buffer (pH 4.0) containing 1.54 mM ABTS at room temperature for 30 to 60 min. The appearance of dark green colour would indicate the oxidation of ABTS by laccase.

**Delignification of paper pulp**

Delignification of paper pulp was done according to Aslam *et al.* (2016) with some modifications. Three types of pulp (newspaper, office paper and tissue paper) were prepared by first soaking them in distilled water for 2 h. Old newspaper, office paper (A4 paper) that had been used and thrown into a bin, and waste tissue paper collected from the laboratory were used for delignification activity using laccase. The paper was then macerated with a blender and oven-dried at 50 to 70 °C. Each pulp (2 mg) was suspended in 1 mL of distilled water and mixed with 1 mL of the culture filtrate (0.1 U/mL), 1 mL of 2.0 mM ABTS and 1 mL of 50 mM sodium acetate buffer (pH 4.5). The mixture was incubated at 30 °C for 3 h without shaking. After incubation, four drops of trichloroacetic acid were added to the mixture, and the pH was adjusted to pH 9.0. It was then centrifuged for 5 min at 13,000 rpm. Absorbance at 280 nm would indicate the concentration of lignin in the supernatant. If the absorbance was too high for measurement, the samples were diluted accordingly. For control, the mixture was not added to the culture filtrate. We tested the mixture with and without the addition of ABTS.

**RESULTS AND DISCUSSION**

A total of twelve fungal isolates were screened qualitatively for laccase production. Among the fungal strains, *Trichoderma* sp. UBDFT12 showed the largest (average diameter ± standard deviation = 7.86 ± 0.23 cm) and darkest green colour formation after 3 days of incubation (Figure 1) and therefore, was selected for further analysis. A larger diameter likely means that the fungal strain showed superior growth in the specified conditions, which would be advantageous for biotechnological applications. Fungi with higher biomass are likely to produce more laccase. A darker green colour would mean that more ABTS compounds were being enzymatically converted to form green-coloured compounds, suggesting the production of more laccase.

*Trichoderma* sp. UBDFT12 was analysed quantitatively for its extracellular production of laccase and the change in fungal biomass and pH of the culture was also analysed daily over a period of one week (Figure 2). This study found that the optimum laccase activity was observed on the fourth day of incubation, amounting to 103 U/L. Based on the fungal biomass, it suggests that the exponential phase occurred in the first three days, while from the third to fifth day, the culture could be in a stationary phase. This could explain the result observed since it is believed that laccase is produced by microbes during the stationary phase

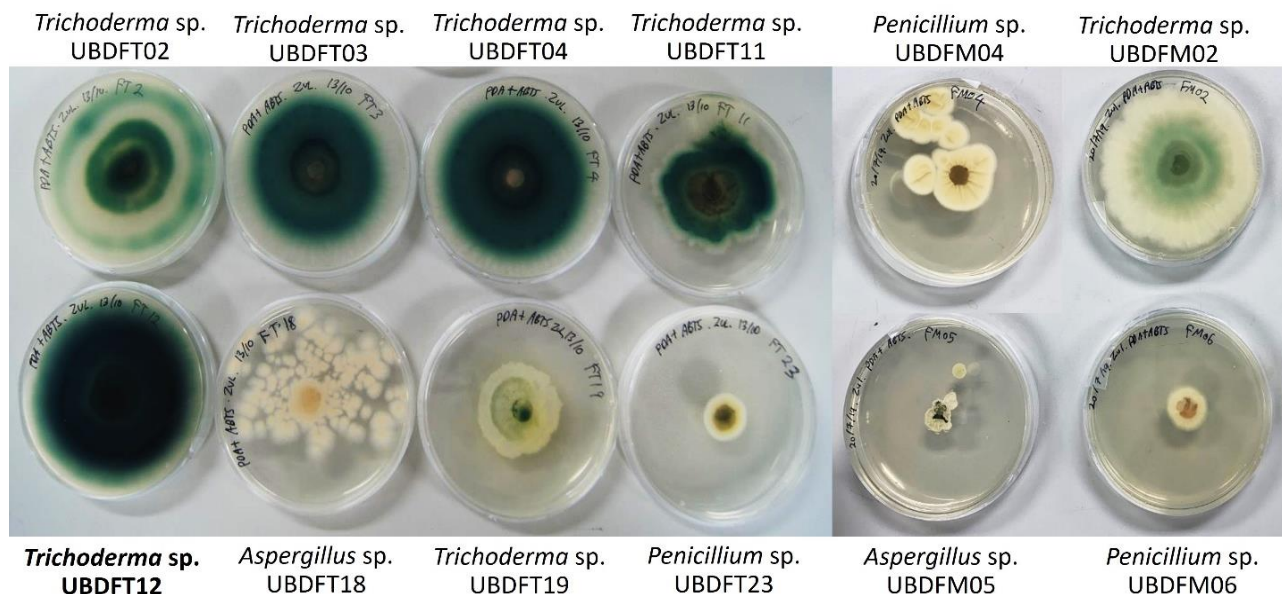


**Figure 1:** Screening for laccase activity of twelve fungal isolates.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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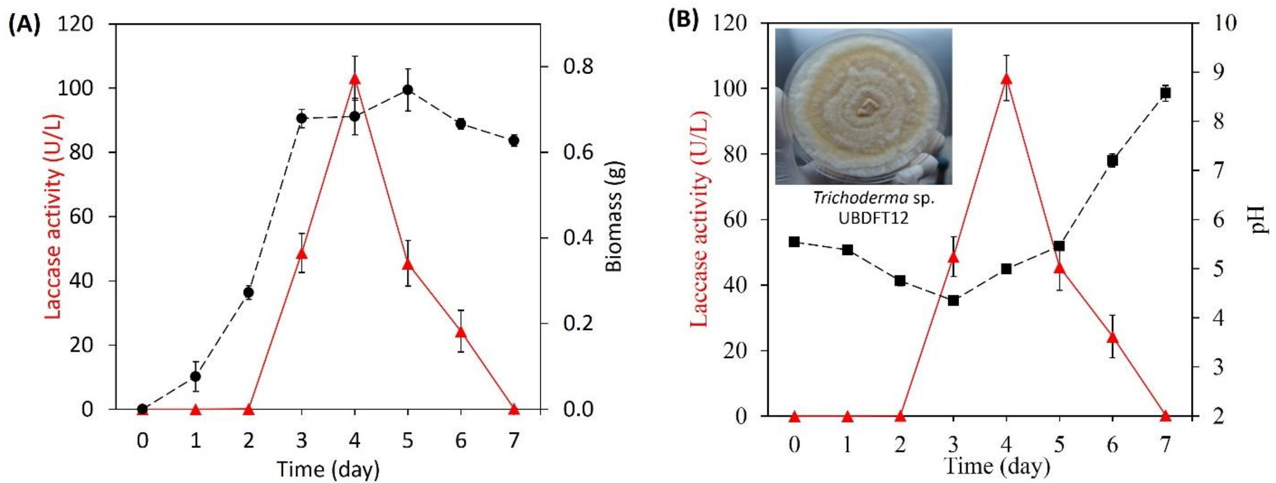


**Figure 2:** Production of extracellular laccase by *Trichoderma* sp. UBDFT12. Laccase activity is shown in both panels for comparison with the change in biomass (A) and pH of the culture (B). In addition, the photo of the fungus is also shown. The average values of two replicates are shown with the standard deviation error bars.

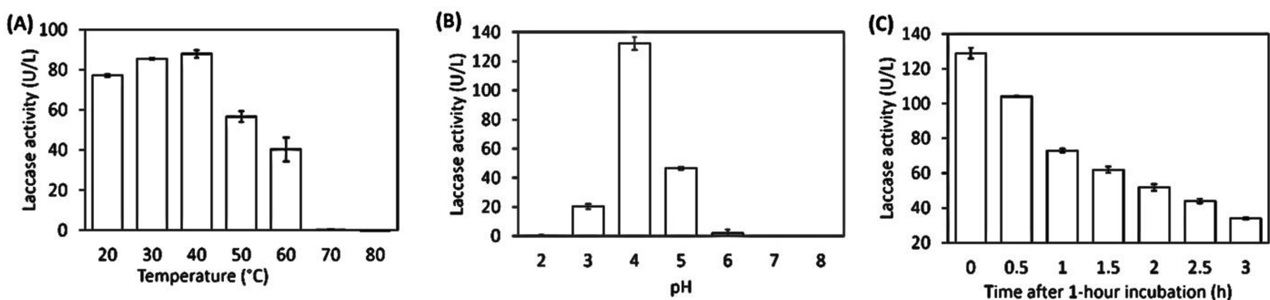


**Figure 3:** Laccase activity of *Trichoderma* sp. UBDFT12 culture filtrates at different temperatures (A), pH values (B) and time intervals (C). The average values of three replicates are shown with the standard deviation error bars.

(Rivera-hoyos *et al*., 2013). After the fifth day, the biomass started to decline slowly, and this suggests that the culture was at the death phase. This could explain the decline in the laccase activity observed during this time period. The drastic increase in the pH of the culture observed after the fifth day could also affect the production of laccase.

The effect of temperature on laccase activity is shown in Figure 3A. *Trichoderma* sp. UBDFT12 culture filtrate showed laccase activity from 20 to 60 °C with the optimum temperature observed at 40 °C. No laccase activity was detected at higher temperatures, suggesting that the enzyme might be denatured by the heat.

In comparison, the laccase from *Marasmius* sp. BBKAV79 is active at 37 to 55 °C with an optimum temperature of 40 °C (Vantamuri and Kaliwal, 2016). Similarly, *Pleurotus sajor-caju* has the same optimum temperature (Murugesan *et al*., 2006). On the other hand, the optimum temperatures of the laccases from *Trametes hirsute* BM-2, *Trichoderma harzianum* and *Ganoderma lucidum* are much higher (50 to 70 °C) (Zapata-Castillo *et al*., 2012; Wang and Ng, 2016; Bagewadi *et al*., 2017). Similarly, the laccase from *Pleurotus* sp. also has a higher optimum temperature of 65 °C and is active at temperatures ranging from 25 to 70 °C (More *et al*., 2011).

The effect of pH on laccase activity of *Trichoderma* sp. UBDFT12 culture filtrate is shown in Figure 3B. It shows substantial laccase activity from pH 3.0 to 5.0, with the optimum pH observed at pH 4.0. Beyond pH 3.0 and 5.0, no substantial laccase activity could be observed, suggesting that the enzyme could be affected at those pH values. Furthermore, it also suggests that the enzyme might not be active in alkaline conditions.

The optimum pH of laccase is known to be affected by different substrates such as ABTS, guaiacol, 2,6-dimethoxyphenol (2,6-DMP) and catechol. The laccase from *Marasmius* sp. BBKAV79 has an optimum pH of 5.5 for guaiacol (Vantamuri and Kaliwal, 2016). *Pleurotus sajor-caju* has an optimum pH of 6.0 for syringaldazine (Zucca *et al*., 2011) but an optimum pH of 4.5 to 5.0 for ABTS (Murugesan *et al*., 2006). The laccase from *Cerrena* sp. HYB07 has an optimum pH of 3.0 for ABTS, pH 3.0 for 2,6-DMP, pH 4.0 for guaiacol and pH 4.5 for

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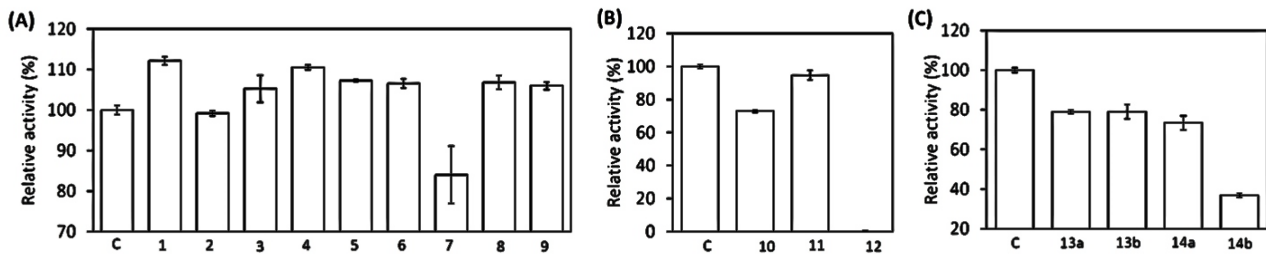


**Figure 4:** Effects of metal ions (A), additives (B) and inhibitors (C) on laccase activity of *Trichoderma* sp. UBDFT12 culture filtrate. The average values of three replicates are shown with the standard deviation error bars. C: Control, 1: KCl, 2: $NH_4Cl$, 3: $MnSO_4$, 4: $CuSO_4$, 5: $CaCO_3$, 6: $AgNO_3$, 7: $FeSO_4$, 8: $ZnSO_4$, 9: $MgSO_4$, 10: NaCl, 11: $H_2O_2$, 12: SDS, 13a: 1 mM EDTA, 13b: 10 mM EDTA, 14a: 1 mM phenanthroline, 14b: 10 mM phenanthroline.

catechol (Yang *et al.*, 2014). For further comparison, when using ABTS as substrate, the optimum pH of laccase is pH 5.0 for *Ganoderma lucidum*, pH 4.0 to 4.5 for *Trametes hirsute* Bm-02, pH 4.5 for *Pleurotus* sp. and pH 4.0 for *Fusarium oxysporum* (El-Fakharany *et al.*, 2016; Wehaidy *et al.*, 2018; Singh *et al.*, 2019). Furthermore, many laccases produced by fungi are active at acidic pH but tend to be inactive at alkaline pH. The laccase from *Mycena purpureofusca* has an optimum pH of 2.2 and is inactive, starting at pH 7 (Sun *et al.*, 2013). The laccase from an edible mushroom, *Lentinula edodes* is only active from pH 3.0 to 7.0 (Nagai *et al.*, 2003).

The stability of laccase from *Trichoderma* sp. UBDFT12 culture filtrates with time is shown in Figure 3C. After 1 h incubation at the optimum temperature and pH (40 °C and pH 4), the laccase activity was measured at 129 U/L. However, the activity reduced to 104 U/L after a further 0.5 h of incubation. Further incubation led to a further decrease in the laccase activity, suggesting the enzyme might not be stable for prolonged incubation at the stated conditions.

Laccase activity of *Trichoderma* sp. UBDFT12 was influenced by metal ions, as shown in Figure 4A. There was a decrease in the laccase activity relative to the control in the presence of $NH_4Cl$ (99%) and $FeSO_4$ (84%), whereas in the presence of KCl, $MnSO_4$, $CuSO_4$, $CaCO_3$, $AgNO_3$, $ZnSO_4$ and $MgSO_4$, there was an increase in the laccase activity to 112, 105, 111, 107, 107, 107 and 106%, respectively. In comparison, an increase in laccase activity was similarly reported in the presence of 10 mM of $K^+$, $Cu^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$ or $Zn^{2+}$ (Yang *et al.*, 2014). However, the presence of 1 mM of $Ag^+$, $Mg^{2+}$, $K^+$, $Cu^{2+}$, $NH^{4+}$ or $Ca^{2+}$ has been reported to decrease the laccase activity of *Aspergillus flavus* (Aslam *et al.*, 2016). $Mg^{2+}$ was reported to increase the laccase activity of *Trametes hirsuta* BM-2 to 186%, while $Cu^{2+}$ and $Mn^{2+}$ reduced the activity to 92 and 40%, respectively (Zapata-Castillo *et al.*, 2012). It was also reported that 1 mM of $MgCl_2$ and 10 mM of $CuSO_4$ increased the laccase activity to 102 and 140%, respectively, while 1 mM $K^+$ and $Zn^{2+}$ reduced the activity to 46 and 36%, respectively (Nagai *et al.*, 2003).

The effect of additives on laccase activity of *Trichoderma* sp. UBDFT12 culture filtrate is shown in Figure 4B. The addition of 1% SDS inhibited the laccase activity completely. However, the addition of 1% NaCl decreased the activity by approximately 30%, while the addition of 1% $H_2O_2$ only showed a slight decrease in activity, which was approximately 6% less. In comparison, it was previously reported that 1% $H_2O_2$ caused a drastic decline in laccase activity to 1.49%, while 1% NaCl reduced the activity by approximately 78% and the addition of 1% SDS reduced it to 33.17% (Vantamuri and Kaliwal, 2016).

The effect of inhibitors on laccase activity of *Trichoderma* sp. UBDFT12 culture filtrate is shown in Figure 4C. The presence of EDTA and phenanthroline was observed to decrease the laccase activity. Increasing the EDTA concentration from 1 to 10 mM did not show any further reduction in the laccase activity, but for phenanthroline, increasing the concentration substantially reduced the activity from 73 to 37%. In comparison, EDTA at 1 mM and 10 mM concentrations and reduced laccase activity to 95 and 83%, respectively (Yang *et al.*, 2014). Another study found that the presence of 1 mM EDTA decreased the laccase activity to 73%, while 10 mM EDTA stopped the activity completely (Zapata-Castillo *et al.*, 2012). However, it was also reported that 20 mM of EDTA and phenanthroline increased the laccase activity to 110 and 105%, respectively (Vantamuri and Kaliwal, 2016). Similarly, 1 mM EDTA was also reported in another study to increase the laccase activity to 101%, but 10 mM EDTA reduced it to 87.8% (Nagai *et al.*, 2003).

The laccase from *Trichoderma* sp. UBDFT12 culture filtrate was partially purified using ammonium sulphate precipitation to remove mostly non-protein compounds from the culture filtrate. The partial purification resulted in the successful recovery of the enzyme, with the specific activity increased from 0.137 to 0.176 U/mg, giving a total purification fold of 1.28 (Table 1).

The partially purified enzyme was analysed using SDS-PAGE (Figure 5A). Several protein bands could be observed, especially after partial purification, including an intense band at approximately 65 kDa. To confirm that the band was laccase, a zymogram of laccase activity was produced (Figure 5B). The appearance of dark green colour on the gel indicated the oxidation of ABTS, confirming the ~65 kDa band as a laccase.

In comparison, laccases from other fungi have molecular weights ranging from 60 to 90 kDa: *Pleurotus*

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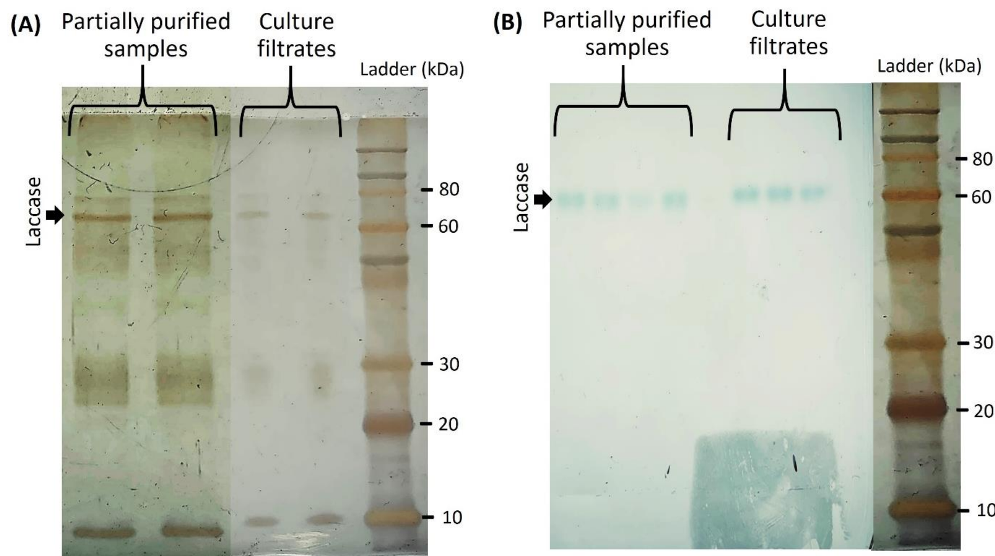


**Figure 5:** SDS-PAGE analysis of laccase (A) and zymogram of laccase (B). Both the culture filtrate and partially purified samples from *Trichoderma* sp. UBDFT12 were analysed in two to four replicates.

**Table 1:** Partial purification of extracellular laccase from *Trichoderma* sp. UBDFT12.

| Sample | Activity (U/mL) | Total protein (mg/mL) | Specific activity (U/mg) | Recovery (%) | Purification (fold) |
|---|---|---|---|---|---|
| Culture filtrate | 0.103 ± 0.007 | 0.75 ± 0.017 | 0.137 ± 0.008 | 100 | 1 |
| Partially purified | 0.178 ± 0.013 | 1.01 ± 0.097 | 0.176 ± 0.023 | 157 | 1.28 |

Average values of six replicates ± standard deviation is shown.

*sajor-caju* (61 kDA) (Murugesan *et al*., 2006; Zucca *et al*., 2011), *Paraconiothyrium variabile* (84 kDa) (Forootanfar *et al*., 2011), *Marasmius* sp. BBKAV79 (75 kDa) (Vantamuri and Kaliwal, 2016), *Trametes hirsute*a Bm-2 (65 kDa) (Zapata-Castillo *et al*., 2012), *Mycena purpureofusca* (61.7 kDa) (Sun *et al*., 2013), *Lentinula edodes* (72.2 kDa) (Nagai *et al*., 2002), *Scytalidium thermophilum* (82 kDa) (Younes and Sayadi, 2011), *Trametes pubescens* (68 kDa) (Galhaup and Haltrich, 2001), *Fusarium solani* MAS2 (72 kDa) (Wu *et al*., 2010) and *Polyporus* sp. (60 kDa) (Guo *et al*., 2011). Smaller laccases from fungi have also been reported: *Cerrena* sp. HYB07 (58.6 kDa) (Yang *et al*., 2014), *Ganoderma lucidum* (38.3 kDa) (Manavalan *et al.,* 2013) and *Pleurotus* sp. (40 kDa) (More *et al*., 2011). The presence of laccase isozymes has also been reported: *Ganoderma lucidum* TVK1 (4 isozymes) (Manavalan *et al*., 2013) and *Ganoderma lucidum* CDBT1 (3 isozymes) (Shrestha *et al*., 2016). However, in the present study, no isozymes were observed since there was only a single green-coloured band on the zymogram.

The culture filtrate from *Trichoderma* sp. UBDFT12 was tested for its delignification activity (Figure 6). The absorbance at 280 nm indicated the amount of lignin being removed from the different types of pulp. There was no substantial delignification activity observed in the control, which is as expected due to the absence of laccase. In the presence of the culture filtrate, delignification activity was observed. The addition of ABTS, which is a laccase mediator, enhanced the amount of lignin being removed by approximately 2 times more.

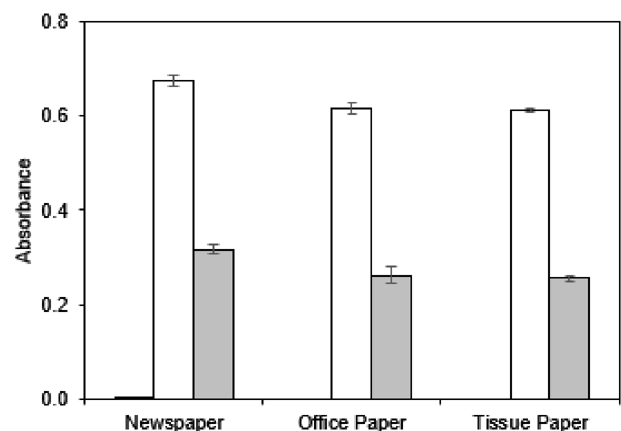


**Figure 6:** Delignification of different types of paper pulp using *Trichoderma* sp. UBDFT12 culture filtrate after 3 h of incubation. Control (black bar), culture filtrate with ABTS (white bar) and culture filtrate without ABTS (grey bar). The average values of three replicates are shown with the standard deviation error bars.


ISSN (print): 1823-8262, ISSN (online): 2231-7538

To scale up the production of laccase for biotechnological applications, further studies are needed to determine its feasibility. However, several factors should be taken into account, such as medium composition, the addition of inducers, culture conditions, culture type (solid state or using broth), static or agitated condition, aeration and incubation time, which could influence the synthesis of the enzyme (Kunamneni *et al*., 2007; El-Shora *et al*., 2008; Rivera-Hoyos *et al*., 2013). Some examples of laccase inducers are ethanol, copper sulfate, gallic acid and ferulic acid (Zucca *et al*., 2011; Manavalan *et al*., 2013). Response surface methodology could be used to identify the factors that could influence the laccase production.

## CONCLUSION

A total of twelve fungal strains were screened for laccase activity, resulting in the selection of *Trichoderma* sp. UBDFT12. The highest laccase activity achieved was at day four of incubation. The enzyme had an optimum temperature and pH of 40 °C and pH 4, respectively. Most of the metal ions tested in this study increased enzyme activity. The enzyme was approximately 65 kDa and with a specific activity of 0.176 U/mg after partial purification. The enzyme was able to remove lignin from different types of paper pulp. Thus, it has the potential for biotechnological and environmental applications. However, further research is required.

## ACKNOWLEDGEMENTS

This study was supported by a research grant from Universiti Brunei Darussalam [UBD/OAVCRI/CRGWG(010)/170501]. All authors would like to thank Prof Abdalla Mohamed Jama, Dr. Monowarul Mobin Siddique, and Dr. Ndanusa Abdullahi Hassan for their assistance with SDS-PAGE.

## CONFLICTS OF INTEREST

The researchers report no conflict of interest.

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