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ORIGINAL ARTICLE

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Cultivation of shiitake in sugi wood meal II: effects of seasoning treatment for wood meal on mycelial growth

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Abstract The effects of seasoning treatment for fresh sugi wood meal on the mycelial growth of shiitake was investigated. The mycelial growth of shiitake in sugi wood meal increased to the same level as that in extract-free sugi wood meal on the 12th day and to approximately 70% of that in konara wood meal on the 28th day of the seasoning treatment. A drastic decrease in extracts of fresh sugi wood meal occurred during the early stage of treatment, and the neutral fraction of methanol extracts decreased to less than 2%. The drainage of sugi wood meal media significantly increased during the later stage of treatment. No changes in the chemical components of sugi wood meal were detected during the seasoning treatment. The mycelial growth of shiitake in the fully seasoned sugi wood meal, which was prepared for commercial mushroom cultivation, was at almost the same level as that in the extract-free sugi wood meal. All of the seasoned sugi wood meals contained a neutral fraction of less than 1%. These results suggested that seasoning treatment can promote mycelial growth of shiitake in sugi wood meal by eliminating inhibitors and improving the physical properties of sugi wood.

Key words Shiitake · Sugi · Seasoning treatment · Extracts · Mycelial growth

Introduction

Miyazaki Prefecture is an important area in Japan for the production of sugi wood (*Cryptomeria japonica* D. Don), and a large quantity of sugi wood meal is produced in sawmills as sawdust. Miyazaki has also been one of the most prosperous prefectures for the production of dried shiitake [*Lentinula edodes* (Berk.) Pegler] on bed logs, but shiitake production using a wood meal medium has recently become prevalent.

As edible mushrooms, hiratake [*Pleurotus ostreatus* (Jacq. Ex Fr.)], enokitake [*Flammulina verutipes* (Curt. Ex Fr.)], and bunashimeji [*Hypsizygus marmoreus* (Peck) Bigelow] are cultivated using sugi wood meal as the main substrate, which is supplied cheaply and abundantly as sawdust. Shiitake, in contrast, is usually cultivated in wood meal of hardwoods such as konara [*Quercus serrata* Thunb.] because of the poor mycelial growth in sugi wood meal.

Nakajima et al. found that the ferruginol in methanol extracts of sugi inner bark has a toxic effect on shiitake mycelial growth.[1] Because hiratake and enokitake growth is not inhibited by ferruginol, Nakajima et al. assumed that the growth inhibition by ferruginol is highly specific to the fungi of *Lentinula* spp. Based on our experiments using thymol as a model of ferruginol, it appears that the inhibitory effect of thymol on enokitake and hiratake mycelial growth is less than that on shiitake because hiratake and enokitake decrease the thymol concentration more rapidly than does shiitake during the early stages of incubation.[2] Matsui et al. also showed that mycelial growth inhibition of shiitake is probably due to a synergistic effect of ferruginol and sandaracopimarinol, which are the major terpenoids in sugi wood.[3] On the basis of these results, it has been thought that the poor shiitake mycelial growth on sugi wood meal compared with that on hardwood such as konara is responsible for the inhibitory effects of extractives contained in sugi wood.

Fresh sugi wood meal is not usually used for mushroom cultivation; instead, it is usually fully seasoned outdoors for several months by sunlight and rain or sprinkles of water. There have been few reports dealing with the effects of this seasoning treatment on the content of sugi wood extractives and the mycelial growth of edible fungi in a sugi wood meal medium.

In the present study, the effects of a seasoning treatment determined by a weather-resistance test on shiitake

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mycelial growth were investigated relative to changes in the extractives content, physical properties, and chemical compositions of sugi wood meal. Shiitake mycelial growth in seasoned sugi wood meal prepared for actual mushroom cultivation was investigated in a similar manner.

Materials and methods

Organism

A commercial dikaryotic strain of shiitake, Mori 465 (Mori Sangyou), maintained at 5°C on potato dextrose agar slants was used in the present study.

Wood meal preparation

Wood meals were prepared from sugi wood and konara wood with a chain saw and the Willey mill and then sieved to a grain size of 0.355–1.000 mm. Some of the fresh sugi wood meal was extracted with methanol for 6 h. Fully seasoned sugi wood meals prepared for cultivating mushrooms such as enokitake, hiratake, and bunashimeji were supplied by five mushroom cultivators in Miyazaki Prefecture. Information about these seasoned sugi wood meals is provided in Table 1. These seasoned sugi wood meals were also sieved to a grain size of 0.355–1.000 mm.

Seasoning treatment

The fresh sugi wood meal (200 g) was placed in a mesh bag, treated with running tap water (1–2 l/min) for 1 h, and then heated at 60°C for 23 h according to the weather-resistance test described in "Method for testing effectiveness of wood preservatives against decay of wood" (JIS A9303).[4] The seasoning treatment continued for 28 days.

Culture conditions

A 25-g portion of the wood meal medium (6.56 g of wood meal, 2.19 g of rice bran, 16.25 g of water) was placed in a petri dish. After autoclaving, the medium was inoculated with an agar disk from stock culture and incubated for 12 days at 25°C and 60% humidity under approximately 200 lux of light (12 h/day).

Table 1. Seasoned sugi wood meals prepared for mushroom cultivation

Symbol	Seasoning treatment		Mushroom[a]
	Method	Period (months)	
A	Rain	Unknown	Enokitake
B	Sprinkled water	12	Enokitake
C	Sprinkled water	6	Enokitake
D	Sprinkled water	3–6	Bunashimeji
E	Sprinkled water	1	Hiratake

[a] These seasoned wood meals prepared for the cultivation of each mushroom were supplied by cultivators

Measurement of mycelial growth

The colony diameter in the wood meal medium was measured at 2-day intervals up to the 12th day of incubation. The shiitake mycelial weight was estimated based on the glucosamine content in the wood meal medium. The glucosamine content was determined by a method similar to that described by Tokimoto and Fukuda[5] and others.[6,7] The shiitake mycelial growth in sugi wood meal medium is presented in relation to the values for konara wood meal medium.

Fractionation of methanol extracts

The fresh sugi wood meal was extracted for 6 h with methanol using a Soxhlet extractor. The methanol extracts were extracted with ethyl acetate. The ethyl acetate-soluble fraction was extracted with 4% NaOH. The liquid layer was acidified with 6 N HCl and then extracted with diethyl ether. After evaporating the organic layer, the residue was obtained as the neutral fraction.[8,9] The methanol extracts, the ethyl acetate-soluble fraction, and the neutral fraction were dried under diminished pressure to calculate their content in the sugi wood meal.

Determination of water retention and drainage

Water retention was determined according to the method of "Determination of water retention value of pulp" (Japan TAPPI, no. 26–78)[10] with some modifications. Sugi wood meal (approximately 1 g) was soaked in water for 1 h with evacuation. Saturated wood meal was put into a 1G1 glass filter and centrifuged at 60 g for 1 min. Water retention was calculated as follows:

$$\text{Water retention (\%)} = (W_a - W_b)/W_b \times 100$$

where W_a is the weight of wet wood meal after centrifugation, and W_b is the oven-dried weight of wood meal.

Drainage of wood meal was determined as follows. Approximately 5 g of wood meal, after adjusting the water content to 65%, was placed in the 1G1 glass filter and centrifuged at 60 g for 1 min.

$$\text{Drainage (\%)} = (W_c - W_d)/W_c \times 100$$

where W_c is the weight of wood meal placed in the glass filter, and W_d is the weight of wood meal after centrifugation.

Determination of chemical components

The alkaline extract, lignin, holocellulose, and α-cellulose contents in sugi wood meal were determined according to standard methods. The hemicellulose content was calculated based on the difference between the holocellulose and

α-cellulose contents. The lignin, holocellulose, and α-cellulose contents in the seasoned sugi wood meal supplied by mushroom cultivators were also determined.

Results and discussion

Effects of seasoning treatment of sugi wood meal on shiitake mycelial growth

Shiitake mycelial growth in sugi wood meal previously treated with water and heat according to the weather-resistance test was compared with that in konara and the extract-free sugi wood meals. The changes in shiitake colony diameter and mycelial weight are shown in Fig. 1. The mycelial growth was significantly inhibited in fresh sugi wood meal (at day 0 of the seasoning treatment in Fig. 1) compared with that in konara and the extract-free sugi wood meal, but it gradually increased with the duration of the seasoning treatment. The colony diameter in fresh sugi wood meal reached the size of that in the extract-free sugi wood meal on the 8th day and almost the size of that in konara wood meal after the 16th day of seasoning treatment. A comparison of shiitake mycelial weight clarified the inhibitory effects of the fresh sugi wood meal. The mycelial weight in sugi wood meal reached that which occurred in the extract-free sugi wood meal on the 12th day of the seasoning treatment, but it increased only to approximately 70% of that in the konara wood meal even on the 28th day.

The effects of the seasoning treatment on the content of the methanol extracts, the methyl acetate-soluble fraction, and the neutral fraction in sugi wood meal are shown in Fig. 2. A drastic decrease in the methanol extracts in fresh sugi wood meal was observed during the early stage of the seasoning treatment. It is well known that the neutral fraction of methanol extracts of sugi wood contains ferruginol, sugiol, sandaracopimarinol, and so on, and that these terpenoids significantly inhibit shiitake mycelial growth.[3,8,9] On the 12th day of the seasoning treatment, the neutral fraction decreased to 2% on a dry-weight basis, and the mycelial growth was roughly equal to that in the extract-free sugi wood meal. These results suggest that shiitake mycelial growth is not substantially inhibited if the neutral fraction is kept below 2% in sugi wood meal.

The effects of the seasoning treatment on the physical properties, water retention, and drainage of sugi wood meal are shown in Fig. 3. It seems that the difference in water retention between the fresh and seasoned sugi wood meal was not large enough to affect shiitake mycelial growth. The drainage of sugi wood meal was significantly increased during the course of the seasoning treatment, especially during the later stages. This can be explained by the fact that the fluffy surface of wood meal just after preparation becomes smooth as a result of the repeated seasoning treatment, so the water-holding ability of the surface is weakened, resulting in improved drainage. These results suggest that the mycelial growth in sugi wood meal is promoted more than that in extract-free wood meal because of the increased drainage that occurs with seasoning. The drainage of konara wood meal was found to be 23.1% – almost five times greater than that of the sugi wood meal previously seasoned for 28 days. The difference in the physical properties of wood meal seemed to be one of the reasons extract-free sugi wood meal was inferior to konara wood meal with regard to shiitake mycelial growth.

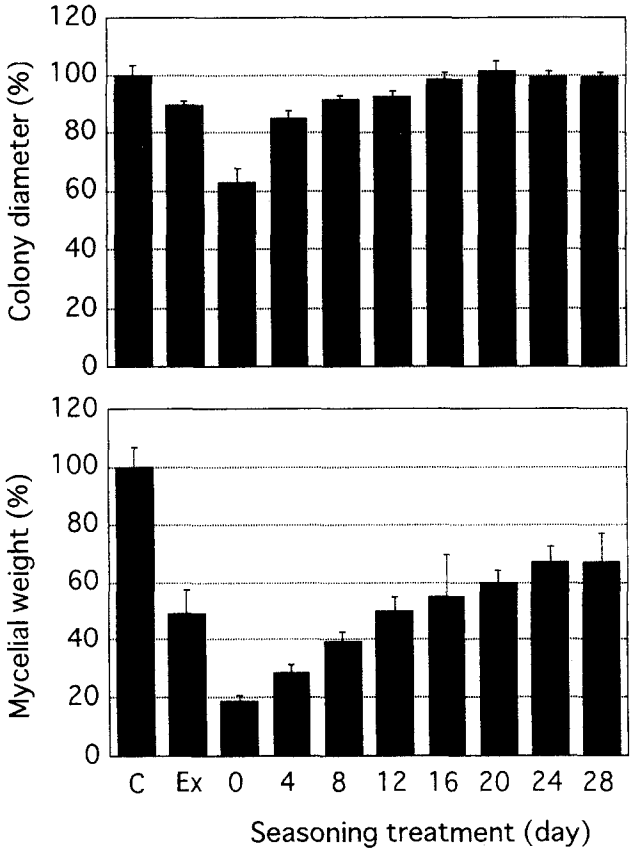

Fig. 1. Effects of the seasoning treatment for sugi wood meal on shiitake mycelial growth. *C*, fresh konara wood meal; *Ex*, extractive-free sugi wood meal. Shiitake was incubated for 12 days at 25°C

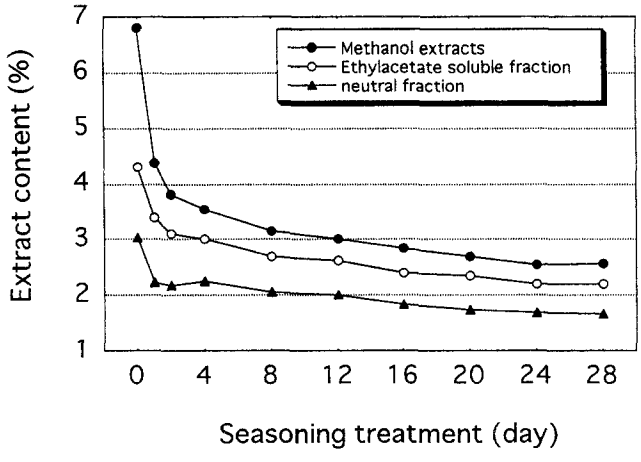

Fig. 2. Effects of seasoning treatment on the content of extracts in sugi wood meal

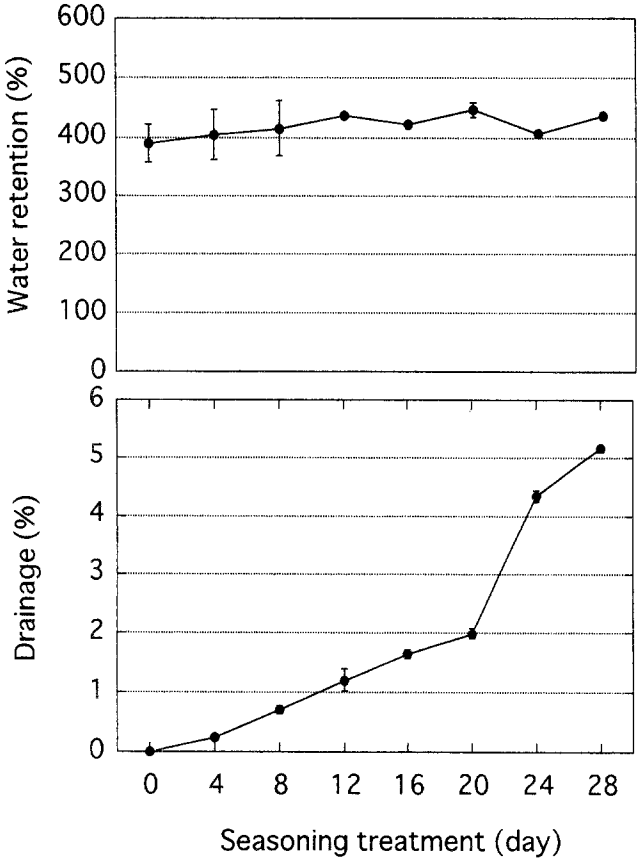

Fig. 3. Effects of the seasoning treatment of water retention and drainage of sugi wood meal

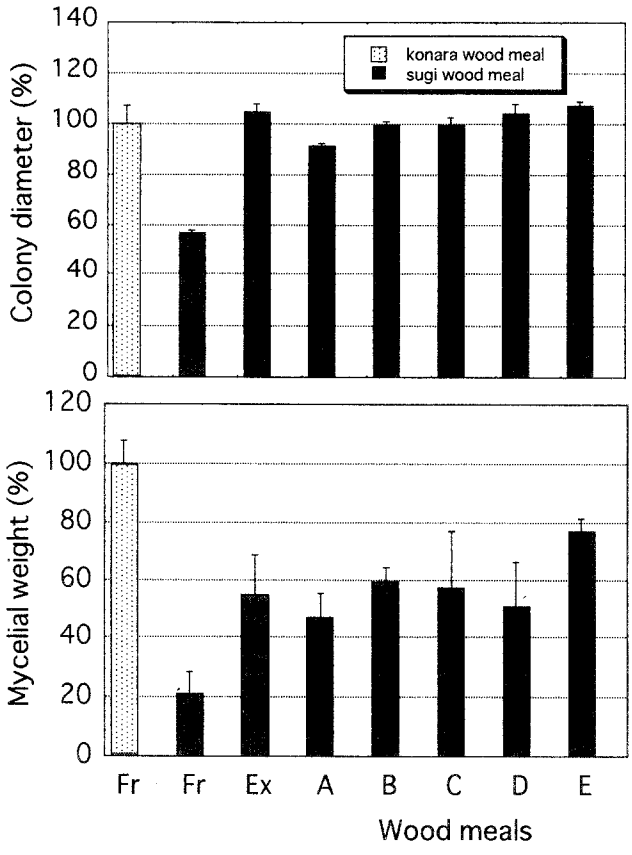

Fig. 4. Shiitake mycelial growth in seasoned sugi wood meal. *Fr*, fresh wood meal; *Ex*, extract-free wood meal. Other symbols are the same as in Table 1

Table 2. Chemical components of fresh and seasoned sugi wood meals

Component	Fresh wood meal	Seasoned wood meal[a]
Alkaline extracts	6.0 (0.04)	5.6 (0.11)
Lignin[b]	36.1 (0.04)	35.6 (0.12)
Cellulose[b]	42.5 (0.96)	43.3 (0.73)
Hemicellulose[b]	22.4 (0.56)	22.5 (2.13)

Data represent the means (SD) of three samples
[a] Seasoning treatment was continued for 28 days
[b] Based on extractive-free wood meal

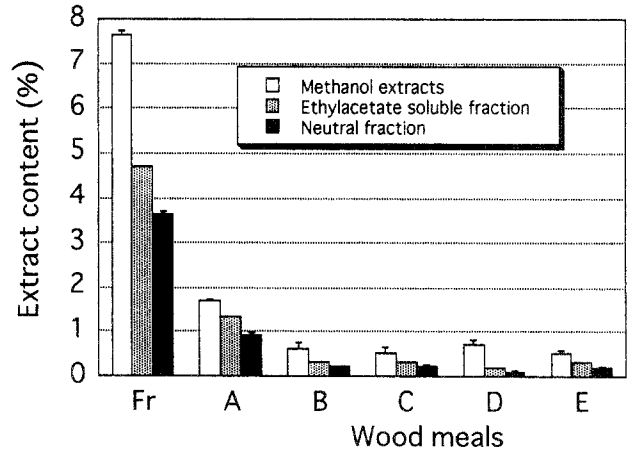

Fig. 5. Extract content in seasoned sugi wood meal. Symbols are the same as in Fig. 4

The chemical components of the fresh and seasoned sugi wood meals are shown in Table 2. There was no significant difference between the two wood meals in terms of the content of the alkaline extracts, lignin, cellulose, or hemicellulose, suggesting that the seasoning treatment in this experiment did not change these chemical components of sugi wood meal enough to affect the shiitake mycelial growth.

Shiitake mycelial growth in seasoned sugi wood meal supplied by mushroom cultivators

The mycelial growth of shiitake in five seasoned sugi wood meals (A–D) prepared for mushroom production was compared with that in konara and extract-free sugi wood meal (Fig. 4). The colony diameters in the seasoned sugi wood meals were large compared with those in fresh sugi wood meal and were almost the same as those in konara and the extract-free sugi wood meal. The mycelial weights in the seasoned sugi wood meals were similar to or higher than those in extract-free sugi wood meal but were only approximately 50%–60% of those in konara wood meal, except for that in wood meal E (77.1%). The extractives content in seasoned sugi wood meal was significantly decreased compared with that in fresh sugi wood meal (Fig. 5). The neutral

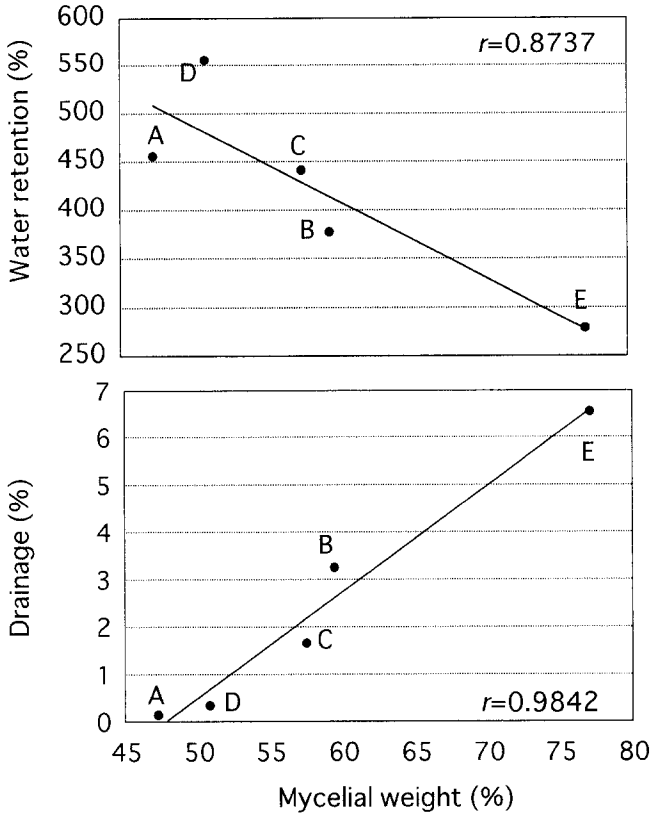

Fig. 6. Effects of water retention and drainage of seasoned sugi wood meal on shiitake mycelial growth. Symbols are the same as in Table 1

fraction content was less than 1% in all of the seasoned sugi wood meals. Judging from the results obtained with the weather-resistance test, the effects of inhibitors on shiitake mycelial growth were negligible, as in the extract-free sugi wood meal.

The relations between shiitake mycelial growth and the physical properties (water retention and drainage) of seasoned sugi wood meal are shown in Fig. 6. Shiitake mycelial growth was increased in seasoned sugi wood meal E, which had the least water retention and the greatest amount of drainage. The water retention and drainage of the fresh sugi wood meal were 389% and 0.03%, respectively (Fig. 3). These results show that the seasoning treatment for sugi wood meal with sunlight and rain (or a sprinkling of water) not only eliminates the inhibitor but also improves the physical properties of wood meal for shiitake mycelial growth.

The cellulose, hemicellulose, and lignin contents in seasoned sugi wood meals were examined, but there was no statistically significant relation between these chemical components and shiitake mycelial growth (Table 3). Because the sugi wood meal medium was supplemented with rice bran for nutrition and the duration of incubation was only 12 days, the difference in wood composition did not directly affect shiitake mycelial growth. It appears, however, that the seasoning treatment can promote shiitake mycelial growth in sugi wood meal by eliminating the inhibitor and improving the physical properties of sugi wood meal.

Table 3. Chemical components[a] of fresh and seasoned sugi wood meals

Component	Fresh	Seasoned wood meal[b]				
		A	B	C	D	E
Lignin	36.1 (0.04)	35.6 (0.24)	34.3 (0.11)	37.6 (0.27)	34.4 (0.28)	34.4 (0.10)
Cellulose	42.5 (0.96)	48.7 (1.57)	51.6 (0.61)	35.4 (0.74)	46.1 (0.04)	50.1 (0.77)
Hemicellulose	22.4 (0.56)	26.8 (0.65)	26.0 (0.56)	25.7 (1.03)	24.9 (0.40)	26.6 (0.84)

Data represent the mean (SD) of three samples
[a] Based on extractive-free wood meal
[b] Symbols A–E are the same as in Table 1

It has been thought that the poor shiitake mycelial growth in sugi wood meal compared with hardwood (e.g., konara) is responsible for the inhibitory effects of extractives contained in the sugi wood.[1–3] In the present study, we found that almost all inhibitory substances are easily eliminated by an ordinal seasoning treatment consisting of sunlight and rain. It was difficult, however, to bring the mycelial growth in sugi wood meal to a level comparable to that in konara wood meal by the seasoning treatment alone. An investigation of factors other than inhibitors affecting shiitake mycelial growth in sugi wood meal, such as the physical properties of sugi wood, is necessary to establish the practical use of sugi wood meal as a culture medium for shiitake cultivation.

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