The degradation of wood and pulp by wood-degrading fungi

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Introduction

We have been using the white rot fungus *Pycnoporus cinnabarinus* as a model white-rot fungus for studying mechanisms of fungal degradation of lignin because of its simple ligninolytic enzyme system. Only one of the three ligninolytic enzymes, laccase, is produced in the degradation of lignocellulosic materials by *P. cinnabarinus* although the fungus can degrade lignin efficiently(2, 5, 6, 9). It is well established that laccase can only oxidize non-phenolic lignin substructures in the presence of a redox mediator(3, 4). Our recent studies revealed that *P. cinnabarinus* was able to degrade non-phenolic lignin substructures (7). However, identification of a natural mediator compound that enables laccase to degrade non-phenolic lignin substructures remains elusive despite extensive efforts (11). We are also investigating two newly isolated wood-degrading fungi, *Trichophyton* sp. LKY-7 and *Trichophyton* sp. LSK-27, for their ability to degrade lignin. In this study, we investigated the abilities of *P. cinnabarinus* and *T.* LSK-27 to degrade wood and unbleached kraft pulp. We analyzed the changes of various functional group contents in pine lignin and kraft lignin during the degradation of wood and unbleached kraft pulp by these fungi in an effort to gain a better understanding of how white-rot fungi degrade lignin,

Materials and methods

2.1. Microorganisms.

2.2. Degradation of wood blocks and pine flour by two fungi.

2.3. Isolation of lignin from wood.

2.4. Degradation of unbleached softwood kraft pulp by *P. cinnabarinus*.

2.5. Isolation of lignin from pulp.

2.6. Quantitative analysis of lignin functional groups with ^{31}P NMR.

Rsults and discussion

Weight loss of decayed wood blocks

After three months of degradation, the weight losses of pine blocks caused by *P. cinnabarinus*, *T.* LSK-27 were 87.6% and 39.2%, respectively, for these two fungi (Table 1). Because lignin content in pine is at least 30%, a weight loss of 87.6% implies that *P. cinnabarinus* efficiently degrade all wood components including lignin.

Table 1. Weight loss of pine blocks caused by wood-degrading fungi after a three-momth incubation*

	P. cinnabarinus	*T.* LSK-27	Control
Weight loss (%)	87.6±2.7	20.8±1.5	2.3±0.5

*data were the mean of results from nine wood samples.

The ability of these two fungi to degrade the hardwoods yellow poplar and sweet gum was also investigated (Table 2). The weight losses of yellow poplar blocks after two months of degradation by *P. cinnabarinus* and *T.* LSK-27 were 30.1%

and 39.2%, respectively. Statistically, *P. cinnabarinus* and *T.* LSK-27 degraded yellow poplar blocks at the same rate. It is perplexing that *P. cinnabarinus* degraded pine blocks much more efficiently than *T.* LSK-27, whereas *P. cinnabarinus* and *T.* LSK-27 degraded hardwood blocks (yellow poplar and sweet gum) at the same rate.

Table 2. Weight loss of hard wood blocks caused by wood-degrading fungi after a two-month incubation*

	P. cinnabarinus	*T.* LSK-27	Control
Yellow ceder	30.1±9.5	39.2±1.5	0.9±0.3
Sweet gum	24.8±4.6	28.4±4.4	0.23±0.1

*data were the mean of results from nine wood samples.

Fungal modification of lignin structures in wood

Activities of three ligninolytic enzymes, lignin peroxidase, manganese peroxidase, and laccase were detected during the incubation of pine flour with *T.* LSK-27, whereas only laccase activity was detected with *P. cinnabarinus* (data not shown). It appeared that these two fungi used different ligninolytic enzyme systems for lignin degradation. To gain a better understanding of the mechanisms by which these three fungi degraded lignin, we investigated the structural changes of lignin during the course of the fungal degradation of wood.

As shown in Figure 1, aliphatic hydroxyl groups in lignin mainly refer to those hydroxyl groups of compound Ⅰ. CompoundⅡin Figure 1 is a representative lignin substructure with condensed phenolic hydroxyl groups. CompoundⅢ is a representative lignin substructure containing guaiacyl phenolic hydroxyl groups. When compared with the control, the contents of condensed phenolic OH groups and carboxylic acid groups

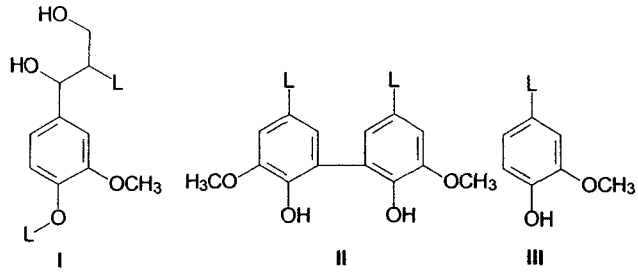

Figure 1. Representative lignin substructures (L = lignin matrix)

increased, the content of guaiacyl phenolic OH groups decreased, and the contents of aliphatic OH groups remained statistically the same after a three-months degradation of pine flour by *P. cinnabarinus*. For *T. rubrum* LSK-27, the trends in functional group changes were the same as those for *P. cinnabarinus*, i.e., the contents of the aliphatic OH groups were basically the same, the contents of condensed phenolic OH groups and carboxylic acid groups slightly increased; and the content of the guaiacyl phenolic hydroxyl groups decreased. The ^{31}P NMR results indicated that three fungi used in this study all caused decreases in the guaiacyl phenolic OH group content and increases in the condensed phenolic hydroxyl group content, which implied that these fungi first degraded the easily oxidizable lignin substructures with guaiacyl phenolic OH group and left the condensed lignin substructures behind regardless of the ligninolytic enzymes they secreted for lignin degradation. Little change of the aliphatic OH groups suggested that the oxidization of the phenylpropane side chain in the pine lignin was not the important action mode in the degradation of lignin by these three fungi. The increase in the carboxylic acid content probably resulted from the ring-opening reactions in the oxidation of the guaiacyl phenolic substructure.

Table 3. The contents of various functional groups(mmol/g lignin) after a three-month degradation of pine flour by three fungi as determined by ^{31}P NMR techniques*

		Phenolic OH		
	Aliphatic OH	Condensed	Guaiacyl	R-COOH
Control	3.80±0.37	0.35±0.20	1.19±0.04	0.07±0.04
P.c[a]	4.31±0.34	0.54±0.10	0.78±0.03	0.20±0.04
T.[b] LSK-27	3.78±0.34	0.39±0.02	0.80±0.01	0.16±0.01

*data were the mean of results from nine wood samples.

[a] *P.c* refers to Pycnoporus cinnabarinus

[b] *T.* refers to Trichophyton sp.

Additional studies were performed to determine the effect of incubation time, i.e., the degree of wood degradation. Results in Table 4 revealed that the incubation time had little effect on the change of the aliphatic OH content. The condensed phenolic OH content significantly increased in the first month of the incubation, remained at the increased level during the second and the third months of the incubation, and then decreased after the fifth month of the incubation. In contrast with the significant increase of the condensed phenolic structures, there was a significant reduction of the guaiacyl phenolic OH content after one months degradation. The guaiacyl phenolic OH content remained unchanged between one month and three months of incubation. However, the guaiacyl phenolic OH content further decreased when the pine flour samples were incubated with *P. cinnabarinus* for five months. Carboxylic acids content gradually increased in the first two months of the fungal degradation and remained constant thereafter.

Table 4. Effects of incubation time on the contents of various functional groups (mmol/g lignin) in the degradation of pine flour by *P. cinnabarinus

Incubation time (month)	Aliphatic OH	Phenolic OH		
		Condensed	Guaiacyl	R-COOH
Control	3.80±0.37	0.35±0.20	1.19±0.04	0.07±0.04
1	3.74±0.33	0.54±0.10	0.81±0.04	0.17±0.04
2	3.75±0.33	0.58±0.00	0.80±0.00	0.21±0.03
3	4.31±0.34	0.54±0.10	0.78±0.03	0.20±0.01
5	3.71±0.14	0.38±0.01	0.67±0.00	0.20±0.01

*data were the mean of results from two wood samples.

Fungal modification of lignin structures in unbleached softwood kraft pulp

The structures of lignin in unbleached softwood kraft pulp (USKP) were significantly altered during the Kraft pulping process. We investigated whether the alteration of lignin structures had effects on the action modes of degradation for *P. cinnabarinus* (Table 5). We investigated whether the alteration of lignin structures had effects on the action modes of degradation for *P. cinnabarinus*. In contrast to the initial increase in the condensed phenolic OH group content in the degradation of pine flour, the condensed phenolic OH group content gradually decreased in the degradation of USKP. The aliphatic OH group content first increased and then decreased during the fungal degradation of USKP, which is also contrary to the small changes observed in the aliphatic OH group content in the fungal degradation of pine flour. The trends of the content change for guaiacyl phenolic OH group and carboxylic acids group during fungal degradation were the same for both pine flour and USKP. It is worth noting that both the increased rate of loss of the guaiacyl phenolic OH group content and the decreased rate of loss of the carboxylic acid group content in the fungal degradation of USKP were higher than those in the fungal degradation of pine flour. This is probably due to the fact that the guaiacyl phenolic substructures in USKP were more accessible than those in pine flour.

Table 5. The contents of various functional groups (mmol/g lignin) in the degradation of bleached softwood kraft pulp by *P. cinnabarinus

		Phenolic OH		
	Aliphatic OH	Condensed	Guaiacyl	R-COOH
Control	3.80±0.37	0.35±0.20	1.19±0.04	0.07±0.04
P.c	4.31±0.34	0.54±0.10	0.78±0.03	0.20±0.04
T. LSK-27	3.78±0.34	0.39±0.02	0.80±0.01	0.16±0.01

*data were the mean of results from three pulp samples.

Treatment of USKP with the fungus *P. cinnabarinus* led to decrease in Kappa number (Table 6), which implied that the fungus selectively degraded lignin. The Kappa number decreased from 23.34 to 5.46 after a 25-day-incubation. However, an increase of the incubation time from 25 to 40 days resulted in little change of the Kappa number.

Table 6. Kappa number of pulp before and after treatment of unbleached softwood kraft pulp by *P. cinnabarinus

Incubation time (day)	Kappa number
Control	23.34±0.10
25	5.46±0.30
40	5.67±0.39

* data were the mean of results from three pulp samples.

Conclusion

Degradation of pine, yellow poplar and sweet gum by two fungi *Pycnoporus cinnabarinus* and *Trichophyton* sp. LSK-27 was investigated. *P. cinabarinus* degraded pine block samples much faster than *T.* LSK-27, whereas *P. cinnabarinus*

and *T. rubrum* LSK-27 degraded yellow poplar and sweet gum at almost the same rate. In an effort to get a better understanding of how fungi degrade lignin in wood, contents of various functional groups were analyzed. After three-months of degradation of pine flour by the three fungi, the following changes were observed: an increase in condensed phenolic OH group content and carboxylic acid group content, a decrease in the guaiacyl phenolic OH content, and little change of aliphatic OH group content. Further studies in the degradation of pine flour by *P. cinnabarinus* revealed that the increase in condensed phenolic OH group content and the decrease in guaiacyl phenolic OH group content occurred in the first month of the degradation. The changes of functional group contents in the degradation of unbleached softwood kraft pulp by *P. cinnabarinus* had the same trends as those in the degradation of pine flour.

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