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RESEARCH ARTICLE

Xin LIU, Min ZHAO, Qiuyu WANG

Biological characteristics of five wood-rotting fungi and wood-decaying ability to *Betula platyphylla*

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Abstract In this paper, the growth rate of five wood-rotting fungi, i.e., *Coriolus versicolor*, *Irpex lacteus*, *Fomes fomentarius*, *Piptoporus betulinus* and *Pholiota adiposa*, in solid medium and their biomass in liquid culture medium were compared by measuring mycelium length and dry mass. The activity of three main ligninolytic enzymes in those fungi, namely LiP, MnP and Lac, were also tested by colorimetry. At the same time, these fungi were used to decay the wood samples from 300 natural trees of white birch, to study their wood-decaying ability by measuring wood mass loss. The result showed that the growth rate, biomass, ligninolytic enzyme activity, and wood-decaying ability of the fungi were incompletely correlated. The growth rates of *C. versicolor* and *I. lacteus* were faster than those of *P. betulinus* and *F. fomentarius*; *P. adiposa* was the slowest in growth. The biomass of *P. betulinus* was the highest; *C. versicolor*, *I. lacteus and F. fomentarius* were in the middle, and *P. adiposa* was the lowest. There existed LiP, MnP and Lac activities in all fungi except *P. betulinus*, and the enzyme activities induced by wood powder were all higher than those of the control. The Lac of *I. lacteus* and the LiP of *F. fomentarius* and *P. adiposa* were only expressed in wood powder medium; the longer the fungi were cultured, the higher activity the enzyme had. The decomposition ability of *C. versicolor* to wood samples was the highest, followed by *F. fomentarius* and *P. betulinus*; *I. lacteus* and *P. adiposa* were the lowest.

Keywords wood-rotting fungi, biological characteristics, mass loss rate, ligninolytic enzymes

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Xin LIU, Min ZHAO, Qiuyu WANG (✉)
College of Life Science, Northeast Forestry University, Harbin 150040, China
E-mail: yingchuixue@yahoo.com.cn

1 Introduction

The wood decayed in the forest is usually eroded by wood-rotting fungi. Different fungi have different physiological characteristics. They excrete distinct enzymes and these enzymatic activities are various. Therefore, different fungi decay dissimilar components of wood, and their rates may differ (Buswell and Odiere, 1987). White rot fungi can decompose lignin of the wood, which causes the wood to turn into white rot. Brown rot fungi can decompose cellulose and hemicellulose, which makes the wood turn into brown rot. Extracellular enzymes of white rot fungi, such as peroxidase and phenoloxidase, can decompose lignin and other organic pollutants with similar structural units as lignin. They are worth researching and have potential in water pollution control, soil remediation, paper industry, and so on. At present, most researches have reported the biological characteristics and activity of ligninolytic enzymes, the wood-decaying ability of fungi. Some researches abroad were most focused on the molecular biology and proteomics of ligninolytic enzymes. In China, however, the kinetic activity of ligninolytic enzymes and pollutants' decomposition by rot fungi were recently reported. Systematic studies on relations among physiological characteristics, enzyme activity, and the wood-decaying abilities of some rot fungi are seldom seen. *Betula platyphylla* is a vulnerable decadent species that is widely distributed in the northeast area of China. In this paper, five wood-rotting fungi that always appear on *B. platyphylla* were selected to be used in the study of the biological characteristics and activity of ligninolytic enzymes of the fungi, and to decay the birch wood in order to research the relationships among the fungi growth rate, biomass, the activity of ligninolytic enzymes and wood-decaying ability. The purpose is to provide some useful information to select high-efficiency wood-rotting fungi for papermaking, their cultivation and utilization, as well as wood protection and reforestation in the future.

2 Materials

Five wood-rotting fungi were obtained from the Moershan Forest Farm of Northeast Forestry University in Heilongjiang Province, China. The sporophore of fungi was collected in September 2005. Mycelia were isolated from the sporophore and stored at 4°C in PDA and wood powder medium (Zhang et al., 1992). The basic information is listed in Table 1.

Three hundred plus trees over 20 years old were selected from natural forest in the Moershan Forest Farm of Northeast Forestry University. The wood samples of increment borer were taken from the breast high of the trees, with 5 mm in diameter, one per tree, and stored at −20°C.

3 Methods

3.1 Biological characteristics of mycelia of fungi

The measurement of the growth rate of mycelia of fungi: wood powder media were put into test tubes and sterilized at 121°C for 2 h. The mycelia were inoculated on the media and incubated at 23°C, whose lengths were measured per 48 h.

PDA medium (90-mm petri dish) was used for plate culture. The mycelia were inoculated at the middle part of the plate medium, and then incubated at 23°C or 28°C, after which the lengths of the mycelia were measured per 12 h.

The measurement of the dry mass of mycelium: small amounts of the mycelia were inoculated into 100-mL bottles with 40 mL potato-glucose medium (200 g/L potato, 20 g/L glucose), and then incubated at 28°C and kept shaking at 150 r/min for 48 h. The mycelia were filtrated by filter paper, rinsed twice with distilled water, and then held to dry at 100°C in the oven until to the constant mass.

3.2 Measurement of ligninolytic enzymes

By the testing method of the dry mass of mycelia, the mycelia were incubated in the liquid medium with 1 g wood powder of white birch, and the samples were cultured in the medium without wood powder as the control. The activity of ligninolytic enzymes was tested on the 3rd and the 6th day, separately. Extracellular liquid was taken out and centrifuged at 4°C, 15000 r/min for 10 min, and then the supernatant liquid could be tested as crude enzymes (Li, 2005; Li and Wen, 2005) after wiping off mycelia and spores. Each sample had three replications. The activities of LiP, MnP, and Lac were also tested (Li and Wen, 2005; Fu and Zhou, 2005).

3.3 Wood-decaying experiment

Five fungi were inoculated into the 90-mm petri dish with 20 mL sterilized (at 121°C for 20 min) PDA medium (200 g/L potato, 20 g/L glucose, 20 g/L agar), and then incubated in a growth chamber at (28±2)°C, 75% to 85% of relative humidity, till the mycelia were spread over the whole dish.

Three hundred wood samples from increment borer were respectively cut into chips of approximately 5 mm × 5 mm × 5 mm, three replications for each sample. All chips were held to dry at (100±5)°C in the oven till a constant mass, sterilized at 121°C for 1 h, and then put on the dishes full of mycelium for decaying experiment, incubating for 38 to 105 d (*Coriolus versicolor* rotted for 38 d, *Fomes fomentarius* for 50 d, *Irpex lacteus* and *Piptoporus betulinus* for 98 d, and *Pholiota adiposa* for 105 d). The chips were taken out from the mycelia and cleaned up, dried, and weighed.

3.4 Data processing and analyses

The data were analyzed by SPSS.

4 Results and analyses

4.1 The changes of mycelia growth of five fungi

The mycelia growth of wood-rotting fungi in the medium with birch wood powder is described in Fig. 1. The growth rates of mycelia of the five fungi were all even. The growth rates of *C. versicolor* and *I. lacteus* were faster than the others, with average growth rates of 7.1 and 6.1 mm/d, respectively. There was no significant variation in growth

Table 1 The main characteristics of five wood-rotting fungi (Chi, 2003)

wood-rotting fungi	classification	decay type	habitat and host
Fomes fomentarius	Aphyllophorales: Polyporaceae: Fomes	white rot	*B. platyphylla*, *B. castata*, etc.
Coriolus versicolor	Aphyllophorales: Polyporaceae: Coriolus	from cavernous white rot to spot mixed rot	*Populus*, *Betula*, etc.
Irpex lacteus	Aphyllophorales: Polyporaceae: Irpex	sapwood white rot	*Acer ukurunduense*, *Almus sibirica*, etc.
Pholiota adiposa	Agaricales: Strophariaceae: Pholiota	spot mixed brown rot	*Populus*, *Betula*, etc.
Piptoporus betulinus	Aphyllophorales: Polyporaceae: Piptoporus	brown rot	*Betula*

rate among them. The two fungi were part of fast-growth species. The average growth rates of mycelia of *P. betulinus* and *F. fomentarius* were in the middle, at 5.2 and 4.5 mm/d, respectively, which placed great difference between them and *C. versicolor* as well as *I. lacteus* (F = 13.033**). The average growth rate of *P. adiposa* mycelium was the slowest at 1.6 mm/d. There was a significant difference between *P. adiposa* and the other four fungi (F = 35.297**).

The growth of the fungi in liquid medium can be seen in Fig. 2. The biomasses of the five fungi were significantly different in period of growth platform. The biomass of *P. betulinus* was the highest, achieving 15.76 g/L on the 12th day; the biomasses of *F. fomentarius*, *C. versicolor*, and *I. Lacteus* were in the middle, reaching 44.47% to 54.95% of the biomass of *P. betulinus* during the same growth period; the biomass of *P. adipose* was the lowest, achieving only 22.15% of the biomass of *P. betulinus*. The delayed growth periods of *I. lacteus* and *C. versicolor* were short; they grew faster in logarithm period at 1.76 and 1.45 g/(L·d), respectively. The delayed growth periods of *P. betulinus* and *F. fomentarius* were long at 4 to 6 d, and their growth was fastest in logarithm period, at 3.45 and 2.09 g/(L·d), respectively. The growth rate of mycelia of *P. adipose* in liquid medium was the same with that in wood powder medium, with the slowest growth rate, and both of them did not reach the growth platform on the 14th day. The growth rates of the fungi had a negative correlation with the delayed growth period (Figs. 1 and 2), but no clear correlation with the maximum biomass.

The five fungi had the same growth trends in wood powder and PDA media, forming round colonies after 2 to 3 d. Their mycelia were all white and linear spreading on the medium surface, except that the colony of *F. fomentarius* had a few brown spots.

At temperatures of 23°C and 28°C (Figs. 3 and 4), the growth rate of *I. lacteus* was the fastest, with an average spreading rate of colony radius of 6.9 mm/d at 23°C and 5.3 mm/d at 28°C. The growth rate of *C. versicolor* was second at 5.6 mm/d at 23°C and 4.7 mm/d at 28°C. *F. fomentarius* grew more slowly than the others, at 3.75 mm/d at 23°C and 3.00 mm/d at 28°C. There were significant

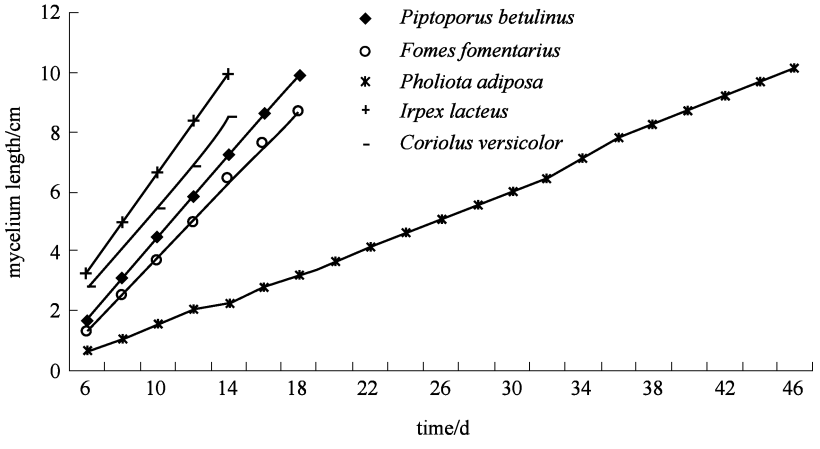

Fig. 1 The growth of fungi in wood powder medium

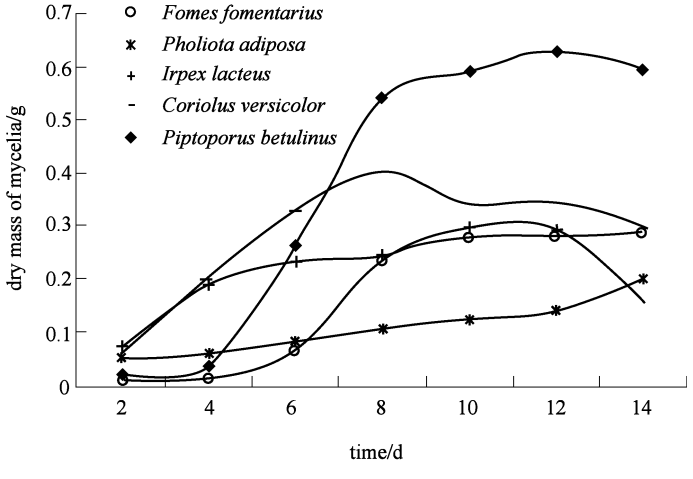

Fig. 2 The growth of fungi in liquid medium

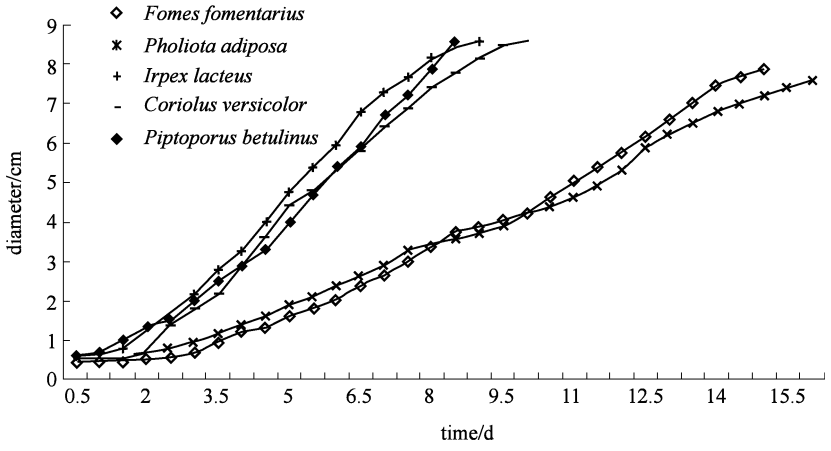

Fig. 3 The growth of fungi at 28°C

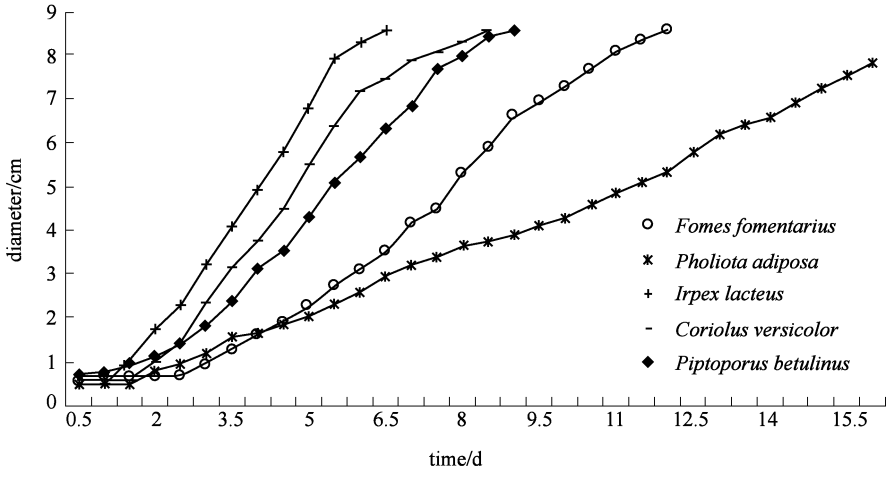

Fig. 4 The growth of fungi at 23°C

differences in the growth rates of *I. lacteus*, *C. versicolor*, and *F. fomentarius* at 23°C and 28°C, with $t = 3.119^{**}$, 4.525^{**}, and 2.077^{*}. This meant that these three fungi were sensitive to temperature, and 23°C was more suitable than 28°C for their growth.

The growth rate of *P. betulinus* was faster, with an average growth rate of 5.3 mm/d at both 23°C and 28°C. The growth rate of *P. adipose* was the slowest, with an average growth rate of 2.1 mm/d at both 23°C and 28°C. There was no significant difference in growth rates at 23°C and 28°C for both *P. betulinus* ($t = -0.070$) and *P. adiposa* ($t = -0.911$). This shows that different fungi have different sensitivities to temperature.

4.2 The changes of ligninolytic enzymes activity of the five fungi

The LiP, MnP and Lac activities of the five fungi induced by wood powder of birch were detected. The results showed that the activities of the three enzymes were higher than or similar to the control (Fig. 5), which indicates that those enzymes were all related to wood decomposition. Among *F. fomentarius*, *C. versicolor*, and *I. lacteus*, the Lac of *C. versicolor* had the highest ability to decompose wood, achieving 1068.63 IU after 6 d of wood powder induction; *I. lacteus* had the lowest ability to decompose wood, achieving 0.728 IU in the same situation. It can be seen that the wood-decaying ability of the fungi was in positive correlation with their Lac activity.

The LiP activity of *C. versicolor* was in the middle. There was no significant difference between the LiP activities of *C. versicolor* and the other three fungi (without *P. betulinus*) under the wood powder inducement; however, the MnP and Lac activities of *C. versicolor* were significantly higher than those of the other three fungi (without *P. betulinus*). After 6 d of wood powder inducement, the MnP and Lac activities of *C. versicolor* were the highest, at 8.34 and 1068.63 IU, respectively. Moreover, the MnP and Lac activities of the control fungus treated without any inducement for 3 d were also higher

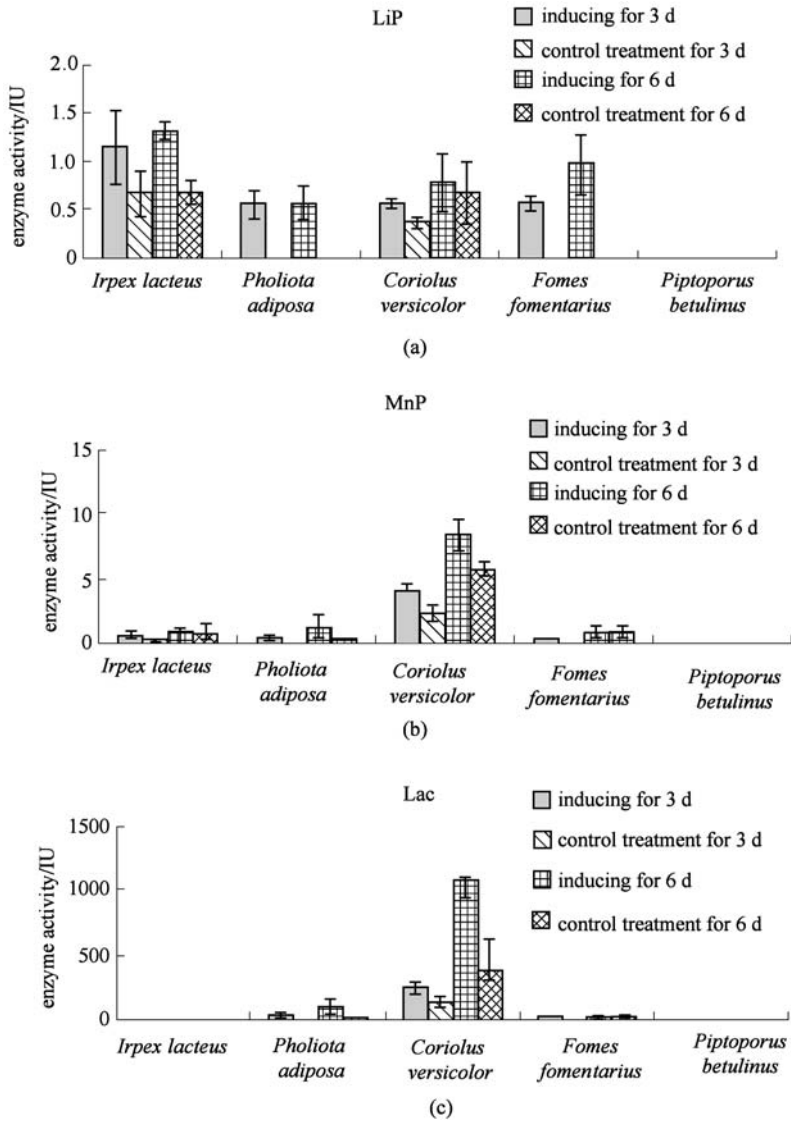

Fig. 5 LiP, MnP, and Lac activity of five fungi

Table 2 ANOVA analysis of LiP, MnP and Lac activity of five fungi

species	LiP		MnP		Lac	
	F (between different growth periods)	*F* (between different treatments)	*F* (between different growth periods)	*F* (between different treatments)	*F* (between different growth periods)	*F* (between different treatments)
I. lacteus	0.058	11.983**	5.514*	2.524		
P. adiposa	−0.092		−1.706		3.744	7.769*
C. versicolor	4.234	1.538	72.099**	24.478**	20.191**	11.566**
F. fomentarius	−2.638		−2.964*	−0.259	32.563**	6.501*
P. betulinus						
among fungi species		13.817**		41.052**		942.111**

than those of the other fungi induced by wood powder of birch for 6 d. There were very significant variations among different growth times, as well as between wood powder inducement and the control for the MnP and Lac activities (Table 2). The reason might be that *C. versicolor* had the best wood-decaying ability.

The LiP of *F. fomentarius* only appeared under wood powder inducement, and was 0.567 for 3 d and 0.995 IU

for 6 d, while the activity of the control was 0 IU. The MnP of *F. fomentarius* just appeared after 3 d of inducement by wood powder, with the activity of 0.28 IU. The average activity of the fungus ranged from 0.28 to 0.928 IU in 3 and 6 d growth, with significant difference; the MnP activity induced by wood powder was a little higher than that of the control, without significant difference; the Lac activity of *F. fomentarius* was lower than those of the other three fungi except *P. betulinus*, but there existed a significant difference in Lac activity between 3 and 6 d of treatment, as well as between wood powder inducement and the control. These indicate that the LiP of *F. fomentarius* was mostly affected by wood powder, followed by the Lac, with the MnP the least, while the Lac of *F. fomentarius*, was mostly influenced by growth period, followed by the MnP, with the LiP the least.

There was no significant difference in the LiP and MnP activities of *I. lacteus* among different growth periods as well as between different treatments. The LiP activity of *I. lacteus* was much higher than those of the other three fungi, except *P. betulinus*. There was no significant difference between the MnP activities of *I. lacteus* after 6 days of inducement by wood powder and *F. fomentarius*, *P. adiposa*, $F = 0.393$. The Lac of *I. lacteus* only appeared after 6 days of inducement by wood powder, but just in a low level, which might be related to the lowest wood-decaying ability of the fungus.

The Lac activity of *P. adiposa* was much higher than that of *F. fomentarius*, but much lower than that of *C. versicolor*. There was great difference in the Lac activity among different growth periods after 6 d of inducement by wood powder, $F = 942.111^{**}$, and also between wood powder inducement and the control, $F = 7.769^{*}$ (Table 2). The LiP and the MnP of *P. adiposa* were similar with those of *F. fomentarius*, which implied that they might have the same way in wood decomposition.

P. betulinus, as a brown rot fungus, did not express the above three enzymes. *P. adiposa* had higher Lac activity, but its MnP activity only appeared after 3 d of inducement by wood powder, which means it is probably not a real brown rot fungus. In a word, there was a complicated relationship between the enzyme activity and the wood-decaying ability of the fungi, indicating that the wood decomposition of fungi is a complex course and other enzymes may be involved. So we cannot affirm the wood-decaying ability only by some enzyme activity.

4.3 The difference in mass loss rate of the wood samples after wood decaying

Three hundred wood samples of birch were decomposed by five fungi, and their mass loss rates were statistically analyzed (Table 3). The durations for wood decomposition by the five fungi were different. The better wood-decaying ability they had, the shorter duration they spent in wood decomposition. The total decomposition durations for all fungi ranged from 38 to 105 d. Given the condition that the duration differences were ignored, the basic characteristics of wood mass loss can be seen in Table 3. The wood-decaying ability of *C. versicolo*r was the highest, with the lowest variation among different samples; *F. fomentarius* was second, with great variation among the samples; third was *P. betulinus*, with variation among the samples also much lower; the wood-decaying abilities of *I. lacteus* and *P. adiposa* were poor, with the variation among the samples higher for *I. lacteus* and lower for *P. adiposa*.

The correlation analysis on mass loss rates of wood samples decayed by the five fungi is listed in Table 4. There was very significant positive correlation between *F. fomentarius* and *P. adiposa*, which means that the birch samples easily decayed by *F. fomentarius* were liable to be invaded by *P. adiposa*, too. Generally, white rot fungi and brown rot fungi have different decomposition ways, with different metabolic pathways and different degrading enzymes. However, both *F. fomentarius* and *P. adiposa* expressed three ligninolytic enzymes after wood powder inducement, while the control did not express MnP after 3 d of wood inducing, and neither LiP after 3 and 6 d. It seems that *F. fomentarius* and *P. adiposa* had a similar process in wood decaying, or they were sensitive to similar wood materials. Their relation should be examined in a future study.

The decomposition rates between *I. lacteus* and *P. betulinus* were in significant negative correlation; in other words, the birch easily decayed by *I. lacteus* was not easily invaded by *P. betulinus*. From enzyme sorts and activity, *I. lacteus* was white rot fungi, in which the three enzymes had been expressed, while *P. betulinus* was brown rot fungi that did not express the above three enzymes as a rule. That means they had different wood-decaying pathways or were not sensitive to the same wood materials.

There were different correlation relationships in wood mass loss rate among *I. lacteus*, *C. versicolor*, and *F.*

Table 3 Basic characteristics of mass loss of birch samples after wood decomposition

species	decomposition duration/d	the number of valid data	average mass loss rate/%	std. deviation	coefficient of variation	range
I. lacteus	98	297	0. 3497	0. 08640	24.71	0.55
P adiposa	105	297	0. 3748	0. 05812	15.51	0.29
C. versicolor	38	295	0. 7047	0. 06827	9.69	0.39
F. fomentarius	50	293	0. 6255	0. 10828	17.31	0.71
P betulinus	98	292	0.5677	0.08458	14.90	0.65

Table 4 Correlation among mass losses of birch samples after wood decomposition

species	*P. betulinus*	*F. fomentarius*	*P. adiposa*	*I. lacteus*
F. fomentarius	0.103			
P. adiposa	0.056	0.333**		
I. lacteus	−0.224**	−0.004	0.010	
C. versicolor	−0.011	−0.077	0.029	0.052

fomentarius. A positive correlation existed between the wood mass loss rates of two brown rot fungi, but was not significant. This suggests that there were many complicated wood-decaying pathways in the fungi, no matter what kind of fungi they belonged to.

5 Conclusions and discussion

It was found that in this experiment the wood-rotting fungi were more suitable to grow at 23°C, but not at 28°C. The reason might be that all tested fungi came from Heilongjiang Province and adapted to grow at lower temperature for a long time. The growth rate of the fungi in solid medium was incompletely relevant with the biomass and wood-decaying ability in liquid medium. The growth rates of four fungi, i.e., *C. versicolor*, *F. fomentarius*, *P. betulinus*, and *P. adipose*, were positively related with their biomass and wood-decaying ability, while *I. lacteus* showed reverse results. Its growth rate was the fastest one, its biomass in the middle, and its wood-decaying ability at a poor level.

Ligninolytic enzymes were the critical enzymes during the lignin decomposition of the wood (Li, 2005). It is generally considered that the higher enzyme activity the fungus had, the better wood-decaying ability it had. In this experiment, all four fungi, except *P. betulinus*, could express Lac, MnP and LiP, but with a tanglesome relation between the enzyme activity and the wood-decaying ability. It suggests that the wood-decaying course of the fungi is not a simple metabolic reaction, which might involve many other enzymes. The wood-decaying ability of the fungus could not be affirmed only by some enzyme activity. The similar results were obtained by Moredo et al. (2003).

The longer the fungi grew in wood powder medium, the higher activity the enzyme achieved, and significant differences could be found in some fungi. It was basically proved by Iakovlev (2000) that the Lac activity of wood-rotting fungi in the late phase was commonly higher than that in early phase during a persistent wood-decaying treatment. In this experiment, the LiP activity of *F. fomentarius* and *P. adiposa*, and the Lac activity of *I. lacteus* are all expressed in wood powder inducement, but not in the control. All ligninolytic enzyme activity in the four fungi could be improved after wood powder treatment, which also showed significant difference among the Lip of *I. Lacteus*, the MnP and the Lac of *C. versicolor*, and the Lac of *F. fomentarius* and *P. adiposa*. Similar results were obtained by Zhang (2005) in studying the white rot fungus F_2.

Chi (2003) described the basic characteristics of *P. adipose*, found that different spreading patterns existed in gallic acid or tannin culture medium, and considered *P. adiposa* and *P. betulinus* as the brown rot fungus that did not express any ligninolytic enzymes. Wang (2001) also reported that *P. adiposa* did not express Lac and peroxide enzyme, but *Pholiota nameko* and *Pholiota squarrosoides* in the same genus as *P. adiposa* expressed Lac and peroxide enzyme. In this experiment, *P. betulinus* did not express any ligninolytic enzymes, such as LiP, MnP and Lac, but *P. adiposa* expressed all three enzymes. The wood chips decayed by *P. betulinus* presented red and brown, easy to be crushed. However, the wood samples decayed by *P. adiposa* were shallow yellow and white, whose characteristics were similar to white rot fungus, hard to be crushed. Liu et al. (2006) tested the change trend of Lac and polyphenol oxidase activity of *P. adipose* during different growth stages, then considered that the estimate method by gallic acid and tannin had some limitation. Davidson et al. studied the growth behavior of 210 fungi cultured in gallic acid or tannin medium, in which seven species showed special results, six species out of the seven were brown rot fungi, one species was white rot fungus. *P. adiposa* might belong to white rot fungus according to our experiment.

Among the five fungi, the wood-decaying ability of *C. versicolor* was the best to birch, *F. fomentarius* was the next, *P. betulinus* was the third, and *I. lacteus* and *P. adiposa* were the least. *P. betulinus* as a brown rot fungus had some correlations with the other four white rot fungi in wood-decaying ability, some positive or negative. However, no significant correlations in wood-decaying ability between white rot fungi and brown rot fungi were detected, as well as between wood-decaying strategy of the fungi and wood resistance to decay. The same results were achieved by Bhat et al. (2005). There was a significant positive correlation between *F. fomentarius* and *P. adiposa* in wood decomposition rates, and a significant negative correlation between *I. lacteus* and *P. betulinus* in that. The relationships among the other fungi would be much complicated.

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