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NOTE

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Reutilization of culture wastes of *Pleurotus ostreatus* and *Pholiota nameko* for cultivation of *Lyophyllum decastes*

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Abstract Possible reutilization of fresh and aged culture wastes of mushrooms for cultivating *Lyophyllum decastes* was investigated, although bark compost has commonly been used as a substrate for cultivating this fungus. The culture wastes studied were obtained after harvesting *Pleurotus ostreatus* and *Pholiota nameko* mushrooms. Mycelia of *L. decastes* grew in the media containing both the fresh culture waste of *P. nameko* and bark compost. However, it did not grow in the medium containing only the fresh culture waste of *P. nameko* or in any media containing the fresh culture waste of *P. ostreatus*. The mycelial growth inhibition in the fresh culture wastes of *P. ostreatus* might be caused by the water-soluble inhibitors present. Mycelia of *L. decastes* grew in all the media with aged culture wastes of both *P. ostreatus* and *P. nameko*, which had been left outdoors for 6 months, regardless of whether bark compost was mixed. Fruit bodies were produced on all the tested media with aged culture wastes of both mushrooms, which had been left outdoors for a year. The aged culture waste of *P. nameko* gave greater yields than the bark compost. This investigation shows that the aged culture wastes of *P. ostreatus* and *P. nameko* could be reutilized for producing *L. decastes* mushrooms.

Key words *Lyophyllum decastes* · Culture waste · *Pleurotus ostreatus* · *Pholiota nameko* · Outdoor treatment

Introduction

Lyophyllum decastes (Fr.:Fr) Sing., which occurs widely in Japan, is a commercially promising mushroom because of its great popularity due to its good taste. The industrial production of this mushroom has started recently. A number of patents for the cultivation method of this mushroom have been obtained, and applications are still being submitted. One is for "the indoor cultivation method of this fungus," which engenders cultivation in a vessel with an opening bedded with mineral matter.[1] A method for its optimal cultivation has not yet been established.

Wood sawdust has been used as a substrate for cultivating many species of wood-rotting fungi, such as *Lentinula edodes* (Berk.) Pegler and *Flammulina velutipes* (Curt.:Fr.) Sing. However, as Shoji and Watanabe reported,[2] the mycelia of *L. decastes* grow much better in a medium with bark compost than in one with wood sawdust. Bark compost has commonly been used as substrate for cultivating the fungus. In view of the fact that the bark compost is formed after a long duration of microbial decay treatment, the culture wastes obtained after harvesting mushrooms may be used as an alternative substrate for cultivating *L. decastes*, because these wastes are similar to the bark compost for microbial treatment.

The culture wastes produced by the mushroom farms are mostly discarded but are partly used for making compost. If we can reutilize the culture wastes for producing fruit bodies of *L. decastes*, it can reduce the cost of mushroom production and achieve effective use of wood resources. Although a few studies on reutilizing culture wastes for mushroom cultivations have been reported recently,[3–5] the possibility of producing the mushroom of *L. decastes* with culture waste has not been examined. In this study we investigated the possible reutilization of culture wastes obtained after harvesting the mushrooms of *Pleurotus ostreatus* (Jacq.:Fr.) Kummer and *Pholiota nameko* (T. Ito) S. Ito et Imai in Imai for cultivating *L. decastes*.

Materials and methods

Organism

Two strains (Ld2 and Ld18) were isolated from the tissue of the wild *L. decastes* fruit bodies. The latter had been

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collected from Shiga and Yamaguchi Prefectures, respectively, and stocked at Fukui Prefectural General Green Center.

Mycelial growth of *Lyophyllum decastes*

Preparation of culture media

Culture wastes of *P. ostreatus* and *P. nameko*, obtained from the Kono and the Maeda Mushroom Growing Companies in Fukui Prefecture, respectively, were used as the main substrate. The sawdusts used for cultivating *P. ostreatus* and *P. nameko* were from softwood (*Cryptomeria japonica* D. Don) and hardwood (not identified), respectively. The culture wastes were piled and left outdoors under natural conditions. Bark compost was obtained from Hotta Co. (Nagoya, Aichi Prefecture) and used as the control substrate.

Rice bran was added to the substrate as supplement. Moisture contents of the media were adjusted to 68% on a wet basis. The pH was not adjusted. Each medium (20 g) was placed in the test tubes (ϕ2.5 × 15.0 cm) and was autoclaved at 121°C for 30 min.

Culture conditions

Each medium was inoculated with a piece of a potato-dextrose-agar disk (0.5 cm diameter) on which *L. decastes* had been grown for 20 days, and the fungus was cultured in the dark at 23°C for 4 weeks. The mycelial growth rates were determined at weekly intervals.

Water extract experiment

The culture waste or bark compost (25 g wet weight) was soaked in water (100 ml) for 1 h at room temperature. The water extract (weight equivalent to 2.5 g each of the wet waste per petri dish) was added to the medium containing 2% glucose, 0.1% peptone, 2% malt extract, and 1.5% agar. The medium was adjusted to pH 6.0 with 1 N KOH before autoclaving, and the sterilized medium (20 ml each) was placed in petri dishes (ϕ8 × 2 cm). Each medium was inoculated with a piece of agar disk (0.5 cm diameter) containing the same components on which *L. decastes* (Ld 18) had been grown for 20 days and subjected to culture in the dark at 23°C for 2 weeks. The diameters of the mycelial colonies were measured.

Production of *Lyophyllum decastes* fruit bodies

Preparation of culture media

Media were prepared in the same way as described above. Each medium (1.2 kg) was placed in a polypropylene bag (14 × 12 × 12 cm) equipped with filter paper obtained from Nisho Co. It was autoclaved at 121°C for 60 min.

Culture conditions

Lyophyllum decastes (Ld 18) was incubated in a medium composed of bark compost and rice bran (10:1, v/v) for 2 months for preparation of the inoculum. After each bag was inoculated with about 50 ml of spawn, the fungus was grown in the dark at 23°C until the media in the bags were completely colonized by the mycelia; the cultivation was then continued for another 10 days. The top of the mycelial bed was covered with soil (about 1.5 cm in thickness), and the excess parts of the polypropylene bags above the media were cut off. For fruit body production, the treated culture beds were transferred to a room where the temperature and relative humidity were maintained at 17° ± 2°C and 85%–95%, respectively. Light intensity was kept at about 1000 lux. The numbers and weights of harvested fruit bodies were measured.

Results and discussion

Lyophyllum decastes mycelial growth in mushroom culture waste media

Fresh waste

Mycelial growth of *L. decastes* was measured in the medium with fresh culture wastes obtained immediately after harvesting the mushrooms (Table 1). The mycelium of *L. decastes* did not grow in any medium containing the culture waste of *P. ostreatus* when the medium was supplemented with rice bran. The fungal mycelium grew in the *P. ostreatus* culture waste medium containing neither wheat bran nor dried soybean-curd (tofu) refuse as a supplement instead of rice bran (data not shown). However, mycelia of the fungus grew in the *P. nameko* culture waste media mixed with bark compost, although it did not grow in the medium containing

Table 1. Mycelial growth of *L. decastes* in various media based on the fresh mushroom culture waste

Medium composition ratios[a]: Culture waste/bark compost/rice bran	Growth rate (mm/d), mean ± SD	
	Ld2	Ld18
P. ostreatus		
10:0:0	0	0
10:0:1	0	0
10:0:2	0	0
10:0:3	0	0
8:2:1	0	0
5:5:1	0	0
P. nameko		
10:0:1	0	0
8:2:1	1.9 ± 0.1	1.9 ± 0.1
5:5:1	2.1 ± 0.4	2.1 ± 0.1
Control		
0:10:1	2.7 ± 0.1	2.2 ± 0.2

Four replicates were tested.
[a] On a volume basis

only the *P. nameko* culture waste as substrate. The more culture waste that was contained in the substrate, the more slowly did the mycelia grow.

Effect of water extracts of fresh culture wastes

It was presumed from the above experiment that fresh culture wastes contain substance(s) to inhibit mycelial growth of *L. decastes* because *L. decastes* mycelium showed no or little growth on the media that contained the fresh culture waste of *P. ostreatus* or *P. nameko*. Therefore, effects of water extracts of fresh culture wastes on the mycelial growth of *L. decastes* on the agar media were examined.

Table 2 shows the diameters of the *L. decastes* colonies incubated for 2 weeks in media to which water extracts of fresh culture wastes had been added. The growth of the mycelial colony in the medium containing the water extract of *P. ostreatus* culture waste was reduced to 72% that of the control (to which water extract had not been added). However, the diameter of the mycelial colony in the medium containing water extract of *P. nameko* culture waste or bark compost was not significantly different from that of the control.

These results indicate that (some) substance(s) contained in fresh culture waste of *P. ostreatus* may inhibit mycelial growth of *L. decastes*.

Aged waste

Mycelial growth of *L. decastes* in the media with the aged culture wastes (prepared by a 6-month outdoor treatment) was measured (Table 3). *L. decastes* mycelia grew in all the media tested that contained the aged culture waste of *P. ostreatus* or *P. nameko*. The growth rates did not differ significantly between *P. ostreatus* culture waste media mixed with bark compost and those not so mixed. However, mycelia grew slower in the *P. nameko* culture waste media not mixed with bark compost than in those that were mixed. When the culture waste and bark compost were mixed in the same ratio, the 10% rice bran addition yielded better mycelial growth than the 20% addition.

The reason outdoor treatment of culture wastes permitted the fungus to grow is not clear. Probably growth inhibitors were removed or wood components such as hemicellulose and cellulose were degraded into substances more easily available to *L. decastes* by microbial actions during the 6-month period.

It was reported that during *Lentinus edodes* cultivation carried out by use of *Flammulina velutipes* culture waste the growth in fresh culture waste medium was suppressed more strongly than that obtained in aged culture waste medium by adding rice bran to the substrate.[3] In this case, Ohga et al. explained that excess nutrient contained in the fresh culture waste inhibited the mycelial growth of *L. edodes*.

Fruit body formation of *Lyophyllum decastes* on mushroom culture waste media

Fruit bodies of *L. decastes* were produced on every tested medium with culture waste subjected to outdoor treatment for 12 months (Table 4). The *P. ostreatus* culture waste medium (A, in Table 4) gave the least fruit body yield among all the media tested. The mycelium of *L. decastes* grew as normally in the *P. ostreatus* culture waste medium as in the *P. nameko* culture waste medium until about 50 days into the cultivation. Thereafter mycelia grew extremely slowly, taking 115 days to colonize completely. Mixing *P. ostreatus* culture waste with bark compost (B, in Table 4) reduced the time for colonization of mycelia and increased the fruit body yield to the same level as that for the control (E, in Table 4).

The *P. nameko* culture waste medium (C, in Table 4) gave the greatest fruit body yield among all the media tested, although the time required for complete colonization of mycelia was longer than that for the control. Mixing the *P. nameko* culture waste with bark compost (D, in Table 4) reduced the time required for colonization of mycelia and the yield, which was nevertheless slightly greater than that of the control.

These results show that outdoor treatment of culture waste is useful for producing *L. decastes* fruit bodies. The aged culture waste of *P. nameko*, in particular, can be utilized as an alternative substrate for cultivating *L. decastes* under

Table 2. Effect of water extracts of fresh culture waste on the growth of *L. decastes* (Ld18)

Additions	Colony diameter (mm), mean ± SD
Water extracts from *P. ostreatus* culture waste	36.0 ± 0.6
Water extracts from *P. nameko* culture waste	47.3 ± 1.4
Water extracts from Bark compost	51.2 ± 1.4
Absence	50.0 ± 1.3

Four replicates were tested

Table 3. Mycelial growth of *L. decates* in various media based on the aged mushroom culture waste

Medium composition ratios[a]: Culture waste/bark compost/rice bran	Growth rate (mm/d), mean ± SD	
	Ld2	Ld18
P. ostreatus		
10:0:1	2.5 ± 0.1	2.5 ± 0.0
10:0:2	2.2 ± 0.1	2.1 ± 0.0
5:5:1	2.4 ± 0.0	2.6 ± 0.0
5:5:2	2.2 ± 0.0	2.4 ± 0.1
P. nameko		
10:0:1	1.8 ± 0.0	1.9 ± 0.0
10:0:2	1.6 ± 0.0	1.8 ± 0.0
5:5:1	2.6 ± 0.1	2.7 ± 0.1
5:5:2	2.5 ± 0.2	2.4 ± 0.1
Control		
0:10:1	2.5 ± 0.1	2.6 ± 0.1

Culture wastes were left outdoors for 6 months. Four replicates were tested.

[a] On a volume basis

Table 4. Production of *L. decastes* (Ld18) fruit bodies by sawdust cultivation with aged mushroom culture waste

Parameter	*P. ostreatus* culture waste		*P. nameko* culture waste		Control
	A	B	C	D	E
Days for complete colonization of mycelia	115.3 ± 12.2	49.8 ± 1.8	76.2 ± 3.6	61.4 ± 0.9	51.8 ± 3.8
Days to croppings after treatments for fruiting	42.0 ± 4.7	31.6 ± 2.2	30.8 ± 1.1	34.0 ± 0.0	39.2 ± 4.4
No. of fruit bodies per bag	19.5 ± 9.0	74.8 ± 16.1	78.8 ± 17.3	41.8 ± 7.5	29.0 ± 15.6
Yields of fruit bodies (g/bag)	62.3 ± 8.9	134.2 ± 3.8	166.8 ± 11.8	140.2 ± 19.3	131.3 ± 40.5

Culture wastes were left outdoors for 12 months. Five replicates were tested; results are the mean ± SD.
A–E: culture waste/bark compost/rice bran, on a volume basis: A, 10:0:1; B, 5:5:1; C, 10:0:1; D, 5:5:1; E, 0:10:1

appropriate culture conditions. Reutilization of the culture waste may lead not only to the reduction of cost for fruit body production but also to the recycling of important woody biomass resources in the future. Research is still being conducted to determine the time required for outdoor treatment and to screen for strains that produce better yields.

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