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The Artificial Cultivation of *Oudemansiella mucida* on the Oak Sawdust Medium

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To produce fruiting bodies of *Oudemansiella mucida*, porcelain fungus, on the oak sawdust medium, additives suitable for the mycelial growth and fruiting body formation were screened. In general, the mycelial growth of the three strains of *O. mucida* used in this study have been good on oak sawdust mixed rice bran of 20~30%. The mycelia incubated in potato dextrose broth for 7 days were inoculated on oak sawdust medium supplemented with various ratios of rice bran and incubated for 30 days at 25°C in the dark condition until the mycelia of *O. mucida* fully colonized the media from top to bottom. Then, top surface of the media in the bottles were horizontally scratched with a spatula and filled with tap water for 3 hours. To induce the primordial formation of *O. mucida*, the bottles were transferred to the mushroom cultivating room under 12 hrs of light (350 lux) and dark condition with relative humidity of 95% at 17°C. The primordia of *O. mucida* were formed on the surface of oak sawdust media after 7 days of incubation. The mature fruiting bodies were observed 5 days after primordial formation. The fruiting bodies *O. mucida* were formed on oak sawdust medium mixed with 5 to 30% rice bran. However, abundant fruiting-bodies of *O. mucida* were produced in oak sawdust medium supplemented with 20% rice bran. This is the first report associated with an artificial fruiting body production of *O. mucida* in Korea.

KEYWORDS: Additives, Artificial cultivation, Fruiting bodies, *Oudemansiella mucida*, Sawdust medium

In nature, the fruiting body of *Oudemansiella mucida*, one of the edible and medicinal mushrooms belonging to Trichlomataceae, Agaricales, develops on the rotten wood of the broadleaf trees from summer to early autumn (Lee, 1988). *O. mucida* is collected occasionally from mountains and national parks in Korea (Park and Lee, 1999). Fruiting bodies of *O. mucida* have been known to possess medically important antifungal substances such as mucidin (strobilurin A) and oudemansin. In the USA, derivatives of mucidin are synthesized for controlling various plant fungal diseases. The derivatives of strobilurin known to kresoxim methyl and azoxystrobin were developed and globally sold in agricultural fungicide markets (Deacon, 2006). Mucidin is also isolated from fruiting bodies of *Oudemansiella radicata* (Anke *et al.*, 1979, 1983, 1990). The oudemansin from *O. mucida* contained not only an antifungal activity but also an outstanding inhibitory effect on the sarcoma 180 and Erhrlich carcinoma of mice (Ying *et al.*, 1987).

Although *O. mucida* has been considered as one of the promising edible and medicinal mushrooms (Ying *et al.*, 1987), there is no published study on the artificial cultivation of the fungus in Korea. As part of preliminary experiment for producing fruiting bodies of *O. mucida*, the optimal culture conditions of mycelia such as temperature, pH, nutrients and culture media were studied (Jaysinghe *et al.*, 2007). Therefore, the aims of this study are to determine suitable additive contents of rice bran or wheat bran for the mycelial growth and developing fruiting of *O. mucida* in the sawdust substrate.

Materials and Methods

Culture. Three strains of *O. mucida* such as IUM 688, IUM 929 and IUM 2345 were obtained from the Culture Collection of Wild Mushroom species (CCWM) in the Department of Biology, University of Incheon (Table 1). To facilitate the experiments in oak sawdust media, three strains of *O. mucida* were transferred to PDA plates and incubated at 25°C in the dark condition until they showed a full growth and then kept at 4°C for further use. Unless otherwise stated, the tests were performed at least 5 times. To prepare each liquid culture of three strains of *O.*

Table 1. Strains of *Oudemansiella mucida* used in this study

Strains	Geographical origin
IUM 688	Deokyusan, Korea
IUM 929	Jeoksangsan, Korea
IUM 2345	Hyanjeokbong, Deokyusan, Korea

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mucida, 100 *ml* of potato dextrose broth (PDB) were poured into an Erlenmeyer flask (250 *ml*) and autoclaved for 15 minutes at 121°C. Then, 5 pieces of 5mm mycelial plugs were removed with cork borer from 7 days old PDA plates, inoculated PDB within the Erlenmeyer flask (250 *ml*) and incubated for 10 days at 25°C in shaking incubator (150 rpm).

Screening suitable additive contents of rice bran or wheat bran for the mycelial growth of *O. mucida*. Oak (*Quercus variabilis*) sawdust, rice bran and wheat bran were purchased from a Yongsan sawdust company located in Namwon city, Jeonbuk. To determine suitable additives for mycelial growth in oak sawdust medium, each additive was added in the ratios of 5%, 10%, 15%, 20%, 25% and 30% (v/v) in oak sawdust media, adjusted moisture content of 65~70%, put into the round glass columns (2 × 22 cm) and steam-sterilized for 90 minutes at 121°C.

Three strains of *O. mucida* were inoculated on the sawdust media in glass columns. A 5 mm agar plug was removed with cork borer from 7 days old PDA plate culture of *O. mucida*, and placed on the top surface of sawdust media in the glass column. The columns were incubated for 30 days at 25°C under dark condition. The mycelial growth of *O. mucida* in oak sawdust media supplemented with rice bran or wheat bran was measured after 30 days of incubation. After 30 days of incubation, a suitable additive was selected and then used for supplements to produce fruiting bodies of *O. mucida* in the oak sawdust medium.

Cultivation condition of *O. mucida* on oak sawdust media. After rice bran was selected as a suitable additive for the mycelial growth of *O. mucida*, oak sawdust medium was prepared to produce fruiting body of *O. mucida*. The oak sawdust was mixed thoroughly with 5%, 10%, 15%, 20%, 25% and 30% of rice bran (v/v), adjusted the moisture content of 65~70%, put into polypropylene bottles (850 *ml*), making a hole with glass bar (diameter 1.5 × depth 8 cm) in the center of the media and autoclaved for 90 minutes at 121°C.

About 10 *ml* of each inoculum was removed from 10 days old liquid culture, inoculated on the top surface of oak sawdust media within polypropylene bottles (850 *ml*), and incubated for 30 days at 25°C under 70% relative humidity in the dark condition.

When the mycelia of *O. mucida* completely colonized the sawdust media from top to bottom, the top surface of the media was horizontally scratched with spatula, filled with tap water for 3 hours and transferred to another room to induce the primordial formation. The bottles were cultured for 6 days at 17°C under the 12 hours of light (350 lux) and darkness at 95% relative humidity.

After 6 days of incubation at 17°C, primordia of *O. mucida* were started to form on the top surfaces of the saw dust media. Then, the bottles were transferred to cultivating room and cultured at 20°C under the 12 hours of illumination (350 lux) and darkness with relative humidity of 95%. Fruiting body formations were examined once a day.

Result and Discussion

Screening of suitable additive contents of rice bran for the mycelial growth of *O. mucida*. After 30 days of incubation in the glass column at 25°C, the mycelial growth of *O. mucida* was measured. Of two additives for screening mycelial growth of three strains of *O. mucida*, rice bran showed a little better mycelial growth than that of wheat bran. The oak sawdust which was supplemented with rice brain of 5~30% showed a good mycelial growth (Table 2). In general, the mycelial growth and density of *O. mucida* on oak saw dust mixed with rice bran were better than that of wheat bran. All three strains of *O.*

Table 2. Effect of two additives on myclial growth of *Oudemansiella mucida*[a]

Content of additives (%) \ Strain[b]	Mycelial growth (cm/30 days)					
	Rice bran			Wheat bran		
	IUM 688	IUM 929	IUM 2345	IUM 688	IUM 929	IUM 2345
0	5.1 ± 0.22[c]	4.2 ± 0.26	3.4 ± 0.28	6.2 ± 0.26	5.5 ± 0.38	4.2 ± 0.22
5	12.4 ± 0.37	13.2 ± 0.38	11.7 ± 0.58	11.6 ± 0.49	13.3 ± 0.55	12.2 ± 0.29
10	14.4 ± 0.26	15.4 ± 0.51	13.8 ± 0.73	13.8 ± 0.41	14.5 ± 0.77	13.7 ± 0.41
15	15.6 ± 0.51	16.8 ± 0.28	14.5 ± 0.41	15.1 ± 0.56	16.2 ± 0.55	14.6 ± 0.63
20	18.2 ± 0.36	19.6 ± 0.48	17.4 ± 0.39	17.4 ± 0.31	18.6 ± 0.49	17.3 ± 0.37
25	18.4 ± 0.72	19.5 ± 0.53	17.5 ± 0.53	17.8 ± 0.54	19.2 ± 0.64	17.6 ± 0.59
30	18.9 ± 0.68	19.3 ± 0.84	17.8 ± 0.63	17.4 ± 0.69	18.5 ± 0.56	17.3 ± 0.49

[a]Each of 2 additives was mixed with oak (*Quercus variabilis*) sawdust at the ratio of 5%, 10%, 15%, 20% and 25%, respectively and then, put into the glass column (2 × 22 cm) and autoclaved at 121°C for 15 min.
[b]Three strains were inoculated on the sawdust media and cultured for 30 days at 25°C under dark condition to measure the mycelial growth.
[c]Value is an average of 5 replications.

mucida showed good mycelial growth on oak sawdust mixed with 20% rice bran. Even though mycelial growth of oak sawdust supplemented with rice bran and wheat bran was not significantly different, rice bran is cheaper and easier to purchase in the market. Therefore, rice bran was used for supplements of oak sawdust to produce fruiting body of *O. mucida*. Rew *et al.* (2004) showed that the mycelial growth of *Phellinus baumii* was best on oak sawdust mixed with 20% of rice bran (v/v). Shim *et al.* (2006a) reported that mycelial growth and density of *O. radicata* were also good on oak sawdust mixed with 10% rice bran. Therefore, rice bran might contain some ingredients to facilitate the good mycelial growth of *O. mucida* and the other mushrooms.

Fruiting body production of *O. mucida*.

Conditions for primordial formation: After scratching of mycelia on oak sawdust media, the polypropylene bottles were filled with tap water for 3 hours and then, kept for 7 days at 17°C under 12 hours of light (350 lux) and dark conditions with relative humidity of 95%. Then, white-milky colored primordia have been started to form on the top surface of the media. The primordia have observed firstly from *O. mucida* IUM 929, IUM 688 and IUM 2345, respectively.

Conditions for fruiting body formation: After formation of the primordia, the bottles were transferred to cultivation room, cultured at 20°C under 12 hours of illumination (350 lux) and darkness with relative humidity of 95%. The fruiting bodies of *O. mucida* were formed in the media supplemented with rice bran of 5~30% (Table 3). Of three strains, mature fruiting bodies of IUM 929 were developed 5 days after occurrence of primordia (Fig. 1).

In general, fruiting bodies of three strains of *O. mucida* have been produced in oak sawdust media mixed with rice bran of 5~30%. But, large numbers of fruiting bodies were produced in oak sawdust media mixed with rice bran of 20~30% (Table 3). Even though good fruiting bodies of *O. mucida* were also produced in the media supplemented with 25~30% rice bran, the contamination rate in this media was relatively higher than those of the media mixed with rice bran of 5~20%. Shim *et al.* (2006b) reported that fruiting bodies of *Armillaria mellea*

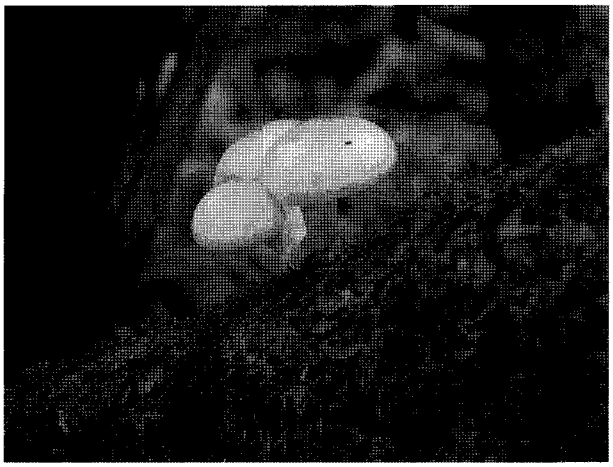

Fig. 1. The fruiting body of *Oudemansiella mucida* in nature.

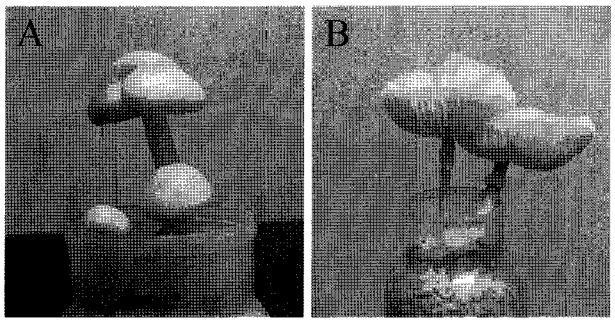

Fig. 2. The artificial fruiting bodies of *Oudemansiella mucida* (IUM 929) produced in the oak sawdust medium supplemented with rice bran (A, young; B, old).

were produced abundantly in the oak saw dust media mixed with 10% rice bran and contamination rate in the saw dust media seemed to be increased in proportion to the higher contents of rice bran. Shim *et al.* (2006a) and Semerdzieva *et al.* (1988) also reported that fruiting bodies of *O. radicata* were formed very well in the oak sawdust media mixed with 10% rice bran.

Now, we are trying to improve the yield and quality of *O. mucida* through a new innovation experimental method by changing substrates of the media. Then, we are able to produce commercially feasible basidiocarps of *O. mucida* in a few years. After all, this is the first report associated with fruiting body production of *O. mucida* in Korea.

Table 3. The fruiting body production of *Oudemansiella mucida* on oak sawdust media

Strains[b]	Content of Rice bran (%)[a]						
	0	5	10	15	20	25	30
IUM 688	–	44.3 ± 2.34	56.4 ± 2.43	69.5 ± 2.34	69.4 ± 2.25	70.2 ± 3.55	68.5 ± 3.15
IUM 929	–	47.2 ± 1.89	58.3 ± 3.95	75.6 ± 3.95	76.7 ± 2.89	75.8 ± 2.71	76.3 ± 4.01
IUM 2345	–	43.5 ± 2.32	55.7 ± 2.88	65.4 ± 3.51	66.9 ± 3.17	65.8 ± 3.88	65.7 ± 4.21

[a]The sawdust of oak (*Quercus variabilis*) was supplemented with of 5%, 10%, 15%, 20%, 25% and 30% rice bran (v/v), respectively.
[b]Each of 3 strains was treated by 10 replications.

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