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Changes in lignocellulolytic enzyme activity during the degradation of *Picea jezoensis* wood by the white-rot fungus *Porodaedalea pini*

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ABSTRACT

The extracellular lignocellulolytic enzymes secreted by *Porodaedalea pini* were investigated for their ability to degrade the wood of *Picea jezoensis* over various time periods. In addition, changes in wood chemical component contents were also investigated. Enzyme production and wood degradation by *P. pini* significantly increased starting at 60 days of incubation. The total lignin, holocellulose, and α-cellulose contents, as well as pH, decreased during the degradation process, while hot water, 1% NaOH, and ethanol-toluene extract contents significantly increased. These results indicate that *P. pini* simultaneously degraded the lignin and polysaccharides of *P. jezoensis* wood. Additionally, *P. pini* continuously produced xylanase, β-glucosidase, and endoglucanase with higher activities than those of exoglucanase and cellobiose dehydrogenase. Manganese(II)-dependent peroxidase showed the highest ligninolytic activity, followed by lignin peroxidase and laccase. These results indicate that *P. pini* produces a variety of lignocellulolytic enzymes, and that the produced enzymes contribute to the degradation of *P. jezoensis* wood components.

1. Introduction

The great capability of white-rot fungi to degrade wood components is mainly results from the activites of different kinds of extracellular enzymes (Baldrian, 2003; Levin et al., 2007; Dashtban et al., 2009; Irbe et al., 2014). Some white-rot fungi removed lignin, cellulose, and hemicelluloses simultaneously, while others selectively degraded lignin and hemicelluloses first, followed by cellulose (Eriksson et al., 1990; Yoshizawa et al., 1992; Machuca and Ferraz, 2001; Tanaka et al., 2009). These fungi secrete oxidative enzymes, such as lignin peroxidase, manganese(II)-dependent peroxidase, and laccase, to degrade lignin, as well as hydrolytic enzymes, such as cellulase, hemicellulases, and pectinase (Machuca and Ferraz, 2001; Ferraz et al., 2003; Levin et al., 2007).

Much research is needed to fully understand the degradation of wood by white-rot fungi, especially of fungal enzyme production. Such information could help to elucidate the biochemical mechanisms of wood degradation by fungi and, consequently, to facilitate fungal strain selection for biopulping and other industrial biotechnological applications (Machuca and Ferraz, 2001).

Porodaedalea is a genus of white-rot fungi that belongs to the *Hymenochaetaceae* family, and it is widely distributed in North America, East Asia, and Europe (Tomšovský et al., 2010). The fungus is a conifer parasite (Szewczyk et al., 2014) and an economically damaging pathogen of *Pinus* and *Picea* trees (Tomšovský et al., 2010). *Porodaedalea pini* is distributed in East Asia on indigenus *Picea jezoensis* (Siebold et Zucc.) Carrière (Dai, 1999), which is an important plantation tree species in Hokkaido, Japan (Narukawa et al., 2003).

Studies of wood degradation by *P. pini* and its enzyme production have been conducted by several researchers (Yamashita et al., 1978; Blanchette, 1980; Yoshizawa et al., 1992). Yoshizawa et al. (1992) examined the degradation of *Chamaecyparis obtusa* Endl sapwood by *P. pini*. They found that this fungus produces a cellulase whose activity is greater than that of ligninolytic enzymes, whereas it degrades lignin to a greater extent than carbohydrates (Yoshizawa et al., 1992). However, detailed relationships between the amounts of remaining wood chemical components and

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enzymatic activities have not been established, especially for cellulose, hemicellulose, and ligninolytic enzymes during wood degradation by this fungus.

In the present study, *P. jezoensis* wood was degraded by *P. pini* under laboratory conditions. During the degradation, the types and amounts of enzymes produced were assayed. In addition, the relationships between enzymes and mass loss, wood chemical components, and pH change are discussed.

2. Materials and methods

2.1. Microorganism and culture conditions

A white-rot fungus, *P. pini* strain WD1174, which was originally provided from the Forestry and Forest Products Research Institue, Tsukuba, Japan, was used in this study. The fungus was pre-cultured on potato-dextrose-agar (PDA; Difco, Becton, Dickinson and Company, USA) medium in 9-cm diameter Petri dishes at 26 °C for 27 days. To study enzyme production and wood degradation, polypropylene bottles (volume, 850 ml; mouth diameter, 58 mm) containing 100 ml of medium (4% glucose, 0.3% peptone, 1.5% malt extract, and 2% agar) were sterilized by autoclaving (HV-110, Hirayama, Japan) at 121 °C and 1.2 atm for 20 min. Seven-millimeter-diameter mycelial disks were punched from pre-cultured PDA plates using a cork borer, and inoculated into the medium in bottles. Then, the fungus was incubated at 26 ± 2 °C and 70% relative humidity.

2.2. Wood samples

Small wood blocks (approximately 2.0 cm × 2.0 cm × 1.0 cm in radial, tangential and longitudinal directions, respectively) without any defects, such as resin, gum, or knots, and no visible evidence of decay, were prepared from wood samples collected from the heartwood of a single *P. jezoensis* tree. Before degradation, the specimens were dried at 60 °C for 48 h and then weighed to determine their initial dry mass. The specimens were sterilized with propylene oxide gas for 2 days in a desiccator. After sterilization, the moisture content of the blocks was adjusted to 50–70% by dipping them into sterilized distilled water. The blocks were put onto the medium in the bottles (three blocks per bottle) and then incubated at 26 ± 2 °C in the dark.

After certain period of incubation (30, 60, 90, or 120 days), the mycelium covering the wood blocks was carefully removed. The blocks were air-dried at room temperature for 24 h and then dried at 60 ± 2 °C for 48 h. The percentage of mass loss was calculated by dividing the mass of the wood block after degradation by its initial mass.

2.3. Analysis of wood chemical components

The amounts of wood chemical components were determined before and after degradation. The wood samples were ground with a rotary speed mill (P-14; Fritsch, Germany) and then sieved through 42–82 mesh. The amounts of hot water extracts, 1% NaOH extracts, ethanol-toluene extracts, Klason lignin, acid soluble lignin, holocellulose, and α-, β-, and γ-cellulose were determined by standard methods (Dence, 1992; The Japan Wood Research Society, 2000; Carrier et al., 2011). The ash content was also determined by heating the wood meal in a muffle furnace (FO 100; Yamato, Japan) at 600 °C for 2 h. After cooling in a desiccator, it was heated again at 600 °C for 1 h, followed by cooling and weighing the residue. All chemical quantifications were performed in triplicate.

2.4. pH measurement

The pH was measured according to the method described by Koenigs (1974). Air-dried wood meal (0.5 g) was placed in a test tube (1.6-cm diameter and 10-cm length) containing 3.5 ml of distilled water. The tubes with wood meal were heated in boiling water for 20 min and then cooled to room temperature. The pH was determined with a pH meter (pH meter M-12, Horiba, Japan). The measurements were performed in triplicate.

2.5. Enzyme extraction and enzymatic activity assay

Enzymes were extracted according to the method described by Elissetche et al. (2007). For the enzyme extraction, the wood blocks were collected from the bottles after incubation (30, 60, 90, or 120 days). The collected blocks were cut into small chips. The chips were transferred to a 300-ml Erlenmeyer flask, and then the enzymes were extracted with 80 ml (4 ml per g of wood chips) of 50 mM sodium acetate buffer (pH 5.0) supplemented with 0.01% Tween-80. The extraction was conducted using a rotary shaker (NR-150, TAITEC Corp., Japan) at 120 rpm for 24 h at 20 ± 2 °C. The crude extract was obtained by filtration through a glass filter (1G3) and subsequently through a 0.45-μm membrane filter (Advantec, Japan).

Endoglucanase, exoglucanase, and xylanase were assayed using carboxymethylcellulose, Avicel, and xylan as substrates, respectively (Wood and Bhat, 1988; Bailey et al., 1992). The reducing sugars were determined by dinitrosalicylic acid (DNS) method (Miller, 1959). β-Glucosidase activity was determined by measuring the *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (Wood and Bhat, 1988). For oxidizing enzymes, cytochrome C, vanillylidene acetone, veratryl alcohol, and syringaldazine were used to assay cellobiose dehydrogenase (Samejima and Eriksson, 1992), manganese(II)-dependent peroxidase (Paszczyński et al., 1988), lignin peroxidase (Tien and Kirk, 1988), and laccase activities (Bollag and Leonowicz, 1984; Cho et al., 2008), respectively. All enzymes were assayed photometrically, in triplicate, using a spectrophotometer (V-650, Jasco, Japan), and the enzymatic activities were expressed as nkat/mg of enzyme.

3. Results

3.1. Wood degradation

A thin mycelial mat was first observed on the wood on the 15th day of incubation. Wood blocks were completely covered with a white mycelial mat after incubation for 60 days. After 60 days of incubation, the mycelial mat on the wood block surface began to turn reddish-brown, which was similar to the color of the mycelia grown in the culture medium.

Table 1 shows the percentage of mass loss of *P. jezoensis* wood blocks after degradation by *P. pini*. Significant mass loss of the wood was first observed at 60 days of incubation, and it increased with increasing incubation time. Finally, the mass loss was 21.2 ± 3.0% after incubation for 120 days.

Table 1 also shows the changes in the amounts of wood chemical components of *P. jezoensis* after degradation by *P. pini*. No significant differences were found in the amounts of wood chemical components between days 0 and 30 of incubation. The amounts of Klason lignin, total lignin, holocellulose, and α-cellulose significantly decreased with increasing degradation time. The increasing trends at the later stages of degradation were similar to those of acid soluble lignin and β-cellulose. However, the amount of γ-cellulose did not significantly change.

Table 2 shows the changes in the amounts of the extracts, as

Table 1
Changes in mass loss and the amounts of chemical components of *P. jezoensis* wood during degradation by *P. pini*.

Degradation period (days)	Mass loss (%)	Chemical component (%)						
		Klason lignin	Acid soluble lignin	Total lignin	Holocellulose	α-Cellulose	β-Cellulose	γ-Cellulose
0	0.0 ± 0.0 a	26.3 ± 0.5 d	0.4 ± 0.1 a	26.8 ± 0.5 d	70.1 ± 1.3 d	46.9 ± 0.6 d	3.5 ± 0.2 a	19.7 ± 0.1 a
30	0.7 ± 0.4 a	26.0 ± 0.4 d	0.4 ± 0.0 a	26.4 ± 0.4 d	70.1 ± 1.3 d	45.9 ± 0.8 d	4.1 ± 0.1 a	19.6 ± 0.0 a
60	5.6 ± 2.2 b	23.3 ± 0.3 c	0.6 ± 0.0 b	24.0 ± 0.3 c	64.6 ± 1.3 c	40.4 ± 0.7 c	4.4 ± 0.0 a	18.7 ± 0.2 a
90	16.1 ± 1.6 c	18.6 ± 0.1 b	0.7 ± 0.0 b	19.4 ± 0.3 b	57.5 ± 0.9 b	31.2 ± 0.2 b	6.3 ± 0.7 b	18.4 ± 1.2 a
120	21.2 ± 3.0 d	17.2 ± 0.1 a	0.7 ± 0.1 b	17.9 ± 0.2 a	54.1 ± 0.3 a	28.5 ± 0.4 a	6.5 ± 0.3 b	18.4 ± 0.3 a

Note: Values represent the means of three replicates ± standard deviations. The same letter indicates that there was no significant difference value between row in the column by Tukey's honestly significant difference (HSD) test at the 5% level. n for mass loss = 45; n for chemical components = 15.
The same letter shows no significant difference between row in the column by Tukey's HSD test at the 5% level. n = 15.

Table 2
Changes in the amounts of extracts, the ash content, and the pH of *P. jezoensis* wood during degradation by *P. pini*.

Degradation period (days)	Extracts (%)			Ash (%)	pH value
	Hot water	1% NaOH	Ethanol-toluene		
0	3.1 ± 0.5a	11.6 ± 0.3 a	1.0 ± 0.3 a	0.4 ± 0.1 a	6.2 ± 0.3 d
30	3.9 ± 0.4 a	13.0 ± 0.3 a	1.6 ± 0.1 ab	0.3 ± 0.0 a	5.1 ± 0.1 c
60	10.4 ± 0.2 b	21.2 ± 0.4 b	1.0 ± 0.4 bc	0.5 ± 0.1 ab	4.2 ± 0.1 b
90	22.1 ± 0.5 c	33.7 ± 0.7 c	2.2 ± 0.2 bc	0.5 ± 0.1 ab	3.7 ± 0.0 a
120	27.7 ± 0.3 d	38.4 ± 0.8 d	2.3 ± 0.4 c	0.6 ± 0.0 b	3.7 ± 0.0 a

Note: Values represent the means of three replicates ± standard deviations. The same letter indicates that there is no significant difference value between row in the column by Tukey's HSD test at the 5% level; n = 15.

well as the ash content, due to degradation by *P. pini*. No significant differences were found in the amounts of hot water and 1% NaOH extracts between 0 and 30 days of incubation. However, the amounts significantly increased after 30 days of incubation. In contrast, the amount of the ethanol-toluene extract, as well as the ash content, gradually increased from 1.0 to 2.3% and from 0.4 to 0.6%, respectively, after 30 days of incubation. The pH of *P. jezoensis* wood significantly decreased from 6.2 to 3.7 during fungal growth and degradation process up to 90 days of incubation. However, after this period, the pH values became relatively constant (Table 2).

3.2. Lignocellulolytic enzyme activity

As shown in Table 3, *P. pini* produced various types of lignocellulolytic enzymes during the degradation of *P. jezoensis* wood. The enzymatic activities varied among the enzyme types throughout the degradation process. Among the different types of enzymes, the highest enzymatic activity was observed for xylanase (89.788 nkat/mg) on the 60th day of incubation. The endoglucanase and exoglucanase activities also reached their maximum levels on the 60th day, and significantly decreased thereafter. In addition, the endoglucanase activity was always higher than the exoglucanase activity throughout the entire degradation period. The β-glucosidase activity fluctuated and reached its maximum (78.563 nkat/mg) at the end of the incubation period. The cellobiose dehydrogenase activity did not significantly differ after 60 days of incubation.

Although the laccase activity was very low throughout the entire incubation period, compared with the activities of the other ligninolytic enzymes (Table 3), it reached its maximum level at the early stage of incubation (30 days of incubation). In contrast, the activities of lignin peroxidase and Mn(II)-dependent peroxidase increased with increasing degradation time, and reached their maximum levels at the end of the incubation period (Table 3). Of the ligninolytic enzymes assayed in this study, Mn(II)-dependent peroxidase showed the highest activity during the degradation of *P. jezoensis* wood by *P. pini*.

4. Discussion

4.1. Changes in mass loss and the amounts of wood chemical components due to degradation by P. pini

The mass loss and the amounts of the main wood chemical components (lignin, cellulose, and hemicelluloses) significantly decreased after 60 days of incubation (Table 1), suggesting that the degradation of *P. jezoensis* wood by *P. pini* primarily began after 30 days of incubation. According to Yoshizawa et al. (1992), mycelium growth of *P. pini* was relatively slow in wood degradation process, suggesting that wood components were slowly decomposed by this

Table 3
Lignocellulolytic enzyme activities of *P. pini* during *P. jezoensis* wood degradation.

Degradation period (days)	Enzymes activities (nkat/mg)							
	Xylanase	Endoglucanase	Exoglucanase	β-Glucosidase	Cellobiose dehydrogenase	Laccase	Lignin peroxidase	Mn(II)-dependent peroxidase
0	0.000 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.000 a
30	0.000 ± 0.000 a	16.759 ± 0.463 b	0.000 ± 0.000 a	4.025 ± 0.458 b	0.550 ± 0.076 a	0.044 ± 0.005 c	0.107 ± 0.002 b	0.573 ± 0.079 b
60	89.788 ± 10.79 d	70.425 ± 2.645 e	15.559 ± 0.435 d	67.461 ± 0.090 d	1.173 ± 0.146 ab	0.006 ± 0.002 b	0.147 ± 0.023 b	0.782 ± 0.276 b
90	57.605 ± 1.011 b	60.250 ± 0.605 d	6.267 ± 0.898 c	34.000 ± 1.387 c	1.970 ± 1.071 c	0.002 ± 0.001 ab	0.176 ± 0.008 c	1.498 ± 0.084 c
120	76.536 ± 9.576 c	24.769 ± 0.885 c	2.927 ± 0.510 b	78.563 ± 1.916 e	1.556 ± 0.119 bc	0.001 ± 0.000 ab	0.368 ± 0.007 d	2.100 ± 0.070 d

Note: Values represent the means of three replicates ± standard deviations. The same letter indicates that there is no significant difference value between row in the column by Tukey's HSD test at the 5% level; n = 15.

fungus. In addition, it has been widely accepted that in the initial wood degradation process by white-rot fungi, the wood cell components are only degraded by low-molecular-mass non-enzymatic compounds from the fungi (Srebotnik et al., 1988; Blanchette et al., 1997). In this stage, the extracellular enzymes are not able to degrade the wood components because the size of enzymes is too large to infiltrate the pores of wood cell walls (Srebotnik et al., 1988; Messner and Srebotnik, 1994). White-rot fungi can produce a variety of low molecular-mass oxidative compounds to initiate changes of the lignin structure and enlarge the pore size of wood substance, subsequently allowing the large enzymes to diffuse and degrade wood components (Srebotnik et al., 1988; Tanaka et al., 1999).

Decrease ratios of the amount of total lignin, holocellulose, and α-cellulose gradually increased as the degradation time increased. Although the decrease ratio on day 120 of incubation was not the same among the wood components (total lignin = 33.0%, holocellulose = 22.9%, and α-cellulose = 39.2%), ratio-increase trends in relation to degradation period were almost the same among the wood chemical components. These results indicate that *P. pini* simultaneously degrades all the main wood components (lignin, cellulose, and hemicelluloses) in *P. jezoensis*. Yoshizawa et al. (1992) reported that *P. pini* could degrade both lignin and carbohydrates in *C. obtusa* wood, and that the decrease ratio in lignin was higher than those in carbohydrates. In addition, *P. pini* degrades lignin prior to carbohydrates in *C. obtusa* wood (Yoshizawa et al., 1992). When the wood of *Pinus strobus* was degraded by *P. pini*, the degradation rates of lignin, cellulose, and hemicelluloses were 43.5%, 10.7%, and 76.9%, respectively, after 12 weeks (Eriksson et al., 1990). It is known that the degradation patterns of wood components differ among species of white-rot fungi, as well as wood species (Eriksson et al., 1990; Tanaka et al., 2009). However, our results for the degradation of *P. jezoensis* wood by *P. pini* are very similar to those reported by Yoshizawa et al. (1992).

It has been reported that the amounts of hot water and 1% NaOH extracts increased after decay by a white-rot fungus, as soluble, low-molecular-weight compounds were produced during the degradation of lignin, cellulose, and hemicelluloses (Malakani et al., 2014). Similar results were reported for the degradation of *Abies bornmülleriana* and *Fagus orientalis* by the white-rot fungus *Phanerochaete chrysosporium* (Istek et al., 2005). In the present study, as shown in Table 2, the amount of the hot water extract increased with increasing degradation time. Therefore, the degradation of wood chemical components, such as lignin, cellulose, and hemicelluloses by *P. pini* also resulted in increases in the amounts of the extracts and a decrease in pH.

4.2. Lignocellulolytic enzyme activity

In the present study, wood blocks were completely covered with a mycelial mat by the 60th day of incubation. At this time, the activities of almost all the lignocellulolytic enzymes tested here increased (Table 3). Among the cellulases and hemicellulases, xylanase, endoglucanase, and β-glucosidase were the predominant enzymes during the wood degradation process, while the activities of exoglucanase and cellobiose dehydrogenase were relatively low (Table 3). In addition, the activities of xylanase, endoglucanase, and exoglucanase reached their maximum values in the middle of the incubation period (60 days), whereas β-glucosidase and cellobiose dehydrogenase reached their maximum activities after 120 and 90 days of incubation, respectively (Table 3). Our results are similar to those obtained in other studies (Machuca and Ferraz, 2001; Ferraz et al., 2003). When *Eucalyptus grandis* wood was decayed by the white-rot fungi *Poria medulla-panis*, *Pycnoporus coccineus*, *Phlebia tremellosa*, *Trametes versicolor*, and *Ceriporiopsis subvermispora*, higher activities of total cellulase and xylanase were found at the initial stage of incubation (15 days), while β-glucosidase increased with increasing incubation time, and the highest β-glucosidase activity was observed at the late stages of incubation (120 and 150 days) (Machuca and Ferraz, 2001; Ferraz et al., 2003). Based on the results of the present and other studies, it is suggested that at the early and middle stages of degradation, *P. pini* secretes enzymes that degrade polysaccharides to produce oligosaccharides, which are further converted to glucose by cellobiose dehydrogenase and β-glucosidase (Dashtban et al., 2009). Additionally, the activities of ligninolytic enzymes were lower than those of cellulases and xylanase (Table 3). The higher cellulose activity, compared with the ligninolytic activity, during wood degradation was also recognized in several white-rot fungi, such as *C. subvermispora* (Tanaka et al., 2009), *Pleurotus ostreatus*, *Pholiota nameko*, *Grifola frondosa* (Yoshizawa et al., 1990), and *P. chrysosporium* (Yoshizawa et al., 1992).

Table 4 shows the correlation coefficients between mass loss and enzymatic activity. Significant positive correlations were found between mass loss and cellobiose dehydrogenase, lignin peroxidase, and Mn(II)-dependent peroxidase activities. Therefore, in *P. pini*, these enzymes are important for degrading wood components. In contrast, highly negative relationships were found between the residual amounts of the main wood chemical components (total lignin, holocellulose, and α-cellulose) and the cellobiose dehydrogenase (except for total lignin), lignin peroxidase, and Mn(II)-dependent peroxidase activities. However, among the ligninolytic enzymes, no significant correlations were found between laccase activity and the residual amounts of wood chemical components, suggesting that lignin degradation by *P. pini* largely results from the actions of lignin and Mn(II)-dependent peroxidases, rather than laccase. Highly negative correlations were obtained between the activities of peroxidases and the residual amounts of holocellulose or α-cellulose (Table 4). In general, when lignin is degraded, a concomitant degradation of cellulose and hemicelluloses occurs via cellulases and hemicellulases (Eriksson et al., 1990). In addition, highly negative correlations were found between the cellobiose dehydrogenase activity and the residual amounts of holocellulose and α-cellulose (Table 4). These results suggest that the greater the cellobiose dehydrogenase activity, the greater the degradation of cellulose and hemicelluloses. Therefore, cellobiose dehydrogenase might play an important role in the degradation of cellulose and hemicelluloses by *P. pini*. Henrikkson et al. (1995) suggested that the cellobiose dehydrogenase from white-rot fungi could attack many different substrates, such as cellulose, hemicelluloses, and lignin. These results suggest that cellobiose dehydrogenase from *P. pini* plays an important role in wood component degradation. Therefore, we suggest that *P. pini* produces a complex lignocellulolytic enzyme system during *P. jezoensis* wood degradation, and that the produced enzymes contribute to the degradation of wood chemical components.

5. Conclusion

Changes in the lignocellulolytic enzyme activities of *P. pini* were investigated during the degradation of *P. jezoensis* wood. In addition, the alteration of wood chemical components was also investigated. The activities of xylanase, β-glucosidase, and endoglucanase that were produced continuously by *P. pini* were higher than those of exoglucanase and cellobiose dehydrogenase. Mn(II)-dependent peroxidase had the highest activity of the ligninolytic enzymes produced by *P. pini*, followed by lignin peroxidase and laccase. All of the main chemical components of wood (lignin, cellulose, and hemicelluloses) started to be degraded after

Table 4
Correlation coefficients between lignocellulolytic enzyme activities and the residual amounts of chemical components in *P. jezoensis* wood during degradation by *P. pini*.

Factor	Xylanase	Endoglucanase	Exoglucanase	β-Glucosidase	CDH	Laccase	LiP	MnP
Mass loss	0.709 ns	0.391 ns	0.158 ns	0.746 ns	0.879*	−0.486 ns	0.898*	0.972**
Total lignin	−0.706 ns	−0.413 ns	−0.168 ns	−0.729 ns	−0.874 ns	0.493 ns	−0.878*	−0.965**
Holocellulose	−0.741 ns	−0.425 ns	−0.205 ns	−0.768 ns	−0.887*	0.519 ns	−0.889*	−0.963**
α-Cellulose	−0.724 ns	−0.443 ns	−0.198 ns	−0.741 ns	−0.888*	0.483 ns	−0.879*	−0.967**

Note: CDH, cellobiose dehydrogenase; LiP, lignin peroxidase; MnP, Mn(II)-dependent peroxidase; n = 5; ns, not significant; *, significant at the 5% level; **, significant at the 1% level; n = 5.

60 days of incubation.

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