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Pretreatments of Softwood Sawdust for Mycelial Growth of *Lentinus edodes*[*1]

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ABSTRACT

Mycelial growth of *L. edodes* by pretreatments of softwood was studied on a sawdust medium. The sawdust used was from the following softwood species : *Larix leptolepis*, *Pinus densiflora* and *Pinus koraiensis*. The pretreatment consisted of cold-water (48 h), hot-water (3 h) and steam extractions (3 h) at a ratio of 500 g : 3,000 mL (sawdust : distilled water). The sawdust medium was a mixture of 76% sawdust, 20% rice bran, 3% glucose, 0.4% potassium nitrate and 0.6% calcium carbonate. Following sawdust pretreatments proved most suitable : *L. leptolepis* (steam extraction), *P. densiflora* (hot-water extraction) and *P. koraiensis* (hot-water extraction). Mycelial growth on *P. koraiensis* sawdust increased in proportion to an increase in hot-water extraction time. Mycelial growth was optimum on the sawdust extracted for 12 hours, hot-water extraction beyond this period proved unsuitable. With the exception of *P. densiflora* at 100 μg/mL, antifungal activity occurred in every sample. Maximum inhibition of mycelial growth was obtained from following concentration of hot-water extractives : *P. densiflora* (10^4 μg/mL) and *P. koraiensis* (10^4 μg/mL). This study has provided useful preliminary information for the cultivation of *L. edodes*.

Keywords : pretreatment, extractives, sawdust, cold-water extraction, hot-water extraction, steam extraction, mycelial growth, *Lentinus edodes*

1. INTRODUCTION

Lentinus edodes (Berk.) Sing., (common name – black forest mushroom; Chinese name – shiang-gu; Japanese name – shiitake), belongs to the basidiomycetes, order *Agaricales*, and family.

The first species cultivated was *Auricularia auricula* around 600 A.D., almost 1400 years ago, the next was *Flammulina velutipes* which

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was first cultivated about 800 to 900 A.D., and the third was *L. edodes*, first cultivated about 1000 to 1100 A.D. (Chang & miles, 1989). The importance of *L. edodes*, the second most popular edible mushroom in the global market, is attributed not only to its nutritional value, but also to possible medical and food industrial applications (Hatvani, 2001).

L. edodes is most commonly grown on felled, aged logs of oak, beech, chestnut and alder, although other hardwood species can support the growth of this fungus (San Antonio, 1981). In more than 90 percent of cases, this fungus is cultivated on *Quercus* species. The cultivation of *L. edodes* on softwoods does not yield the same quantity and quality of the harvested mushroom (Shieh *et al.*, 1991a).

Therefore, proportion of softwood utilization for *L. edodes* cultivation is very low as compared to *Quercus* species, in spite of a large resource of *Larix leptolepis*, *Pinus densiflora*, *Pinus koraiensis* in Korea.

However, the greater abundance of softwoods in Korea as compared to hardwoods, and the opportunities to make use of thinnings and sawmill by-products should encourage greater use of softwoods for growing mushroom fungi, such as *L. edodes*.

Consequently, utilization of softwoods in favor of *Quercus* species, however, in order to utilize softwoods for mushroom cultivation is growing (Shieh *et al.*, 1991b). The component of wood which are inhibitory to *L. edodes* cultivation should be removed by appropriate methods mycelial growth (Matsui *et al.*, 2001; Kawachi *et al.*, 1991; Nakajima *et al.*, 1980). Also, the pretreatment method to be used has to be cost-effective.

The objective of this study was to determine experimentally the effects of various pretreatments of softwood sawdust, including cold-water, hot-water and steam extractions on the mycelial growth of *L. edodes*.

2. MATERIALS and METHODS

2.1. Fungal Strain

L. edodes strain (Sanlim No. 5) was obtained from the Forest Research Institute in Seoul, Korea. The fungus was maintained on a medium containing potatoes (infusion from 300 g/L), bacto dextrose (20 g/L) and bacto agar (15 g/L) and grown on agar plates.

2.2. Culture Conditions

2.2.1. Preparation of Sawdust Medium

The sawdust of *Larix leptolepis*, *Pinus densiflora* and *Pinus koraiensis* was screened to size of 10 mesh pass-60 mesh on. this sawdust fraction included bark. The pretreatment methods to remove mycelial growth inhibition components included cold-water (48 h), hot-water (3 h) and steam extractions (3 h) at a ratio of 500 g : 3,000 mL (sawdust : distilled water). After pretreatment the sawdust was separated from the extraction solution using filter paper filter (No. 2).

All experiments were performed on the sawdust medium : 76% sawdust, 20% rice bran, 3% glucose, 0.4% potassium nitrate and 0.6% calcium carbonate (air-dry weight). This sawdust medium included moisture contents about 65%.

2.2.2. Growth of L. edodes Mycelium

The sawdust medium was placed in test tubes (180 mm × Φ1.8 mm) and petridishes (90 mm inside diameter). A 10 mm diameter of fungal mycelium was placed in the center of each test tube and petridish, and then incubated at 25°C.

Mycelial growth was measured at intervals up to 11 days. The diameter of the mycelium colonies was recorded in mm.

Earlier studies have shown that measurement of mycelial growth contain several methods that in both woody and other substrates (Aidoo *et al.*, 1981; Boyle & Kropp, 1992; Braid & Line, 1981; Matcham *et al.*, 1985).

Mycelial growth was measured in the vertical direction relative the inoculation surface in test tubes, and in the horizontal direction relative to the inoculation surface in petridishes. The experiment was performed in five replicates.

2.3. Assay of Antifungal Activity

Freeze dried powder of extractives was dissolved in distilled water and the appropriate aliquots of the solution were added to sterilized PDA to give 100, 10^3, 10^4 μg/mL concentrations of extractives.

These potato dextrose agar (PDA) plates were inoculated with strain (Sanlim No. 5) of *L. edodes* after sterilization (autoclaving by 1.2 kg/cm^2 during 15 minutes). The antifungal activity was measured via determination of the mycelial growth of the samples. The mycelial growth was measured after incubation of the plates for 10 days at 25°C.

Antifungal activity was calculated by the following equation :

$$\textit{Antifungal activity}(\%) = \left\{1 - \frac{\textit{mycelial growth diameter(mm) in test petridish}}{\textit{mycelial growth diameter(mm) in control petridish}}\right\} \times 100$$

2.4. Statistical Analysis of Experimental Data

Microsoft Excel was used for the statistical analysis of mycelial growth data to determine the significance of the test conditions. The general linear models procedure of SAS (1987) was used for analysis of mycelial growth by pretreatment. If the main effects were significant, the means were separated using Duncan's multiple range test.

3. RESULTS and DISCUSSION

3.1. Pretreatment of the Softwood Sawdust Medium

The pretreatment of the softwood sawdust medium had a marked effect on mycelial growth of *L. edodes*. The data on the effect of different pretreatment methods of the softwood sawdust medium on the mycelial growth are shown in Table 1. The pretreatment of sawdust from *P. densiflora* and *P. koraiensis* had a pronounced positive effect on the mycelial growth. However, the mycelial growth was lower for *L. leptolepis* ($P < 0.01$).

The pretreatments which resulted in positive mycelial growth were : medium *L. leptolepis* (steam extraction), *P. densiflora* (hot-water extraction), and *P. koraiensis* (hot-water extraction), the corresponding mycelial growth being 33.1, 27.9 and 27.8 mm, respectively.

The highest mycelial growth was obtained from steam extraction of *L. leptolepis*, although the difference from the control was only mar-

Table 1. Influence of pretreatment on mycelial growth of *L. edodes* in the sawdust medium (Unit; mm)

Species	Control	cold-water extracted	hot-water extracted	Steam extracted
Larix leptolepis	32.6 a	27.9 c	30.2 b	33.1 a
Pinus densiflora	22.1 c	24.2 b	27.9 a	26.7 a
Pinus koraiensis	18.9 c	25.3 b	27.8 a	24.3 b

[a] Means followed by the same letters within each column are not significantly different by Ducan's multiple range test ($P < 0.01$).

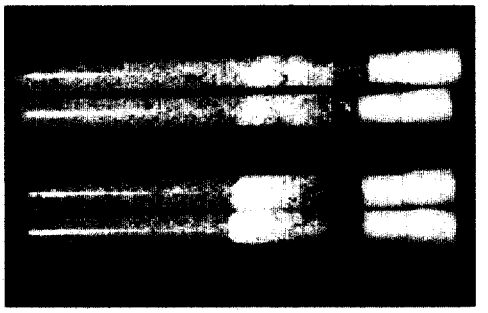

Photo 1. Effect of pretreatment on mycelial growth of *L. edodes* in the softwood sawdust medium of *P. koraiensis* (5 days incubation at the 25°C). * (A) Test tube; before pretreatment, (B) Tetst tube; after pretreatment.

ginal. The mycelial growth on the cold and hot-water extincted media from this wood species. Depletion in nutrients due to about pretreatments is the most likely reason for the observed decrease in mycelial growth on this medium.

Therefore, pretreatment of *L. leptolepis* is unsuitable for mycelial growth of *L. edodes*.

In the cases of *P. densiflora* and *P. koraiensis*, increase in mycelial growth, all pretreatment methods caused on the highest mycelial growth occurring in the hot-water extraction. The observed increase of mycelial growth in this case probably due to pretreatment removal of materials from the sawdust which maybe inhibitory to mycelial growth.

The effect of pretreatment of the sawdust medium of *P. koraiensis* on the growth of *L. edodes* mycelium was also tested using test tubes, and is illustrated in Photo 1.

In photo 1 test tubes A and B show mycelial growth approximately 5 days after incubation. Test tube B shows distinctly higher mycelial growth as compared to test tube A, which clearly demonstrates the advantage of hot water extraction in enhancing mycelial growth.

Table 2. Antifungal activity of hot-water extractives in the PDA medium

Species	Antifungal activity, %		
	100 μg/mL	10^3 μg/mL	10^4 μg/mL
Pinus densiflora	N.D.	6.7	32.4
Pinus koraiensis	4.8	20.7	48.3

Note; N.D. : Not detected

3.2. Antifungal Activity of Hot-water extractives

The hot-water extractives had a marked effect on mycelial growth of *L. edodes*. The results of the antifungal activity of different concentration of hot-water extractives on the PDA medium are shown in Table 2. The hot-water extractives of *P. densiflora* and *P. koraiensis* had a marked effect in terms of the measured antifungal activity for *L. edodes*.

As shown in Table 2, the antifungal activity of hot water extractives from *P. densiflora* was found at 10^3 μg/mL and 10^4 μg/mL concentration, but the activity was not detected at 100 μg/mL. In the cases of *P. koraiensis*, the antifungal activity occurred in all samples of hot-water extractives, with 10^4 μg/mL concentration showing the highest activity. Maximum inhibition was obtained from 10^4 μg/mL concentration of hot-water extractives from both *P. densiflora* and *P. koraiensis*, the corresponding antifungal activity being 32.4 and 48.3%, respectively. These data suggest that the antifungal activity against *L. edodes* was due to hot-water extractives.

The mycelial growth inhibition of *L. edodes* in an earlier work was probably due to a synergistic effect of ferruginol and sandaracopimarinol, which are the major terpenoids in sugi (Matsui *et al.*, 2001). The inhibitory effect of o-isopropylphenol or thymol on the mycelial

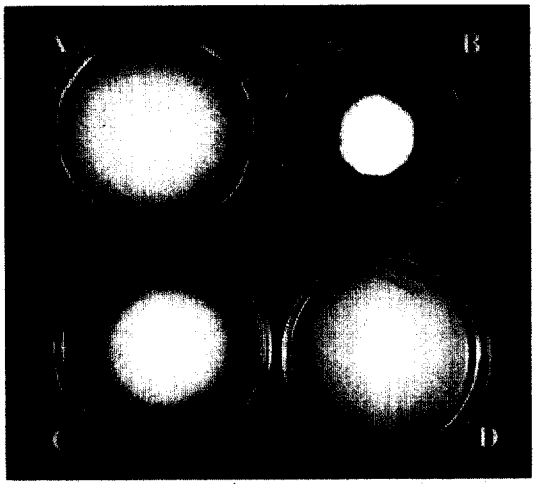

Photo 2. Effect of mycelial growth inhibition of *L. edodes* in the PDA plate medium by hot-water extractives of *P. koraiensis* (10 days incubation at the 25°C). * A; control, B; 10^4 μg/mL, C; 10^3 μg/mL, D; 100 μg/mL.

growth of *L. edodes* was almost the same as that of ferruginol, suggesting that the o-isopropylphenol moiety of ferruginol may be the main functional moiety of the inhibitors (Kawachi *et al.*, 1991). Ferruginol was found to have toxic effect on the growth of *L. edodes* fungus, because the more ferruginol was added to the medium, the lesser the fungus grew (Nakajima *et al.*, 1980). On the other hand, the results of our experiments showed that the mycelial growth inhibition components derived by the hot-water extractives in the antifungal activity had similar effect.

As shown in Photo 2, four different concentration by hot water extractives of *P. koraiensis* were tested with regard to the antifungal activity, judging by the growth of *L. edodes* mycelium on the PDA plate medium. The petridish A of the control medium prepared for comparison of the mycelial growth inhibition with others sample, added hot-water extractives to medium.

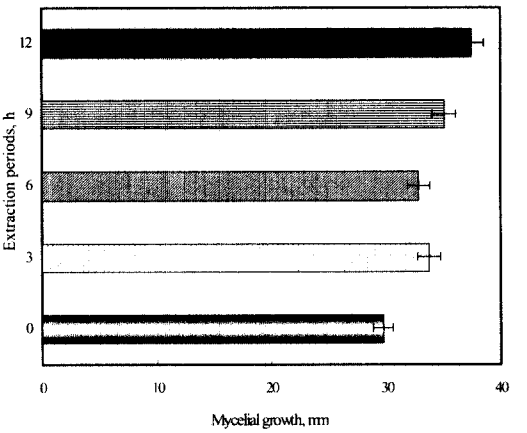

Fig. 1. Effect of hot-water extraction time onr mycelial growth of *L. edodes* in the sawdust medium of *P. koraiensis* (11 days incubation at 25°C).

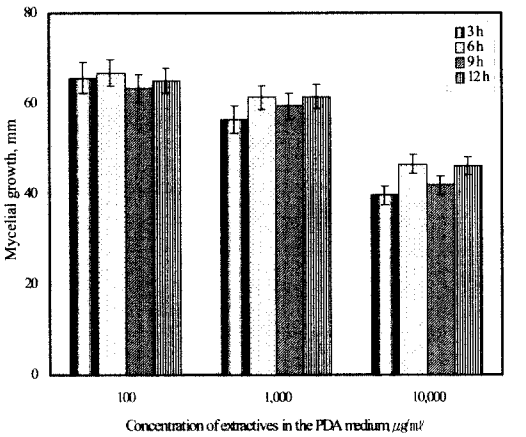

Fig. 2. Effect of hot-water extractives of *P. koraiensis* on growth inhibition of *L. edodes* mycelia in the PDA plate medium (10 days incubation at 25°C).

As shown in Fig. 1, the hot-water extraction time for *P. koraiensis* had only marginal effect on mycelial growth of *L. edodes*. The results of the mycelial growth by extraction time on the sawdust medium of *P. koraiensis* are described in detail below.

The mycelial growth was enhanced in proportion to extraction time, with the exception of 6

hours extraction which showed to slight decrease as compared to 3 hours extraction. The highest mycelial growth was observed on the medium extracted for 12 hours, which is regarded as the most suitable extraction time with regard to the mycelial growth of *L. edodes*.

The results of the antifungal activity by different concentration of hot-water extractives and extraction time on the PDA medium are shown in Fig. 2. The concentration of hot-water extractives from *P. koraiensis* had much effect on the mycelial growth of *L. edodes*. However the hot-water extraction time had only marginal effect.

4. CONCLUSIONS

The results of this study have shown that the mycelial growth of *L. edodes* can be enhancing under removal of toxic extractives on readily available softwoods in a extraction pretreatments. It is suggest that increase of mycelial growth was probably due to removal of inhibitory materials from the softwoods sawdust. Earlier studies have shown that softwoods contain several compounds that exert inhibitory effects on the mycelial growth of *L. edodes* (Matsui *et al.*, 2001; Kawachi *et al.*, 1991; Nakajima *et al.*, 1980).

Removing of toxic extractives play a very important part in utilization of softwoods for mycelial growth of *L. edodes*. The extraction pretreatment of the softwood sawdust had a marked effect on mycelial growth of *L. edodes*. The mycelial growth was optimum on the sawdust extracted for 12 hours, hot-water extraction beyond this period proved unsuitable.

The antifungal activity effects were found in the hot water extractives, and hot water extractives from *P. densiflora* and *P. koraiensis* was found at 10^3 μg/mL and 10^4 μg/mL concentration. These data suggest that the antifungal activity against *L. edodes* was due to hot-water extractives.

This study has provided useful preliminary information which would be helpful in the cultivation of *L. edodes*.

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