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The Recycling of Enokitake Cultural Waste and the Potentiality of 2nd Flush for Enokitake Production

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ABSTRACTS

The recycling method of enokitake cultural waste and the potentiality of second flush for enokitake were determined, because this fungus is not as prolific as the more commonly cultivated white rot fungi in the conversion of sawdust to mycelial mass.

The mycelial growth of *F. velutipes* on several substrates, variously treated with rice bran was promoted at ratios of 10~20% (w/w) on all substrates, but suppressed at above ratios, although some difference was there. The mycelial densities generally increased correlated to the supplementation contents of rice bran. It could be concluded that *F. velutipes* preferred mild acidic to acidic conditions for mycelial growth, considering that the mycelial growth rate was highest on waste of pH 6.01, treated with 0.1% $Ca(OH)_2$ and on populus mixed waste of pH 6.02, non treated. The ranges of substrate bulk densities, which was pertinent for mycelial linear growth were from B.D. (g/cc) 0.17 to 0.23 on waste and populus mixed waste all. The pertinent contents of rice bran supplementation in bottle cultivation was from 20 to 30% on waste and 20% on populus mixed waste, considering the requried duration for pinheading and fruiting yields. Standard bulk density for filling and utilizing the waste and populus mixed waste for commercial F. velutipes cultivation were B.D.(g/cc) 0.19~0.23, and 0.23~0.25, which could be conversed to 510~540g/900ml and 520~570g/900ml, respectively. The second flush of F. velutipes was tried and the re-inoculation by sawdust and liquid spawn showed somewhat good results, indicating the potentiality of second crop and suggesting further research for it.

Key Words **:** *Bulk Density, Flammulina velutipes, Growth rate, Liquid spawn, Mycelial density, Populus sawdust, Re-inoculation, Rice bran.*

INTRODUCTION

Flammulina velutipes, which has been widely industrialized in Korea, is a commercially promising mushroom because of its great popularity. The

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production of these edible mushrooms is rapidly increasing in every district, and they compete with other mushrooms in the market as a regular commodity in Korea. A wide variety of hardwoods (oak, alder, poplar, cottonwood, aspen, etc.) and some softwoods (Douglas fir) could be used (Stametes, 1993). Although softwoods might nearly allow the fungus to grow, not to be supplemented, they have been dominantly employed as enokitake cultural substrates. This fungi are generally considered as white rotter, although some different argument was (Zadrazil, 1985). It is well known that white rot fungi decay hardwoods in preference to softwoods. Guaiacyl lignin-rich species of softwoods and some hardwoods showed higher decay resistance to some white rotter (e.g., *Trametes versicolor*) than syringyl-rich hardwoods (Highley, 1982). One frequently observed method of cell wall degradation by white rot fungi in gymnosperms is an erosion of the cell wall that occurs from the cell lumen toward the middle lamella (Eriksson, et al., 1990). However, it could be supposed that sawdust components, especially, middle lamella and most of cell wall components not be touched considering the short cropping cycle, the rice bran dependent mycelial growth characteristics and the sawdust type.

There is so much cultural waste of enokitake in Korea as enokitake bottle culture has been fully commercialized. The cultural waste produced by the mushroom farms are mostly discarded but are partly recycled for making compost. If the cultural waste can be recycled and the second flushes are possible, the effective use of wood resources and the production of lower cost enokitake can be achieved. Hence, the present work was undertaken to determine the reutilizing method of enokitake cultural waste and the potentiality of the enokitake second flush

MATERIALS and METHODS

1. Organisms

Commercial strains of *Flammulina velutipes* were employed as inoculants in the media of agar (PDA, Acumedia Manufactures, Inc), sawdust (*Quercus serrata*) and liquid (PD Broth, Acumedia Manufactures, Inc).

2. Reutilization of Enokitake Cultural Waste for *F. velutipes*

Preparation of substrates

Cultural waste after enokitake harvesting was employed as a major cultivating substrate. Aging mill waste, which was naturally fermented outdoors, sawdust of *Quercus serrata*, sawdsut of *Populus alba* × *P. deltoides* and *Populus* mixed waste supplemented with *Populus* sawdust were empolyed. Cultivating substrates were mixed with rice bran in certain ratios and watered (M.C. 65%). These mixtures were filled into the test tube and polypropylene bottle (P.P. Bottle, 900ml) with a screw cap, allowing air supply through a filter. After capped, the media were sterilised at 121℃ for 30 min. in case of tubes and for 90 min. in bottles. After inoculation by *F. velutipes*, they were placed in room of constant air temperature (25℃) and humidity (75%) and incubated in darkness.

Growth Characteristics of *F. velutipes* on Enokitake Cultural Waste

As an additive, rice bran was supplemented in ratios of 0～40% and the growth rates (mm/24h) were calculated to select the pertinent content of rice bran. The pH ranges for *F. velutipes* was examined by $Ca(OH)_2$, which was treated in weight ratios of 0～0.5 percent to the dried substrate. And substrate bulk density was kept at bulk density (B.D., g/cc) 0.17～0.27 to determine the pertinent compaction levels for *F. velutipes.*

Filling Weights of Substrate and Harvesting

To analyse the effects of rice bran supplementation and substrate bulk density on the duration required for pinheading and fruiting yields of *F. velutipes*, rice bran was added in several ratios and the filling weights was varied keeping the bulk density from 0.17 to 0.29. When mycelia spread to 90% of the bottle space, the cap was pulled off, the inoculated spawn was removed, and the surface of the media is made smooth for fruiting. Bottles are then placed in the dark at a temperature of 10～12℃ and the humidity is maintained at 80～85%. After 10～15 days after low-temperature treatment, most of the primordia were formed. Thereafter, the growth of fruit bodies was limited by lowering the temperature to 3～5℃ and providing air movement (3～5 m/sec) to control the uniform formation and differentiation of pinhead for 5～7 days. When the stem were approximately 2cm long, the fruit bodies are placed where the temperature was maintained at 5～8℃ and the humidity is maintained at 75～80℃ to encourage vigorous fruit body growth. When the stem get long, about 2～3cm from the mouth of the bottle, a plastic film was rolled around the mouth to hold the fruit body upright. When the fruit bodies were from 13～14 cm, the rolled paper was removed and fruit bodies were pulled up from the crop. The harvesting room was kept at 13±2℃(temp.) and 75-80%(R.H.) and the tiny fruit bodies were cultivated and harvested.

3. Second Flush Potentiality of Enokitake

Preparation of substrates

After enokitake harvesting, the bottles, which were not contaminated were divided with the naked eye and the remains of fruit bodies were removed. And then, several treatments written in the following section were done.

Second Flush, treated with several carbon, nitrogen sources and natural extracts

Distilled water of 50ml, containing several carbon (Dextrin, Dextrose; 0, 0.1, 0.5, 1%, w/v), nitrogen sources (Peptone, Glutamic Acid; 0.01, 0.1, 1%, w/v) and natural extracts (Rice bran) was treated in liquid for the mycelial mass not to shrink, which cause the division hole between mycelial mass and bottle and, which allow the pre-pinheading from the division hole. And thenafter, the bottles were treated with cold shock. After pinheading, they were coursed the growing procedures, mentioned above.

Second Flush after Re-inoculation and Re-incubation

Just after removation of fruiting remains, the bottles were capped with a sterilized cap and re-inoculated in a quite clean room, which is sterilized before. The re-inoculated bottles were incubated for additional 10～20 days for the newly introduced mycelia to join together with the old mycelial mass. And then, they were coursed the same growing courses, mentioned above.

RESULTS and CONSIDERATIONS

1. Recycling of Enokitake Cultural Waste

Mycelial Growths

Mycelial growth of *F. velutipes* is shown Table 1.1. The mill waste and cultural waste, which were aging treated outdoors gave poor or nearly no mycelial growth when not supplemented with rice bran. However, cultural waste, oak sawdust, Populus sawdust and Populus mixed waste without rice bran allowed mycelial growth, although the growth rate and the compactness of mycelia were not so good and dense. Rice bran at above 10% (to the dry sawdust weight) accelerated mycelial growth on all substrates without any significant difference. Mycelial density, however,

Table 1.1. Effects of Rice Bran on the Mycelial Growth and Densities of *F. velutipes* on Several Substrates variously treated.

Substrates \ Contents of Rice Bran(%)	Mycelial Growth Rate (mm/24h)					Mycelial Density				
	0	10	20	30	40	0	10	20	30	40
Aging Mill Waste	0.3[d]	45.5[b]	50.4[a]	41.8[c]	42.8[c]	-	+	++	+++	+++
Waste	36[b]	44.2[a]	42.9[a]	43.6[a]	41.4[a]	+	+	+++	++	++
Aging Waste	0.3[c]	45.5[a]	45.6[a]	41.8[b]	41.8[b]	-	+	++	+++	+++
Q. serrata	36[b]	44.2[a]	44.2[a]	43.6[a]	41.4[a]	+	+	++	+++	++
P. alba × P. deltoides	37.2[d]	52.3[a]	47.9[b]	43.9[c]	40.2[c]	+	++	+++	+++	+++
Populus Mixed Waste	38.1[d]	53.8[a]	47.2[b]	42.6[c]	41.1[c]	+	++	+++	+++	+++

Notes. Data are mean of 10 replicates and values followed by the same letter in the same column do not differ significantly at P=0.01 according to Duncan's multiple range test. Mycelial density was determined with naked eye and presented as (-) very poor; (+) poor; (++) dense; (+++) very dense.

Table 1.2. Effects of $Ca(OH)_2$ Treatment on the Mycelial Growth of *F. velutipes* on Waste and Populus Mixed Waste.

Substrates / Per. of $Ca(OH)_2$	Mycelial Growth Rate (mm/24h)			
	Waste	pH	Poulus Mixed Waste	pH
0	45.1[b]	5.90	53.5[a]	6.02
0.1	52.6[a]	6.01	49.9[a]	6.13
0.2	40.4[c]	6.10	42.2[b]	6.27
0.3	41.7[c]	6.15	39.9[b]	6.42
0.4	37.2[d]	6.21	36.7[c]	6.58
0.5	43.3[d]	6.23	37.2[c]	6.70

Notes. are shown in Table 1.1

increased correlated to the supplemented contents of rice bran.

Mycelial growth responses to $Ca(OH)_2$ were examined to improve the pH of cultural waste. *F. velutipes* showed a direct response to the substrate pH, as indicated in Table 1.2. Mycelial growth was most accelerated in 0.1% $Ca(OH)_2$ (to the dry substrate weight), which allowed growth rate of 52.00±6.0mm per 24h and maintained the hydrogen ion concentration at 6.01. It was addressed by Kinugawa (1993) that the pertinent hydrogen ion ranges for *F. velutipes* was at pH 6~7. Song et al.(1995) indicated that pH 6.5 was pertinent. Recently, Kim (1997) showed a little difference with them, stating that mycelial dry weight at pH 6.0 was mostly increased and was decreased at above or below ratios. Generally, the fungi are able to adjust the pH values by forming acids or ammonia for the purpose of forming the optimum pH value during a certain stage of developement (Han et al., 1981; Jablonsky, 1981). And most of the edible fungi prefer mild acidic or acidic substrates for mycelial growth and fruiting, although a few prefer more alkalic conditions (e.g., pH 7.0 for mycelial growth of *Agaricus* sp.)(Chai et al., 1999; Tokimoto & Komatsu, 1978). The result in this study is similar to Kim's (2000) although not executed at more acidic conditions. In all treatments except for pH 6.01 and 6.03, however, mycelial growths

Table 1.3. Effects of Substrate Bulk Density on the Mycelial Growth of *F. velutipes*

Mycelial Growth Rate (mm/24h) / Bulk Densities (g/cc)	Waste		Populus Mixed Waste	
	Growth Rate[1)]	Densities	Growth Rate[2)]	Densities
0.17	40.2	++	53.8	++
0.19	39.2	++	52.8	++
0.21	41.5	+++	53.5	+++
0.23	39.4	++++	52.8	+++
0.25	35.2	+++	41.6	+++
0.27	33.1	+++	37.2	+++

Notes are shown in Table 1.1 [1)] R.E. $Y=0.104+3.09X-0.86X^2$ [2)] $-0.15+5.64X-14.33X^2$

Table 1.4. Duration required for Pinheading and Fruiting Yields of *F. velutipes* according to the Rice Bran Supplementation.

Rice Bran (%)	Waste		Populus Mixed Waste	
	Duration for Pinheading(day)	Yields (g)	Duration for Pinheading (day)	Yields (g)
0	50.1[a]	90.65[c]	56.2[a]	89.1[c]
10	43.4[b]	119.89[b]	42.2[b]	130.2[b]
20	39.9[b]	156.44[a]	39.2[b]	162.3[a]
30	41.8[b]	160.97[a]	43.7[b]	149.3[a]
40	43.4[b]	155.49[a]	43.9[b]	150.7[a]

Notes. Data are average of 100 replicates and values followed by the same letter in the same column do not differ significantly at P=0.05 according to Duncan's multiple range test.

were suppressed. Moreover, the hydrogen ion concentration of waste was pH 5.8 (not shown datum). It could be concluded that *F. velutipes* prefer mild acidic conditions to acidic conditions.

To select the filling weights of substrates, which was economic and allowing the fast and most prolific conditions, the effect of substrate bulk densities on the mycelial growth and fruiting pattern was analysed (Table 1.3). *F. velutipes* showed the negative correlation curves (R.E.; $Y=0.104+3.09X-0.86X^2$, $Y=-0.15+5.64X-14.33X^2$) in its mycelial growth rates. The ranges of substrate bulk densities, which was pertinent for mycelial linear growth were from B.D.0.17 ~ B.D.0.23 on both substrates. At above B.D. 0.25, mycelial growth was suppressed (The waste particles was so small that the bulks could not be formed at below and the *Populus* particles was so large that the bulks could not above densities). The mycelial densities, however, was higher at above B.D. 0.21. Chai et al. (1999) showed, in their research on the pertinent bulk densities of black locust for several edible fungi, that the mycelial growths decreased proportionally when the bulk densities increased. And they suggested the need of further studies whether the optimum bulk density of different substrates for different fungi would be varied or not. Kim et al.(2000) indicated that there was negative correlation between mycelial growth of *F. velutipes* and substrate bulk densities but mycelial densities of *F. velutipes* on substrates with bulk

densities below 0.20 g/cc were decreased and no significant at above bulk densities.

Fruiting Yields

Rice bran was added in ratios of 0～40%, as a supplementation to waste and Populus mixed waste and cultured for harvesting in polypropylene bottle (900cc). Table 1.4 shows the duration (days) for pinheading and yields (g) accor ding to the supplementation contents. Generally, the duration periods for pinheading was shortened with rice bran addition, although not shown a significant difference between addition contents. And the populus mixed substrates promoted the mycelial growth in bottles. The fruiting yields increased to the contents of rice bran 30% on waste and 20% on populus mixed waste. However, the yields a

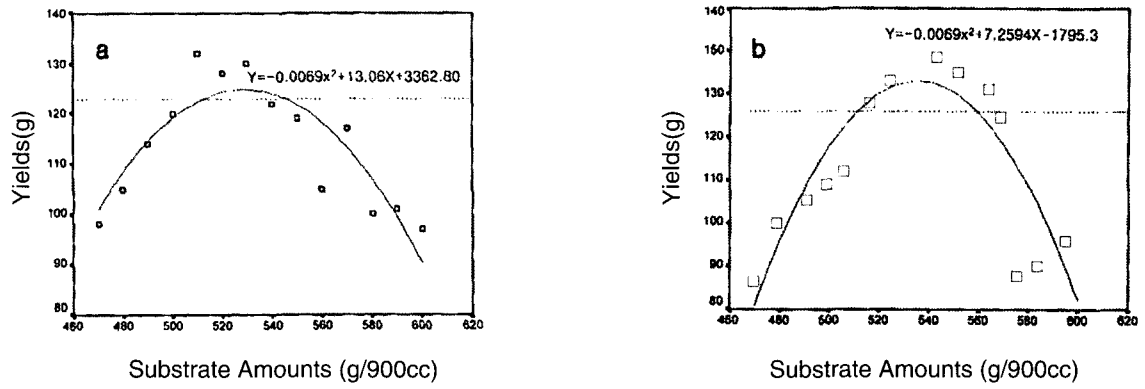

Fig. 1.1. Effect of Substrate Amount on the Fruiting Yields of *F. velutipes.*

Table 2.1. Second Flushes of *F. velutipes* on waste substrate, treated with several carbon, nitrogen sources and natural extracts.

Additives	*Concentration (%)	Yields (g)	%
Distilled Water	-	68.55	
Dextrin	0.1	54.33	
	0.5	57.24	
	1	80.35	
Dextrose	0.1	68.34	
	0.5	48.78	
	1	61.76	
Peptone	0.01	67.88	
	0.1	48.90	
	1	30.22	
Glutamic Acid	0.01	64.33	
	0.1	63.31	
	1	38.93	
Rice Bran Extracts	0.1	60.55	
	1	92.06	
	3	63.01	

Table 2.2. Second Flush of *F. velutipes* on Waste Substrate, re-inoculated with sawdust and liquid spawn.

Reinoculation / Incubation Periods	Distilled Water		Sawdust Spawn		Liquid Spawn	
	Yields(g)	Contamin.	Yields(g)	Contamin.	Yields(g)	Contamin.
10	72.35	3	0	1	85.35	0
15	87.05	5	105	2	121.22	1
20	74.88	7	114	2	123.06	2

little decreased at above ratios.

Effect of substrates amount on the fruiting yields and regression equation between substrate bulk densities and fruiting yields are determined (Fig. 1). There were the pertinent filling weight ranges of 510~540g/900ml with waste and 520~570g/900ml with populus mixed waste. The ranges could be conversed as B.D. 0.19~0.21 and 0.23~0.25, respectively. It should be noted that approximately 540g/900ml is widely filled, maybe with mill waste (Tonomura, 1978). Our results might be caused by smaller sizes of waste owing to biological deterioation and mechanical puverlization during substrate preparation process. Anyway, our results could be used as the ranges of standard bulk density, when filling and utilizing the waste and populus mixed waste for *F. velutipes* cultivation.

2. Potentiality of Second Flush

The potentiality of second flush for *F. velutipes* after first flush harvesting was examined. In our experimental results from the testing, executed for determining the supplementation content of distilled water, second crop usually amounted to 68.55g/bottles when distilled water of about 50ml was poured. When carbon, nitrogen sources and natural extracts of rice bran were treated in liquid, the second crop amounted to 80.35g with 1% dextrin and 92.06g with 1% rice bran extracts. It could not be used commercially in yields and qualities. When re-inoculated with sawdust and liquid spawn and re-incubated for additional days, the new mycelia recolonized the surface, although some contamination was occurred. When sawdust spawn was used, the additional re-incubation periods was 20days, and when liquid spawn was used, the periods was 15~20 days. Further studies are needed for the potentiality of second flush for *F. velutipes*.

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