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**Effects of Nutritional Component on Mycelial Growth
and Fruit Body Yield of the Shiitake Mushroom, *Lentinus edodes*,
on Sawdust Substrate**

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A pulping waste liquor-component, consisting mainly of reducing sugar, lignin sulfonic acid, and sugar-derivatives was tested for its ability affect mycelial growth and basidiocarp formation of shiitake mushroom, *Lentinus edodes* (Berk.) Sing.. Sawdust substrate supplemented with wheat bran and this component were inoculated with spawn plugs, that is small cylinders of wood colonized by mycelium of *L. edodes*. The supplementation of nutrients with this component resulted in greater mycelial biomass in substrates, shorter spawn run time, and furthermore, increase in the amount of fruit bodies in succeeding generations. These results suggest a potential use of the waste liquor-component from pulping to produce a large amount of fruit bodies with much shorter periods of cultivation time.

INTRODUCTION

The cultivation of shiitake, *Lentinus edodes* (Berk.) Sing., on natural logs is an established industry, especially in Japan (San Antonio, 1981). Shiitake production in 1983-84 was estimated at 234,436 metric tons in terms of fresh equivalent weight and now accounts for 15.4% of the world's total production of edible mushrooms. Shiitake is the second most important edible mushroom in the world from the standpoint of production, and it is the most popular fungus cultivated in Japan, China, and other East Asian countries. Japan, the major producer, accounted for 67.8% of the world total in that year. This mushroom has been valued for its unique taste and flavor as well as a medicinal tonic. For all these reasons the demand for shiitake has greatly increased in recent years, followed by an increase in production. With new production methods it is anticipated that there will be a continuous expansion of production in the world (Farr, 1983 ; Chang and Miles, 1989).

In an attempt to develop a more efficient and dependable method for the production of shiitake mushrooms, it has recently been tried to produce it on supplemented sawdust. Indoor cultivation on supplemented sawdust substrates provides a more rapid and controled method of cultivation than the present one using the traditional outdoor logs method. Approximately 3 to 4 times as much production can be obtained in one-tenth of the time on supplemented sawdust as compared to the natural logs. The technology for the cultivation of shiitake on enriched sawdust media in plastic bags was originally developed in Japan, Taiwan and mainland China. American and European growers have adapted much of this technology in their operations. As a

result, the cultivation of shiitake is a rapidly growing industry in the U. S. and Europe (Miller and Jong, 1986).

Substrates usually consist of sawdust mixed with rice bran, wheat bran or nutrient supplements. The sawdust should be composed of hardwood species, preferable *Quercus* spp., *Acer* spp. and *Betula* spp.. The wood is supplemented with a sugar source such as grain corn powder, glucose, and a nitrogen-vitamin-mineral such as oatmeal, rice bran or wheat bran. A ratio of 8 : 1 : 1 of sawdust to wheat bran to grain on a dry weight basis is reported to give the optimum yield (Roysse, 1985).

Water is added to the mixture so that the final moisture content is between 55-68% depending on the ability of the sawdust to absorb and retain the water. Badham (1989) studied the relationships between sawdust and water, water availability in terms of water activity or water potential. Factors such as concentration and type of nutritional supplements and moisture content may greatly affect yields (Ohga, 1989).

The study reported here was conducted to determine the effects of selected nutritional components on spawn run time and yield of *L. edodes* mushroom.

MATERIALS AND METHODS

Substrates and preparation

Beech, *Fagus crenata* B1. was obtained from the Kyushu University Forest at Shiiba, Miyazaki Prefecture and freshly milled sawdust was used as the main substrate ingredient. Sawdust grade contains particle sizes between 20 mesh and 48 mesh of U. S. standard sieve size. Moisture content of the initial sawdust used in these experiments was 33.6%.

After mixing the sawdust with wheat bran (6 : 1), a supplemented component dissolved in distilled water was added. The component tested was a sulfite pulping waste liquor-component made from softwood, consisting of 30% of lignin sulfonic acidcalcium salt, 34% of reducing sugar (mannose, xylose, glucose etc.), 34% of sugar-derivatives (sulfonated monosaccharides, aldonic acid) and 2% of inorganic substances ; commercial name "Sanpearl CP" (product of Sanyokokusaku Pulp Co., Tokyo).

One kg (wet weight) of the mixture was bagged in unused polypropylene bags (ϕ 12 cm x 12 cm) and a cavity for spawn inoculation was made in the center, then stoppered with a microporous filter patch for gas exchange during spawn growth. This medium was composed of 300 g (dry weight) of beech sawdust and 50 g (dry weight) of wheat bran, and distilled water was added, containing three concentrations of the component (1.75, 3.50, 7.00 g per bottle ; that is 0.5, 1.0, 2.0% of substrate based on dry weight, respectively).

The bags were placed on metal carts and autoclaved at 121°C until the center of the bags reached 121°C for at least 15 min. Following heat treatment the substrate was allowed to cool to 25°C overnight.

Five spawn plugs of selected strains were added to the center inoculation hole of the cooled substrate. Shiitake (*Lentinus edodes* (Berk.) Sing.) spawn plugs, small cylinders of wood colonized by mycelium of *L. edodes*, obtained from a commercial source were used. The strains used in these experiments were Mori Institute no. 121, no. 290, no. 465, no. 505, no. 701, Akiyama Spawn Co. no. 20, no. 567 and Hokken

Institute no. 600, no. 601.

Spawn run and determination of mycelial biomass

Inoculated substrates, contained in bags, were transferred to an incubator for vegetative growth at $23 \pm 1^\circ\text{C}$ with 12 h of light per day from cool-white fluorescent bulbs. The biomass and mycelial growth degree were determined by measuring the area of expanding colonies with a slide caliper.

It was thought that chitin assay and whiteness measurement would be useful for the estimation of fungal biomass in sawdust substrates (mature degree) at this stage. The chitin assay method has three basic steps : (i) Acid hydrolysis of chitin-containing wood samples, and dilution of the hydrolysates ; (ii) Ion-exchange chromatography ; (iii) Estimation of the resultant glucosamine concentrations by colorimetric method (Tsuji et al., 1969 ; Sharma et al., 1977 ; Braid and Line, 1981). Whiteness measurement of substrate surface parts was done with a photoelectric colorimeter (Minolta CR-200). There was a positive linear correlation between whiteness and fungal biomass, physiological maturity (Ohga, 1989).

Fruit body formation

On the 40th day, the polypropylene bags were removed completely and the colonized substrates (synthetic log) were transferred to an outdoors laying yard. A hinoki (*Chamaecyparis obtusa* Endl.) forest was used as the synthetic logs laying yard (Kyushu University Forest at Kasuya, Fukuoka Prefecture).

L.edodes mushrooms were harvested (picked from the substrates) from each synthetic log when the veil had broken and the gills were fully exposed. The picked mushrooms were counted and weighed.

RESULTS AND DISCUSSION

Evaluation of unique genotypes on the supplemented substrates

Differences in mycelial growth by addition of the component among the nine strains were observed after fifteen days in the incubator. Strains no. 701 and no. 567 had excellent growth of the nine strains evaluated (Table 1).

Data collection was continued for the two productive strains. Best growth occurred in the second concentration level (1.0% level) at the 20th day of culture (Table 2). Towards the end of the spawn run (about the 30th day), the surface of the bag began

Table 1. Effect of additive on the mycelial growth of *Lentius edodes* among the nine strains (on the 15th day after inoculation, concentration of additive is 0.5 %.).

<i>Lentius</i> strains								
Low temp. type		Middle temp. type			High temp. type			
M-121	M-290	M-505	M-701	M-465	A-20	A-567	H-600	H-601
136 ^(a)	114	126	176	112	122	137	132	130

^(a) : Growth ratio (%) = $A/B \times 100$. A : Length of colony extension growing on the medium containing additive, B : Length of colony extension growing on the basal medium.

Table 2. Effect of additive on the mycelial growth of *Lentiuus edodes* (on the 20th day after inoculation of spawn no. 567.).

Control	Addition		
	0.5 %	1.0 %	2.0 %
6.8cm (3.6cm) ^(a)	8.9 (4.8) 130.9 ^(b) (133.3)	9.4 (5.5) 138.2 (152.8)	8.3 (4.5) 122.1 (125.0)

^(a): Particular mature zone.

^(b): Growth ratio : Refer to Table 1.
Mean of 20 blocks.

Table 3. Glucosamine content and surface whiteness of the sawdust substrate of *Lentinus edodes* (on the 40th day after spawn inoculation.).

Additive concentration (%)	Glucosamine content (mg/g dry wt)	Surface whiteness (Z ^(a) /1.18)
0	5.26	22.41
0.5	6.76*	31.04*
1.0	7.32**	40.12**
2.0	7.41**	41.55**

*: * *: Significant difference from the control at $P < 0.05$, $P < 0.01$, respectively.

^(a): Z: CIE standard colorimetric system.

to form a thick white mycelial mat and began to swell. This tissue is analogous to the bark of a natural log and it is this tissue through which the primordia will emerge. All concentration levels of the additive tested showed significant increases in growth over the control.

Mycelial biomass

The amount of mycelia in substrates can be estimated from several data in Table 3. According to Tokimoto et al. (1987), the glucosamine content in a bed long was usually 2.75 to 3.41 mg/g. The present results suggested that mycelial biomass would be increased in sawdust substrates compared with bed logs. Glucosamine contents were increased by supplementation with the component. Surface whiteness of several substrates gave similar results as compared with glucosamine. This suggest that much more mycelia are present in the supplemented substrates than in the control.

Environment on fruit body production

After the spawn run and swelling cycle (about the 40th day), the surface became dark brown and took on a leathery-like texture. The plastic bag was removed and the substrates were placed in contact with forest floor in a natural environment outdoor laying yard.

Fruiting of *L. edodes* under the Kasuya forest conditions were essentially determined by the temperature and precipitation. The daily record of temperature and precipitation during these experiments has been summarized as daily maximum-

minimum temperatures and monthly total precipitation (Fig. 1). The fruiting of *L. edodes* requires a drop in temperature, increased humidity, and a certain degree of light. Major *L. edodes* production began in October at minimum-maximum temperatures of 6.2°C to 25.3°C and ended in December. Precipitation was consistent during October-December.

Effects of nutritional component on fruit body production

The first reliable flush occurred on the 50th day after spawn inoculation (Fig. 2). No. 567 strain fruited spontaneously without any treatment. After pin formation, the sporocarp will mature within about one week. During the experiments, normal primordia may form and produce a considerable amount of good quality mushrooms.

All concentration levels of addition tested showed significant increases in yield over the control, without decreasing uniformity of mushroom size (Fig. 3). Three levels of addition (0.5%, 1.0%, 2.0%) showed significant increases in yield over the control of 132.7%, 153.1% and 162.6%, respectively. There appeared to be a progressive increase in yield with increasing concentrations of the additive. The highest concentration showed most significant increases. Variance analysis showed significant difference from the control at $P < 0.01$ and $P < 0.05$, respectively, at the two higher concentrations, but no differences found at the lowest concentration. The effects of

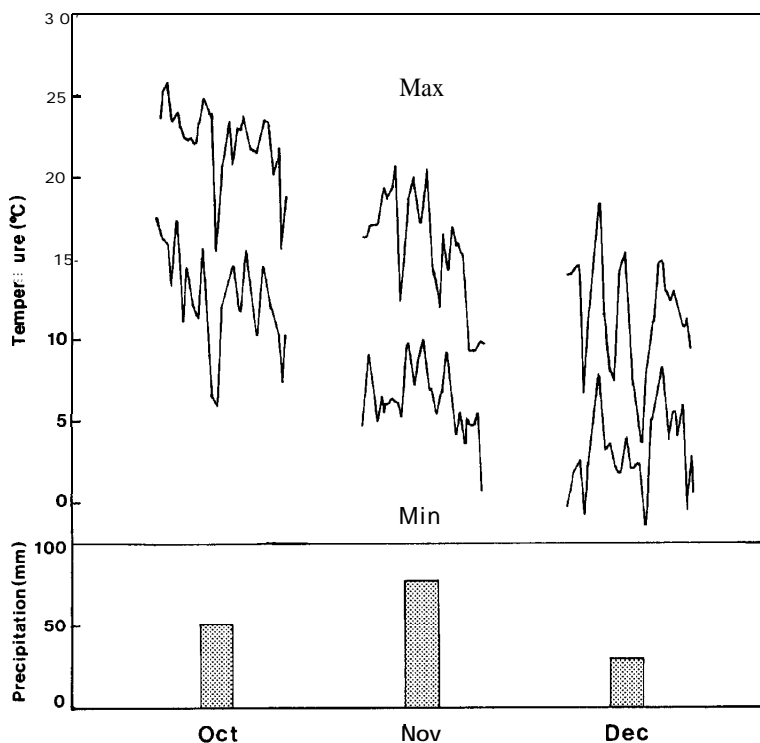


Fig. 1. Daily minimum and maximum temperatures with total monthly precipitation for *Lentinus edodes* experiments in open forest plot at Kasuya, Fukuoka from October to December 1988.



Fig. 2. Mature fruit bodies of *Lentinus edodes* grown in sawdust substrate. Since the plastic bags were removed at a later stage of mycelial development, most of the fruit bodies flushed on the top (first flush ; on the 50th day after spawn inoculation.).

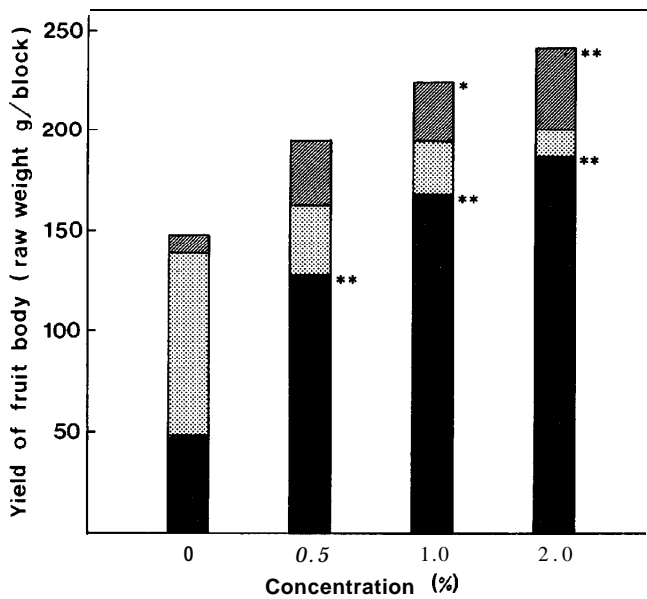


Fig. 3. Effect of additive on yield of *Lentinus edodes* fruit body.

*, **: Significant difference from the control $P < 0.05$, $P < 0.01$, respectively.

■: 1st flush (after 40-50 days from spawn inoculation), ▨: 2nd flush (60-70 days), ▩: 3rd flush (85-100 days).

Mean of 20 blocks.

analogy compounds to the growth of mycelium and formation of fruit bodies were studied ; that is on *Pleurotus* spp. (Zadražil, 1974) and on *L. edodes* (Inaba *et al.*, 1979 ; Kawamura *et al.*, 1983). Iijima *et al.* (1986) reported that this component stimulated the growth of mycelia of *P. ostreatus* suitable as protoplast sources, and markedly

increased the regeneration frequency of the protoplasts.

The reasons for increased yield from substrates with nutritional supplement may include greater mycelial biomass, increased levels of enzymes present, increased solubility of wood components, or combinations of these factors. The results reported in this work show that waste liquor component from sulfite pulping, has marked effects on the mycelial growth and fruit body yield of the *L. edodes* mushroom. Also, basidiocarp formation, fruit body yield, was positively correlated with both mycelial initial growth rate and its biomass in the sawdust substrate.

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