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Fruitbody Development of *Pleurotus ostreatus* via Bottle Cultivation Using Recycled Substrate

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This study was carried out to determine the possibility of bottle cultivation utilizing recycled oyster mushroom culture waste as a cultivating substrate for *P. ostreatus*. Total nitrogen percentage was 0.76%, 1.13%, 1.16%, 1.36%, and 1.38% in the 1-, 2-, 3-, 4-, and 5-time mixed substrate, respectively; 0.95%, 1.04%, 1.34%, 1.36%, and 1.25% in the 1-, 2-, 3-, 4-, and 5-time postharvest substrate, respectively; and 0.72% and 0.68% in the 2- and 3-time nonadditive substrate, respectively. Weight of the fresh fruiting body harvest was 115 g, 120 g, 117 g, 118 g, and 114 g on 1-, 2-, 3-, 4-, and 5-time mixed substrate, respectively; and 105 g and 45 g on 2- and 3-time nonadditive substrate, respectively. The first mixed substrate (fresh) and recycled substrates generated no significant difference in the weight of fresh fruiting bodies harvested.

KEYWORDS : Cultivation, *Pleurotus ostreatus*, Recycling substrate

Oyster mushroom, *Pleurotus ostreatus*, is one of the most widely cultivated mushrooms in the world (Baars and Sonnenberg, 2000) and are primary decomposers of hardwood trees. Asians have popularly consumed *P. ostreatus*, and it has been cultivated for many years using artificial cultivation methods developed in 1980's (Cha *et al*., 1989). Its yield ratio is almost 32% among all mushrooms produced and 45,782 metric tons are harvested in Korea (Ministry of Agriculture & Forestry, 2007). Mushrooms are economically important biotechnological products, and the mushroom market has markedly expanded worldwide over the past few decades. Mushrooms have been traditionally consumed as a food and considered as potential nutraceuticals (Chang and Buswell, 1996). In mushroom cultures, one of the most advantageous aspects is that they are grown on agricultural wastes. This enables us to acquire substrate materials at low prices or for free, and promotes conservation of our environment by recycling waste materials. Most of all, oyster mushrooms can utilize various kinds of substrate materials, more so than any other mushroom. It was reported in a worldwide survey that about 200 different substrates are available for oyster mushroom cultivation (MushWorld, 2004). Softwood sawdust (*Cryptomeria japonica*, *Pseudotsuga menziesii*, *Populus* spp., and *Pinus* spp.; widely used for commercial cultivation) has been chiefly used as a substrate for both *F. velutipes*. and *P. ostreatus*. The waste produced by mushroom farms is mostly discarded or partly recycled in compost and feed (Cho *et al*., 2008). *P. ostreatus* colonizes the substrate quickly and then fruits. This characteristic implies that most of the lignocellulosic material is not degraded. If the cultural waste can be recycled and reutilized as a cultivating substrate, this would promote the effective use of wood resources and lower operating costs. The purpose of this research is to explore the reutilization of oyster mushroom cultural waste as a cultivating substrate for *P. ostreatus* bottle culture.

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Materials and Methods

Substrate analysis. Chemical compositions of substrates were analyzed by RDA Soil Physicochemistry Analysis (Han, 1988). CaO, MgOm, and K_2O content of the mushroom substrates was analyzed with an atomic absorption spectrometer (Perkin Elmer 2380). Carbohydrate, nitrogen, and P_2O_5 were analyzed using Tyurin, Kjeldahl, and Colorimetric assays, respectively.

Fungal isolates. *P. ostreatus* (strain Sinnong 73) was obtained from Plantech Agricultural Product Company and cultured at 20°C on potato dextrose agar (PDA) medium. The medium consisted of 0.4% potato extract, 2% dextrose, and 1.5% agar. Subcultures were made routinely every 10 days.

Inoculation. The PDA medium was sterilized for 20 minutes at 121°C and poured into a Petri dish under sterile conditions. After cooling, a piece of mycelia was inoculated onto the PDA medium plate to be used as an inoculum for the next step.

Mother spawn. Popular sawdust and rice bran were mixed at 90% and 10% (v/v), respectively. The mixed

media was adjusted to a 65% water content and put in a Erlenmeyer flask (250 *ml*) and sterilized at 121°C (1.2 kg/cm^2) for 90 minutes. After cooling to 20°C, a piece of mycelia from the PDA plate was inoculated into the sawdust medium to be use as an inoculum for the planting spawn. The isolate was inoculated and was maintained at 20 ± 1°C for 15 days (Oh *et al*., 2003).

Planting spawn. The planting spawn medium was prepared by the same method used for the mother spawn. The medium containing sawdust and rice bran was put into a 850 *ml* polyethylene bottle and sterilized at 121°C for 90 minutes and cooled at 20°C. Mother spawn was inoculated into the sawdust culture medium in a 850 *ml* polyethylene bottle. The inoculated sawdust media was incubated at 20 ± 1°C for about 20 days until mycelia spread, and then this was used as an inoculum for cultivation.

Sawdust cultivation process. The cultivation method for *P. ostreatus* was ordered as follows; substrate preparation, transfer substrate to polyethylene bottle, sterilization, inoculation, spawn run, initiation of primordium, and growing of basidiocarps.

Substrates and preparation. Popular (*Populus* spp.) sawdust was collected from a local sawmill (Namwon City, Jeollabuk-Do province). The sawdust was collected and stored in an enclosed warehouse until it was used. Culture substrates, 80% of popular sawdust and 20% additives (v/v), were prepared and used to screen suitable culture media for mycelial growth. The mixed media (530~550 g) was adjusted to a 65% water content and put in a polypropylene bottle (850 *ml*) using an automatic substrate injection machine. Then the substrates were pasteurized at 121°C for 90 minutes (Jo *et al*., 2008). After cooling the pasteurized substrates to 15~20°C, they were inoculated with 8~10 g of pre-cultured spawn. The inoculated media was then incubated in a dark room for 25 days at 20 ± 1°C, and the duration of mycelial growth and mycelial density were examined. In 2-, 3-, 4-, and 5-time cultures, substrates were mixed with recycling substrate and 20% additives (5% beet pulp, 5% cottonseed hull, 4% rice barn, 1% calcium carbonate, and 5% rice hull). Sterilization and inoculation were performed using the same method as previously described.

Experimental condition. After the completion of the spawn run (25 days), the upper 5% of the aging spawn in polypropylene bottles was removed, and the synthetic media was placed in the cultivation house. Relative humidity was maintained at 90~95% during the initiation of primordium and at 80~90% during fruiting body growth. The temperature was maintained at 15 ± 1°C throughout the experiments. The fruiting body yields of *P. ostreatus* mushroom on various sawdust media are displayed as days for pinhead formation, days for fruit body formation, and weight (g) of fresh fruiting bodies (Jang *et al*., 2003).

Results and Discussion

Physicochemical analysis of substrates. Chemical investigation showed similar values for T-C (total carbon) and T-N (total nitrogen) among the substrate components. The T-C percentage was 43.7% for Popular (*Populus* spp.) sawdust, 39.7% for beet pulp, 42.2% for cottonseed hull, 42.6% for rice barn, and 37.9% for rice hull. T-N percentages were 0.16% for Popular sawdust, 1.47% for beet pulp, 0.87% for cottonseed hull, 2.23% for rice barn, and 0.39% for rice hull (Table 1).

Heavy metal examination showed that the value for Cu was 6.5 ppm for Popular sawdust, 9.1 ppm for beet pulp, 7.2 ppm for cottonseed hull, 6.6 ppm for rice barn, 3.1 ppm for rice hull, 0.7 ppm for calcium carbonate; and the Zn values were 37.8 ppm for Popular sawdust, 22.8 ppm for beet pulp, 26.0 ppm for cottonseed hull, 62.1 ppm for rice barn, 26.9 ppm for rice hull, and 18.7 ppm for calcium carbonate (Table 1).

Chemical analysis of 1-, 2-, 3-, 4-, and 5-time mixed substrate; 1-, 2-, 3-, 4-, and 5-time postharvest substrate; and 2- and 3-time nonadditive substrate indicated similar values for T-C (0.76%, 1.13%, 1.16%, 1.36%, and 1.38%; 0.95%, 1.04%, 1.34%, 1.36%, 1.25%; and 0.72% and 0.68%; respectively). These results were similar to the

Table 1. Chemical compositions of substrates

Substrate	T-C[a]	T-N[b]	C/N	P_2O_5	K_2O	CaO	MgO	Cu	Zn	Pb	Cd	Cr	As	Ni
	(%)							ppm						
Popular sawdust	43.7	0.16	273	0.03	0.40	0.21	0.09	6.5	37.8	0.2	0.2	14.9	1.1	8.7
Cottonseed hull	42.2	0.87	48.5	0.17	1.50	0.29	0.26	7.2	26.0	3.9	0.1	10.9	0.3	6.4
Beet pulp	39.7	1.47	27.0	0.11	0.55	0.63	0.42	9.1	22.8	1.4	0.5	13.7	7.5	5.9
Rice barn	42.6	2.23	19.1	3.54	1.89	0.09	1.00	6.6	62.1	0.6	0.1	16.7	0.3	7.9
Rice hull	37.9	0.39	97.3	0.14	0.61	0.10	0.05	3.1	26.9	0.5	0.1	13.7	1.1	4.7
Calcium carbonate	11.23	–	–	0.13	0.07	7.04	0.27	0.7	18.7	0.1	0.1	5.0	2.5	1.9

[a]Total carbon, [b]total nitrogen.

Table 2. Changes in chemical compositions of recycling substrates

Substrate	T-C[a]	T-N[b]	C/N	P_2O_5	K_2O	CaO	MgO	Cu	Zn	Pb	Cd	Cr	As	Ni
	(%)							ppm						
1st mixed	45.4	0.76	59.7	0.33	0.90	0.71	0.26	5.7	34.7	1.9	0.3	14.4	2.4	5.2
2nd mixed	44.0	1.13	38.9	0.41	1.05	0.96	0.40	6.3	39.9	2.2	0.3	12.1	3.7	5.6
3rd mixed	43.7	1.16	37.7	0.65	1.24	0.95	0.48	5.6	36.5	0.9	0.2	14.2	2.3	2.1
4th mixed	42.9	1.36	31.5	0.78	1.34	1.10	0.57	6.5	43.3	1.9	0.2	23.3	3.4	15.8
5th mixed	41.6	1.38	30.2	0.83	1.25	1.13	0.53	6.3	35.2	1.1	0.2	23.9	3.5	13.6
1st mixed (postharvest)	44.0	0.95	46.4	0.30	0.91	0.91	0.32	5.0	40.5	2.3	0.2	2.9	2.3	1.1
2nd mixed (postharvest)	43.4	1.04	41.7	0.56	1.11	0.98	0.49	6.0	48.7	2.9	0.3	6.9	1.5	2.9
3rd mixed (postharvest)	42.1	1.34	31.4	0.56	1.25	1.10	0.54	6.8	42.0	1.2	0.3	15.6	2.5	7.9
4th mixed (postharvest)	41.2	1.36	30.3	0.72	1.35	1.18	0.57	6.8	45.0	1.9	0.2	14.3	2.9	8.3
5th mixed (postharvest)	42.2	1.41	29.9	0.64	1.31	1.20	0.50	7.7	44.7	1.6	0.3	14.9	2.8	8.8
2nd mixed (nonadditive)	44.3	0.70	61.5	0.86	1.28	1.17	0.55	6.6	36.8	1.4	0.3	24.8	1.2	14.1
3rd mixed (nonadditive)	40.9	0.69	59.3	0.69	1.16	0.95	0.53	4.7	32.9	2.1	0.2	36.2	2.6	16.3

[a]Total carbon, [b]total nitrogen.

chemical characteristics of enokitake culture waste, and they indicated that the cultural wastes were only slightly degraded and suggested that the rice bran component was mostly lost and consumed by *F. velutipes* after harvesting (Chai, 2000). In our analysis, 2-time nonadditive and 3-time nonadditive substrate had the lowest T-N concentration. From the above results, mixed and postharvest substrates did not show any differences in their general and elemental compositions (Table 2).

Growth of mycelium on various sawdust substrates. To study the possibility of recycling substrates for cultivation of *P. ostreatus*, we investigated *P. ostreatus* mycelium growing status using seven kinds of mixed and nonadditive substrates. It took about 17~18 days to complete mycelial growth (Table 3). Mycelial densities on nonadditive substrates were lower than those of mixed substrates. These results were similar to previous results indicating satisfactory mycelial growth of *Pleurotus eryngii* using cultivation media wastes (Kim *et al*., 2007).

Character of fruiting body in various substrates. The current experiments were conducted to determine the possibility of artificial bottle culture using recycling substrates of *P. ostreatus*. The days for primordium formation were similar at 5~6 days on both mixed substrates and nonadditive substrates (Fig. 1). Furthermore, the days required for growing basidiocarps were similar at 4~5 days on both mixed substrates and nonadditive substrates. The weights of fresh fruiting body harvest on 1-, 2-, 3-, 4-, and 5-time mixed substrate were 115 g, 120 g, 117 g, 118 g, and 114 g, respectively; and 2- and 3-time nonadditive substrate were 105 g and 45 g, respectively (Table 3, Fig. 2). Among these, the yields from different mixed substrates were similar, whereas the 3-time nonadditive substrate was the poorest. Chai (2000) reported that the second flush *F. velutipes* was tried utilizing the waste Populus mixed waste for commercial *F. velutipes* cultivation, indicating the potentiality of second crop and suggesting further research for it. This is in agreement with our experimental results. Using recycling substrates is economical because the first mixed substrate and recy-

Table 3. Comparison of mycelial growth, mycelial density, primordium formation, fruitbody, and fruitbody yields of *P. ostreatus* with various medium

Substrate	Duration of mycelial growth (days)	Mycelial density[a]	Days for fruitbody primordium formation	Days for fruitbody formation	Wt. of fresh fruitbody (g/850cc bottle)
1st mixed	17	+++	6	5	$115.3 \pm 7.5^{a,c}$
2nd mixed	18	+++	5	5	119.7 ± 9.1^{a}
3rd mixed	17	+++	5	6	117.0 ± 8.5^{a}
4th mixed	17	+++	6	5	117.7 ± 13.8^{a}
5th mixed	18	+++	5	5	114.0 ± 7.9^{a}
2nd mixed (nonadditive)	18	+++	5	5	104.7 ± 7.0^{a}
3rd mixed (nonadditive)	17	++	5	4	44.7 ± 9.3^{b}

[a]Mycelial density: ++ = low, +++ = medium, ++++ = high.
[b]Fresh weight.
[c]Values in the same line w.ith different literal differ at Duncan's multiple range test ($P < 0.05$) and the results are mean ± standard deviation of ten replicates.

Fig. 1. Primordium formation of *P. ostreatus*. A, 1st mixed; B, 3rd mixed (nonadditive); C, occurrence of *P. ostreatus* on waste substrate under wild conditions in May.

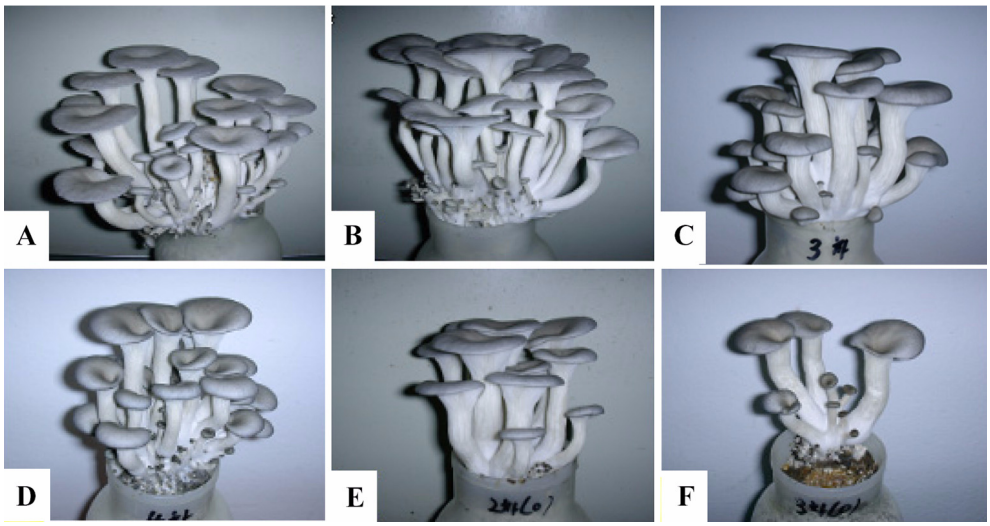

Fig. 2. Effect of recycled substrates on fruitbody development of *P. ostreatus*. A, 1st mixed substrate; B, 2nd mixed substrate; C, 3rd mixed substrate; D, 4th mixed substrate; E, 2nd mixed (nonadditive); and F, 3rd mixed (nonadditive).

cling substrates show no substantial difference in the weight of fresh fruiting body harvested (Table 3).

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