

Cultivation of some important edible mushrooms using the sawdust from waste Shiitake bed logs.

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► Abstract

By using sawdust of waste bed logs, some important edible mushrooms, the commercial strains of *Pleurotus ostreatus* (Fr.) Kummer and *P. cornucopiae* Rolland var. *citrinopileatus*, and the wild strain of *Flammulina velutipes* (Fr.) Sing. were cultivated. In the cultivation of *P. ostreatus* and *F. velutipes*, the yield of fruit bodies on the substrate using the sawdust of waste bed logs was little lower than that on the substrate using the sawdust from softwood, *Abies Sachalinensis* Mast. The days required for the spawn running and for the harvest on waste bed logs were significantly shorter than those on the latter sawdust substrate, so that the net productivity in year was slightly increased in the former substrate. The days required for the primordia formation and for the harvest of the former substrate were almost same as on the latter substrate in cultivation of *P. cornucopiae*. On the other hands, the yield of fruit bodies and the net productivity on the former substrate were significantly higher, about twice, than that on the latter substrates. These results suggested that the sawdust of waste bed logs could be used as a resource for the cultivation of these mushrooms, and it would be more suitable sawdust substrate than that softwood material to be generally used in the cultivation of these mushrooms.

► Introduction

Many kinds of edible mushrooms are cultivated by the sawdust-based cultivation methods throughout year, and their total quantities are increasing year by year. Though, the growers' price is falling down in wholesale market, and the price of raw materials and personal expenses are raising up, so that the management of growers is falling into financial difficulties. On the other hand, after cultivation of those mushrooms, a great amount of culture waste is discharged. The waste culture media can be converted into animal feed or organic fertilizer, though, now a great amount is abandoned for their handling complication. If these culture waste can be reused as materials for the cultivation of mushroom, it might be reduced the cost of production and might be recycled of resources. The cultivation of some edible mushrooms by using wastes from agriculture, food industries and culture waste of mushrooms was reported (Ohga et al. 1991, Jwanny et al. 1995, Eguchi et al. 1996, Okamura et al. 1996, Ragunathan et al. 1996, Akamatsu 1998, Harada et al. 1999). In this report, we described waste shiitake bed logs could be reused as substrates for the sawdust-based cultivation of *P. ostreatus* (Jacq:Fr.) Kummer, *P. cornucopiae* (Paulet) Rolland var. *citrinopileatus* (Sing.) Ohira., and *Flammulina velutipes* (Fr.) Sing.

► Materials and methods

1. Strain

The strains of *P. cornucopiae*, *P. ostreatus*, and *F. velutipes* used throughout these experiments

were our laboratory's stock number Pc76-5, Po89-1 and Fv84-1, respectively.

2. Substrates and preparation of media

After cultivation of *L. edodes*, the waste bed logs were collected, and their sawdust was prepared in our laboratory. The sawdust of soft wood, *Abies sacchalinensis*, was also prepared as a control. The elemental analysis of the media for the cultivation was shown in Table 1. The media for *P. cornucopiae* and *P. ostreatus* were consisted of sawdust and rice bran as an additive nutrient and for *F. velutipes* was added wheat bran as the nutrient (dry weight ratio of sawdust and nutrient was 1:1). Moisture contents of all media were adjusted to 65%. Four hundred and sixty grams of each medium was placed into an 850ml of plastic bottle, and were sterilized by autoclaving at 121°C, for 30 min.

3. Culture conditions

Five grams of sawdust spawn was inoculated aseptically into the cooled media. Inoculated media were incubated in the dark, at 22°C. The relative humidity was kept at 70±5%. In the cultivation of *P. cornucopiae*, when the primordia were formed, the cultivating bottles were transferred to a fruit body production room which was maintained to keep at 16±1°C and relative humidity at 85±5%, CO₂ level below 2,000 PPM and intermittently light condition (300 lx, 12 hours/day) through the experiments. When the diameter of basidiocarps became approximate 2 cm, the fruit bodies were harvested and weighed. In the cultivation of *P. ostreatus* and *F. velutipes*, scraping treatment of the surface and 2 hours of soaking treatment were done one by one when the spawn was run completely in each cultivating bottle. Then the cultivating bottles were transferred to a fruit body production room was maintained to keep at 12±1°C and relative humidity at 85±5%, CO₂ level below 2,000 PPM and intermittently light condition (300 lx, 12 hours/day) through the experiment. When the diameter of basidiocarps became approximate 2 cm, the fruit bodies were harvested and weighed. In the cultivation of *P. cornucopiae*, the days required for primordia formation and for fruit bodies harvest were measured. The days required for spawn running and for fruit bodies harvest were also measured in the cultivation of *P. ostreatus* and *F. velutipes*. Net productivity in year was calculated from the following an expression in the cultivation of these mushrooms.

► Results

1. Cultivation of *P. cornucopiae*.

Normal shaped fruit bodies were formed on even the medium using sawdust of waste bed logs (data was not shown). Results of cultivation tests were shown in Table 2. When the sawdust of waste bed logs was used, the days required for primordia formation and for the harvesting of fruit bodies were shortened slightly. Though, the yield of fruit bodies and net productivity in year increased about 88% and about 100%, restrictively.

2. Cultivation of *P. ostreatus*.

Normal shaped fruit bodies were also formed in the cultivation of *P. ostreatus* by using sawdust of waste bed logs. Results of cultivation tests were shown in Table 3. When the sawdust of waste bed logs was used, the yield of fruit bodies was slightly decreased. Though, the days required for spawn running and for the harvesting of fruit bodies were obviously shortened. So, net productivity in year increased about 20% than that of control medium.

3. Cultivation of *F. velutipes*.

In cultivation of *F. velutipes* by using sawdust of waste bed logs, normal shaped fruit bodies were also formed like as *P. cornucopiae* and *P. ostreatus*. The results of cultivation tests were shown in Table 4. The yield of fruit bodies was slightly decreased like as the cultivation of *P. ostreatus*, and the days required for spawn running and for the harvesting of fruit bodies were also slightly decreased as the cultivation of *P. cornucopiae*. So, net productivity in year was comparable with those of the control medium.

► Discussion

Cultivation of some mushrooms by using wastes from agriculture and food industries has been reported. Recently, there have been proposed novel cultivation methods using culture waste for the substrate of media. The sawdust-based cultivation of *L. edodes* by using cultural waste of *F. velutipes* (Ohga et al. 1993), and the cultivation of *Lyophyllum decastes* by using cultural waste of *P. ostreatus* and *Phoriota nameko* (Akamatsu, 1998) were reported. For cultivation of these mushrooms by using the waste media, though, aging and/or water extraction treatments were required. In this study, we described the availability of waste bed logs of *L. edodes* for the substrate of media without aging and/or water extraction treatments, because of saving time and labor. In general, both nitrogen and carbon concentration influenced the growth of mushrooms, and between 20 and 40 of carbon-nitrogen ratios were suitable for the cultivation of mushrooms (Kitamoto et al. 1974, Yamanaka and Ohta 1998). All the media used in this study, the CN ratio was between 30 and 40 that was suitable range for the cultivation of *P. cornucopiae*, *P. ostreatus*, and *F. velutipes*. Cultivation of *F. velutipes* by using the sawdust of waste shiitake bed logs, the productivity of fruit bodies was comparable with that of control medium, though, days required for primordia formation or *ostreatus*. So, net productivity in year was increased about 20% and 100%, in cultivation of *P. cornucopiae* and *P. ostreatus*, respectively. The composition and physical properties of the medium including such as moisture contents, particle size of sawdust, amount of packing into the bottle, influenced the characteristics of mushroom cultivation. Specific gravity of sawdust, waste bed logs was smaller than that of *A. saccharalinensis*. So, it was considered that the medium used waste bed logs was jam-packed in the cultivation bottles. Wood components i.e. lignin, cellulose and hemicellulose of the sawdust of waste bed logs had been partially decomposed by *L. edodes* once, so we considered that the inside of logs became more porous. Furthermore, we considered that the structure of lignin was cut off and crystallization degree of cellulose was decreased, so that wood components became to decompose easily by those mushrooms. Because of these consideration, the days required for spawn running and the days from primordia formation to fruit bodies harvest were shortened and the yield of fruit bodies was increased by using the waste bed logs. In conclusion, these results showed that the sawdust of waste bed logs could be reused for cultivation of *P. cornucopiae*, *P. ostreatus*, and *F. velutipes* without aging or water extract treatment, and also suggested that wood components affected the characteristics of cultivation of these mushrooms.

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Table 1. Elemental analysis of substrate materials.

Substrate materials	H %	C %	N %	CN ratio
Sawdust of waste bed logs	5.54	48.9	0.40	111.8
Sawdust of <i>A. sacchalinensis</i>	6.26	46.4	0.42	419.4
Rice bran	6.98	50.2	2.63	18.3
Wheat bran	5.98	48.1	2.26	20.5
Waste bed logs_rice bran				31.0
Waste bed logs_wheat bran				34.6
<i>A. sacchalinensis</i> _rice bran				35.8
<i>A. sacchalinensis</i> _wheat bran				40.6

Table 2. Cultivation tests on the sawdust medium of waste Shiitake bed logs and soft wood medium in the bottle cultivation of *P. cornucopiae*.

Sawdust material	Days required for primordia formation	Days required for fruit bodies harvest	Yield of fruit bodies*	Net productivity in year**
Waste bed logs	13.9±1.1***	19.0±0.6	98.8±3.7	1.90±0.22
<i>A. sacchalinensis</i>	14.3±0.5	20.2±0.4	52.8±2.1	0.96±0.13

*: g/bottle. **: kg/bottle/year. ***: Mean with standard error by sixteen replicates.

Table 3. Cultivation tests on the sawdust medium of waste Shiitake bed logs and soft wood medium in the bottle cultivation of *P. ostreatus*.

Sawdust material	Days required for spawn running	Days required for fruit bodies harvest	Yield of fruit bodies*	Net productivity in year**
Waste bed logs	13.6±0.83***	27.8±0.72	99.9±5.11	1.31±0.07
<i>A. sacchalinensis</i>	18.6±1.00	35.0±1.04	107.3±2.1	1.12±0.15

*: g/bottle. **: kg/bottle/year. ***: Mean with standard error by sixteen replicates.

Table 4. Cultivation tests on the sawdust medium of waste Shiitake bed logs and soft wood medium in the bottle cultivation of *F. velutipes*.

Sawdust material	Days required for spawn running	Days required for fruit bodies harvest	Yield of fruit bodies*	Net productivity in year**
Waste bed logs	18.8±0.83***	42.8±1.24	142.3±6.86	1.22±0.06
A.sacchalinensis	20.1±1.54	46.0±1.03	146.7±9.73	1.16±0.08

*: g/bottle. **: kg/bottle/year. ***: Mean with standard error by sixteen replicates.