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Evaluation of White-rot Fungi for Biopulping of Wood

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Ergosterol involves in fungal cell growth as a major component in fungal cell membranes. It can be an indicator that shows the fungal activity, and its content depends on the fungal strains, culture, growth conditions and so on. In this study, fungal activities and growth patterns of three white-rot fungi strains isolated in Korea were evaluated by determination of ergosterol contents during the incubation. Wood decay test and chemical analyses of wood were also performed to verify the relationship between fungal activity and wood degrading capacity of white-rot fungi for 60 days. In the results of experiments, it is considered that the test strains selectively degrade large amount of lignin in wood at the early stage of decay. Especially, *Phanerochaete chrysosporium* showed the best capability on selective degradation of lignin among the test fungi. It is suggested that the determination of ergosterol content in the fungal culture during the incubation is the simple and effective screening method of white-rot fungi for the application to biopulping of wood.

KEYWORDS: Biopulping, Decay, Ergosterol, Fungal activity, Lignin, White-rot fungi, Wood

Wood is mainly composed of lignin, cellulose, and hemicelluloses. It is well known that the white-rot fungi selectively degrade the lignin in wood. Biopulping is defined as the treatment of wood chips with lignin-degrading fungi prior to pulping. There are about 10,000 species of white-rot fungi, with varying capacities to degrade lignin, cellulose, and hemicelluloses. To find appropriate species and strains for biopulping, a screening program was initiated to identify fast-growing strains that could selectively remove lignin from wood (Akhtar *et al.*, 1999). Although there are many different methods of screening white-rot fungi, one of the most appropriate procedure appears to be an assessment of decay (chemical analyses of lignin and wood sugar content) from inoculated wood blocks placed in accelerated decay chambers. The species selected with this method also have been shown to be successful candidates for biological pretreatment of wood for mechanical pulping processes or as an alternative to chemical pretreatments in high-yield wood pulping (Blanchette *et al.*, 1992).

Ergosterol is an indicator of fungi and involves in fungal cell growth as a major component in fungal cell membranes (Weete and Gandhi, 1996; Ekblad *et al.*, 1998). Thus, this chemical can be used to estimate live fungal biomass within various solid substrates (Pasanen *et al.*, 1999; Hart and Brokes, 1996; Seitz *et al.*, 1979; Pennanen *et al.*, 1998).

In this study, three whit-rot fungi, *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, and *Trametes versicolor*, were tested to evaluate the screening method of white-rot fungi for application to biopulping of wood. These fungi are well known as the wood degrading fungi, and have excellent capacity of lignin degradation in wood. The fungal growth patterns and activities of each test strain during the incubation were evaluated by determination of ergosterol contents in fungal culture to compare them with the decay patterns.

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Materials and Methods

Fungal Strains and Growth Medium. Three white-rot fungi strains, *P. chrysosporium* KCCM 34740, *T. versicolor* KCCM 11258, and *C. subvermispora* KFRI 21078, were obtained from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) and the Korea Forest Research Institute (KFRI, Seoul, Korea). The strains were inoculated and grown in YM broth (Difco 271120) and potato dextrose liquid medium at 29 ± 1, RH 70 ± 1% for 7 days with shaking. The composition of growth medium is shown in Table 1.

Wood Blocks. Twenty year old pine (*Pinus densiflora* S. et Z.) and poplar (*Populus alba* x *glandulosa*) were obtained from Dongguk University Research Forest in Namyangju, Korea in January 2001. The logs were debarked and then sawed immediately after felling. The ensuing lumbers were subsequently dried in the air. The air-dried sapwood lumbers were collected and then sawed to a regular hexahedron wood block, 20 ± 1 mm each side, according to the Korean Standard Test Methods KS F 2201. The wood blocks were weighed after oven-drying and gas sterilized with ethylene oxide for 24 hours prior to fungal inoculation.

Table 1. Composition of growth medium used in this study

Fungus	Medium	Composition		
Phanerochaete chrysosporium	YM broth (Difco 271120)	Yeast extract	3.0 g	Distilled water 1.0 *l*
		Malt extract	3.0 g	
Ceriporiopsis subvermispora		Peptone	5.0 g	
		Dextrose	10.0 g	
Trametes versicolor	Potato dextrose	Diced potatoes	300.0 g	
		Dextrose	20.0 g	

Determination of Ergosterol Contents. The ergosterol contents in the fungal culture were determined to evaluate the fungal growth patterns and activities of test strains during the incubation according to the method of Koo *et al.* (2000). Following the growth of every 24 hours, the mycelia were collected by filtration, and dewatered in the blotting paper. The collected mycelia were ground by the pestle and mortar, and then dissolved in 8 *ml* of 10% KOH (w/w in 95% methanol). The solution was subsequently warmed up in a double boiler at 80 ± 1°C for 60 minutes. And then the solution was warmed up for 30 more minutes with the additional adding 2 *ml* of 10% KOH to prevent the solution from drying. The saponificated solution was cooled in the room temperature, and 3 *ml* of D.I. water was added in the solution. After the vortex of the solution, 2 *ml* of hexane was added in it. The clear supernatant after the mixing was collected with the Pasteur pipette. After the adding the additional 2 *ml* of hexane in the supernatant, the separated supernatant was collected from it. The white crystals obtained from evaporation of hexane were dissolved in 2 *ml* of 99.5% ethanol, and then filtered by 0.2 μm syringe filter. The filtered sample (10 μl) was analyzed by HPLC (Waters 510, USA) with the Nova-Pak C18 (Waters, USA) reverse-phase column for determining ergosterol contents.

Wood Decay Test. Wood decay test for each strain was performed according to the Korean Standard Test Methods KS F 2213. All tests were performed in triplicate.

Total test period was 60 days. Every 10 days from the start of the decay test, wood blocks were collected to determine the weight loss. The collected wood blocks were cleaned and oven-dried to measure the oven-dry weight. The weight loss of each wood block was calculated by the equation as follows.

$$\text{Weight loss (\%)} = (W_1 - W_2)/W_1 \times 100$$

Where W_1 is the oven-dry weight of wood block before test, W_2 is the oven-dry weight of decayed wood block after test.

Alkali Solubility and Chemical Analyses of Wood Blocks. Chemical composition of wood blocks from the decay test was analyzed to characterize the wood degrading pattern of test fungi. It was performed according to the TAPPI Standard Test Methods T 212 om-93 (1% NaOH solubility) and T 222 om-88 (Holocellulose and lignin contents). The sound wood block was used as the control. All tests were performed in triplicate.

Results and Discussion

Ergosterol Contents in Fungal Culture. The test fungi were incubated in the optimum growth condition for 15 days. The result of ergosterol contents measurement according to the incubation time is shown in Fig. 1. It could be regarded as the fungal activity and growth pattern of each test fungi. *C. subvermispora* showed the highest value of ergosterol content as 1.22% at the 6th day of incubation. It should be considered that *C. subvermispora* has the excellent growth capability in the early stage of incubation. This result is in accord with the result by Messner *et al.* (1998) that referred to the growth pattern of this fungus. On the other hand, *P. chrysosporium* showed the highest value as 1.31% among the test fungi at the 10th day of incubation. In the case of *T. versicolor*, the highest value was 1.07% at the 14th day of incubation. In the result of ergosterol contents measurement, all test fungi showed the excellent growth capability in the early stage of incubation. It was verified that *P. chrysos-*

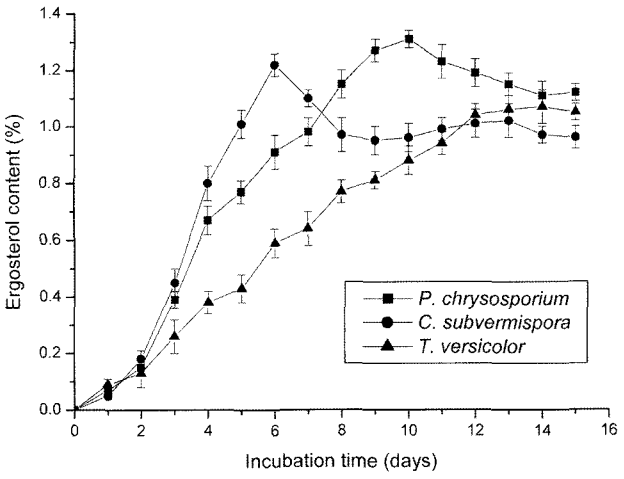

Fig. 1. Changes in ergosterol contents of test fungi according to the incubation time.

porium and *C. subvermispora* especially have high fungal activity and growth capability within 10 days of incubation in the optimum growth condition.

Weight Loss of Wood Blocks. In the wood block decay test during total 60 days, all test fungi showed the highest increasing rate of weight loss in the first 10 days of test period (Fig. 2 and 3). According to the progress of decay, the weight loss of wood was increased, but the increasing rate of weight loss showed a gentle slope after 10 days of test period. The weight loss of poplar wood block was higher than pine wood block. Therefore, it is considered that all test fungi prefer the hardwood to the softwood. *P. chrysosporium* showed the highest value of weight loss among the test fungi in the first 10 days of test period. It was 6.12% and 7.23% in pine and poplar wood block, respectively.

In the results of ergosterol content measurement and wood decay test, all of three test fungi showed the excellent ability to degrade the wood by the high fungal activity during the early stage of decay. Especially, *P. chrysosporium* showed the most excellent ability for wood degrading with the stable growth curve.

Alkali Solubility of Wood Blocks. To determine the grade of wood decay, alkali solubility of wood blocks was measured. The alkali solubility of wood block decayed by *P. chrysosporium* was the highest in both of pine and poplar (Fig. 4 and 5). It indicates that *P. chrysosporium* has the best ability of wood degrading among the test fungi. *C. subvermispora* showed the higher increasing rate of alkali solubility in poplar than in pine. In the case of *T. versicolor*, it showed relatively low capability in wood degrading with the lowest value among the test fungi,

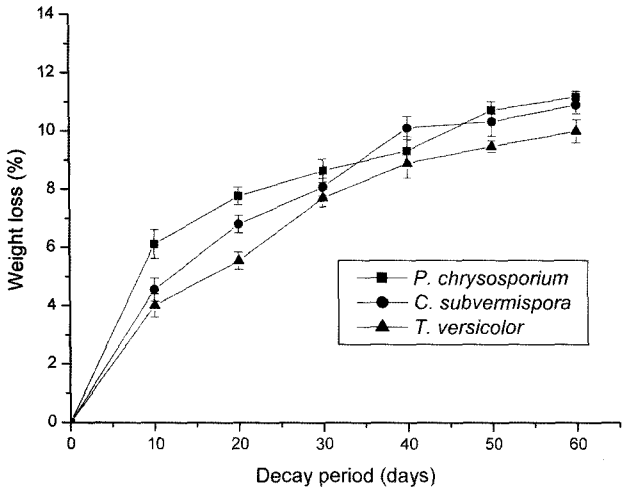

Fig. 2. Changes in weight loss of pine wood blocks in wood decay test.

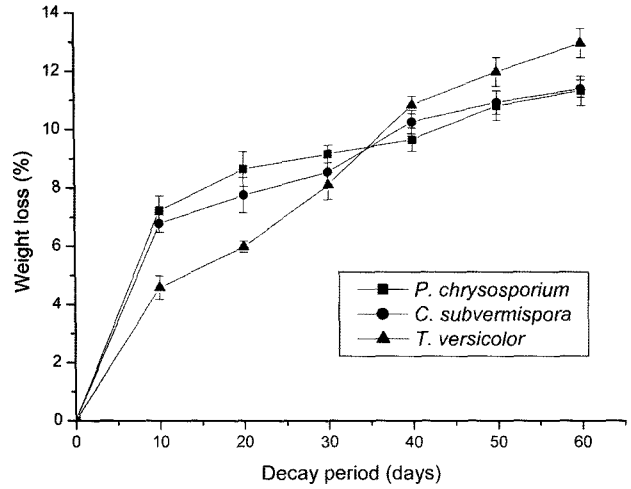

Fig. 3. Changes in weight loss of poplar wood blocks in wood decay test.

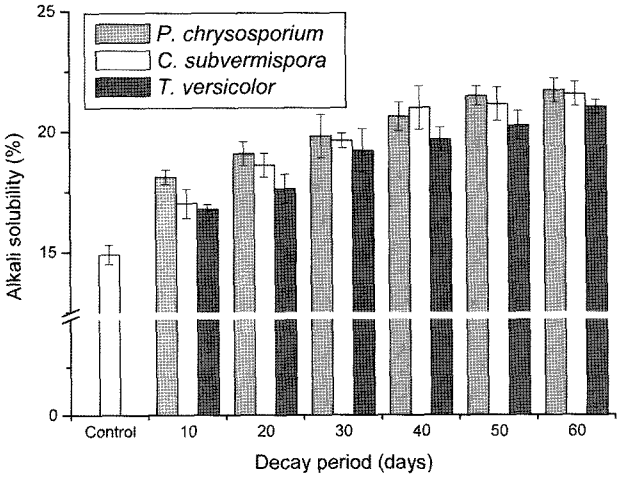

Fig. 4. Changes in alkali solubility of pine wood blocks in wood decay test.

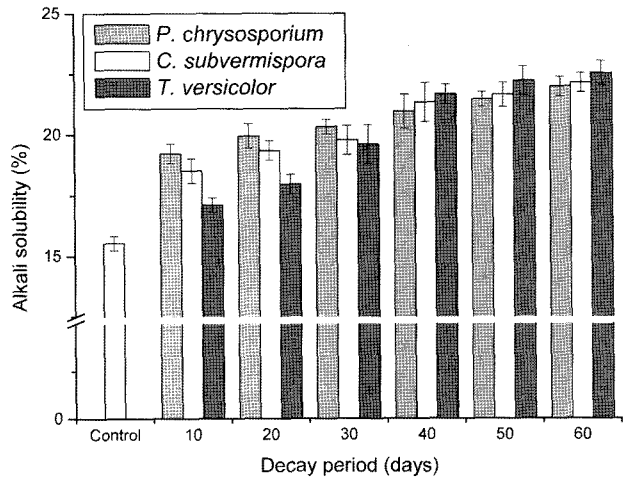

Fig. 5. Changes in alkali solubility of poplar wood blocks in wood decay test.

Table 2. Changes in chemical components of wood during the wood decay test

Wood	Fungi	Component	Decay period (days)						
			Ctrl.	10	20	30	40	50	60
Pine	*P. chrysosporium*	Holocellulose	58.2	57.0	56.2	55.4	53.2	51.6	50.3
	C. subvermispora			56.8	54.5	53.1	51.9	49.4	48.2
	T. versicolor			55.4	53.9	52.2	50.3	47.7	46.1
	P. chrysosporium	Lignin	24.7	19.3	16.6	13.7	11.1	9.5	8.1
	C. subvermispora			20.2	16.9	14.1	11.7	10.1	9.3
	T. versicolor			21.8	17.1	15.5	13.6	11.3	10.0
Poplar	*P. chrysosporium*	Holocellulose	66.5	64.9	63.1	61.5	59.3	57.9	54.4
	C. subvermispora			64.3	61.8	59.7	58.1	55.2	53.6
	T. versicolor			63.6	62.1	60.2	57.9	54.3	51.7
	P. chrysosporium	Lignin	17.2	13.2	11.8	10.4	9.8	8.3	7.9
	C. subvermispora			14.9	12.4	11.9	9.4	8.2	7.8
	T. versicolor			16.0	15.5	12.1	9.1	7.2	6.9

while the highest value in the latter stage of decay in poplar. These results are in accord with the previous results of weight loss and alkali solubility (Blanchette *et al.*, 1992).

Chemical Composition of Wood Blocks. The changes in chemical components of wood blocks during the decay test were shown in Table 2. The klason lignin contents of pine and poplar were rapidly decreased within 10 days of the decay test. After 50 days of the decay test, the decrement of klason lignin contents was less than 1%.

The decreasing rate of holocellulose in the early stage of the decay test was less than that of klason lignin. Holocellulose content was decreased in a steady rate, and the decreasing rate was less than 1% after 50 days of the decay test. Therefore, it is considered that the test fungi selectively degrade large amount of lignin in wood at the early stage of decay. Especially, *P. chrysosporium* showed the best capability on selective degradation of lignin among the test fungi. It is in accord with the result of Akhtar *et al.* (1993).

Conclusions

The screening of fungi can be the most important factor for biopulping of wood. Fungal activity and growth pattern of a fungus should be considered during the screening of fungi for biopulping. In this study, we tried to suggest a method how to evaluate the fungal activity and growth pattern to make a successful fungal pretreatment of wood. A method that measures the ergosterol content in fungal culture during the incubation was very effective for screening the white-rot fungi.

The results presented here show the importance of screening for selecting superior strains to be used in pretreating wood chips for biopulping or for other industrial purposes. In the results of the experiments, *P. chrysosporium* have excellent colonization and delignification capabilities on a variety of different substrates, softwood and hardwood. Therefore, it is considered that *P. chrysosporium* is the most effective fungus for biopulping among the test fungi and can be used to pretreat wood chips.

To conclude, it is definitely suggested that the determination of ergosterol content in the fungal culture during the incubation is one of the simple and effective screening method of white-rot fungi for the application to biopulping of wood.

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