Decolorization of Synthetic Dyes by Tropical Fungi Isolated from Taman Eden 100, Toba Samosir, North Sumatra, Indonesia

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ABSTRACT

Taman Eden 100 is one of the tourist parks located in Toba Samosir, North Sumatra, Indonesia that provides a wide range of biodiversity. The objective of this research was to obtain the potential fungal isolate with high laccase activity to decolorize synthetic dyes. The results show that six isolates of *Perenniporia subtephropora* EDN 050, *Trametes hirsuta* EDN 082, *T. hirsuta* EDN 084, *T. hirsuta* EDN 085, *Deconica coprophila* EDN 114, and *T. pavonia* EDN 134, which were confirmed by molecular identification using 5.8 rDNA/ITS analysis, exhibited decolorization activity. Six potential isolates showed the ability to decolorize textile dyes of Acid Blue 129, RBBR,Orange II, and Reactive Black 5. The highest decolorization rate of 100 ppm Acid Blue 129, RBBR, Orange II, and Reactive Black 5 dyes by using *T. pavonia* EDN 134 were 98.87%, 98.26%, 100%, and 98.11%, respectively after 96 h incubation. Almost all positive isolates also decolorized dyes at higher concentrations (1,000 ppm). This study offers the potential of Indonesian tropical fungal isolates for synthetic dyes waste treatment.

1. Introduction

Synthetic dyes are widely used in many industries such as textile dyeing, paper, cosmetics, pharmaceuticals, and food industries. In Indonesia, the textile industry is one of the highest export earners. With the growth of the textile industry, the demand for synthetic dyes increases too. These phenomena have shown a high pollutant potential. It is estimated that around 5-15% of the dyes are lost in the wastewater during the dyeing processes. Most of them are toxic and recalcitrant pollutants (Arabaci and Usluoglu 2014; Asgher *et al.* 2017).

Removal of dyes in wastewater has been made by physical, chemical, and/or biological processes. In the physical method, dyes are removed by adsorption, membrane filtration, ion exchange technique, and activated carbon while in the chemical method, chromophore has been modified through chemical reactions such as coagulation process, electrochemical, and ozonation. Although both methods demonstrate good results, they have major disadvantages such as the formation of hazardous by-products, high cost, and high consumption of energy. A biological method such as microbial degradation, adsorption and bioaccumulation by microbial biomass, and enzymatic mineralization (lignin peroxidase (LiP), manganese peroxidase (MnP), and laccases) is considered as a cost-effective and eco-friendly method for color removal from textile wastewater (Jebapriya and Gnanadoss 2013; Parmar and Shukla 2018).

Fungi, especially the Basidiomycota group, are the main group of lignocellulosic degrading microorganisms. The fungi produce extracellular enzymes in the form of ligninolytic enzymes, including LiP, MnP, and laccase (Lac). Ligninolytic enzymes are produced by fungi to decompose complex lignin compounds. In addition to the decomposition of lignin compounds, ligninolytic enzymes can be applied to degrade various pollutants, such as synthetic dyes, and polyaromatic hydrocarbons. Several white-rot fungi are effective in degrading pollutans such as *Trametes hirsuta* (Abadulla *et al.* 2000), *Ganoderma* sp. (Fazli *et al.* 2010), *Pestalotiopsis* sp. (Yanto *et al.* 2014), and *Schyzophyllum commune* (Singh *et al.* 2017).

Taman Eden 100 is one of the tourist parks in Toba Regency, Samosir, North Sumatra province, Indonesia located at an altitude of 1,100 to 1,750 meters above

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sea level. Tropical forest conditions in Taman Eden are natural and provide a wide range of biodiversity both flora and fauna (Toba Samosir Regency Government 2014). Microorganism such as fungi are also commonly found at Taman Eden 100. Wahyudi *et al.* (2016) explained that tropical rain forests are a habitat for flora and fauna including fungi which are the second largest species after insects. The potential of fungus as an agent of synthetic dyes degradation is very important, considering that Toba is a tourism area with a variety of craft products including Ulos crafts that also use synthetic dyes in their coloring. In this study, we explored fungi at Taman Eden 100, isolated, and screened them on agar and liquid medium containing synthetic dyes. Through this series of processes, a database will be produced in the form of the potential of fungi isolated from the Toba region as dyes degradation.

2. Materials and Methods

2.1. Materials

Acid Blue 129 (AB 129), Remazol Brilliant Blue R (RBBR), Orange II/Acid Orange 7 (O II), and Reactive Black 5 (RB5) dyes, lignin, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma (USA). Peptone and chloramphenicol were purchased from Hi-Media (India). All other chemicals were provided by Merck (Germany).

2.2. Sample Collection and Isolation

The fungal species used in this study were isolated from fungal bodies collected from Taman Eden 100, Toba Regency, Samosir, North Sumatra, Indonesia (Figure 1). Samples of the fungal bodies were taken from twigs or stems, both live and dead wood or litter then cleaned and dried using tissue paper. All

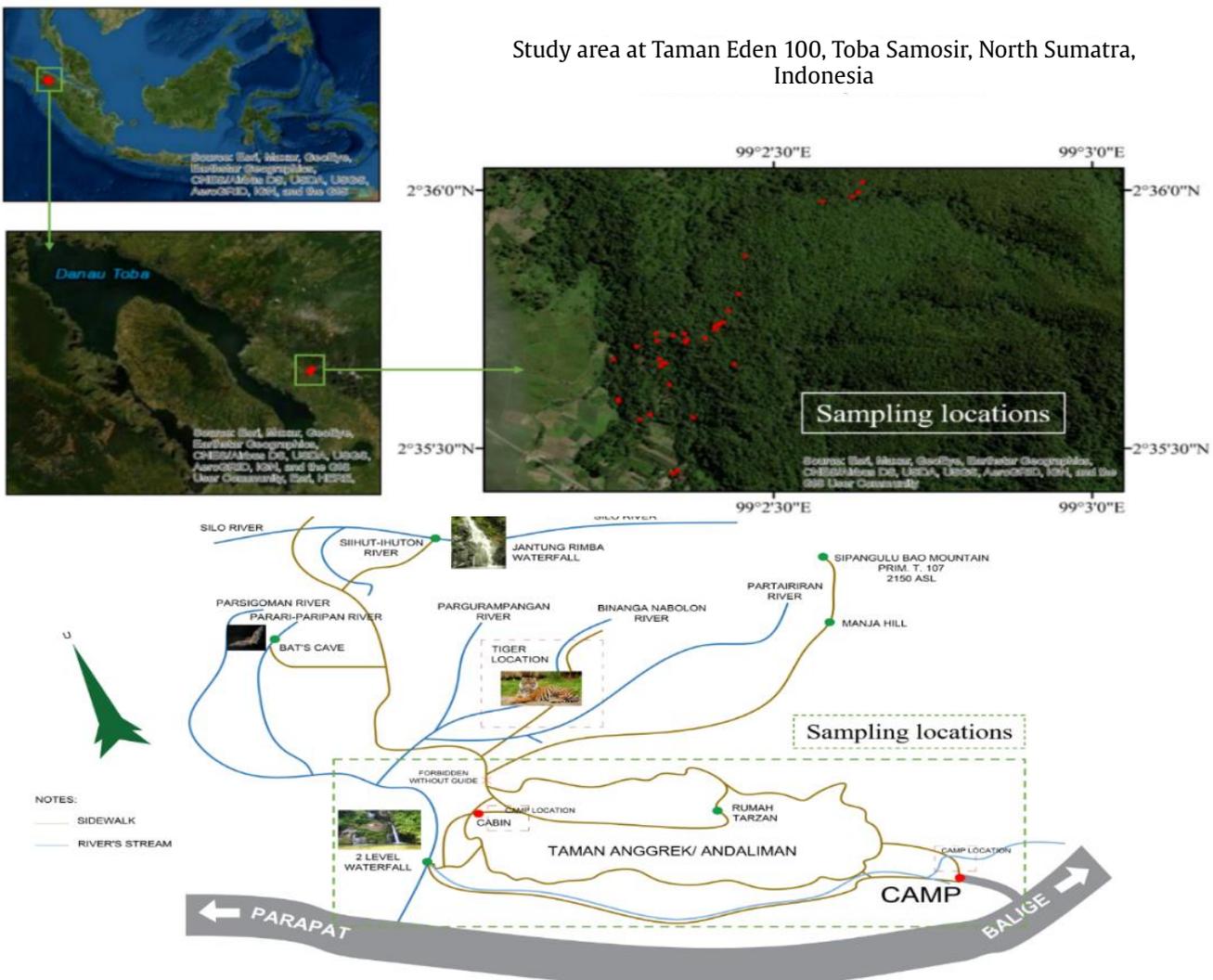Study area at Taman Eden 100, Toba Samosir, North Sumatra, Indonesia

Figure 1. Map of sampling locations in Taman Eden 100, North Sumatra, Indonesia

samples were put into aluminum foil and marked with a number for further examination at Research Center for Biomaterials, National Research and Innovation Agency (BRIN). Isolation was carried out by spraying the surface layer of fungal sporocarps using 75% ethanol and drying using sterile tissue. Next, the basidiomata were torn with a sterile blade, then a small piece (<2 cm) of the inner part was transferred to Malt Extract Agar (MEA) medium using a sterilized tweezer. The cultures were incubated at room temperature for 3-7 days. This process was repeated until a pure isolate was obtained.

2.3. Fungal Screening for Ligninolytic Enzyme-Producer

The fungal screening was conducted by using two layers of medium agar on a petri dish. The composition of medium (g/L), bottom layer: Dzapek-Dox broth (35), KH_2PO_4 (1), yeast extract (2), peptone (2), agar (20), lignin (2), glucose (10), top layer: malt extract (5), chloramphenicol (0.4), RBBR (1), agar (10). One plug (5 mm in diameter) of 7 days old pure fungal isolate was inoculated on two layers of screening medium and incubated at room temperature for 7 days. Positive isolates were shown by the formation of a clear zone around the fungi growth (Oktaviani and Yanto 2015). AB 129, O II, and RB5 were also used for screening. Each isolate was screened based on growth and decolorization rate in the agar medium.

2.4. Identification of Fungi

Fungal mycelia were grown on MEA for 7 days at room temperature as a fungal genomic DNA source. Mycelia were scrapped and put into a 1.5 ml plastic tube by using a scalpel blade. DNA was extracted using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, USA) according to manufactured protocol. The fungal strains were identified using an internal transcribed spacer (ITS region) of ribosomal RNA genes, amplified by polymerase chain reaction (PCR) using ITS1(5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') primers (White *et al.* 1990). Confirmed PCR products were then Sanger-sequenced and compared to the database with National Center for Biotechnology Information (NCBI)-BLAST. The phylogenic tree was constructed with the maximum likelihood (ML) method using MEGA 7 software (Kumar *et al.* 2016). The Bootstraps 1000 replication (BS) is used to test the strength of the internal branches of the phylogenetic trees (Felsenstein 1985). Other parameters used in the ML analysis were

selected according to the default standard in MEGA 7 software. Bootstrap values of 40% or higher were shown. Gen Bank accession number, sequence name, and strain code used in the phylogenetic analysis were shown in Table 3.

2.5. Decolorization of Dyes in Liquid Medium

Three plugs (5 mm in diameter) of 7 days old positive isolate growth on MEA agar were inoculated into 20 ml malt extract-glucose-peptone (MGP) liquid medium that contained 20 g/L malt extract, 20 g/L glucose, and 1 g/L peptone. Flasks were then incubated at room temperature for 7 days. After finished, 100 µl of each dye (RBBR, AB129, O II, and RB5) was added into the culture, so that the final concentration of dyes becomes 100 ppm and 1,000 ppm. MGP liquid medium with the addition of dyes was used as a control. All reactions were performed in triplicate and incubated without shaking at room temperature for 96 h. Samples were taken at intervals 24 h for decolorization and enzymatic assay.

2.6. Decolorization Assays

The decolorization process was monitored at 592.5 nm for RBBR, 629 nm for AB 129, 482.5 nm for O II, and 598 nm for RB 5 by UV-Vis spectrophotometer, UV-1800 Shimadzu, Japan. Decolorization was calculated by the following Eq. (1) (Anita *et al.* 2020):

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance of dye} - \text{Final absorbance of dye}}{\text{Initial absorbance of dye}} \times 100\% \quad (1)$$

2.7. Enzymatic Assays

Laccase activity was observed spectrophotometrically by monitoring the oxidation of 2 mM ABTS in 0.1 M acetate buffer pH 4.5 at 420 nm for 1 min. The assay mixture contained 100 µl of culture filtrate, 400 µl of 0.1 M acetate buffer, and 500 µl of 2 mM ABTS. Enzyme activities were calculated by the following Eq. (2) with a molar absorptivity (ε) of 36,000 $M^{-1}cm^{-1}$ (Yanto *et al.* 2021):

$$\text{Enzyme activity}\left(\frac{U}{ml}\right) = \frac{(\text{Abs. }(t) - \text{Abs. }(0)) \times V\text{mixture (ml)} \times 10^3}{\varepsilon \times V\text{enzyme (ml)} \times t \text{ (min)}} \quad (2)$$

Where Abs.(t) and Abs. (0) are the final and the initial absorbance, respectively; 10^3 is the correction factor (µmol/mol).

3. Results

3.1. Isolation and Screening

A total of 175 samples were collected from fungal bodies that lived on wood or litter from Taman Eden 100. Twenty-five samples were successfully isolated and cultured on MEA (Table 1). Out of 25 isolates, six fungal isolates performed ligninolytic enzyme activity indicated by the formation of the clear zones on the medium containing RBBR dye. Positive isolates were EDN 050, EDN 082, EDN 084, EDN 085, EDN 114, and EDN 134 (Figure 2).

Six positive isolates were then measured for growth and decolorization rate on solid medium (Table 2). The results showed that six isolates were able to grow on medium with different types of dye e.g. AB 129, RBBR (anthraquinone), O II (monoazo), and RB 5 (diazo) dyes. The growth rate of six positive isolates were in a range from 0.09 cm/days to 1.27 cm/days. It means that the fungal mycelium diameter ranged from 0.63 cm to 8.89 cm in 7 days. The decolorization rate was calculated based on the

formation of clear zone around the fungal growth, showing the results in a range from 0.59 cm/days to 1.25 cm/days and the total decolorization were about 4.13 cm to 8.75 cm in 7 days. However, all isolates did not show decolorization activity on solid medium with O II (monoazo) and RB 5 (diazo) dyes.

3.2. Fungal Identification

The results from the BLAST search of the ITS rDNA showed that the amplicons of the six potential isolates had similarity >99% compared to the species in GenBank (Table 3). Hence, to confirm the identification of BLAST results, phylogenetic analyses of six isolates of positive fungi were carried out by comparing their ITS rDNA region sequences with their closest species from NCBI using the Maximum likelihood (ML) method. All sequences with the closest relationship to each analyzed isolate were obtained from GenBank to verify the phylogeny of these fungi. The ML tree of isolate strain EDN 82, EDN 84, and EDN 085 showed that three new

Table 1. Screening results of isolate which has ligninolytic enzyme activity

Sampling location	Number of samples	Number of pure isolates	Number isolates with ligninolytic producer	Isolate code
Camp	21	2	-	
Taman Anggrek/ Andaliman	85	18	4	EDN 050, EDN 082, EDN 084, EDN 085
2 Level waterfall	60	5	2	EDN 114, EDN 134
Rumah Tarzan	9	-	-	
Total	175	25	6	

Figure 2. Screening isolates on selective medium uninoculated control (A), clear zone of positive isolate: EDN 050 (B), EDN 082 (C), EDN 084 (D), EDN 085 (E), EDN 114 (F), EDN 134 (G). Bar = 1 cm

Table 2. Growth rate and decolorization rate of six potential fungi isolates for 7 days incubation at 27±3°C

Isolate code	AB 129		RBBR		RB 5		O II	
	Growth rate (cm/days)	Decolorization rate (cm/days)	Growth rate (cm/days)	Decolorization rate (cm/days)	Growth rate (cm/days)	Decolorization rate (cm/days)	Growth rate (cm/days)	Decolorization rate (cm/days)
EDN 050	0.63±0.10	0.59±0.13	0.80±0.09	0.74±0.10	0.95±0.13	n.d.	0.09±0.08	n.d.
EDN 082	0.84±0.01	0.91±0.04	1.26±0.13	1.25±0.14	1.13±0.08	n.d.	0.16±0.02	n.d.
EDN 084	0.62±0.09	0.86±0.07	1.04±0.06	1.02±0.05	1.13±0.17	n.d.	0.13±0.06	n.d.
EDN 085	1.00±0.11	0.99±0.07	1.27±0.11	1.23±0.09	1.14±0.09	n.d.	0.16±0.04	n.d.
EDN 114	0.58±0.15	0.65±0.12	1.05±0.05	0.95±0.04	1.01±0.05	n.d.	0.47±0.09	n.d.
EDN 134	0.85±0.11	0.90±0.11	0.95±0.06	1.04±0.02	0.88±0.03	n.d.	0	n.d.

nd: not detected

sequences of the fungi nested in the same clade with *T. hirsuta* with 99% BS. This demonstrated that strain EDN 082, EDN 084, and EDN 085 belong to *T. hirsuta* (Figure 3). The same result was shown in analyses strain EDN 134. The strain is nested in one clade to species *T. pavonia* with 100% BS. It suggested that the sequences of isolate EDN 134 are high similar to *T. pavonia.* On the other hand, strain EDN 50 is one clade to *Perenniporia subtephropora*, and strain EDN 114 is one clade to *Deconica coprophila*. However, the bootstrap value from those strains was low (<90 %).

3.3. Decolorization in Liquid Medium

Six positive isolates were tested on a liquid medium containing 100 ppm of dyes. Figure 4 shows a representative photo for decolorization of RBBR by positive isolate. With increasing incubation time, the blue color of RBBR in a liquid medium disappeared and left the natural color of the MGP medium. Fungal biomass also appeared white, indicating that the loss of the blue color was due to the enzyme degradation process secreted by the fungus.

Table 3. Best matches of ITS rDNA sequences of potential fungi using BLAST

| Isolate code	Sequence length (bp)	Homology	Accession number	Max. score	Query coverage (%)	Max indent. (%)	E. value
EDN 114	572	*Deconica coprophila* isolate A2S6-16	KJ780773	1029	100	99.13	0
EDN 082	609	*Trametes hirsuta* strain SYBC-L19	JX861099	1120	100	99.84	0
EDN 050	611	*Perenniporia subtephropora* isolate 2_2C210_3N_636 bp	MK209003	1129	100	100.00	0
EDN 085	618	*Trametes hirsuta* strain SYBC-L19	JX861099	1118	100	99.35	0
EDN 134	626	*Trametes pavonia* voucher AJ519	KF573032	1125	99	99.36	0
EDN 084	629	*Trametes hirsuta* strain SYBC-L19	JX861099	1138	99	99.36	0
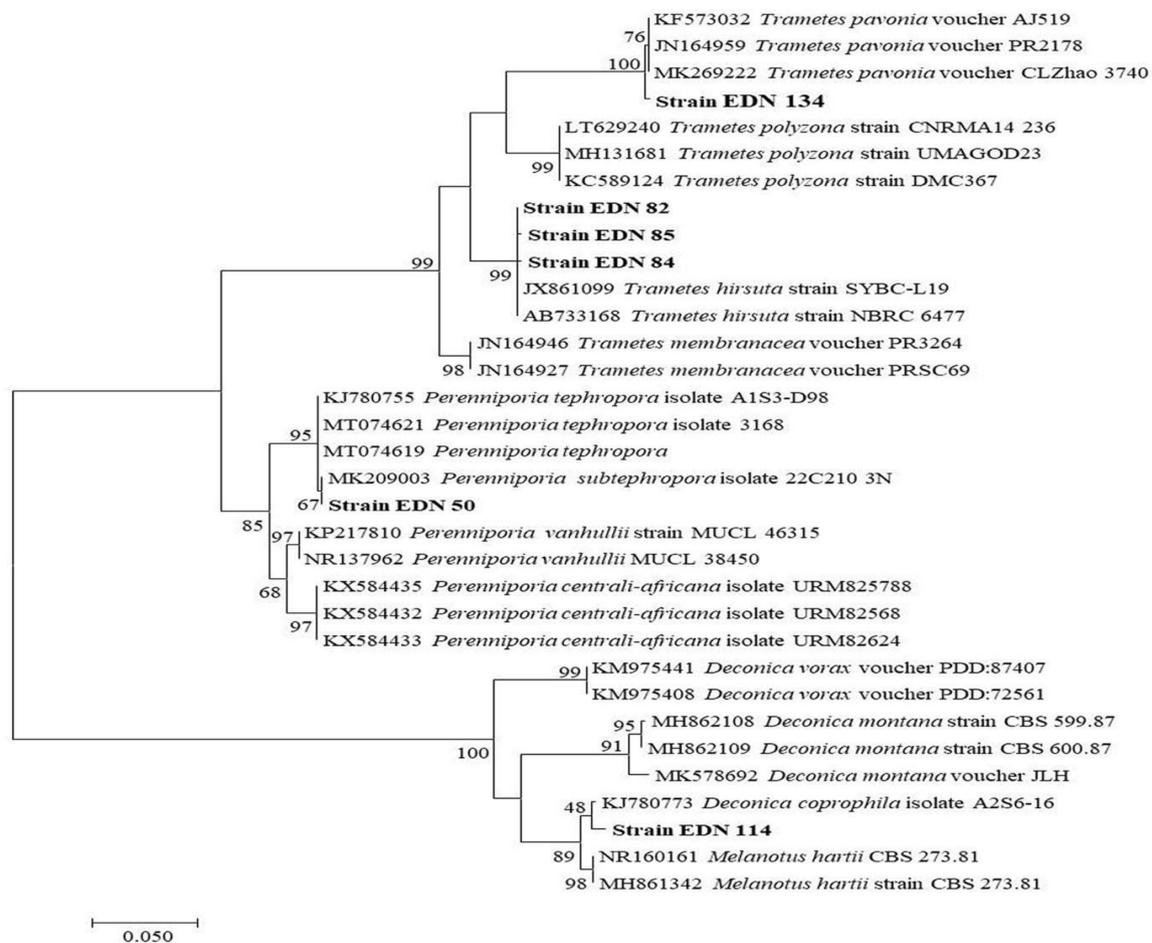
Figure 3. Phylogenetic tree for strain EDN 50, EDN 82, EDN 84, EDN 85, EDN 114, EDN 134, and related species based on ITS rDNA sequence. Bootstrap values >40% are indicated at the nodes (1,000 replication). The scale bar represents an evolutionary distance of 0.05

The effect of initial dye concentration on the different types of dyes was also tested in a liquid medium. Figure 5 shows that six isolates were capable of decolorizing four types of dyes at a concentration of 100 ppm in liquid media. The decolorization process occured rapidly at 24 h incubation and increased slowly or even remained stable with increasing incubation time. It was found that *T. pavonia* EDN 134 exhibited the highest decolorization activity, followed by *T. hirsuta* EDN 082. At the concentration of 100 ppm, the maximum dye decolorization by *T. pavonia* EDN 134 was 98.87%, 98.26%, 100%, and 98.11% for AB129, RBBR, O II, and RB5 dyes, respectively, at 96 h incubation. It was surprising that almost positive isolates decolorized dyes at higher concentrations (1,000 ppm). However, *T. pavonia* EDN 134 showed the best dyes decolorization compared to other isolates with values of 96.10%, 91.96%, 99.03%, and 19.63% for AB129, RBBR, O II, and RB5 dyes, respectively, at 96 h incubation. All isolates were not able to decolorize diazo dyes at a concentration of 1,000 ppm. This can be seen from the percentage of decolorization produced below 50% (Figure 6).
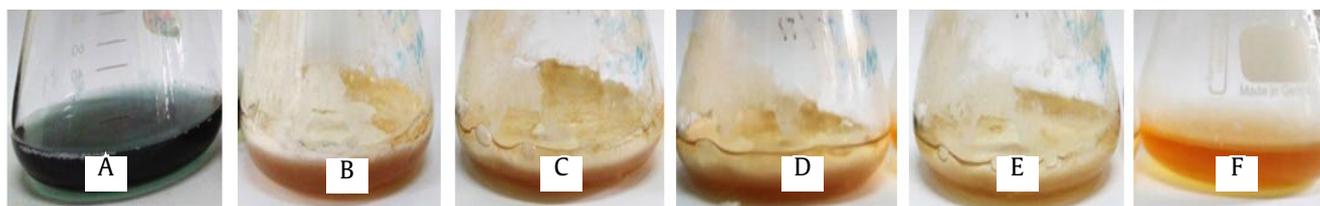

Figure 4. Decolorization of positive isolate on RBBR liquid medium, (A) negative control (RBBR+MGP medium), (B) 24 h, (C) 48 h, (D) 72 h, (E) 96 h of incubation, (F) positive control (MGP medium)
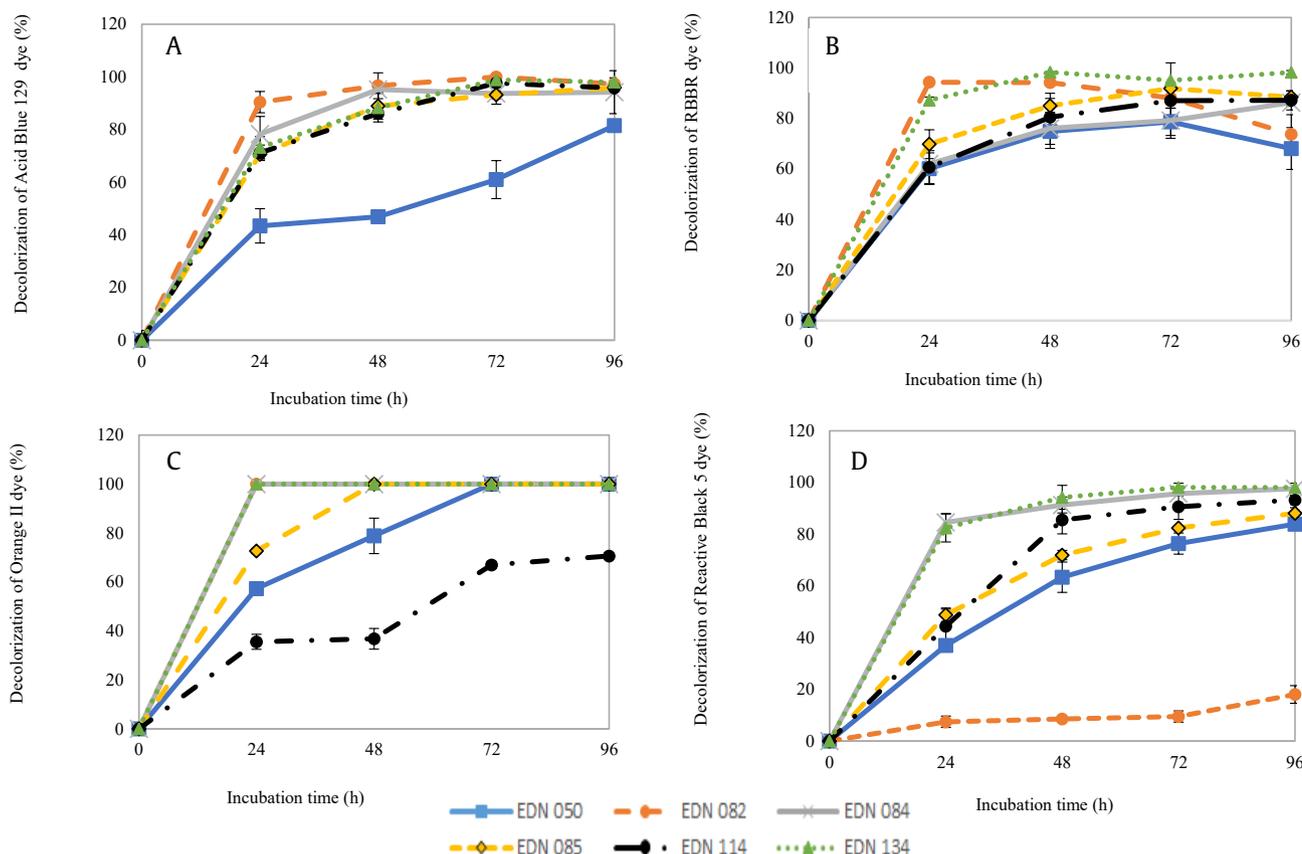

Figure 5. Decolorization of 100 ppm dyes, (A) AB 129, (B) RBBR, (C) O II, (D) RB 5 by six potential fungal isolates for 96 h incubation at 27±3˚C
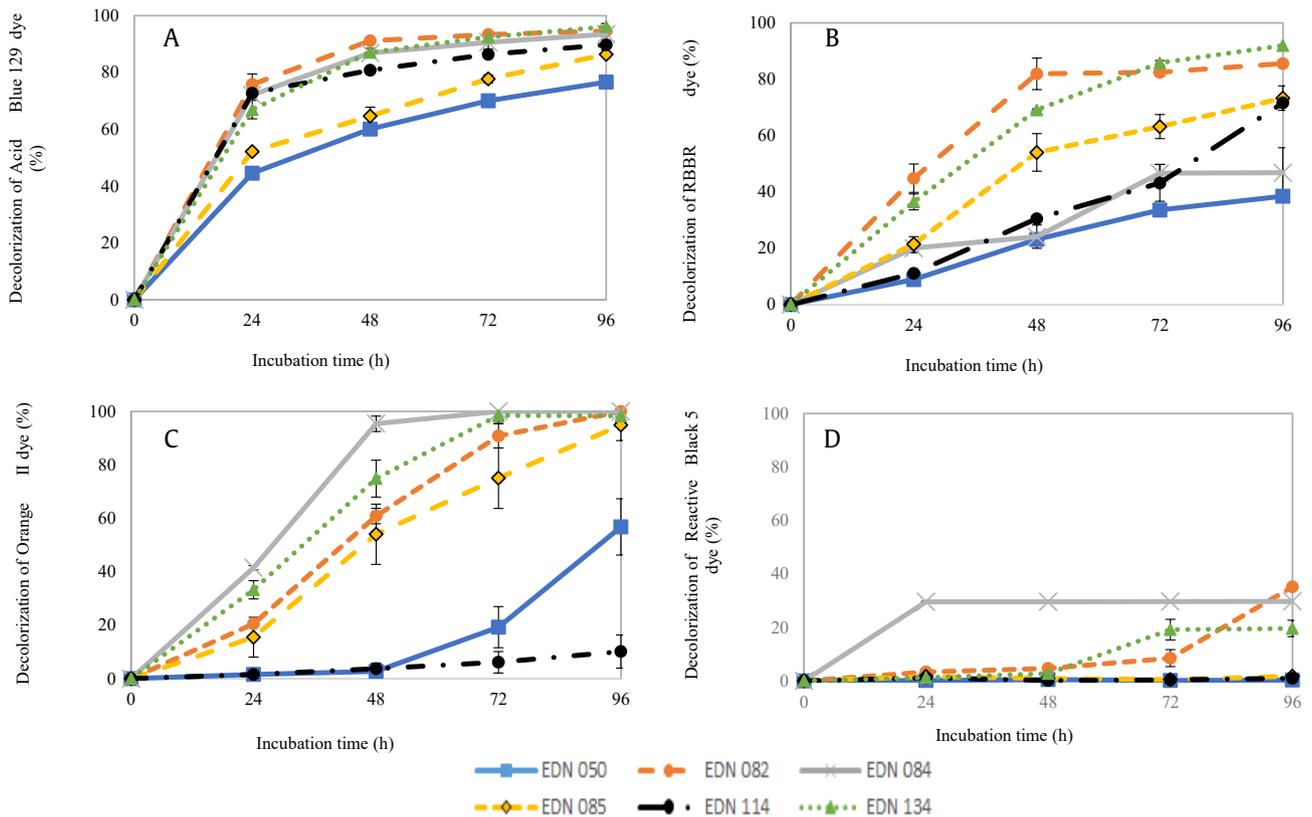
Figure 6. Decolorization of 1,000 ppm dyes, (A) AB 129, (B) RBBR, (C) O II, (D) RB 5 by six potential fungal isolates for 96 h incubation at 27±3°C

3.4. Laccase Activity

Production of laccase enzyme during decolorization varied depends on the incubation time, type of dyes, and dyes concentration. The results showed that laccase activity increased at 24 h and decreased after 48 h at a concentration of 100 ppm for each type of dye. The highest activity was 0.262 U/ml in AB 129 and obtained by *T. hirsuta* EDN 082 at 24 h incubation (Figure 7). Whereas the concentration of 1,000 ppm dyes showed that the enzyme activity of laccase tended to increase between 48 h and 72 h then decreased or remained stable until the end of the incubation time. The highest activity was 1.136 U/ml in AB 129 and obtained by *Deconica coprophila* EDN 114 at 72 h incubation (Figure 8).

The laccase produced by *Deconica coprophila* EDN 114 has the same high activity as *T. hirsuta* EDN 082 and even higher at concentration dyes of 1,000 ppm. Meanwhile, the laccase activity of *T. pavonia* EDN 134 is relatively low compared to the two.

4. Discussion

The isolation process from 175 samples resulted in 25 pure isolates. Through the screening process, six isolates showed the ability to produce ligninolytic enzymes, especially laccase. In this study, screening was carried out by using an agar medium containing lignin and RBBR synthetic dye. The reddish-brown color arising on the alkali-lignin medium as the fungal growth indicated the presence of ligninolytic enzymes, while the decolorization ability of the fungus was demonstrated by removing the blue color of the RBBR or by the formation of the clear zones on the medium. In the selective medium containing lignin and dyes, fungi could secrete ligninolytic enzyme activity and form a brown zone around the fungus growing colony. Anita *et al.* (2011) reported that the brown layer formed around the growth of fungal colonies indicates that the fungus produces ligninolytic enzymes so that it can oxidize lignin contained in the medium. Whereas the decolorization process occurs when the color around
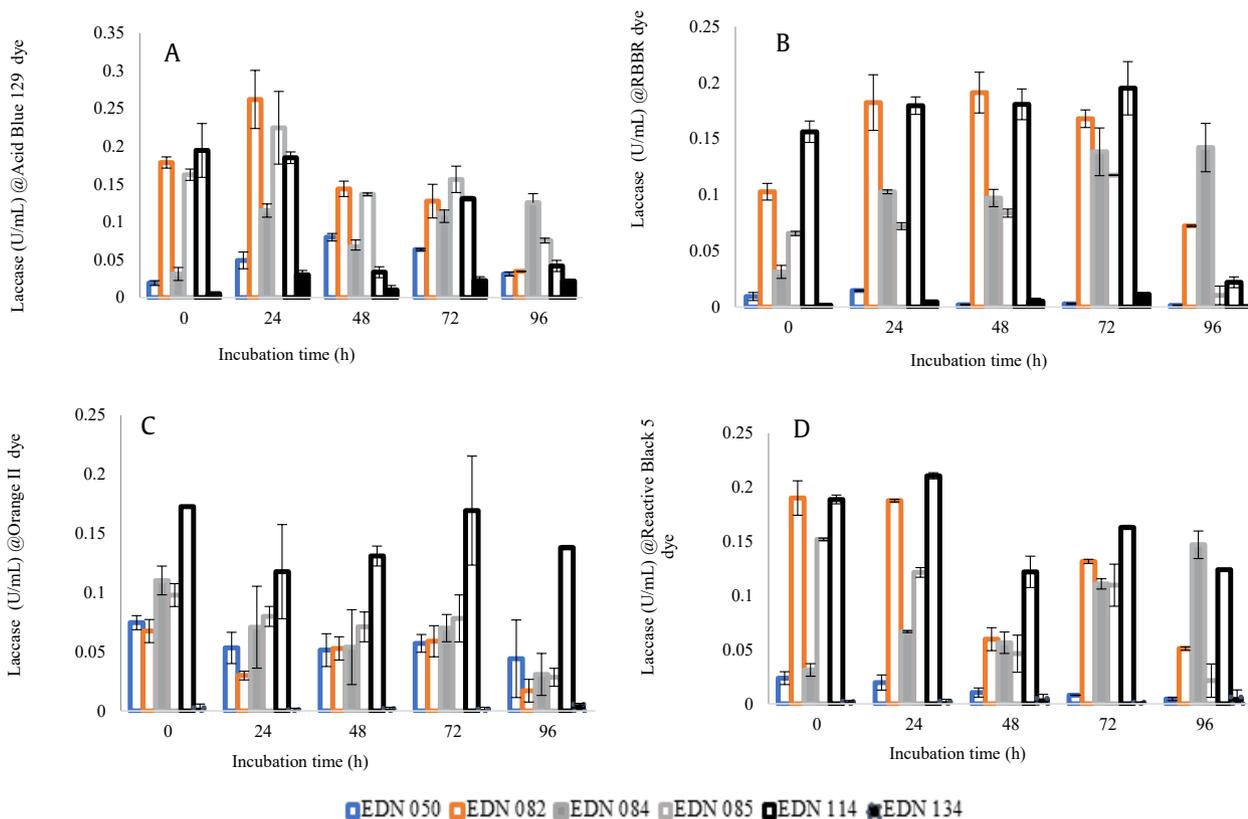
Figure 7. Laccase activity during decolorization process of dyes (100 ppm) (A) AB 129, (B) RBBR, (C) O II, (D) RB 5 by six potential fungi isolate for 96 h at 27±3°C
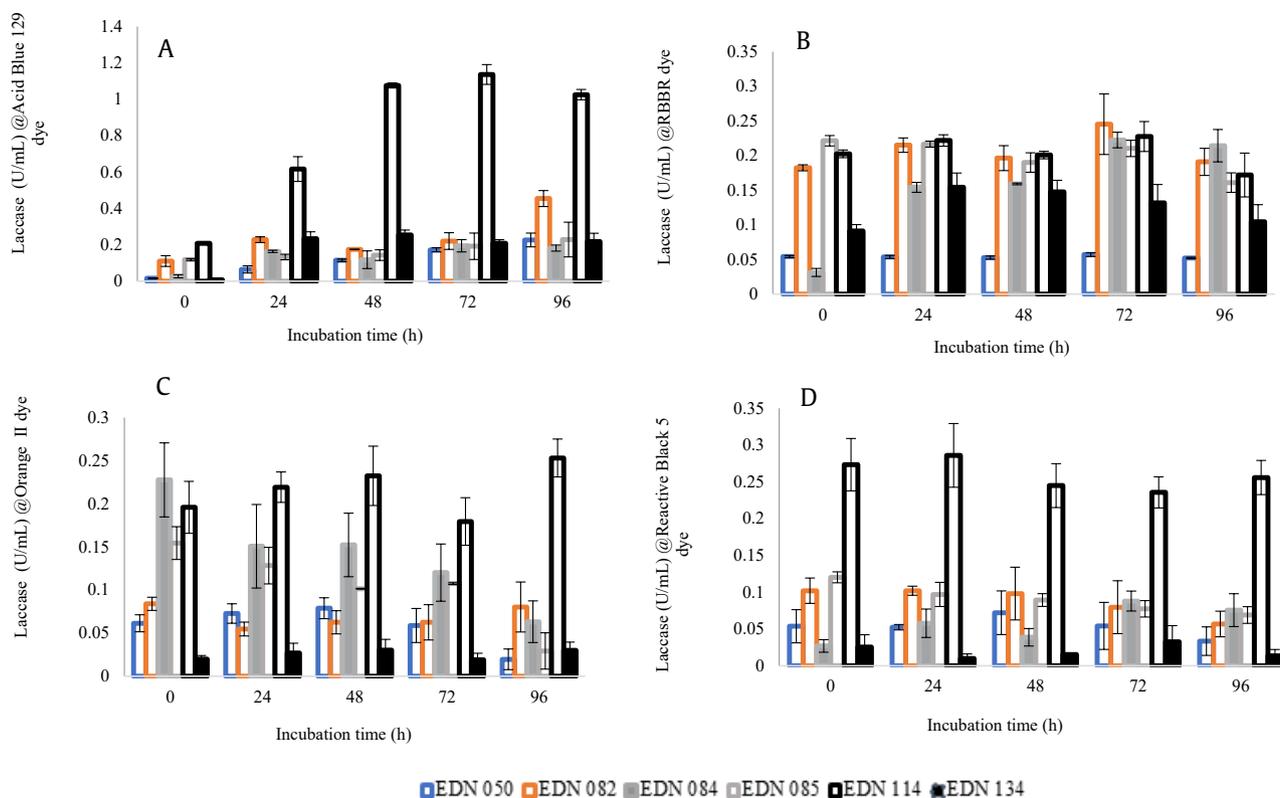

Figure 8. Laccase activity during decolorization process of dyes (1,000 ppm) (A) AB 129, (B) RBBR, (C) O II, (D) RB 5 by six potential fungi isolate for 96 h at 27±3°C

the fungus colony grows becomes clear or colorless. Various types of dyes can be used as a selective medium for the screening process of color-degrading fungi isolates. Koyani *et al.* (2014) used reactive golden yellow HRLN and reactive yellow FG for color decolorization tests. In addition, methyl orange dye has been used as a selective medium for fungi (Sharma *et al.* 2017).

The phylogenetic analysis of ITS rDNA gene sequence showed that isolate EDN 082, 084, and 085 were *T. hirsuta*, while isolate EDN 134 was *T. pavonia* due to the bootstrap values from those isolates being at the high level of support of sequence similarities (95-100%). On the other hand, strain EDN 114 is one clade to *Perenniporia subtephropora* and strain EDN 50 is one clade to *Deconica coprophila*. However, the bootstrap value from those strains was low support (<70%) that indicating the sequence is possibly different from the species in the GenBank but still related to genus level. Bootstrap value is the number of branch patterns in the replication tree that are formed again at the node similar to the original tree. Bootstrap values of 50–69% indicate low support, 70–75% suggest moderate support, and 76–94% suggest strong support for the topology at that particular node. While the bootstrap value of 95-100% indicates a very high level of confidence in the topology of the phylogenetic tree (Felsenstein 1985). Further sequence analyses from the partial fragments of the glyceraldehyde-3-phosphate dehydrogenase and the translation elongation factor 1-a genes are necessary to determine the identity of sequences strain EDN 114 and EDN 50 to the species level. All positive isolates in this study, except *Deconica*, are white rot-fungi. Most white-rot fungi have been used widely as dye-degrading fungi (Wang *et al.* 2018, 2020).

Hefnawy *et al.* (2017) reported that the boundary of the decolorization zone in the solid medium could not be determined significantly at higher dye concentrations. Therefore, the determination of six isolates decolorization ability was carried out not only in solid medium but also in liquid medium. In this study, decolorization of synthetic dyes on a liquid medium caused color losses but the color did not remain in the fungus mycelium. Fungus mycelium remained white and its mycelium did not change to blue color as a result of the presence of dyes in the medium. This result showed that the decolorization process occured not only by the adsorption process but also by the degradation process using extracellular enzymes produced by the fungus. Jebapriya and Gnanadoss (2013) explained that decolorization of dyes with some fungi occurred by adsorption mechanism but both adsorption and degradation mechanism can occur simultaneously or sequentially by white rot fungi.

Six positive isolates could decolorize four types of dyes at a concentration of 100 ppm in liquid media with a decolorization rate ranging from 78-99%. However, the decolorization rate of six isolates at 1,000 ppm was lower than at 100 ppm. The decolorization rate of six isolates at 1,000 ppm of anthraquinone (AB 129 and RBBR) dyes ranged from 38-96%, monoazo (O II) dyes range from 10-94% while for diazo dyes (RB 5) ranges from 1-35% for 96 h incubation. Each fungus isolate produced a different decolorization rate and it was also observed that the rate of degradation depends on the concentration of dyes (Jebapriya and Gnanadoss 2013). In addition to the type of fungus and concentration of dyes, the type of dyes also significantly affected the decolorization rate (Chaturvedi 2019). Dyes with a simpler structure were known to be easier to degrade. Based on this, decolorization of AB 129> RBBR> O II> RB 5, so that the anthraquinone dye can be completely decolorized using six positive isolates for 96 h incubation. As azo dyes (monoazo and diazo), it takes longer than anthraquinone dyes to complete the decolorization process. Lavanya *et al.* (2014) explained that the process of color removal in monoazo-dyes occurs faster when compared to diazo or triazo dyes.

This study also found that *T. pavonia* EDN 134 exhibited the highest decolorization activity, followed by *T. hirsuta* EDN 082. Ramadhan *et al.* (2021) reported that *T. polyzona* H18 could decolorize more than 90% of RBBR, AB129, and RB5 and over 70% of AO7/O II after 96 h incubation. White-rot fungi from the genus *Trametes* have known as decolorization agents since they are good laccase producers (Wang *et al.* 2018).

The decolorization of dyes was greatly influenced by ligninolytic enzymes such as laccase produced by fungi. In this study, the laccase production increased at 24-72 h incubation and decreased at the end of incubation. The distribution of the laccase enzyme also varied among isolates. *T. hirsuta* EDN 082 had a positive correlation between the production of laccase enzymes and synthetic dye decolorization. However, this phenomenon occurred on the contrary in *T. pavonia* EDN 134 and *Deconica coprophila* EDN 114. Eichlerova and Baldrian (2020) reported that in some strains, Orange G was decolorized by laccase and RBBR by MnP, and in other strains vice versa.

Further research should be carried out to determine whether only laccase or other enzymes play a role in decolorizing the dyes AB129, RBBR, O II and RB5.

In conclusion, this study successfully isolated and characterized the six potential fungal from Taman Eden 100, Toba Samosir, North Sumatra, Indonesia. The isolates were identified as *Trametes hirsuta* (three isolates), *Trametes pavonia* (one isolate), *Perenniporia subtephropora* (one isolate), and *Deconica coprophila* (one isolate). All isolates showed the ability to decolorize textile dyes of anthraquinone (AB 129, RBBR), monoazo (O II), and diazo (RB 5). *T. pavonia* EDN 134 exhibited the highest decolorization activity, followed by *T. hirsuta* EDN 082. Almost all isolates demonstrated a positive correlation between the production of laccase enzymes and synthetic dye decolorization. The study suggests that six fungal isolates could be a prospective microorganism for further use in textile wastewater treatment.

Acknowledgements

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