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Effect of Supplement nutrition on the Mycelial Growth of *Lentinus edodes*[*1]

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ABSTRACT

Mycelial growth of *L. edodes* by supplement nutrition 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 added nutritions consisted of carbon nutritions(sucrose, active carbon, xylose, glucose, paper pellet), nitrogen nutritions(potassium nitrate, ammonium chloride, asparagine, glutamic acid) and vegetable oil(rice bran oil). The sawdust medium was a mixture of 76% sawdust, 20% rice bran, 3% carbon nutrition, 0.4% nitrogen nutrition and 0.6% calcium carbonate. Following addition of carbon and nitrogen nutritions on the sawdust medium proved most suitable : *L. leptolepis* (glucose, glutamic acid), *P. densiflora* (active carbon, asparagine) and *P. koraiensis* (xylose, glutamic acid). The highest mycelial growth was obtained from sawdust medium of optimum condition with 97% of *L. leptolepis*, 110% of *P. densiflora* and 98% of *P. koraiensis*. This study has provided useful preliminary information for the cultivation of *L. edodes*.

Keywords : *Lentinus edodes*, mycelial growth, sawdust medium, nutritions, vegetable oil

1. INTRODUCTION

Lentinus edodes (Berk.) Sing., (common name - black forest mushroom), belongs to the basidiomycetes, order *Agaricales*, and pleurotaceae family.

The increasing demands for *L. edodes* are due to healthy foods, poor in calories and in fat, rich in vegetable proteins, chitin, vitamins and minerals. For a long period of time, this mushroom has been valued for its unique taste, flavor and as a medicinal tonic (Manzi *et al.*, 1999; Buswell *et al.*, 1993; Campbell *et al.*, 1999; Chang & Miles, 1989).

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; Tautorus, 1985; Ito, 1978; Nutalaya *et al.*, 1981). On the other hand, the cultivation of *L.*

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edodes on softwoods does not yield the same quantity and quality of the harvested mushroom (Shieh *et al.*, 1991). 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, addition of nutrition play a very important part in utilization of softwoods for mycelial growth of mushroom (Kadiri *et al.*, 1994; Alberghina, 1973; Fisidi *et al.*, 1994; Jandaik *et al.*, 1976; Boyle, 1998; Shen *et al.*, 2001). Calcium and magnesium were the best macro-elements while micro-elements (copper and zinc) enhanced optimum growth (Jonathan *et al.*, 2001). Calcium, magnesium, sodium, potassium and zinc significantly enhanced growth whereas hormones and vitamins did not (Fasidi *et al.*, 1996). Also, addition of nutrition method to be used has to be cost-effective.

The objective of this study was to determine experimentally the effects of supplement nutrition on the softwood sawdust medium, including carbon nutrition, nitrogen nutrition and vegetable oil for the mycelial growth of *L. edodes*.

2. MATERIALS and METHODS

2.1. Wood Species and Fungal Strain

The softwood species used to *Larix leptolepis*, *Pinus densiflora* and *Pinus koraiensis*. At this time, control sample used to *Quercus variabilis*. The used softwood species and *L. edodes* strain (sanlim No. 5) were obtained 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 *L. leptolepis*, *P. densiflora* and *P. 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, hot-water and steam extractions at a ratio of 500 g : 3000 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. Application of Nutrition

The sawdust medium contained 3% carbon nutrition, 0.4% nitrogen nutrition and 5% vegetable oil (air-dried weight). Carbon nutritions were applied to sucrose, active carbon, xylose, glucose and paper pellet. Nitrogen nutritions were applied to potassium nitrate, ammonium chloride, asparagine and glutamic acid. Vegetable oil was applied to rice bran oil.

2.3. Growth of L. Edodes Mycelium

2.3.1. Measurement of Mycelial Growth

The sawdust medium was placed in test tubes (180 mm × Φ1.8 mm). A 10 mm diameter of fungal mycelium was placed in the center on the sawdust medium of each test tube, and then incubated at 25°C. Mycelial growth was measured at intervals up to 3 days. The growth length of the mycelium colonies was recorded in mm. Mycelial growth was measured in the

Table 1. Influence of carbon nutrition for mycelial growth of *L. edodes* in the softwood sawdust medium

(Unit: %)

Species	Sucrose	Active carbon	Xylose	Glucose	Paper pellet
Larix leptolepis	16.90	16.31	15.61	18.49	15.66
Pinus densiflora	14.74	17.93	14.39	17.01	16.96
Pinus koraiensis	16.82	16.93	20.25	16.18	17.04

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

vertical direction relative the inoculation surface in test tubes. The experiment was performed in five replicates.

2.3.2. 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. Influence of Supplement Nutrition

3.1.1. Effect of Carbon Nutrition

Addition of carbon nutritions (sucrose, active carbon, xylose, glucose, paper pellet) on the softwood sawdust medium had a marked effect on mycelial growth of *L. edodes*. The data on the effect of different carbon nutrition of the softwood sawdust medium on the mycelial growth are shown in Table 1.

Addition of carbon nutrition which resulted in positive weight loss of sawdust medium was : medium *L. leptolepis* (glucose), *P. densiflora* (active carbon), and *P. koraiensis* (xylose), the corresponding ratio of weight loss being 18.49, 17.93 and 20.25%, respectively. The highest weight loss of sawdust medium was obtained from xylose of *P. koraiensis*, although the mycelial growth was only marginal.

Utilization of glucose by some other tropical edible macro fungi has been reported (Jonathan *et al.*, 2001; Fasidi *et al.*, 1994; Hong, 1978; Chandra *et al.*, 1977; Oso, 1977). Especially, Jandaik *et al.* reported to the preference of glucose over other carbon compounds may be due to the ease with which this sugar was metabolized to produce cellular energy (Jandaik *et al.*, 1976).

Addition of carbon nutrition from softwood sawdust medium had a pronounced positive effect on the mycelial growth. However, the carbon nutrition for mycelial growth on the sawdust medium appeared differently within each species ($P < 0.01$). The observed an increase of weight loss in this case due to supplement of nutrition for the sawdust medium which maybe help to mycelial growth.

The effect of carbon nutrition 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.

Photo A to E show mycelial growth approximately 120 days incubation. Test tube B shows distinctly higher mycelial growth as compared to the others, which clearly demonstrates the advantage of carbon nutrition in enhancing mycelial growth.

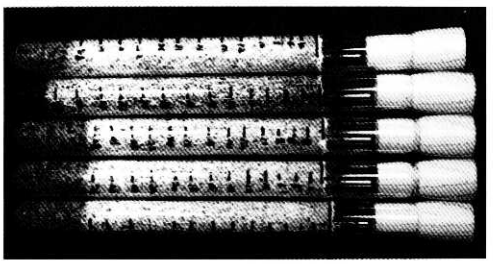

Photo. 1. Effect of carbon nutrition for mycelial growth of L. edodes in the softwood sawdust medium of P. koraiensis (120 days incubation at the 25°C). * A; sucrose, B; active carbon, C; xylose, D; glucose, E; paper pellet

3.1.2. Effect of Nitrogen Nutrition

Addition of nitrogen nutrition (potassium nitrate, ammonium chloride, asparagine, glutamic acid) on the softwood sawdust medium had a marked effect on mycelial growth of *L. edodes*. The results of mycelial growth by different nitrogen nutrition on the sawdust medium are shown in Fig. 1.

Addition of nitrogen nutrition which resulted in positive mycelial growth was : medium *L. leptolepis* (glutamic acid), *P. densiflora* (asparagine), and *P. koraiensis* (glutamic acid), the corresponding mycelial growth being 67.7, 67.4 and 57.1 mm, respectively. The highest mycelial growth was obtained from glutamic acid of *L. leptolepis*, although the mycelial growth was only marginal.

Utilization of nitrogen nutrition by some other tropical edible macro fungi has been reported. Garcha *et al.*(1979) obtained a substantial growth of *V. volvacea* in NH_4NO_3, whereas the other inorganic nitrates tested inhibited growth and ammonium nitrate was the most utilizable among the inorganic nitrogen sources tested. Also, Yusef *et al.*(1967) reported that the glutamic acid and leucine was as good nitrogen for edible mushroom.

Accordingly, nitrogen nutrition for mycelial growth of *L. edodes* is assumed to difference within species same as nitrogen nutrition.

3.1.3. Effect of Vegetable Oil Nutrition

Addition of vegetable oil (rice bran oil) on

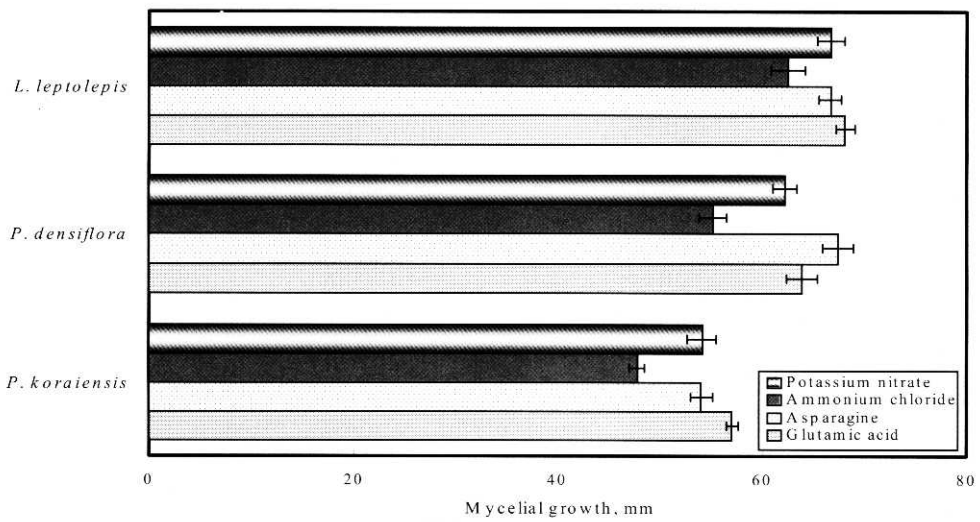

Fig. 1. Effect of nitrogen nutrition for mycelial growth of *L. edodes* in the softwood sawdust medium of *L. leptolepis, P. densiflora* and *P. koraiensis* (12 days incubation at the 25°C).

Table 2. Optimum condition of sawdust medium for mycelial growth of *L. edodes*

Species	Carbon nutrition	Nitrogen nutrition	Vegetable oil
Larix leptolepis	Glucose	Glutamic acid	–
Pinus densiflora	Active carbon	Asparagine	–
Pinus koraiensis	Xylose	Glutamic acid	Rice bran oil

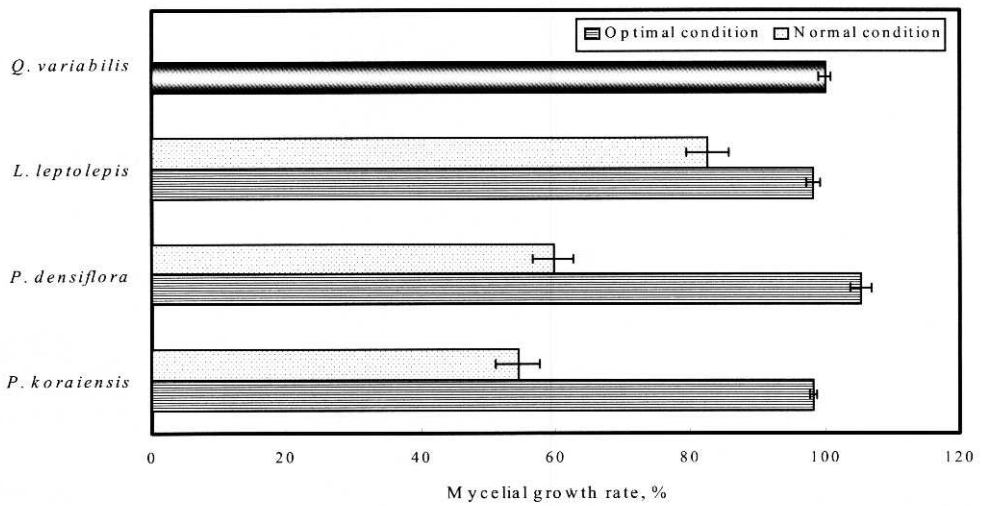

Fig. 2. Comparison of control condition(non pretreatment and nutrients exception) and optimal condition in the mycelial growth of *L. edodes* in the softwood sawdust medium of *L. leptolepis, P. densiflora* and *P. koraiensis* (12 days incubation at the 25°C).

the softwood sawdust medium had a marked insufficient effect on mycelial growth of *L. edodes*. Because, addition of vegetable oil for mycelial growth had a marked little bit increase or same growth compare to control samples. Accordingly, addition of vegetable oil is assumed to disadvantage for mycelial growth of *L. edodes* on the softwood sawdust medium.

3.2. Effect of Optimum Condition of Softwood Sawdust Medium

Addition of nutrition on the softwood sawdust medium had a marked effect on mycelial growth of *L. edodes*. The softwood sawdust medium of optimum condition for mycelial growth of *L. edodes* are shown in Table 2.

As shown in Table 2, the optimum condition of sawdust medium from *L. leptolepis* was found at glucose as carbon nutrition and glutamic acid as nitrogen nutrition, but it was not found at pre-treatment and addition of vegetable oil. In the case of *P. densiflora* and *P. koraiensis*, the mycelial growth increased in all samples after pretreatment of sawdust medium with active carbon, xylose as carbon nutrition and asparagine, glutamic acid as nitrogen nutrition showing the highest mycelial growth, respectively.

As shown in Fig. 2, the sawdust medium of optimum condition had much effect on the mycelial growth of *L. edodes*. The mycelial growth rate increased in all sawdust medium of optimum condition with 97% of *L. leptolepis*, 110% of *P. densiflora* and 98% of *P. koraiensis*

showing the highest mycelial growth. The highest mycelial growth was obtained from sawdust medium of optimum condition, although the difference from the control was only marginal.

It means that the softwood sawdust medium will be possible to use for production of *L. edodes* if this study has a mark effect in the fruit body production as well as mycelial growth.

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 (kim *et al.*, 2002). 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 hs, 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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