マイタケ廃菌床を用いたきのこの栽培

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論　文

Mushroom Cultivation Using Maitake (*Grifola frondosa* (Fr.) S.F. Gray) Cultural Wastes*1

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SUMMARY

Chemical components on the cultural wastes of Shiitake (*Lentinus edodes*) and Maitake (*Grifola frondosa*) mushrooms were examined to research the possible use of the wastes for further mushroom cultivation. Relatively large amounts of wood components remained undegraded in the Maitake cultural wastes compared to those after Shiitake cultivation. The use of cultural wastes retarded the mycelial growth rate in the cultivation of Maitake and Hiratake (*Pleurotus ostreatus*) mushrooms. However, primordium formation was promoted remarkably after full colonization of mycelia through the sawdust beds in both mushrooms, compared to the control medium. This led to considerable shortening of the cultivation period for two mushrooms. As the results, relatively high yields of fruiting bodies were obtained with some media containing the cultural wastes. In the cultivation of Maitake mushrooms the yields of fruiting bodies slightly decreased, whereas those of Hiratake increased in all the media containing the cultural wastes. In addition, the quality of the fruiting bodies obtained was almost similar to that of the control media in both mushrooms. These results obtained here indicate the availability of the Maitake mushroom cultural wastes for use as a substrate in the sawdust-based cultivation of mushrooms, promising a shortened cultivation period and a reduction of the substrate costs.

Keywords : cultural wastes, fruiting bodies, *Grifola frondosa, Lentinus edodes*, sawdust-based cultivation.

要　旨

シイタケおよびマイタケ栽培後の廃菌床について、残存化学成分を分析し、廃培地使用の可能性を検討した。シイタケに比べてマイタケ廃培地には化学成分が比較的多く残されており、他のきのこの栽培に再利用できる可能性が示された。廃培地の混合割合を変えて菌床を作成し、マイタケとヒラタケの栽培を行ない、次の結果が得られた。

廃培地を主体とした菌床では菌回りに時間を要したが、菌糸まん延後の子実体原基の形成が促進されるため栽培日数が短縮された。廃培地を混合した菌床栽培において、マイタケでは子実体の収量はわずかに減少したが、ヒラタケではすべての培地で増加した。マイタケ、ヒラタケ共に得られた子実体の形質には差は見られなかった。これらの結果から、マイタケ栽培後の廃培地は他のきのこの栽培に十分使用することができ、さらに栽培期間の短縮と栽培経費の節減が期待できる。

キーワード：廃菌床、子実体、マイタケ、シイタケ、菌床栽培

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1. INTRODUCTION

Recently, mushrooms are becoming very popular as one of the healthy foods, because they have certain medical virtue. In the 1990's, mushrooms of about two-hundred-thousand ton have been produced by sawdust-based cultivation every year in Japan (Yamanaka 1995).

The indoor cultivation of mushrooms using the sawdust beds has an advantage that mushrooms can be produced throughout the year. Therefore, techniques of sawdust-based cultivation have been developed gradually for many kinds of mushrooms. However, the shortage of hardwood sawdust is now a serious problem for mushroom cultivation.

Although there might be possibilities for the mushroom cultural wastes to be converted into many useful products, most of them is thrown away in the fields or forests at present. Several attempts to use the cultural wastes for mushroom cultivation have been done so far (Okuyama 1988 ; Oizumi 1990 ; Ohga et al. 1994; Togashi 1995). The cultural wastes have been shown to be available sufficiently to the cultivation of certain mushrooms as a substitute for hardwood sawdust. High yields of fruiting bodies can be obtained with a shortened production period as well as the reduction of substrate costs. More research is required to reutilize the cultural wastes for the cultivation of various mushrooms.

In this paper, the residual chemical components in the cultural wastes of the Shiitake (*Lentinus edodes*) and Maitake (*Grifola frondosa*) mushrooms were determined to compare between them. Based on the results, the availability of the cultural wastes was discussed in regard to a substrate in sawdust-based cultivation of Maitake and Hiratake (*Pleurotus ostreatus*) mushrooms.

2. MATERIALS AND METHODS

2.1 Microorganism

Shiitake (*Lentinus edodes* (Berk.) Sing.) strain used in this experiment was Hokken Institute No. 600. Strains of Maitake (*Grifola frondosa* (Fr.) S. F. Gray) and Hiratake (*Pleurotus ostreatus* (Fr.) Quel.) were Mori Institute No.51 and No.39, respectively, which are suitable strains for the sawdust-based cultivation.

2.2 Preparation of sawdust beds

Cultural wastes of Shiitake were obtained from the Forest Research Center of Tochigi Prefecture and those of Maitake from Ogura Mushroom Co. (Imaichi, Tochigi Pref.), respectively. Sawdust beds, 2.5kg in fresh weight, for the cultivation of those mushrooms consisted of beech sawdust and wheat bran with a moisture content of 65% (Table 1). The sawdust beds inoculated with spawns were cultured for certain periods (about 6 months for Shiitake and 3 months for Maitake) before harvesting mushrooms. After being analyzed chemically, only the cultural wastes of Maitake were used for the cultivation of Maitake and Hiratake mushrooms.

Sawdust beds for the cultivation of Maitake and Hiratake mushrooms were prepared by mixing fresh beech sawdust and cultural wastes of Maitake with

Table 1. Weight losses of sawdust beds and yields of fruiting bodies per 1kg sawdust bed.

Mushroom	Weight of sawdust bed (kg)	Moisture content (%)	Weight loss(%)	Yield(g)
Shiitake	2.0	65	62.5	295
Maitake	2.5	65	26.4	180

Sawdust bed consists of fresh beech sawdust and wheat bran with the ratio of 4 : 1. Yields of fruiting bodies indicate fresh weight per 1kg sawdust bed.

Table 2. Preparation of sawdust beds for cultivation of mushrooms using the Maitake cultural wastes.

Sawdust bed	pH	Fresh sawdust (g)	Cultural waste (g)	Mixing ratio
A	5.9	280	0	—
B	5.5	210	70	3 : 1
C, (c)	5.4	187	93	2 : 1
D, (d)	5.2	140	140	1 : 1
E, (e)	5.1	93	187	1 : 2
F, (f)	5.0	70	210	1 : 3
G, (g)	4.9	0	280	—

Each sawdust bed contains 70g wheat bran. Moisture content was adjusted to 65%. Beech sawdust was used as substrate of mushroom cultivation. Parenthesized sawdust beds (c-g) were adjusted to pH 5.5.

the various ratios as listed in Table 2. Moisture content of the substrates was adjusted to 65% of the fresh weight. Each substrate of 1kg was packed in a polypropylene bag equipped with a porous sterile filter, and was sterilized by autoclaving at 120 ℃ for 90 min.

2.3 Culture conditions

Spawn was inoculated aseptically into the sawdust beds, and then the beds were incubated for vegetative growth, as shown in Table 3. After the culture of certain periods (50-80 days for Maitake and 50 days for Hiratake), the upper part of polypropylen bag was cut according to the progress of colonization, and the colonized sawdust beds were transferred to a fruiting room. Temperature was maintained at 18 ±2℃ for Maitake and at 13±2℃ for Hiratake, respectively. Relative humidity was maintained at 95% by overhead misting. After the culture of certain periods, developed fruiting bodies were harvested from the beds and their fresh weights were measured.

2.4 Chemical analysis of cultural wastes

Weight losses of sawdust beds were measured after the harvesting of fruiting bodies, and the chemical components (cellulose, holocellulose, lignin and pentosan) were determined according to the ordinary methods (Nakano 1956). Residual amounts of main chemical components were calculated from the results of chemical analysis, and then the amounts of chemical components required for producing fruiting body of 1g fresh weight were obtained.

Table 3. Culture and fruiting conditions.

Culture condition	Maitake	Hiratake
Temperature (℃)	20	20
Relative humidity (%)	70	70
Period (days)	50—80	50
Fruiting condition		
Temperature (℃)	18	13
Relative humidity (%)	95	95
Period (days)	10-50	15-30

3. RESULTS AND DISCUSSION

3.1 Chemical analysis of cultural wastes

After mushroom cultivation, Shiitake sawdust beds showed a greater weight loss with a higher

Table 4. Contents of respective chemical components in sawdust beds before and after fruiting.

Component	Shiitake			Maitake		
	Before fruiting(%)	After fruiting(%)	Residual rate(%)	Before fruiting(%)	After fruiting(%)	Residual rate(%)
Cellulose	52.5	44.0	31.4	50.4	44.7	77.9
Holocellulose	70.8	63.3	33.5	70.9	57.2	89.2
Lignin	26.2	18.8	26.9	25.3	23.1	80.2
Pentosan	20.3	17.0	31.4	20.9	12.7	53.4

yield of fruiting bodies than those of Maitake. The sawdust beds after cultivation of Maitake mushroom showed only 26.4% in weight loss, whereas the weight loss of Shiitake sawdust beds was 62.5%. This fact suggests the potential for re-utilizing the cultural wastes of Maitake as a substrate for other mushroom cultivation rather than those of Shiitake. Thus, residual amounts of main wood components in the cultural wastes of both mushrooms were determined. The results are shown in Table 4, in which the values indicating the residual amounts of wood components were calculated with taking account of each moisture content and weight loss of the sawdust beds. The residual amounts of main components in the Maitake cultural wastes were all greater than those in Shiitake, especially lignin. This fact indicates that Shiitake fungus apparently degraded large amounts of carbohydrates and lignin compared to Maitake.

Some white-rot fungi simultaneously remove all of the wood components, and others selectively degrade lignin in preference to cellulose (Kirk and Moore 1972; Blanchette 1984; Otjen and Blanchette 1987; Yoshizawa and others 1990, 1992). Maitake and Shiitake used here have been found to have selective lignin-degrading ability in localized areas of decayed wood (Hiroi and others 1986; Yoshizawa and others 1989, 1990, 1992). In decay by both fungi, however, extensive degradation of lignin was accompanied with large carbohydrate losses in advanced stages of decay. In this experiment, large differences in the residual amounts of wood components were found between the cultural wastes of both mushrooms. The ratios of lignin to total carbohydrates present in the cultural wastes after fruiting were also different, the ratio 0.30 for Shiitake and 0.40 for Maitake. This indicates that degradation of lignin in preference to cellulose progressed faster in decay by Shiitake than by Maitake. In addition, lengthened production period in Shiitake cultivation also caused greater degradation of wood components.

In general, when lignin is degraded, a concomitant loss of carbohydrates occurs. Fungi that preferentially degrade lignin also remove large amounts of hemicelluloses (Kirk and Highley 1973 ; Blanchette and others 1987). In addition, hemicelluloses seem to be degraded faster than cellulose as a carbohydrate-nutrient source when lignin is degraded by white-rot fungi (Kirk and Highley 1973; Blanchette and Abad 1988). In this experiment, relatively large amounts of carbohydrates remained undegraded in the Maitake cultural wastes, because of only one-time fruiting. The residual rates of cellulose, holocellulose and lignin were all more than twice those of Shiitake. Apparently, Shiitake degraded more lignin than did Maitake, resulting in the great degradation of hemicellulose prior to degradation of cellulose during the culture. Table 5 also shows a different nutritional requirement in both fungi. The amounts of chemical components degraded for producing fruiting body of 1g fresh weight apparently differed between both fungi. Large

amounts of carbohydrates and lignin were required for the mycelial growth and fruiting in Shiitake compared to Maitake. It is considered that the nutritional requirement for the mycelial growth and fruiting is larger in Shiitake than in Maitake. Mycelia of Maitake may be able to easily use wheat bran supplemented to the sawdust beds as a nutrient prior to causing active degradation of wood carbohydrates.

Table 5. Amounts of chemical components degraded for producing fruiting body of 1 g fresh weight.

Components	Shiitake (g)	Maitake (g)
Cellulose	0.42	0.27
Holocellulose	0.56	0.19
Lignin	0.23	0.12
Pentosan	0.16	0.24

3.2 Mycelial growth

Mycelial growth was measured in a test tube filled with 40g of a substrate. The results are shown in Figs. 1 and 2. Both mycelia of Maitake and Hiratake grew vigorously in the Maitake cultural waste-based substrate. The mycelial growth was faster in Hiratake than in Maitake. Both fungi showed best mycelial growth in the fresh sawdust. In both fungi, however, the mycelial growth gradually decreased with the increase in the mixing ratio of cultural wastes. These results indicate that the use of the cultural wastes hampered the mycelial growth in both mushrooms. Adjusting pH of culture media was also less effective in promoting the mycelial growth of both mushrooms, in which pH of the media had been adjusted to 5.5 (data not shown). As described later, however, this

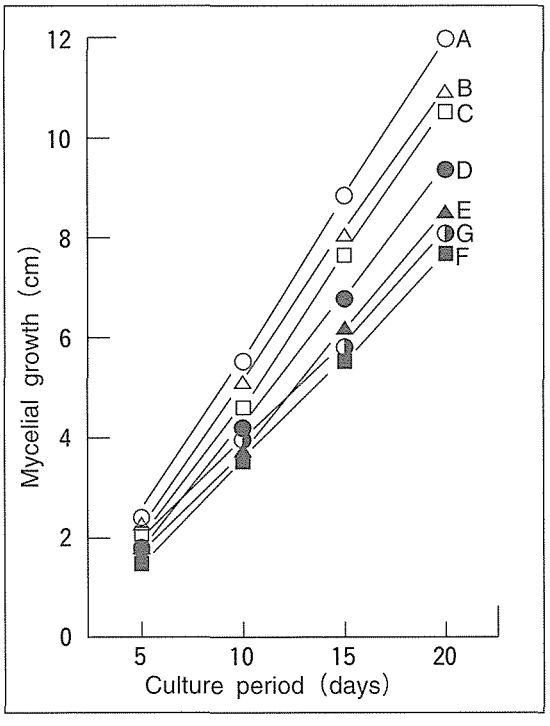

Fig. 1. Mycelial growth of Hiratake in various media mixtured with the cultural wastes. Legends of A-G are reffered to Table 2.

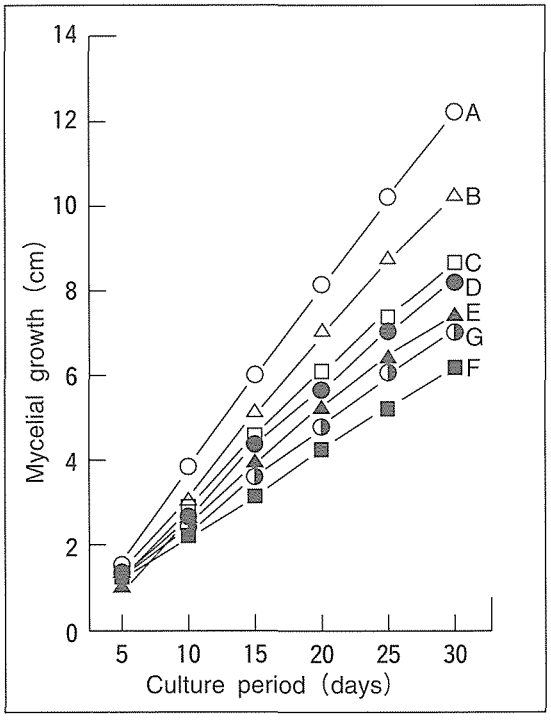

Fig. 2. Mycelial growth of Maitake in various media mixtured with the cultural wastes. Legends of A-G are reffered to Table 2.

delay of the mycelial growth did not affect the fruiting in both mushrooms.

Among the culture media mixed with cultural wastes, culture medium B with a mixing ratio of 3:1 (fresh sawdust to cultural wastes) gave a relatively better result in the mycelial growth of Maitake, while in Hiratake better mycelial growths were observed in the culture media B (mixing ratio; 3 : 1) and C (mixing ratio; 2 : 1).

Ohga and others (1993) reported that corncob meal supplementation is effective in improving air permeability inside the sawdust bed. Water sprinkling treatment of the cultural wastes also gave an effect on shortening the culture period in sawdust-bed cultivation of Maitake mushroom (Okuyama and Mikawa 1988). Improvement of the substrate air-permeability or aging treatment of the cultural wastes should be done in order to promote the mycelial growth.

3.3 Yields of fruiting bodies

Yields of fruiting bodies (fresh weight) in the sawdust-based cultivation of Maitake and Hiratake mushrooms using Maitake cultural wastes are shown in Figs. 3 and 4. In the cultivation of Maitake mushroom, all of the culture media containing the cultural wastes gave lower yields of fruiting bodies than did the fresh sawdust medium. Differences in the mixing ratio of cultural wastes gave almost no great differences in the yields of fruiting bodies. The adjustment of medium pH did not also give remarkable effects on increasing fruiting body yields (Fig. 3 c-g). Among the culture media mixed with cultural wastes, relatively high yields of fruiting bodies were obtained with the media of C and E, the mixing ratios being 2 : 1 and 1 : 2 for medium C and E, respectively. It was reported that in the cultivation of Maitake mushroom re-utilizing the cultural wastes is possible by using the sawdust beds containing 50-70% cultural wastes (Shimizu 1992). These findings indicate that the cultural wastes are available sufficiently for the sawdust-based cultivation of Maitake mushroom, although much yet remains to be improved in order to increase the yield of fruiting bodies.

As described above, although the use of the cultural wastes reduced the mycelial growth rate with the increase in the mixture ratio of cultural wastes, it remarkably promoted the primordium

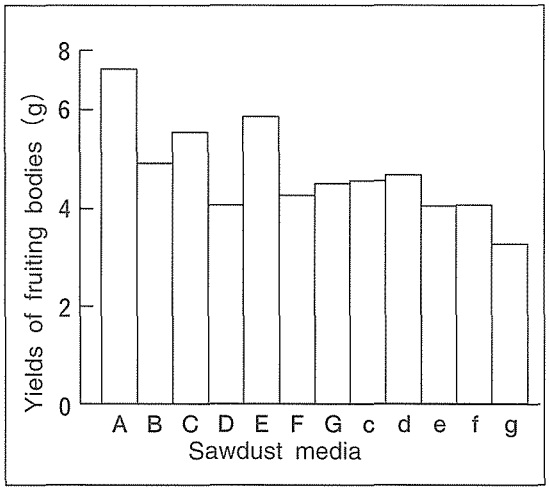

Fig. 3. Yields of fruiting bodies in the sawdust-based cultivation of Maitake. Legends are reffered to Table 2.

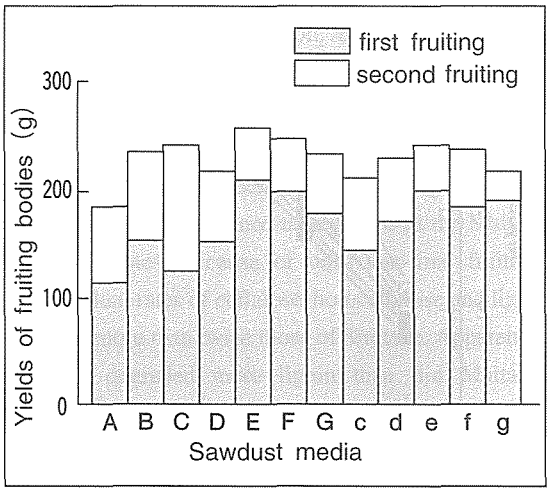

Fig. 4. Yields of fruiting bodies in the sawdust-based cultivation of Hiratake. Legends are reffered to Table 2.

formation after the full colonization of mycelia within a sawdust bed compared to the control medium, resulting in shortening the cultivation period totally up to 5-10 days for Maitake mushroom cultivation. Larger amounts of sugar substances extractable with hotwater are present in the cultural wastes (Ohga and others 1993). These sugar substances easily provide carbohydrates available for primordium formation after the colonization, because the cultural wastes have been degraded to some extent, possibly shortening the time required for fruiting. This was true for the Hiratake mushroom cultivation, as described later. Furthermore, the quality of fruiting bodies was not affected by using the sawdust medium containing the cultural wastes, as shown in Fig. 5.

In the sawdust-based cultivation of Hiratake mushroom using Maitake cultural wastes, better results were obtained compared to the control media containing no cultural wastes. As shown in Fig. 4, all of the media containing the cultural wastes increased the yields of fruiting bodies. Particularly, in the culture media of E, F and G the yields in the first crop stage increased, compared to that of the control medium. Adjustment of the medium pH gave almost the same yields of fruiting bodies as those of the culture media without the pH adjustment, or slightly increased them. These results indicate that in the cultivation of Hiratake mushroom the use of only the cultural wastes also is full promising. Furthermore, primordium formation was largely promoted in the culture media containing the cultural wastes as well as Maitake, although the mycelial growth was retarded to some extent. This reduced the cultivation period from 10-15 days. The quantity of the fruiting bodies was almost similar to those obtained with the control medium (Fig. 5).

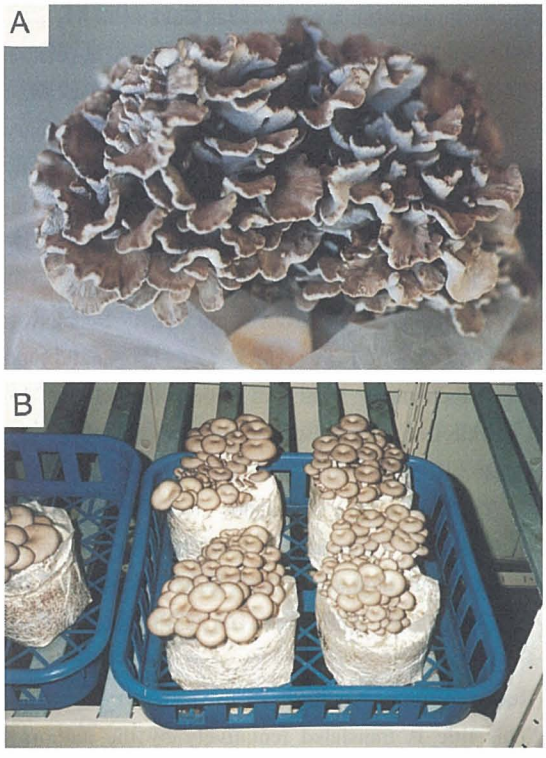

Fig. 5. Fruiting bodies of Maitake and Hiratake cultivated by using the cultural wastes.

Mushroom cultural wastes involve significant amounts of water soluble materials (Ohga and others 1993). It is considered that sufficient nutrients easily available for fruiting are present in the cultural waste. Mycelial growth may be promoted by adding the coarse corncob meal to the culture media, or by using the aged cultural waste. The addition of the corncob meal maintained high O_2 and low CO_2 concentrations in the aged waste-based substrate, resulting in better mycelial growth (Ohga and others 1993). It is expected to yield a large quantity of fruiting bodies with a shortened cultivation period also for other mushroom species, if mycelial growth could be more promoted in the sawdust beds containing mushroom cultural wastes. In addition, with the use of the cultural wastes, of course, it is possible to largely reduce the substrate costs for the cultivation of mushrooms.

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