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Optimization of Culture Media for Solid-state Culture of *Pleurotus ferulae*

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Abstract In order to elucidate the possibility of artificial production of *P. ferulae* by solid-state culture, the optimization of culture conditions was carried out. When $NH_4H_2PO_4$ and $CaCO_3$ were used in the cultures using test tube with 30 g of *Populus* sawdust at 25°C ± 1 in the dark, the favored mycelial growth was observed with 1% of $NH_4H_2PO_4$ and the production of polysaccharide was 7.85 mg/100 mg of mycelium with 1% of $CaCO_3$. The mixtures of 80% of *Populus* sawdust and 20% of rice bran at 60% of water content were determined to be optimal for the production of fruiting bodies in the sawdust culture. When three treatments containing various ratios of garlic powder were conducted, yields of fruiting bodies were drastically higher than those of synthetic mixture without garlic powder. The highest yield (143 g/bag) was obtained with 7% garlic powder. The yield of synthetic mixture containing 7% of garlic powder was 83% higher than that of sawdust culture. The reason why garlic powder did support growth was not clear but it is possible that garlic powder might contain effective components for the formation of fruiting body. The optimal synthetic mixture composition consisted of cotton seed 77%, lime 6.4%, K_2HPO_4 0.2%, KH_2PO_4 0.2%, $CaHPO_4$ 0.2%, corn flour 4%, wheat flour 5%, and garlic powder 7%.

Keywords: polysaccharide, *Pleurotus ferulae,* solid-state culture

INTRODUCTION

Mushroom research and production have received increased attention in recent years because of the recognition that mushrooms are a nutritional food with health-stimulating properties and medicinal effects [1]. Most potent mushroom-derived substances have been known to have antitumor and immunomodulating properties [2-4].

All over the world and especially in developing countries, a problem of shortage of protein has been a problem. Producing cultured mushrooms can be one suitable solution to this problem. Because of rapid industrialization, the amount of waste materials has increased and utilization of these wastes is very important for the government economy and natural valance [5]. The ability of being an edible reproductive structure has been exploited for centuries in Asia for the production of mushrooms like shitake (*Lentinus edodes*) and oyster mushroom (*Pleurotus ostreatus*) [6-9].

Pleurotus spp are flavorful mushrooms that are found throughout the northern temperate zone. These species are characterized by an eccentric stipe. The common name "oyster mushroom" stems from the white, shell-like appearance of the fruiting body. This mushroom represents a valuable source of protein for rural populations and its production has increased significantly in recent years [10]. Because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes and a low demand on resources and technology, several species of *Pleurotus* are cultivated commercially in different parts of the world [11].

However, there were few reports related to artificial cultivation of mushroom production by *Pleurotus ferulae* within our country.

In this study, in order to elucidate the possibility of artificial production of *P. ferulae* by a solid-state culture, the optimization of culture conditions were carried out.

MATERIALS AND METHODS

Strain

Pleurotus ferulae was obtained from Culture Ground of Kaya-Backsong (Chunnam, Korea). The culture was incubated on PDA (potato dextrose agar) at 25°C ± 1 for 7 days, and then stored at 4°C.

Preparation of Spawn

The sawdust of *Populus alba* was mixed with rice bran at a ratio of 4:1 (w/w) and moisture content was ad-

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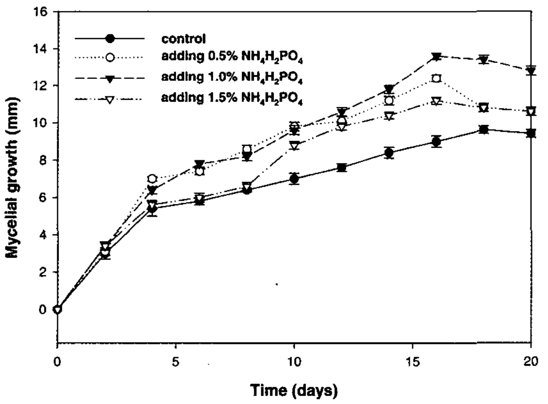

Fig. 1. Effect of ammonium phosphate on mycelial growth by *P. ferulae* in test tube cultures with media containing *Populus* sawdust of 30 g and water content of 60% at 25°C ± 1.

justed to about 65% by adding water. Then the mixed medium was put into a 300-mL flask and was sterilized at 121°C for 40 min. After cooling to 20°C, a piece of mycelia from the agar disc was obtained by sterilization cork borer and inoculated on the *Populus* sawdust medium. The spawn culture was grown at 25°C on a rotary shaker for 7~8 days.

Sawdust Culture

To investigate the feasibility of using *Populus alba* as a sawdust, preliminary tests were performed in test tubes. *Populus alba*, rice bran, $NH_4H_2PO_4$, and $CaCO_3$ were mixed at different ratios to investigate the effect of various components on mycelial growth. The water content was adjusted to 60 or 70% by weighting a sample, drying it, and re-weighting. Thirty gram of the mixture was dispersed into a test tube (Ø2.2 cm × 20 cm) with a silicon plug. After sterilization at 121°C for 40 min and inoculation, the test tubes were incubated upright at 25°C ± 1 in the dark for 20 days. Fruiting body cultures were carried out in propylene bags (diameter 9 cm, length 38.5 cm). *Populus* sawdust, rice bran were mixed at various ratios with the water content of 60% and $NH_4H_2PO_4$ and $CaCO_3$ of 1%. Five hundred gram of the mixture was dispersed into the bag. After autoclave sterilization at 121°C for 40 min and cooling, a 10% inoculation was used in each sample. Inoculated blocks were incubated at 25°C ± 1 in the dark. After 30 days, the substrates were completed colonized by the mycelium. The blocks were then shocked at 4~5°C for 48 h to stimulate production of fruiting bodies. The bags were then incubated at 12~15°C, 80~90% relative humidity, and illuminated for 12 h/day until fruiting bodies developed.

Compositions of Synthetic Mixture

Fruiting body cultures were carried out in a growth chamber. Corn flour, wheat flour, and garlic powder in a mixture containing 77% of cotton seed, 6.4% of lime, 0.2% of KH_2PO_4, 0.2% of K_2HPO_4, and 0.2% of $CaHPO_4$ were mixed at various ratios with the water content of 60%. The method for producing fruiting body cultures was the same as the method that was employed in sawdust cultures.

Analytical Methods

To examine the growth of mycelium, the periphery of a test tube was divided into four sections and the length of mycelia measured. The values are means of triplicated determination. The mushroom yield (g/bag) was calculated by division of fresh weight of fruiting bodies that was obtained from each one bag to the dry weight of 500 g of mixture. For determination of expolysaccharide, dry powdered mycelia were extracted with 1,000 mL of hot distilled water using soxhlet apparatus at 100°C for 2~3 h After standing out overnight at 4°C, the solvent was centrifuged and the supernatant was evaporated and lyophilized, and then the solid mass obtained was measured by constant weight.

RESULTS AND DISCUSSION

Effects of Addition of $NH_4H_2PO_4$ and $CaCO_3$ on Mycelial Growth

Carbon, nitrogen and mineral sources are essential for the growth of fungi. The effect of $NH_4H_2PO_4$ and $CaCO_3$ on mycelial growth was studied in the cultures by using test tube containing 30 g of *Populus* sawdust at 25°C ± 1 in the dark. $NH_4H_2PO_4$ would provide not only nitrogen and phosphorus but also gave a buffering capacity. The results are shown in Fig. 1. $NH_4H_2PO_4$ led to a significant increase in mycelial growth rate and the highest level of mycelial growth by *P. ferulae* was observed with a 1% of $NH_4H_2PO_4$. Therefore, the concentration of $NH_4H_2PO_4$ that was added was then determined to be 1% in the following test.

For buffering the medium, $CaCO_3$ has been used with commercial substrates for various mushroom productions Thus, the effect of $CaCO_3$ concentration on mycelial growth was investigated. As shown in Fig. 2, the mycelial growth was further increased with addition of 1% of $CaCO_3$. At the 20 day of cultivation, the polysaccharide production was 2.35, 5.85, 7.85, and 7.35 mg/100 mg DW at 0%, 0.5%, 1%, and 1.5% of $CaCO_3$, respectively.

Effects of Mixture Composition and Water Content on Mycelial Growth

The optimal water content for the mycelial substratum ranged between 35 and 60% for wood and 60 and 80% for the other substrate [12]. *Populus* sawdust and rice bran were mixed at various ratios and at water contents of 60 and 70% to study their influence on mycelial growth.

The cultivations were carried out in test tubes at 25°C ± 1 in the dark. The results are shown in Table 1. When the water content was 70%, the growth of mycelia

Table 1. Effects of mixture composition and water content on the mycelial growth by *P. ferulae* in test tube cultures

Mixed ratio % (*Populus* sawdust: rice bran)	Mycelial growth (mm/day)[a]	Mycelial growth (mm/day)[b]
100 : 0	33	34
90 : 10	53	51
80 : 20	58	49
70 : 30	52	44
60 : 40	48	45
50 : 50	45	42
40 : 60	42	43

[a] The mean growth rate of mycelium was obtained at water content of 60%
[b] The mean growth rate of mycelium was obtained at water content of 70%

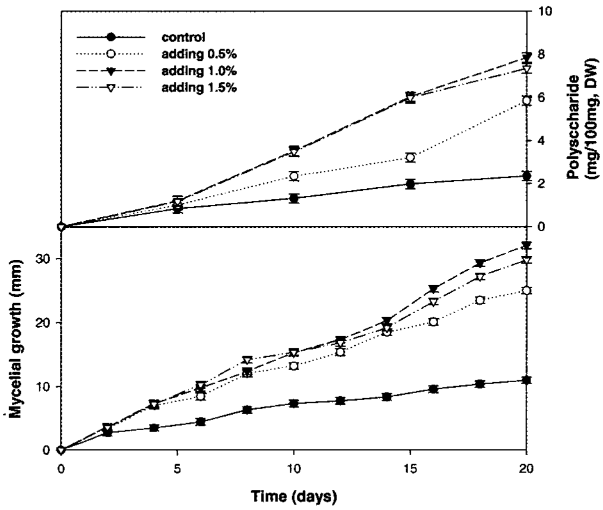

Fig. 2. Effect of carbonate on mycelial growth and polysaccharide by *P. ferulae* in test tube cultures with media containing *Populus* sawdust of 30 g and water content of 60% at 25°C ± 1.

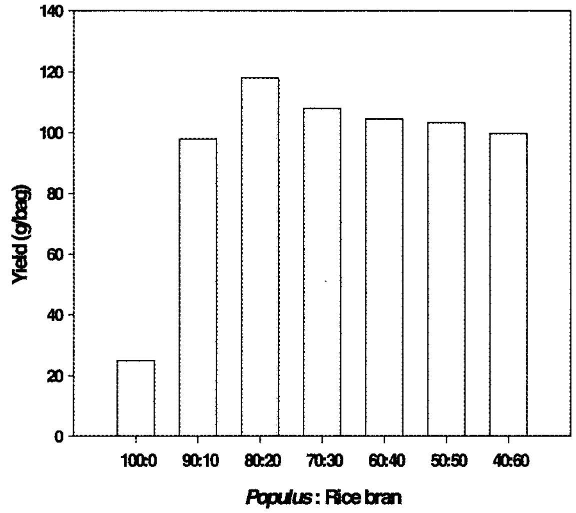

Fig. 3. Effect of the mixture composition on the production of fruiting body by *P. ferulae* in the propylene-bag cultures (*Populus* sawdust: rice bran) at 60% water content, 1% $NH_4H_2PO_4$ and 1% $CaCO_3$ for 2 months.

was unfavorable. This was attributed to the fact that the water holding capacity of the substrate was poor, which caused the accumulation of water in the bottom part of the test tubes. This phenomenon resulted in the diminution of porosity and also resulted in limitation of oxygen transfer.

As for the effect of the mixed ratio on mycelial growth, a level above 20% of rice bran tended to decrease the growth rate, which was attributed to the increase of viscous degree. The mixtures of 80% of *Populus* sawdust and 20% of rice bran at 60% of water content were determined to be optimal for the mycelial growth of *P. ferulae.*

Effect of Mixture Composition on the Production of Fruiting Body

The preliminary tests were carried out in test tubes to determine the ratio of sawdust and rice bran. It is well known that sawdust has been used as a substrate for the commercial production of many mushrooms. The fruiting body cultures were carried out in propylene bags to study the influence of mixture composition on the production of fruiting body in a growth chamber. The results are shown in Fig. 3. When the level of *Polulus* sawdust was lower or higher than 80%, the yield of fruiting bodies was decreased. It is of interest to note that the yield of fruiting body would increase with the addition of rice bran. The yield of fruiting body increased with the increase of rice bran level up to 20% and then it slightly decreased at level past 20%. Therefore, the mixtures of 80% of *Populus* sawdust and 20% of rice bran at 60% of water were determined to be optimal for the production of fruiting body in the sawdust culture.

Table 2. Effect of synthetic mixture composition on the production of fruiting body by *P. ferulae* in the propylene-bag cultures in 2 months

Trt. No.	Ingredients								Mushroom Yield (g/bag)
	Cotton seed (%)	Lime (%)	KH_2PO_4 (%)	K_2HPO_4 (%)	$CaHPO_4$ (%)	Corn flour (%)	Wheat flour (%)	Garlic (%)	
1	77	6.4	0.2	0.2	0.2	8	8	0	121
2	77	6.4	0.2	0.2	0.2	4	12	0	123
3	77	6.4	0.2	0.2	0.2	12	4	0	117
4	77	6.4	0.2	0.2	0.2	6	7	5	135
5	77	6.4	0.2	0.2	0.2	4	5	7	143
6	77	6.4	0.2	0.2	0.2	2.5	3.5	10	128
(Control)	80	20	0.2	0.2	0.2	0	0	0	85

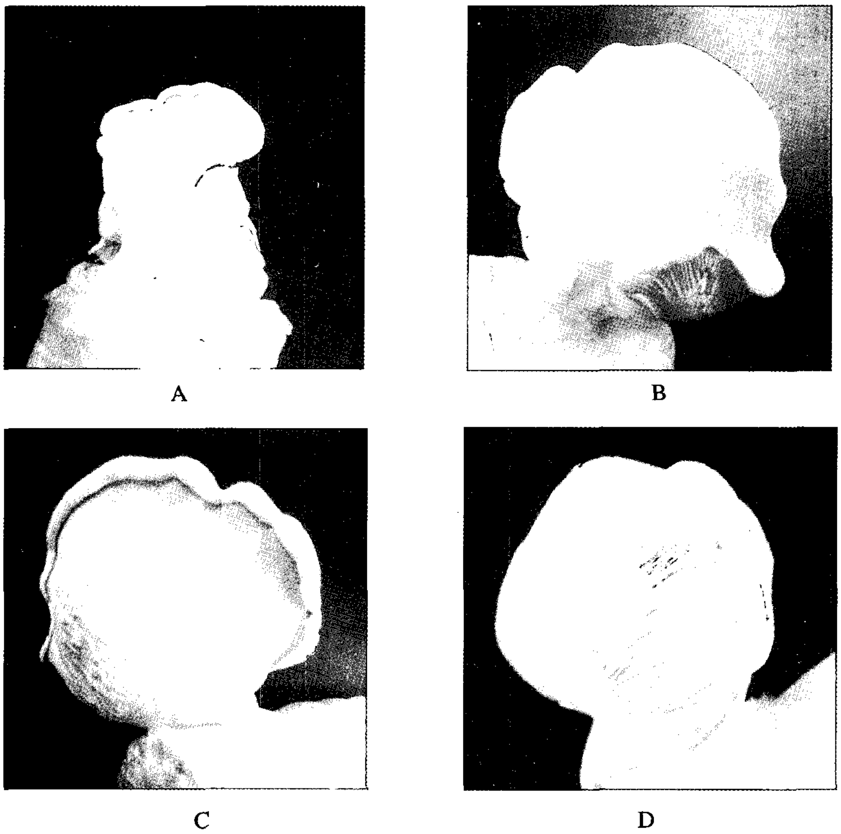

Fig. 4. Fruiting body morphology of *P. ferulae* in the different levels of garlic. A: control, B: 5%, C: 7%, D: 10%.

Effect of Synthetic Mixture Composition on the Production of Fruiting Body

The effect of synthetic mixture composition on the production of fruiting body is shown in Table 2. The yield of fruiting body was on average 85 g/bag on standard. Treatments one, two, and three containing a mixture of corn flour and wheat flour had yields of 121, 123, and 117 g/bag, respectively. Yields of fruiting bodies at various mixtures of corn flour and wheat flour have difference between these three treatments. When the three treatments containing various ratios of garlic powder were conducted, the yields of fruiting bodies were drastically higher than those of synthetic mixture without garlic powder. The highest yield (143 g/bag) was obtained on 7% level of garlic powder (Table 2, Fig. 4). The yield of synthetic mixture containing 7% level of garlic powder was 83% higher than that of sawdust culture (Table 2, Fig. 3). The reason why garlic did support growth was not clear but it is possible that garlic might contain effective components in the formation of fruiting body. Thus, optimal synthetic mixture composition for the production of *Pleurotus ferulae* fruiting body consisted of cotton seed 77%, lime 6.4%, KH_2PO_4 0.2%, K_2HPO_4 0.2%, $CaHPO_4$ 0.2%, corn flour 4%, wheat flour 5% and garlic 7%.

CONCLUSION

This study was an attempt to elucidate the possibility of the artificial production of *P. ferulae* by solid-state fermentation. Cultures were carried out in test tubes or propylene bags. In the case of sawdust cultures, the addition of $NH_4H_2PO_4$ and $CaCO_3$ favored mycelial growth and the highest polysaccharide content (7.85 mg/100 mg) was observed with a 1% of $CaCO_3$. Sawdust supplemented with rice bran at a ratio of 4:1 at 60% of water content was optimal for the production of fruiting body, which is similar to the result obtained by Scarse and Eliot [12]. When the effect of synthetic mixture composition was examined, yields of synthetic mixture containing garlic were drastically increased. The reason why garlic did support growth was not clear but it is possible that garlic might contain effective components in the production of fruiting body. Optimal synthetic mixture composition consisted of cotton seed 77%, lime 6.4%, K_2HPO_4 0.2%, KH_2PO_4 0.2%, $CaHPO_4$ 0.2%, corn flour 4%, wheat flour 5%, and garlic 7%.

While our results are promising, substantially more work is needed. Less expensive and more readily available ingredients may reduce the cost of production and make this technology more competitive with compost-produced mushrooms. Such a development would benefit the specialty mushroom growers wishing to diversify their product line.

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REFERENCES

[1] Mizuno, T., G. Wang, J. Zhang, H. Kawagishi, T. Nishitoba, and L. J. Reishi (1995) *Ganoderma lucidum* and *Ganoderma tsugae*: Bioactive substances and medicinal effects. *Food Rev. Int.* 11: 151-166.

[2] Wasser, S. P. and A. L. Weis (1999) Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. *Int. J. Med. Mushrooms.* 1: 31-62.

[3] Ohtsuka, S., S. Ueno, C. Yoshikumi, F. Hirose, Y. Ohmura, T. Wada, T. Fuji, and E. Takahashi (1973) Polysaccharides having an anticarcinogenic effect and a method of producing them from species of Basidiomycetes. *UK Patent* 1,331,513.

[4] Ooi, V. E. C. and F. Liu (1999) A review of pharmacological activities of mushroom polysaccharides. *Int. J. Med. Mushrooms.* 1: 195-206.

[5] Erkel, I. (1989) Growing Edible Mushrooms: Growing Methods. Ankara University, Ankara, Turkey.

[6] Leatham, G. F. (1982) Cultivation of Shitake, the Japanese forest mushroom, on logs: a potential industry for the united states. *Forest Prod. J.* 32: 29-35.

[7] Zadrazil, F. (1974) The ecology and industrial production *Pleurotus ostreaus*, *Pleurotus florida*, *Pleurotus cormucopia*, or *Pleurotus eryngii*. *Mushroom Sci.* 9, 621-652.

[8] Sang, S. H., S. H. Yeom, S. S. Choi, and Y. J. Yoo (2002) Effect of aeration on denitrification by *Ochrobactrum anthriopi* SY 509. *Biotechnol. Bioprocess Eng.* 7: 352-356.

[9] Nakamura, Y. and G. Mtri (2003) Biodegration of endocrine-disrupting phenolic compounds using laccuase followed by activated sludge treatment. *Biotechnol. Bioprocess Eng.* 8: 294-298.

[10] Chang, S. T. (1999) Gloloal impact of edible and medicinal mushrooms on human welfare in the 21st century: Nongreen revolution. *Int. J. Med. Mushrooms.* 1: 1-8.

[11] Wang, D., A. Sakoda, and M. Suzuki (2001) Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Biosource Technol.* 78, 293-300

[12] Scrase, R. J. and T. J. Eliott (1998) Biology and technology of mushroom culture. pp. 543-584. In: B. J. B. Wood (eds.). *Microbiology of Fermented Food*, Blackie, London, UK.

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