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## Screening of potential lignin-degrading fungi from the tropical forest for lignocellulose biotreatment

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# Screening of potential lignin-degrading fungi from the tropical forest for lignocellulose biotreatment

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**Abstract.** Lignocellulose can be converted into many products replacing products produced from fossil, such as bioenergy, biochemicals and biomaterial products through several treatment. In the bioethanol production, the lignin content in lignocellulose causes the conversion of cellulose to ethanol is less optimal. This research aimed to screen a number of fungal isolated from tropical forests to obtain the lignin-degrading fungi which are potentially used in lignocellulose biotreatment. Screening was conducted by growing fungal isolates on a minimal salt media containing black liquor as a carbon source. Fungal growth ability was observed based on growth index and lignin degradation by fungal isolates was determined by measuring the absorption spectra of the degradation medium at wavelengths of 280 nm and compared to uninoculated control media. The screening results showed that all of 10 isolates had the different ability to grow on minimal media containing black liquor. One fungal isolates identified as *Daldania eschscholtzii* SA2 146 showed the highest lignin degradation ability that can degrade lignin up to 15% of the total lignin in the media. The lignin degradation by five fungal isolates was range between 5% - 15%. This study proved that the obtained fungal isolates can degrade lignin and potentially be used in lignocellulose biotreatment. Further investigation will be conducted to evaluate the selectivity of potential fungi to degrade lignin.

## 1. Introduction

Nowadays, lignocellulose has become a concern of many researchers because it has the potential to be a source of primary renewable raw material hence it can be used sustainably. Lignocellulose can be converted into many products replacing products produced from fossil, such as bioenergy, biochemicals and biomaterial products [1]. Apart from being renewable, products from lignocellulose are also environmentally friendly, for example, the energy produced from bioethanol can reduce greenhouse gas emissions, and biomaterial products such as bioplastics can be decomposed easily. It is different from the case when using products from fossil [2, 3]. The biological method utilizes



biological agents in the form of microorganisms such as bacteria, yeast, and fungi in the processing of lignocellulose biomass to produce derivative products [4].

The presence of lignin in lignocellulose causes this material not to decompose readily in nature. A complex and rigid structure of lignin is the reason why it is difficult to decompose [5]. Related to the problem, some studies have been trying to encounter those issues. One of them is utilizing ligninolytic fungi as lignin biodegrading agents in lignocellulose. Lignolytic fungi are a group of fungi that are known to be able to produce a number of enzymes that can break down the complex structure of the lignin polymer into simple molecules. Generally, this enzyme was owned by many groups of rot fungi, specially white-rote fungi [6]. Until now, many white rot fungi that have been used in lignocellulose processing. For example, the most studied and efficiently degrade lignin strain are *Phanerochaeta chrysosporium*, *Pleurotus ostreatus* and *Ceriporiopsis subvermipora* [7, 8, 9, 10, 1, 12].

Lignolytic fungi have been used in lignocellulose biomass pretreatment for bioethanol production [13]. Lignocellulose pretreatment using ligninolytic fungi was reported to be able to increase bioethanol production [14]. Apart from being able to increase the amount of cellulose that can be converted, pretreatment using ligninolytic fungi also does not produce by products that can inhibit the reaction at a later stage [13]. Lignolytic fungi were also used in the biobleaching process in the paper industry as well as in making compost and bioremediation of xenobiotic compounds [15, 16, 17]. The biobleaching process can reduce the use of chlorine that is commonly used and reduce the production of toxic waste produced from the process. Some types of ligninolytic fungi that are utilized are *Coriolus versicolor*, *Trametes versicolor* and *Ceriporiopsis subvermispora* [18, 19, 20]. The presence of ligninolytic fungi which have high lignin degradation activity can accelerate compost formation time, especially materials that have high lignin content which takes a long time to decompose [16].

Although lignocellulose pretreatment by utilizing lignolytic fungi was considered as a method that is environmentally friendly and less expensive, the level of its use by industry is still low. One of the challenges is at the time of processing which takes longer than chemical or thermal processing, so it is considered less efficient in the production process [21]. Moreover, the problem of consuming cellulose during the treatment process by lignolytic fungi is also a particular problem especially in the lignocellulose pretreatment for bioethanol production. Pretreatment of lignocellulose for bioethanol requires the character of lignolytic fungi which have high lignin degradation ability and low cellulose consumption [22].

Efforts that can be made in overcoming this challenge are through the search for lignolytic fungus candidates with characteristics that are in line with industrial needs, including the rapid and specific lignin degradation ability. In addition, efforts to combine biotreatment processes with thermal processes are considered to increase the degradation reaction rate [13]. However, this process requires the character of lignolytic fungi that are resistant to high temperatures. The rate of degradation can also be increased by the microorganism consortium technique, which uses a combination of several microorganisms in the lignocellulose treatment process. This technique requires the character of microorganisms that can grow together and synergize positively in degrading the target substrate [21].

Based on the problem mentioned above, it is known that research on the search for potential microorganisms that can answer the industrial needs in processing lignocellulose, especially lignolytic fungi is still necessary. Therefore, this study aims to search for potential lignolytic fungi that are suitable for the needs of lignocellulose treatment. The study was conducted by selecting a number of fungal isolates owned by the Environmental Microbiology Laboratory at the Research Center for Biology-LIPI using a selective medium. The indicators used in the selection were the growth index of

fungal isolates in the selection medium and quantification of the level of lignin degradation in the selection medium after being treated with fungal isolates at specified time intervals.

## 2. Material and Methods

### 2.1. Source of microorganisms

The five fungal isolates used in this study were obtained from a collection of Environmental Microbiology Laboratory at the Research Center for Biology-LIPI. All fungal isolates were isolated from wood decay forest located in Central Kalimantan. Fungal isolates stored in preservation media contained paraffin at room temperature.

### 2.2. Pre-culture fungal isolates

All fungal isolates in the preservation media were grown in Potatoes Dextrose Agar (PDA) media at 30 °C. Fungal mycelia surface was first cleaned from paraffin (preservative agent) using sterile aquades several times. After that, mycelia is ready to be inoculated on PDA media. The mycelial growth of each isolate was observed by direct observation.

### 2.3. Black liquor preparation

Black liquor used in the study came from hydrolysis of oil palm empty bunches (OPEFB) using NaOH solution. 25 g of 40 mesh OPEFB were mixed with 1 M NaOH solution. The mixture was homogenized and the resulting suspension (10% (w / v)) was heated at 121 oC for one hour using an autoclave. The resulting hydrolysis product is filtered in a hot state to separate the black liquor from the solid. Black liquor was then neutralized by adding dilute H<sub>2</sub>SO<sub>4</sub> solution to obtain a pH solution ranging from 6.8 to 7.0. Black liquor is stored at 4 oC before used as a carbon source for selection media.

### 2.4. Screening for lignolytic fungi

The screening was carried out using two methods, namely calculating the fungal growth index and measuring the level of lignin degradation in the selection medium.

**2.4.1 Growth Index.** Screening is conducted by growing fungal isolates on solid selection media, namely a minimum salt medium with black liquor as the main carbon source (MSM-BL). The composition of MSM-BL is, 4.5 g / l K<sub>2</sub>HPO<sub>4</sub>; 0.53 g / l KH<sub>2</sub>PO<sub>4</sub>; 0.5 g / l CaCl<sub>2</sub> 2H<sub>2</sub>O; 0.5 g / l MgSO<sub>4</sub>.7H<sub>2</sub>O; 5 g / l NH<sub>4</sub>NO<sub>3</sub>; 0.001 g / l CuSO<sub>4</sub>.5H<sub>2</sub>O; 0.001 g / l FeSO<sub>4</sub> 7H<sub>2</sub>O; 0.001 g / l MnSO<sub>4</sub>.H<sub>2</sub>O; 0.001 g / l ZnSO<sub>4</sub>.7H<sub>2</sub>O; 0.001 g and 10% (v / v) black liquor. The 7-day-old fungal inoculants were cut to a diameter size of 0.8 cm from PDA culture and placed in the middle of the solid MSM-BL test media. The culture was incubated at 30 °C and 40 °C for seven days. The experiment is set by making three replications for each fungal isolate. The fungal growth index is

determined by measuring the diameter of the mycelial and observing the thickness of mycelia. Then compared to controls grown on PDA media. The growth index of fungal isolates on the selection media was calculated using the following equation:

$$\text{Growth Index (\%)} = \frac{\varnothing_s}{\varnothing_c} \times 100\% \quad (1)$$

Note:  $\varnothing_s$  are colony diameter in selection media (cm), and  $\varnothing_c$  is colony diameter on control media (cm).

*2.4.2 Lignin degradation.* Screening is performed on the liquid MSM-BL selection medium with the same composition as the solid MSM-BL, only differing from the black liquor concentration used, which is 1% (v / v) and without agar. Two pieces of 7 days old fungal isolate culture (1 cm diameter) were inoculated in 50 ml of MSM-BL 1% liquid media in 300 ml erlenmeyer. The culture was incubated in dark conditions at room temperature (28-30 °C). The experiment was set by making three replications for each fungal isolate. Observations were carried out periodically on days 7, 14 and 21 by measuring the absorbance of media supernatants at wavelengths of 200-400 nm using a UV-Vis (Varioscan, Thermoscientific) spectrophotometer. Lignin degradation was determined by observing changes in absorptions at wavelengths of 280 nm and compared to controls (MSM-BL media 1% liquid without inoculants). Lignin degradation was expressed in percent relative to controls such as the following formula:

$$\text{Lignin Degradation(\%)} = \frac{A_{cn} - A_{sn}}{A_{cn}} \times 100\% \quad (2)$$

Note:  $A_{cn}$  is the absorption of control on the n-day.  $A_{sn}$  is the absorption of a sample on the n-day.

*2.4.3 Absorbance measurement for lignin degradation.* Three ml of media culture were sampled at each time interval and centrifuged at 8000 rpm for 30 minutes, 4°C. The media supernatant obtained is separated from the cell pellets. Before the absorbance was measured, 250 ul of media supernatant was first diluted to 1000 µl using distilled water. Then 300 diluted supernatants are placed in the microplate reader. Sample absorbance is read in a wavelength range of 200-400 nm at intervals of 10 nm by spectrophotometer (Varioscan, Thermoscientific). The absorbance measurements of each sample were carried out three times repetitions.

## 2.5. Molecular identification of the fungal isolate

DNA isolation was started by growing fungal isolates in liquid media Potato Dextrose Broth (PDB) and incubated for 72 hours. Fungal mycelia were then harvested for DNA extraction. The extraction of fungal DNA was performed using the nucleon PHYTOPure (Amersham LIFE SCIENCE) nucleon

reagent. Strain identification was carried out by PCR amplification in ITS using ITS 4: (5'-TCCTCCGCTTATTGATATGC-3') and ITS 5: (5'-GGA AGT AAA AGT CGT AAC AAG G-3')

[23, 24]. PCR product purification was carried out with PEG precipitation method [25] and continued with the sequencing cycle. The sequencing cycle results are re-purified with ethanol purification method. Sequencing was carried out in Macrogen, South Korea. The DNA sequencing results were analyzed using the ChromasPro version 1.7.5 program (Technelysium Pty Ltd., South Brisbane, Australia) and the homologies were determined for species that already existed in GenBank database using the Basic Local Alignment Search Tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/blast.cgi>) of the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) to identify the approximate species.

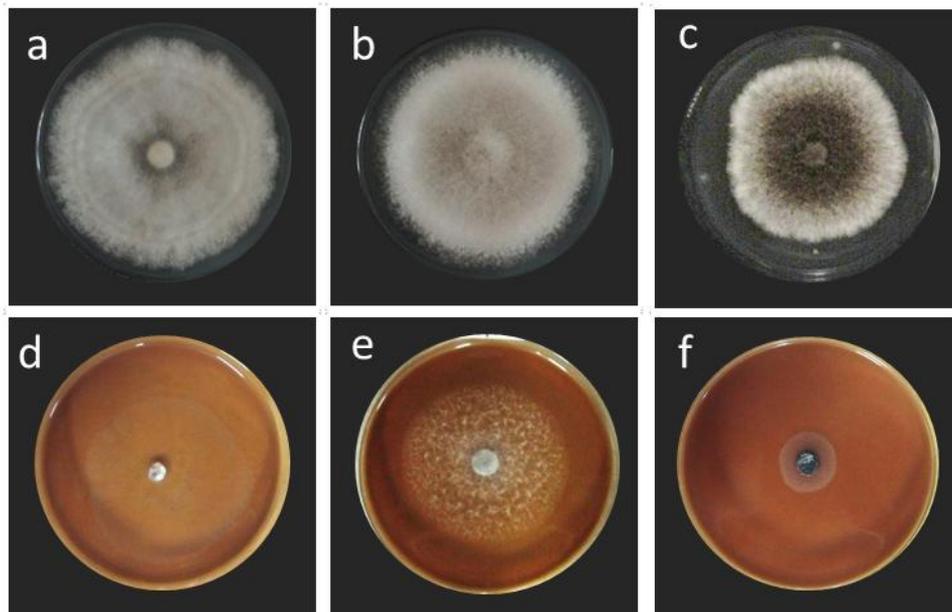
### 3. Results and Discussion

#### 3.1. Fungal growth index in solid media MSM-BL 10%

Until now there have been many media developed in the selection of lignolytic fungi qualitatively. In general, all media use salt media as the base media and type of carbon sources. Some of the compounds used as a single carbon source in a lignin selection medium are alkaline lignin (lignin kraft), guaiacol, tannic acid, Azure-B dye, poly-R dye, and ABTS. Ligninolytic activity was characterized by the presence of clear zones or color zones around the colony depending on the type of compound [26].

In this study we utilize black liquor as a single carbon source in the selection medium for lignolytic fungus selection. Black liquor is a by-product produced by lignocellulose hydrolysis and commonly found in the pulp production process in the paper industry. The use of black liquor in this study is expected to be an alternative source of carbon in selecting lignolytic fungi because it is readily available as well as less expensive. The major content in black liquor is lignin [27]. In principle, only fungi which can utilize lignin as a carbon source that will be able to grow on this medium. This ability is only possessed by fungi that can decompose lignin polymers into simple compounds so that they can be metabolized by fungal cells to produce energy to grow. Numerous studies have also reported that lignolytic fungi can grow well in media containing black liquor as their primary carbon source [28, 29, 30, 31, 32].

The growth observation of selected fungal isolates showed that all fungal isolates tested could grow on MSM-BL 10% media. However, not all isolates can grow optimally in this medium when compared to their growth on PDA media. Based on mycelial diameter and mycelial thickness it was known that two fungal isolates showed rapid growth when grown on selection media. One of them was SA2 146 which had an increase in mycelial diameter larger than control media (PDA) (Figure 1). Besides changes in growth, fungal isolates also showed slight changes in morphology, specifically the color of mycelia appearance which tends to turn white. Previously there were some fungal isolates which were light brown, green and light gray when grown on PDA media. The mycelial thickness all of fungal isolates grown on MSM-BL became thinner than grown on PDA. Data on the measurement of colony diameter and observation of mycelial thickness of each isolate can be seen in Table 1.



**Figure 1.** Fungal growth on PDA and MSM-BL 10% media. a) SA2 80 on PDA, b) SA2 149 on PDA, c) SA2 149 on PDA at 40 °C, d) SA2 80 on BSM-BL 10%, e) SA2 122 on MSM-BL 10%, f) SA2 149 on BSM-BL 10% at 40 °C.

**Table 1.** Growth observation data of fungi on solid medium MSM-BL 1% and PDA

Isolate code	30 °C					40 °C				
	Mycelial diameter (cm)		Mycelia Thickness		Growth Index (%)	Mycelial diameter (cm)		Mycelial Thickness		Growth Index (%)
	PDA	MSM-BL	PDA	MSM-BL		PDA	MSM-BL	PDA	MSM-BL	
SA2 80	7.30 ± 0.20	9.00 ± 0.00	++	+	123.3	0.80±0.00	0.90±0.00	-	-	1.10
SA2 85	7.35 ± 0.35	7.00 ± 0.10	+++	++	95.2	0.80±0.00	0.80±0.00	-	-	1.00
SA2 146	6.75 ± 0.25	9.00 ± 0.00	+++	++	133.3	0.80±0.00	1.15±0.05	-	+	1.40
SA2 149	8.00 ± 0.00	6.30 ± 0.20	+++	++	78.8	6.40±0.03	4.15±0.05	+++	+	64.8
KT2 106	5.15 ± 0.05	2.15 ± 0.10	+++	++	41.7	0.85±0.05	0.80±0.00	-	-	0.94

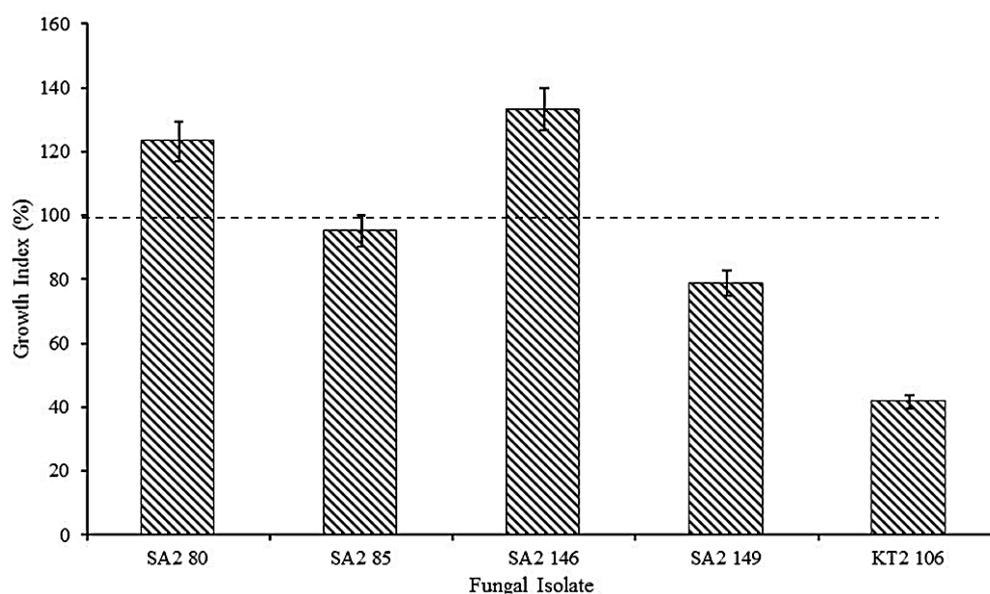
Note: - : not growing, + : thin, ++ : medium; +++ : thick

Besides observing the growth of fungal isolates at 30 °C, this study also observed the growth of fungal isolates at 40 °C. The observation showed that there was only one isolate capable of growing at relatively high temperatures (40 °C), namely SA2 isolates 149. SA2 149 isolate showed good growth in media selection with a growth index of 65%. On PDA media this isolate did not show significant

differences in mycelial thickness between 40 °C and 30 °C (Figure 1 b and c). Otherwise, this isolate grown slower on MSM-BL 10% than on PDA. Moreover, the mycelial color of SA2 149 grown on 40 °C was darker than grown on 30 °C and the mycelial diameter little bit decreased.

The growth index shown in Figure 2 inform that there are only two isolates that have a growth index above 100%, namely SA2 80 and SA2 146. Growth index 100% means the fungi have the same growth to rich media. If the growth index less than 100% it means that the fungi can not grow optimally and vice versa. Two isolates have a growth index in the range of 75% to 95%, namely SA2 85 and SA2 149 and there was only one isolate with low growth in this selection medium which was less than 50%, that is SA2 106 isolate.

The growth index of fungal isolates showed that several fungal isolates were able to utilize lignin as a source of carbon optimally. It is showed by SA2 146 isolate with an increase in growth index up to 133% (Figure 2). It can be assumed that the fungal isolates can produce ligninolytic enzymes that can degrade lignin and utilize it optimally as a source of nutrition for growth [33]. This growth can be observed from the extent of the diameter of the fungal colonies produced. SA2 146 fungal isolates showed the fastest growing ability in 10% MSM-BL media compared to other isolates.

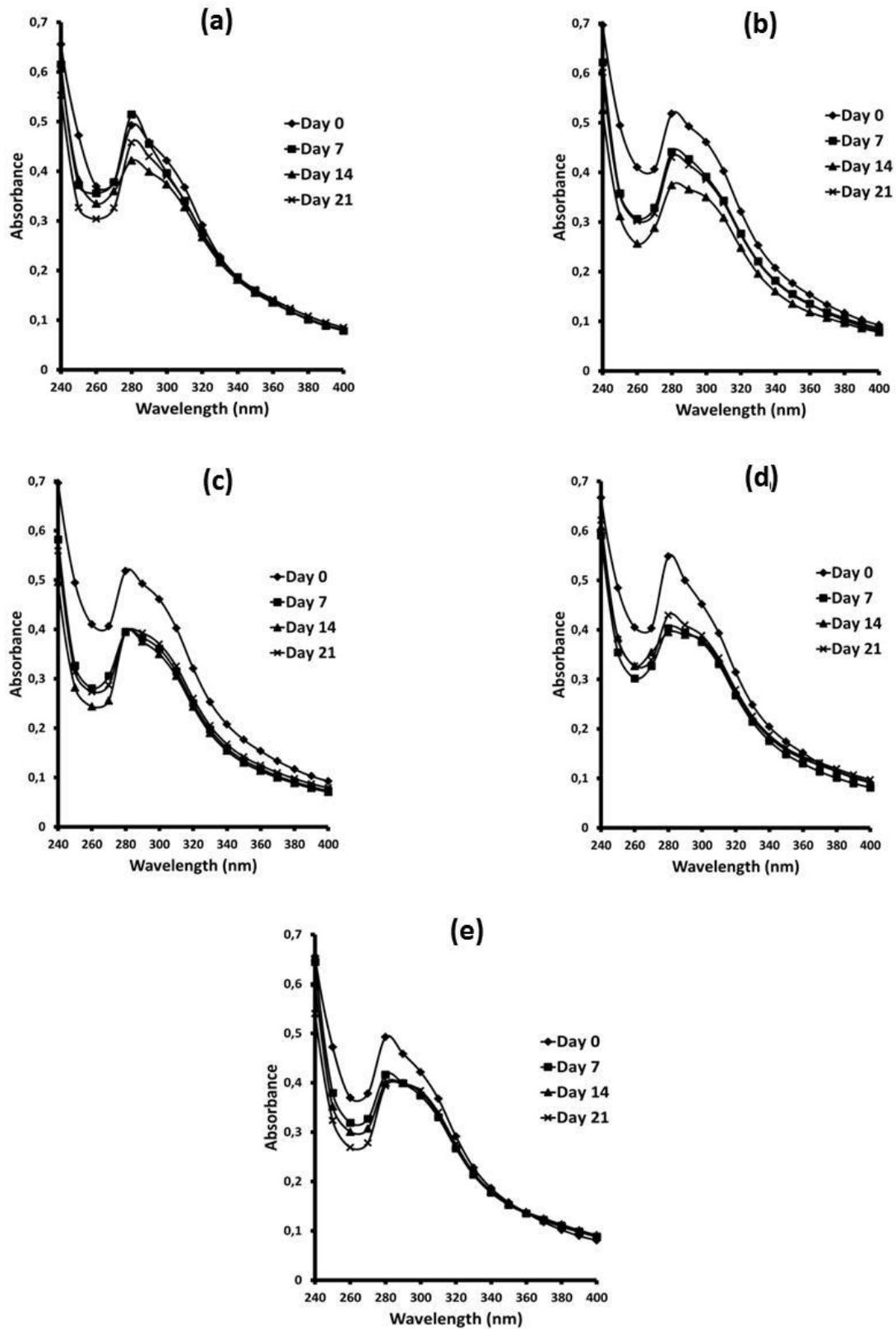


**Figure 2.** Growth Index of fungi on solid minimal salt medium containing black liquor (10%) as a sole carbon source.

### 3.2. Lignin degradation by fungal isolates in liquid media MSM-BL 1%

Screening conducted on solid media was a qualitative method that could not reflect the real performance of each isolate tested. It was used as a preliminary stage to detect the potential for lignin degradation possessed by fungal isolates. Quantitative further testing is needed to measure the capability of lignin degradation precisely [34]. Lignin content can be determined quantitatively through the measurement of the absorbance at a wavelength of 280 nm using a UV-VIS spectrophotometer [35,36]. The absorbance at 280 nm has a linear correlation with changes in the concentration of lignin or follows the Lambert-Beer's law. Therefore, absorption in this area can be

used to determine the concentration of lignin with the help of the standard lignin curve [37]. Maximum absorption at 280 nm is specific to the lignin polymer. It is due to the presence of aromatic ring compounds with an unsubstituted position (conjugated bond) in lignin. Besides, there are hydroxyl ether groups such as those in the guaiacyl lignin polymer constituent group also providing maximum absorption in the 280 nm region [35]. The results obtained in this study also showed maximum absorption in the area of 280 nm and was consistent in each sample measured during the specified time interval (Figure 3).

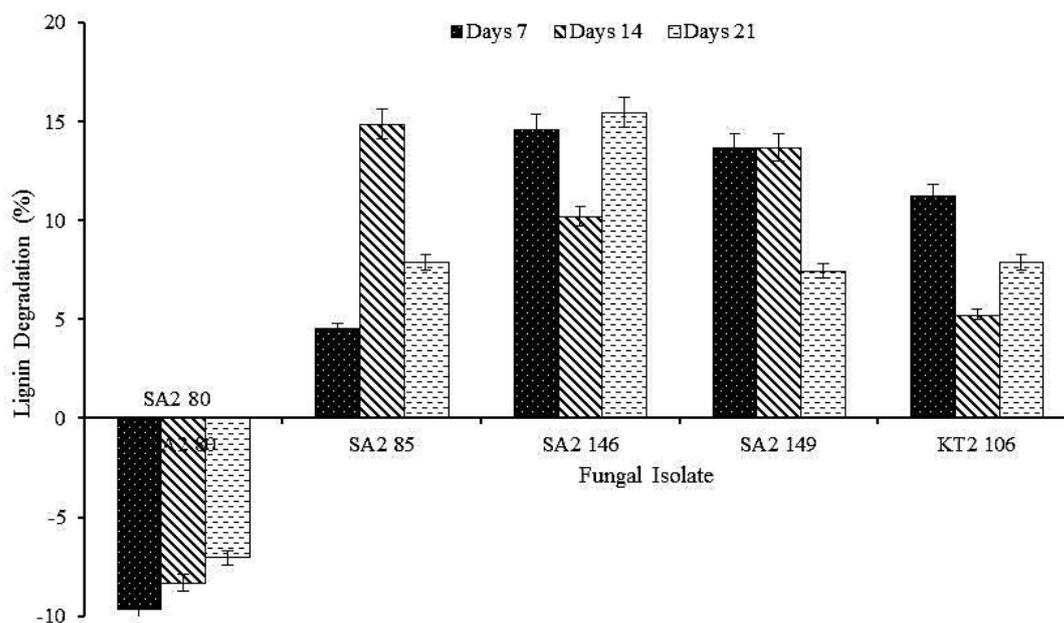


**Figure 3.** Day-wise absorption spectra of MSM-BM 1% medium after inoculated with fungus isolates.(a) SA2 80, (b) SA2 85, (c) SA2 146, (d) SA2 149, and (e) KT2 106.

Observation results of absorption of each media supernatant after being inoculated with fungal isolates showed consistent of absorption changes in the 280 nm area. Most of inoculated media absorption at 280 nm decreased along the incubation time. Chromatograms of changes in absorption of media supernatant from each isolate can be seen in Figure 3. A decrease started to occur on the first seven days of incubation, and after that, no significant decline occurred except those occurring in SA2 85 isolates. SA2 85 isolates decreased absorption more significant on the 14th day of observation compared to the 7th day of incubation. The sharp decrease was showed by SA2 146 and SA2 149 on the first seven days observation. Further incubation did not give a significant change in absorbance intensity.

The decrease in absorption intensity at 280 nm from lignin samples after being treated indicates that the degradation of lignin by fungal isolate has occurred. The different pattern of absorbance decrease of each fungal isolate could be assumed as different fungal strain. This assumption will be revealed by molecular identification analysis. The degradation process occurs involving many enzymes produced by fungi that catalyze the breaking of the bonds on the aromatic ring that composes the lignin polymer. Due to the reduced of aromatic groups that provide absorption at 280 nm region, it resulted in a decrease of absorption intensity [35].

Interestingly enough results were shown by SA2 80 isolates which had different lignin degradation characteristics with four other isolates. The degradation ability is negative because the absorbance of the sample has increased, so it is higher than the control, unlike the other four isolates which experienced a decrease in absorbance value (Figure 3a and 4). It was assumed that the SA2 80 have a different mechanism to degrade lignin compared to other isolates. The increase of absorption intensity at 280 nm might be due to the formation of a product having a rich  $\pi$ -electron cloud of aromatic ring conjugated group, or the aromatic ring compounds of lignin polymer are more exposable after the breakdown of lignin complex structure [38, 39].



**Figure 4.** Lignin degradation on MSM-BL 10% medium after inoculated with five fungus strains at 30 °C.

The lignin degradation by lignolytic fungi involves a combination of several enzymatic reactions. Three main types of enzymes play a role in the degradation of lignocellulose by lignolytic fungi, namely lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. These three enzymes are able to break down the bonds between the monomers that make up lignin so that cellulose can be released from lignin [40]. LiP or ligninase plays a significant role in lignin degradation by lignolytic microorganisms. This enzyme can degrade compounds that have high redox potential which cannot be oxidized by other enzymes either phenolic or non-phenolic compounds. Lignin peroxidase dependent on the presence of H<sub>2</sub>O<sub>2</sub> as an initiator of the oxidation reaction. MnP has the character of catalysis which is almost similar to LiP, but its oxidation ability is low and is unable to oxidize non-phenolic compounds. In addition, MnP involves binding Mn in its catalysis reaction. Laccase or phenol peroxidase is an enzyme capable of oxidizing phenol compounds without the need for H<sub>2</sub>O<sub>2</sub> mediation. The type of compound that is oxidized has an extensive range, and its activity varies in each lignolytic microorganism [41].

The level of lignin degradation by each fungal isolate can be seen in Figure 4. It shows that each isolate has a diverse ability to degrade lignin which ranges from 5-15% relative to the control. The highest lignin degradation was shown by SA2 146 fungi isolates which reached 15% followed by SA2 85 isolate at 14%, SA2 149 by 13%. The remaining two isolates have relatively low lignin degradation ability. This result in line with the growth index of this isolate. SA2 146 isolate also have a higher growth index than other fungal isolates (Figure 2). The highest lignin degradation capability played an important role in biotreatment [42]. The presence of lignin in lignocellulose hampers the degradation of lignocellulose. A complex and rigid structure of lignin is the reason why it is difficult to decompose [5]. Therefore, the fungal isolate SA2 146 that has highest lignin degradation is promising candidate for the biotreatment of lignocellulose.

### 3.3. Fungal isolate identification

Nowadays molecular identification through ITS region has become the most powerful tool to identification of fungi species. ITS region is commonly used as fungal barcode or DNA marker [43]. The result of BLAST analysis according to the ITS sequences revealed that all of the fungal isolates belong to Ascomycetes with high homology  $\geq 99\%$ , except for KT2 106 only has homology 97%. SA2 80 was identified as *Daldinia eschscholtzii*. SA2 85 was identified as *Hypoxylon investiens*. SA2 146 was identified as *Daldinia eschscholtzii*. SA2 149 was identified as *Hypoxylon* sp. and KT2 106 *Nemania primolutea*. Molecular identification result of the fungal isolate was shown in Table 2.

*Daldania eschscholtzii* is well known as endophytic wood-decaying fungi [44]. To date, its secondary metabolite gets more attention than its wood decay activity, especially by natural product researcher. This fungal species has been shown to produce potential bioactive compounds [45, 46]. *Hypoxylon investiens* was known as endophytic fungi which live in the plant without causes any diseases in the host plant [47]. *Nemania primolutea* was first reported in 2005 by Ju et al. [48] that was isolated from dead trunk *Artocarpus communis*. This species is also known as endophytic fungi that was found associated with plants [49] *Hypoxylon* sp. has the ability to produce bioactive volatile organic compounds (VOCs) with fuel potential and it belong to endophytic fungi [50].

**Table 2.** Homology level of fungal isolate based on BLASTN analysis of ITS rDNA gene

Isolate Code	References species (RS)	Accession No. RS	Identity (%)	Phylum
SA2 80	<i>Daldinia eschscholtzii</i>	KY792621	100	Ascomycetes
SA2 85	<i>Hypoxylon investiens</i>	KC968925	99	Ascomycetes
	<i>Daldinia eschscholtzii</i>	KY792620		Ascomycetes
SA2 146	<i>Hypoxylon</i> sp.	GQ334432	100	Ascomycetes
SA2 149	<i>Nemania primolutea</i>	EF026121	100	Ascomycetes
KT2 106			97	

Xylariaceae is well known as a common endophyte in tropical plants and its potential bioactive secondary metabolite compounds attract many researchers to exploit this fungus [51, 49, 45]. Few studies were found related to the ligninolytic ctivity of Xylariaceae. Lignolytic activity from Xylariaceae was first reported by Urairuj at al. in 2003 [52] isolated from the tropical plant. Based on our knowledge, there have been no reports related to the ligninolytic activity of *Daldania eschscholtzii*, *Hypoxylon investiens*, and *Nemania primolutea*. *Hypoxylon* sp. was reported that tolerance to polycyclic aromatic hydrocarbon (PAH) but it is still unclear whether this fungus use ligninolytic enzyme or other peroxidase enzymes [53].

Two fungal isolates in this study were identified as *Daldania eschscholtzii*, but each isolates has different lignin degradation character. This finding revealed that lignin degradation is strain specific. The thermotolerant fungal isolate from this study was identified as *Hypoxylon* sp. Thermotolerant lignolytic enzymes have become a subject of interest in industrial processes due to their ability to

degrade lignocellulosic biomaterial at elevated temperature [54]. Future research is needed to investigate the thermostability of the enzyme produced by this fungal isolate.

#### 4. Conclusion

This study has demonstrated the practical of screening lignin-degrading fungi using black liquor as a sole carbon source. Growth index and lignin degradation level could be used as the indicator of ligninolytic activity. The highest lignin degradation level was shown by *Daldania eschscholtzii* SA2 146 followed by *Hypoxylon investiens* SA2 85 and *Hypoxylon* sp. SA2 149, about 15% ,14%, and13% respectively. To our knowledge, this is the first report for the ligninolytic activity of *Daldania eschscholtzii*, *Hypoxylon investiens*, and *Nemania primolutea*. The lignin degradation of 5 fungal isolates was ranging from 5% to 15%. All of fungal isolates belong to Ascomycetes, Xylareaceae and endophytic fungi. This study proved that those fungal isolates degrade lignin and potentially be used in lignocellulose biotreatment. Further investigation will be conducted to evaluate the selectivity of potential fungi to degrade lignin.

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