Comparative Analysis of the Laccase Secretion Ability of Five White-rot Fungi in Submerged Fermentation with Lignocellulosic Biomass

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Lignocellulosic biomass is widely used in the field of laccase production because it has the advantages of low price and easy availability. Thus, a comparative analysis was performed of the laccase secretion ability of five white-rot fungi in submerged fermentation using single or mixtures of lignocellulosic biomass. Maximum laccase activity of Trametes gibbosa An 360, Vanderbylia fraxinea An 369, Perenniporia pyricola Han 202, Coriolopsis trogii Han 474, and Trametes versicolor Han 1504 was 55.83 ± 0.28 U/L on the mixture of corncob and cottonseed hull, 77.96 ± 1.60 U/L on corncob, 443.33 ± 15.49 U/L on corncob, 686.57 ± 16.49 U/L on corncob, and 162.04 ± 11.33 U/L on cottonseed hull. The mixed lignocellulosic material effectively improved the laccase activity of T. gibbosa An 360 compared with other fungal strains. However, the presence of corncob contributed to the secretion of laccase activity for V. fraxinea An 369, P. pyricola Han 202, and C. trogii Han 474. Meanwhile, cottonseed hull was conducive to the secretion of laccase of T. versicolor Han 1504. Laccase activity of P. pyricola Han 202 was very stable throughout most of the fermentation time. The results from this study provide new methods and increase fungal strains to improve laccase activity.

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Keywords: White-rot fungi; Single lignocellulosic biomass; Mixture lignocellulosic biomass; Laccase activity; Substrate selectivity

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INTRODUCTION

Lignocellulosic biomass, the most abundant renewable resource on earth, is usually treated as a cheap source of raw materials for renewable energy, chemicals, and other valuable products (Xiao et al. 2013). Lignocellulosic biomass has three main polymeric constituents: lignin, cellulose, and hemicellulose (Sánchez 2009). Lignin is dispersed between cellulose fibers, and hemicellulose is present and connected between lignin and cellulose fibers, thus forming a strong cellulose-hemicellulose-lignin structure that is similar to the “reinforced concrete structure” (Mosier et al. 2005). This “reinforced concrete structure” limits the contact between hydrolyase and cellulose, and the insolubility of lignin and the complexity of chemical structure are the main factors leading to the difficulty of lignocellulosic biomass degradation (Zhen et al. 2017; Choi et al. 2019).
Although physical or chemical methods can be used to effectively degrade lignocellulosic biomass, they usually require harsh and extreme conditions (Singh et al. 2015; Ma et al. 2020; Singh and Gupta 2020). The microorganisms can ferment and degrade lignocellulosic biomass by secreting extracellular lignocellulolytic enzymes (Sánchez 2009), which has the advantages of mild reaction conditions and lack of secondary pollution to the environment. It is of great significance to isolate and screen efficient lignocellulosic biomass-degrading microorganisms. Therefore, it is necessary to screen the microorganisms that can effectively break down lignin structure.

There are many kinds of microorganisms in nature, and white-rot fungi are recognized as a large group of microorganisms that can effectively degrade lignin (Dinis et al. 2009; Muthuvelu et al. 2020). Traditional lignin-degrading enzymes primarily include laccase, manganese peroxidase, and lignin peroxidase, among which laccase plays an important role in the process (Arora and Gill 2001). Laccase (EC 1.10.3.2), belongs to a family of blue copper oxidases; it has the capacity of oxidizing phenols and aromatic amines by reducing molecular oxygen to water (Sharma et al. 2019; An et al. 2021a,b). Laccases are widespread among higher plants, fungi, insects, and bacteria (Yadav et al. 2018; Zhao et al. 2018; Nuskern et al. 2021). Since laccase was discovered by Yoshida in 1883, it has become a hot research topic because of its potential application value (Pandi et al. 2019; Song et al. 2020). Today, laccase can be applied in various aspects of the industrial, biotechnological, and environmental fields, such as energy exploitation, biodetection, bio-degradation, bio-synthesis, nanoscience, and the beverage and food industry for the removal of phenolic compounds, and other miscellaneous applications (Baldrian 2006; Kudanga and Le Roes-Hill 2014; Bertrand et al. 2017; Yashas et al. 2018; Bilal et al. 2019; Singh and Arya 2019; Atilano-Camino et al. 2020; Liu et al. 2020; Perez-Montiel et al. 2021; Khatami et al. 2022). However, the production of large amounts of low cost and high activity laccases is the basis for the wide application of laccases (Couto and Sanromán 2005; Couto and Toca-Herrera 2007). Thus, it is necessary to promote the effective production of laccase by white-rot fungi.

Previous studies indicated that there are many factors affecting the laccase activity of fungi, such as carbon/nitrogen sources, lignocellulose materials, exogenous metal ions (Na\(^+\), Ca\(^{2+}\), Cu\(^{2+}\), Fe\(^{2+}\)), pH, fungal species, and so on (Atila et al. 2017; Zhou et al. 2017; Zhuo et al. 2017; Kostadinova et al. 2018; Xu et al. 2018; Lallawmsanga et al. 2019; Jasinska et al. 2019; Rodrigue et al. 2019; Sun et al. 2021). Lignocellulosic materials are good substrates for fungi-producing enzymes because of their low price. Thus, selecting suitable cheap lignocellulosic biomass for microorganisms to efficiently produce laccase is an important and lasting work (Mate and Alcalde 2017; Rodrigues et al. 2019; Habimana et al. 2021; Han et al. 2022a). Furthermore, submerged fermentation is generally recognized as the most economical way for the production of extracellular enzymes because of their significant advantages, such as low water requirements and no dilution of the enzymes (Kapich et al. 2004). There are few studies on the activity of mixed lignocellulosic materials on fungal laccase activity. Xu et al. (2020) investigated the effects of mixed lignocellulosic biomass (corn crop waste) on lignin-degrading enzyme activities, growth, and quality of Lentinula edodes. Han et al. (2021) analyzed the effect of the mixture of Pinus tabuliformis and Firmiana plataniolia on laccase activity secreted by three fungi. Therefore, five newly isolated fungal species were used for detecting the laccase activity. The comparative analysis of the laccase secretion ability of these fungi in submerged fermentation with lignocellulosic biomass was investigated. Meanwhile, the effect of using a mixture of lignocellulosic biomass on laccase activity was investigated.
for five tested fungi at the same time. These results contribute to new methods and increase the available fungal strains to improve laccase activity.

**EXPERIMENTAL**

**Materials**

**Microorganisms**

Five fungal species, *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 were isolated from Zizhen Park in Xiangyang city of Hubei Province (China), Yanghu Mountain in Xiangyang city of Hubei Province, Laoxiying Village in Chengde city of Hebei Province (China), Nature Park in Langfang city of Hebei Province, and Wuling Mountain in Chengde city of Hebei Province, respectively. The strains were purified on complete yeast medium (CYM) (g/L: glucose 20, peptone 2, yeast extract 2, MgSO$_4$·7H$_2$O 0.5, K$_2$HPO$_4$·3H$_2$O 1, KH$_2$PO$_4$ 0.46, and agar 15), maintained on malt extract agar (MEA) medium (g/L: glucose 10, malt extract 20, KH$_2$PO$_4$ 3, and agar 20), and stored in College of Life Science, Langfang Normal University (Langfang, China).

**Lignocellulosic biomass**

Corn cob and cottonseed hull were obtained from Chengde city, Hebei province (China). All of these air-dried lignocellulosic materials were ground to the practical size between 20- and 60-mesh.

**Methods**

**Microbial culture and inoculum preparation**

All fungal strains, *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504, were cultured on CYM at 26 °C to perform the process of activation. After 6 days, five inoculants with a diameter of 1.0 cm were obtained and transferred into 250-mL triangular flask containing 100 mL liquid CYM. All flasks were cultured in shaking incubators at 26 °C with the speed of 150 rpm. After 7 days, the mycelium pellets obtained were homogenized using a blender at 8000 rpm for 2 min to prepare the homogenized inoculum.

**Submerged fermentation for laccase activity**

Triangular flasks (250 mL) containing 100 mL liquid (KH$_2$PO$_4$ 1.5 g, MgSO$_4$·7H$_2$O 0.5 g, and deionized water 1 L) and 2 g lignocellulosic biomass were autoclaved at 121 °C for 30 min. Each component of the experimental group is described in Table 1. Then, 3 mL homogenized inoculum was added into each flask. All flasks were cultured into a rotary shaker with the speed of 150 rpm at 26 °C for submerged fermentation.

**Assay of laccase activity**

The crude enzyme solution was obtained by filtering the fermentation liquid through a filter paper and centrifuging it at 12000 rpm (4 °C) for 20 min. Laccase activity of the crude enzyme solution was detected by the UV-4802 spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China) with the optical density (OD) at 420 nm. The substrate used for laccase assay was 2,2’-azinobis-[3-ethylthiazoline-6-sulfonate] (ABTS).
The UV-determination process was performed according to the method of Han et al. (2021b, 2022a). One activity unit was defined as the amount of enzyme required to oxidize 1.0 µmol ABTS per minute ($\varepsilon_{420} = 3.6 \times 10^4$ M$^{-1}$ cm$^{-1}$).

### Table 1. Description of Each Component of the Experimental Group

<table>
<thead>
<tr>
<th>Species</th>
<th>Lignocellulosic Biomass (g)</th>
<th>Liquid (mL)</th>
<th>Homogenized Inoculum (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Han 474</td>
<td>Corncob 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 474</td>
<td>Cottonseed hull 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 474</td>
<td>Corncob 1 and Cottonseed hull 1</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 202</td>
<td>Corncob 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 202</td>
<td>Cottonseed hull 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 202</td>
<td>Corncob 1 and Cottonseed hull 1</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>An 360</td>
<td>Corncob 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>An 360</td>
<td>Cottonseed hull 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>An 360</td>
<td>Corncob 1 and Cottonseed hull 1</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 1504</td>
<td>Corncob 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 1504</td>
<td>Cottonseed hull 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Han 1504</td>
<td>Corncob 1 and Cottonseed hull 1</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>An 369</td>
<td>Corncob 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>An 369</td>
<td>Cottonseed hull 2</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>An 369</td>
<td>Corncob 1 and Cottonseed hull 1</td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

**Data analysis**

Two-way analysis of variance (ANOVA) was applied to analyze the effects of lignocellulosic biomass and fungal species on laccase activity by following Han et al. (2021b) using and using SPSS 22.0 (PROC GLM, Armonk, NY, USA). All colorful figures were generated by the software of Origin 2016 (OriginLab Corporation, Northampton, MA, USA).

### RESULTS AND DISCUSSION

#### Statistical Analysis Result

Previous studies indicated that fungal species and agricultural and forest residues can significantly affect the laccase activity (Elisashvili et al. 2008; Janusz et al. 2015; An et al. 2016, 2021a,b; Han et al. 2021, 2022a). Similarly, as shown in Table 2, fungal species and lignocellulosic biomass could affect the laccase activities significantly ($P < 0.001$) through the whole submerged fermentation stage. Further, the interactions of fungal species and lignocellulosic biomass could affect the laccase activity significantly ($P < 0.001$).

#### Effect of Various Fungal Species on Laccase Activity

Previous studies evaluated the laccase secretion ability and found that it varies from different *Basidiomycetous* white-rot fungus or different strains belonging to same species (Janusz et al. 2015; An et al. 2016; Han et al. 2021, 2022a). Thus, the assessment of capacity of laccase production from new strains isolated from their natural ecological environment is an important task for obtaining strains with high production capacity. The
levels of extracellular laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 were compared.

**Table 2.** Two-way ANOVA to Analyze the Effects of Fungal Species, Lignocellulosic Biomass, and the Interactions between Fungal Species and Lignocellulosic Biomass on Laccase Activities

<table>
<thead>
<tr>
<th>Incubation Period (d)</th>
<th>Fungal Species</th>
<th>Lignocellulosic Biomass</th>
<th>Fungal Species × Lignocellulosic Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1394.026***</td>
<td>169.469***</td>
<td>222.485***</td>
</tr>
<tr>
<td>2</td>
<td>1989.127***</td>
<td>165.302***</td>
<td>446.185***</td>
</tr>
<tr>
<td>3</td>
<td>1815.177***</td>
<td>361.831***</td>
<td>707.617***</td>
</tr>
<tr>
<td>4</td>
<td>989.416***</td>
<td>49.343***</td>
<td>283.889***</td>
</tr>
<tr>
<td>5</td>
<td>921.434***</td>
<td>32.932***</td>
<td>301.983***</td>
</tr>
<tr>
<td>6</td>
<td>825.474***</td>
<td>70.958***</td>
<td>95.143***</td>
</tr>
<tr>
<td>7</td>
<td>1102.169***</td>
<td>4.123***</td>
<td>62.616***</td>
</tr>
<tr>
<td>8</td>
<td>3226.846***</td>
<td>391.073***</td>
<td>409.607***</td>
</tr>
<tr>
<td>9</td>
<td>9312.340***</td>
<td>641.941***</td>
<td>657.984***</td>
</tr>
<tr>
<td>10</td>
<td>2216.403***</td>
<td>55.879***</td>
<td>99.642***</td>
</tr>
</tbody>
</table>

**Note:** df = 4, 2, 8; ***P < 0.001; The values were the F-value of Two-way ANOVA

In terms of corncob, the laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 at the first day of the submerged fermentation stage was 0 ± 0 U/L, 9.54 ± 0.70 U/L, 38.06 ± 3.06 U/L, 2.87 ± 0.16 U/L, and 10.65 ± 0.16 U/L (Fig. 1), respectively. However, *P. pyricola* Han 202 needs a shorter adaptation time to grow on corncob and produced laccase faster than other fungal species. Han et al. (2022b) analyzed the extracellular laccase activity among *Ganoderma* and *Coriolopsis* species on different lignocellulosic wastes and found that the laccase activity of *Coriolopsis trogii* Han 1211, *Ganoderma lingzhi* Han 1345, *Coriolopsis strumosa* Han 1356, and *Ganoderma applanatum* Han 1578 fermentation on corncob at the 1st day was 1.61 ± 0.17 U/L, 0.30 ± 0 U/L, 0 ± 0 U/L, and 0 ± 0 U/L, respectively. Thus, the laccase activity of *V. fraxinea* An 369 and *P. pyricola* Han 202 fermented on corncob at the 1st day was higher than that from some strains belonging to genus of *Coriolopsis* and *Ganoderma*. Maximum laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 was 12.13 ± 1.12 U/L, 77.96 ± 1.60 U/L, 443.33 ± 15.49 U/L, 686.57 ± 16.49 U/L, and 49.07 ± 4.57 U/L, and the corresponding time was 2nd day, 2nd day, 3rd day, 8th day, and 3rd day (Fig. 1, Table 3), respectively. Based on this, maximum laccase activity of *C. trogii* Han 474 was nearly 56.60-fold, 8.81-fold, 1.55-fold, and 13.99-fold higher than that from *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, and *T. versicolor* Han 1504, relatively. Maximum laccase activity of *C. trogii* Han 1211, *G. lingzhi* Han 1345, *C. strumosa* Han 1356, and *G. applanatum* Han 1578 grown on corncob was 151.50 ± 3.32 U/L at 3rd day, 333.74 ± 11.41 U/L at 8th day, 116.94 ± 2.09 U/L at 6th day, and 35.16 ± 2.93 U/L at 6th day (Han et al. 2022b). Maximum laccase activity of *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751 fermentation on corncob was 1.51 ± 0 U/L, 19.89 ± 1.31 U/L, and 90.92 ± 4.01 U/L, respectively (Liu et al. 2022). Clearly, maximum laccase activity of *P. pyricola* Han 202 and *C. trogii* Han 474 was higher when compared with the laccase activity from other strains in previous studies (Han et al. 2022b; Liu et al. 2022).

With cottonseed hull, the laccase activity of *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 was 7.22 ± 0.48 U/L, 11.76 ± 0.42 U/L, 105.83 ± 7.52 U/L, 9.26 ± 0.16 U/L, and 0 ± 0 U/L for the 1st day of the submerged fermentation (Fig. 2). Thus, the laccase activity from *P. pyricola* Han 202 at the 1st day was 14.66-fold, 9.00-fold, and 11.43-fold higher than that from *T. gibbosa* An 360, *V. fraxinea* An 369, and *C. trogii* Han 474, respectively. The laccase activity of *Coriolopsis trogii* Han 1211, *Ganoderma lingzhi* Han 1345, *Coriolopsis strumosa* Han 1356, and *Ganoderma applanatum* Han 1578 fermentation on cottonseed hull at the 1st day was 28.03 ± 2.09 U/L, 7.03 ± 0.63 U/L, 0 ± 0 U/L, and 19.09 ± 0.76 U/L, respectively (Han et al. 2022b). The values of laccase activity from *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751 fermented on cottonseed hull were 1.51 ± 0 U/L, 1.21 ± 0 U/L, and 1.21 ± 0 U/L on the 1st day, respectively (Liu et al. 2022). Therefore, *P. pyricola* Han 202 showed higher laccase activity on day 1 than the strains previously used and exhibited excellent ability to secrete laccase. Maximum laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 was 29.54 ± 1.12 U/L (3rd day), 65.46 ± 0.32 U/L (4th day), 159.26 ± 3.31 U/L (7th day), 288.80 ± 14.49 U/L (9th day), and 162.04 ± 11.33 (2nd day), respectively (Table 3). Maximum laccase activity of *C. trogii* Han 1211, G. lingzhi Han 1345, C. strumosa Han 1356, G. applanatum Han 1578, P. acerina Han 618, T. hirsuta Han 726, and C. trogii Han 751 grown on cottonseed hull was 223.03 ± 11.51 U/L at 8th day, 84.09 ± 7.69 U/L at 5th day, 203.64 ± 5.89 U/L at 5th day, 41.09 ± 3.60 U/L at 2nd day, 28.03 ± 1.88 U/L at 9th day, 27.43 ± 1.59 U/L at 5th day, and 16.58 ± 0 U/L at 8th day, respectively (Han et al. 2022b; Liu et al. 2022).
Table 3. Maximum Value of Laccase Activity and Occurrence Time of *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 in Submerged Fermentation with Lignocellulosic Biomass

<table>
<thead>
<tr>
<th>Maximum Laccase Activity (U/L)</th>
<th>Lignocellulosic Biomass</th>
<th>Species</th>
<th>Time (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.13 ± 1.12</td>
<td>Corncob</td>
<td><em>Trametes gibbosa</em> An 360</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>29.54 ± 0.32</td>
<td>Cottonseed hull</td>
<td><em>Trametes gibbosa</em> An 360</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
</tr>
<tr>
<td>55.83 ± 0.28</td>
<td>Corncob and Cottonseed hull</td>
<td><em>Trametes gibbosa</em> An 360</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>77.96 ± 1.60</td>
<td>Corncob</td>
<td><em>Vanderbylia fraxinea</em> An 369</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>65.46 ± 0.32</td>
<td>Cottonseed hull</td>
<td><em>Vanderbylia fraxinea</em> An 369</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>42.5 ± 0.96</td>
<td>Corncob and Cottonseed hull</td>
<td><em>Vanderbylia fraxinea</em> An 369</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>443.33 ± 15.49</td>
<td>Corncob</td>
<td><em>Perenniporia pyricola</em> Han 202</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
</tr>
<tr>
<td>159.26 ± 3.31</td>
<td>Cottonseed hull</td>
<td><em>Perenniporia pyricola</em> Han 202</td>
<td>7&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>172.50 ± 15.00</td>
<td>Corncob and Cottonseed hull</td>
<td><em>Perenniporia pyricola</em> Han 202</td>
<td>7&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>686.57 ± 16.49</td>
<td>Corncob</td>
<td><em>Coriolopsis trogii</em> Han 474</td>
<td>8&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>288.80 ± 14.49</td>
<td>Cottonseed hull</td>
<td><em>Coriolopsis trogii</em> Han 474</td>
<td>9&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>379.54 ± 22.46</td>
<td>Corncob and Cottonseed hull</td>
<td><em>Coriolopsis trogii</em> Han 474</td>
<td>8&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>49.07 ± 4.57</td>
<td>Corncob</td>
<td><em>Trametes versicolor</em> Han 1504</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
</tr>
<tr>
<td>162.04 ± 11.33</td>
<td>Cottonseed hull</td>
<td><em>Trametes versicolor</em> Han 1504</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>75.19 ± 4.05</td>
<td>Corncob and Cottonseed hull</td>
<td><em>Trametes versicolor</em> Han 1504</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Maximum values of laccase activity are presented as mean value ± standard deviation for biological triplicates.

It was clear that the laccase secretion capacity of *C. trogii* Han 474 was superior to other strains used in the previous study (Han *et al*. 2022b; Liu *et al*. 2022) due to their maximum laccase activities. In contrast, although the maximum laccase activity of *P. pyricola* Han 202 was not the largest among the five fungi, its laccase activity was very stable and remained above 100 U/L through most of the fermentation time (Fig. 2).

When a mixture of corncob and cottonseed hull was used, the laccase activity of *P. pyricola* Han 202 on the 1<sup>st</sup> day was 57.69 ± 1.89 U/L, which is nearly 12.46-fold and 1.52-fold higher than that from *C. trogii* Han 474 and *T. versicolor* Han 1504, respectively (Fig. 3). Meanwhile, no laccase activity was detected from *T. gibbosa* An 360 and *V. fraxinea* An 369 on 1<sup>st</sup> day. Thus, *P. pyricola* Han 202 could produce higher laccase activity in a short time compared with other fungal species.
Fig. 2. Laccase activity from *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 in submerged fermentation with cottonseed hull

Maximum laccase activity of *C. trogii* Han 474 was 379.54 ± 22.46 U/L at the 8th day, nearly 6.80-fold, 8.93-fold, 2.20-fold, and 5.05-fold higher than that from *T. gibbosa* An 360 (55.83 ± 0.28 U/L, 4th day), *V. fraxinea* An 369 (42.5 ± 0.96 U/L, 2nd day), *P. pyricola* Han 202 (172.50 ± 15.00 U/L, 7th day), and *T. versicolor* Han 1504 (75.19 ± 4.05 U/L, 3rd day), respectively (Table 3).
Laccase activities in tree leaves and mandarin peels in submerged fermentation from *Lentinus edodes* IBB 123, *L. edodes* IBB 363, *Pleurotus dryinus* IBB 903, *P. ostreatus* IBB 8, *P. ostreatus* 2175, *P. ostreatus* 2191, and *P. tuberregium* IBB 624 were respectively 39 ± 2.8 and 4 ± 0.3 U/flask, 89 ± 6.5 and 6 ± 0.6 U/flask, 6 ± 0.5 and 205 ± 2.1 U/flask, 11 ± 0.9 and 24 ± 2.0 U/flask, 5 ± 0.5 and 17 ± 1.7 U/flask, 4 ± 0.5 and 17 ± 1.4 U/flask, and 7 ± 0.6 and 19 ± 1.3 U/flask (Elisashvili et al. 2008). It is easy to find that various fungal species and different strains of the same species have great differences in the secretion of laccase.

### Effect of Various Lignocellulosic Biomass on Laccase Activity

Solid lignocellulosic biomass used for cultivating fungi has the potential not only to reduce the pollution of solid agricultural and forestry waste, but also it could create economic value, such as the enzyme products (Gupta and Jana 2019; Leite et al. 2019). There are many kinds of lignocellulose biomass used in producing chemical products, and poplar, sugarcane bagasse, tree leaves, corncob, and straw were commonly used in previous studies (Gaikwad and Meshram 2019; Atilano-Camino et al. 2020; Malhotra and Suman 2021; Liu et al. 2022). Cottonseed hull and corncob used in the present study are by-products of cotton and corn, which are of great yield and low price in China. Thus, evaluation of the cheap and readily available lignocellulosic biomass materials for fungi to produce enzymes is of interest for the production of low-cost enzyme products.

Laccase activity of *T. gibbosa* An 360 on the 1st day was only detected on cotton hull (7.22 ± 0.48 U/L) (Figs. 1 to 3). However, the maximum value of laccase production from *T. gibbosa* An 360 was obtained with a lignocellulosic mixture of cottonseed hull and corncob, 55.83 ± 0.28 U/L, which was 4.62-fold and 1.89-fold higher than that from corncob and cottonseed hull used alone (Table 3). It was clear that the mixed lignocellulosic material effectively improved the laccase activity of *T. gibbosa* An 360. At the 1st day, laccase activity from *V. fraxinea* An 369 on corncob, cottonseed hull, and the mixture of corncob and cottonseed hull was 9.54 ± 0.70 U/L, 11.76 ± 0.42 U/L, and 0 ± 0 U/L, respectively. Maximum laccase activity from *V. fraxinea* An 369 on corncob (77.96 ± 1.60 U/L) and cottonseed hull (65.46 ± 0.32 U/L) was higher than that on the mixture of corncob and cottonseed hull (35.56 ± 2.37 U/L). The value of laccase activity from *P. pyricola* Han 202 for the 1st day was 38.06 ± 3.06 U/L on corncob, 105.83 ± 7.52 U/L on cottonseed hull, and 57.69 ± 1.89 U/L on the mixture of corncob and cottonseed hull. During the test with various lignocellulosic biomass, maximum laccase activity of *P. pyricola* Han 202 was 443.33 ± 15.49 U/L, which occurred on corncob for the 3rd day. Similar to the phenomenon from *V. fraxinea* An 369, mixture of corncob and cottonseed hull was not useful for improving laccase activity to *P. pyricola* Han 202. However, it is worth noting that the laccase activity of *P. pyricola* Han 202 on each material remained relatively stable. For example, the laccase activity on corncob was greater than 200 U/L for most of the time. Similarly, maximum laccase activity of *C. trogii* Han 474 and *T. versicolor* Han 1504 appeared on corncob (686.57 ± 16.49 U/L) and cottonseed hull (162.04 ± 11.33 U/L), respectively. Highest laccase activity from *Pseudolagarobasidium* sp. PP17-33 fermented on oil palm decanter cake was 5.841 U/g, which was obtained via Plackett–Burman design (Thamvithayakorn et al. 2019). The optimal conditions for *Trametes versicolor* to produce laccase used wheat bran as submerged fermentation substrate at 35 °C and 5 g/L, approximately 200 U/mL on 11 days (Atilano-Camino et al. 2020). Most studies have focused on the effects of single lignocellulosic materials on laccase production by fungi, whereas few studies have investigated the effect of mixed...
lignocellulosic materials on fungal laccase production (Han et al. 2021). Han et al. (2021) found that the mixture of Pinus tabuliformis and Firmiana platanifolia was beneficial for improving the laccase activity by Cerrena unicolor Han 849. Similarly, the mixture lignocellulosic biomass with cottonseed hull and corncob was useful for enhancing the laccase activity of T. gibbosa An 360. Thus, it also can be seen that laccase activity of fungal strains fermented on different lignocellulosic biomass was existent.

CONCLUSIONS

1. Overall, the capacity of secreting laccase by Coriolopsis trogii Han 474 on all tested lignocellulosic biomass materials was superior to other four fungal strains, Trametes gibbosa An 360, Vanderbylia fraxinea An 369, Perenniporia pyricola Han 202, and Trametes versicolor Han 1504.

2. It was demonstrated that the mixed lignocellulosic material effectively improved the laccase activity of T. gibbosa An 360 compared with other fungal strains. However, the presence of corncob contributed to the secretion of laccase from V. fraxinea An 369, P. pyricola Han 202, and C. trogii Han 474. Meanwhile, cottonseed hull was conducive to the secretion of laccase of T. versicolor Han 1504.

3. Although the maximum laccase activity of P. pyricola Han 202 was not the largest among the five fungi, its laccase activity was very stable throughout most of the fermentation time.

4. Maximum laccase activity of T. gibbosa An 360, V. fraxinea An 369, P. pyricola Han 202, C. trogii Han 474, and T. versicolor Han 1504 were 55.83 ± 0.28 U/L on the mixture of corncob and cottonseed hull, 77.96 ± 1.60 U/L on corncob, 443.33 ± 15.49 U/L on corncob, 686.57 ± 16.49 U/L on corncob, and 162.04 ± 11.33 U/L on cottonseed hull.

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