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The Fruiting Body Formation of *Oudemansiella radicata* in the Sawdust of Oak (*Quercus variabilis*) Mixed with Rice Bran

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To screen additives and their mixed ratio suitable for the mycelial growth and fruiting body formation of *Oudemansiella radicata* in the oak sawdust, additives such as rice bran, fermented soybean powder and wheat bran were used. Generally, the mycelial growth of *O. radicata* has been stable on oak sawdust mixed with rice bran of 5~20%. In case that *O. radicata* was cultured for about 30 days at 22 ± 1°C under the illumination (350 lux) of 12 hours and moisture condition of 90 ± 5%, the primordia have been formed gradually from red-brown crusts covering the surface of oak sawdust media. Based on the experimental results from 9 strains of *O. radicata*, fruiting bodies were produced widely on oak sawdust medium mixed with rice bran of 5 to 30%. Even though fruiting bodies of *O. radicata* have been produced well on oak sawdust media mixed with rice bran, fruiting bodies of *O. radicata* were produced intensively on oak sawdust media mixed with rice bran of 10%. Therefore, this result will provide a basic information for commercial production of fruiting body of wild *O. radicata*. This result is the first report associated with an artificial fruiting body formation of *O. radicata* in Korea.

KEYWORDS: ***Oudemansiella radicata*, Additives, Commercial production, Fruiting bodies**

Oudemansiella radicata (Relhan ex Fr.), one of edible and medicinal mushrooms belonging to *Tricholomataceae*, *Agaricales* has been known to be inhabited on the soil surface or rotted woods of the broad-leaved trees from summer to autumn (Lee, 1988). *O. radicata* has been collected occasionally in the remote sites of mountainous area of large parks in Korea (Kim *et al*., 2005). Also, fruiting body of *O. radicata* has been reported to possess oudenone which is one of medicinally important chemical compounds (Umezawa *et al*., 1973; Tsantrizos *et al*., 1999). The oudenone exhibited an outstanding therapeutic and inhibitory effect on the sarcoma 180 and Erhrlich carcinoma (Umezawa *et al*., 1973; Anke and Werle, 1990). In the submerged culture, *O. radicata* has been known to secrete oudemansin which exhibits high antifungal activities to plant pathogenic fungi such as *Penicillium notatum*, *Ustilago nuda* and *Alternaria poli* (Anke and Werle, 1990). Oudemansin was also produced from fruting body of *O. mucida* as well as *O. radicata* (Anke *et al*., 1979, 1983).

Although *O. radicata* has been considered as one of the promising edible fungi (Umezawa *et al*., 1973; Anke and Werle, 1990; Tsantrizos *et al*., 1999), there was no an experiment for producing fruiting bodies of *O. radicata* in Korea. As part of preliminary experiment for realizing an artificial mass production of *O. radicata*, Kim *et al*. (2005) reported the optimal culture conditions such as temperature, pH, nutrients and culture media suitable for its mycelial growth. Therefore, this study was carried out to screen an additive suitable for the mycelial growth of *O. radicata* on sawdust media and to test if the additive could produce fruiting body of *O. radicata*.

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Materials and Methods

Cultures. Nine strains of *O. radicata* were obtained from the Culture Collection of Wild Mushroom Species (CCWM) in the Department of Biology, University of Incheon (Table 1). To facilitate various tests in oak sawdust media, *O. radicata* was transferred to PDA, incubated at 25°C until it exhibited a full growth in the dark condition and kept at 5°C for further use. Unless otherwise stateed, all the tests

Table 1. *Oudemansiella radicata* used in this study

Strains	Geographical origins
O. radicata (2) IUM 762	Korea
O. radicata (2) IUM 767	Korea
O. radicata (3) IUM 781	Korea
O. radicata (1) IUM 766	Korea
O. radicata (1) IUM 779	Korea
O. radicata (1) IUM 780	Korea
O. radicata (2) IUM 770	Korea
O. radicata IUM 1259	China
O. radicata IUM 1260	China

which the strain was used were performed at least twice.

Screening of an additive suitable for the mycelial growth of *O. radicata*.

The preparation of sawdust medium mixed with each of 3 additives: To screen an additive suitable for the mycelial growth and fruiting body formation of *O. radicata*, 3 additives such as rice bran, fermented soybean powder and wheat bran were used. Each additive was mixed with oak sawdust (*Quercus variabilis*) at the ratio of 5, 10, 15, 20, 25 and 30% (v/v), adjusted to the moisture content of 70%, put into the round glass column (2 × 22 cm) and steam-sterilized for 90 minutes at 121°C.

The inoculation and culture of *O. radicata*: Of 9 strains of *O. radicata*, 3 strains such as IUM 770, IUM 767 and IUM 766 were selected randomly and inoculated on the sawdust media. A 5 mm plug of inocula was removed with cork borer from 7 days old cultures of *O. radicata*, placed on the surface of sawdust media in the column (2 × 22 cm) and incubated for 60 days at 24 ± 1°C under the dark condition. Based on sawdust media mixed with each of rice bran, fermented soybean powder and wheat bran, the mycelial growth of *O. radicata* has been measured once in every 5 days for 60 days of incubation. After 60 days of incubation, a suitable additive was screened and then used for producing fruiting body of *O. radicata* in the sawdust media.

Fruiting body formation of *O. radicata* in sawdust media.

The preparation of spawn: A total of 9 strains of *O. radicata* were used to test fruiting body formation (Table 1). To prepare each spawn of 9 inoculum sources, oak sawdust was mixed throughly with 20% rice bran (v/v), adjusted to the moisture content of 70%, put into an Erlenmayer flask (250 *ml*), steam-sterilized for 90 minutes at 121°C. To inoculate each of 9 strains, a 5 mm plug of inocula was removed with cork borer from 7 days old cultures of *O. radicata*, placed on the surface of sawdust media into an Erlenmayer flask (250 *ml*) and cultured for 10 days at 24 ± 1°C.

The preparation of suitable sawdust media: After an additive such as rice bran suitable for the mycelial growth of *O. radicata* was screened, sawdust medium was prepared to produce its fruiting body. The oak sawdust was mixed throughly with 5%, 10%, 15%, 20%, 25% and 30% rice bran (v/v), adjusted to the moisture content of 70%, put into plastic bottles (800 *ml*), marking a hole with glass bar (diameter 1.5 × depth 8 cm) in the center of sawdust medium and steam-sterilized for 90 minutes at 121°C.

The inoculation and incubation of *O. radicata*: About 20 grams of each inoculum was removed from fully grown sawdust spawn, inoculated on the surface of sawdust medium in the polyethylene plastic bottle (800 *ml*), and cultured for 30 days at 24 ± 1°C under the dark condition.

Conditions for primordium formation: When the mycelia of *O. radicata* were colonized completely from the top of cultivation bottle to bottom, the mycelia of *O. radicata* on the top of the medium was scratched slightly with a spatula, transferred to an another room to induce an occurrence of primordia and cultured for 2 days at 15 ± 1°C under the illumination (350 lux) of 12 hours and 90 ± 5% of humidity in a day.

Conditions for fruiting body formation: After 2 days of culture at 15 ± 1°C, the cultures of *O. radicata* were transferred to incubation room, cultured at 22 ± 1°C under the illumination (350 lux) of 12 hours and relative humidity of 90 ± 5% in a day and checked to identify fruiting body formation once in a day.

Results and Discussions

Screening of an additive suitable for the mycelial growth of *O. radicata*. After 60 days of incubation in the columns at 24 ± 1°C, the mycelial growth of *O. radicata* was analyzed. Of 3 additives used for measuring the myceilal growth of 3 strains of *O. radicata*, rice bran stimulated its mycelial growth. The oak sawdust which was mixed with rice bran of 5~20% showed a favorable mycelial growth (Table 2). Generally, the mycelial growth and density of *O. radicata* seemed to be more favorable on oak sawdust mixed with rice bran than fermented soybean powder or wheat bran. Particularly, all 3 strains of *O. radicata* showed their outstanding mycelial growth on oak sawdust mixed with 10% rice bran. Rew *et al*. (2004) reported that the mycelial growth of *Phellinus baumii* was outstanding in oak sawdust mixed with 20% of rice bran (v/v). Therefore, it seems to be reasonable that rice bran contains some ingredients to promote the favorable mycelial growth of *O. radicata* and the other mushroom.

Fruiting body formation of *O. radicata*.

Conditions for primordium formation: After a slight scratch of fully grown mycelia on oak sawdust media filled in the polyethylene plastic bottles, the polyethylene plastic bottles were kept for 2 days at 15 ± 1°C under the illumination (350 lux) of 12 hours and relative humidity of 90 ± 5% in a day. After *O. radicata* was kept for 2 days at 15 ± 1°C, *O. radicata* was transferred to another incubation room and cultured for 30 days at 22 ± 1°C under the illumination (350 lux) of 12 hours and relative humidity of 90 ± 5% in a day. *O. radicata* has been started to form primordia from its red-brown mycelial crusts covering the surface of oak sawdust medium 30 days after the culture (Fig. 1). The primordia have been observed firstly from *O. radicata* IUM 1259 and IUM 1260, respectively.

Conditions for fruiting body formation: After primordia of *O. radicata* were formed, fruiting bodies were pro-

Table 2. Effect of 3 additives for the mycelial growth of *Oudemansiella radicata* in oak sawdust media

Strains / Content of additives (%)	[a]Mycelial growth of *O. radicata* (cm/60 days)								
	Sorts of additives[b]								
	Rice bran			Fermented soybean powder			Wheat bran		
	O. radicata IUM(2)770	*O. radicata* IUM(2)767	*O. radicata* IUM(1)766	*O. radicata* IUM(2)770	*O. radicata* IUM(2)767	*O. radicata* IUM(1)766	*O. radicata* IUM(2)770	*O. radicata* IUM(2)767	*O. radicata* IUM(1)766
0	[c]8.0 ± 0.82 T[d]	9.7 ± 0.16 T	9.1 ± 0.28 T	8.5 ± 0.41 T	9.3 ± 0.28 T	9.4 ± 0.37 T	8.2 ± 0.57 T	9.8 ± 0.36 T	9.7 ± 0.16 T
5	20.0 ± 0.82 C	19.6 ± 0.14 C	20.0 ± 0.82 C	14.3 ± 0.16 C	20.0 ± 0.82 C	20.0 ± 0.88 C	8.3 ± 0.36 C	20.0 ± 2.35 C	20.0 ± 0.82 C
10	20.0 ± 0.86 C	20.0 ± 0.82 C	20.0 ± 1.85 C	14.7 ± 0.16 T	16.3 ± 0.29 T	17.1 ± 0.45ST	20.0 ± 0.82 C	20.0 ± 0.82 C	17.0 ± 2.13 C
15	20.0 ± 0.95 C	20.0 ± 1.82 C	19.7 ± 0.22 C	11.3 ± 0.16ST	13.2 ± 0.14ST	20.0 ± 1.65ST	13.8 ± 0.24ST	17.0 ± 3.6 ST	20.0 ± 0.82 C
20	20.0 ± 1.65 C	17.8 ± 0.14 C	20.0 ± 1.84 C	17.2 ± 0.16ST	13.4 ± 0.14ST	13.2 ± 0.14 C	6.6 ± 0.33 C	14.4 ± 2.1 C	20.0 ± 0.82 C
25	10.6 ± 0.16 C	14.8 ± 0.28 C	13.2 ± 0.14 C	14.9 ± 0.14 C	12.0 ± 0.41ST	16.8 ± 0.57ST	13.5 ± 0.71 C	20.0 ± 1.35 C	15.1 ± 1.35 C
30	20.0 ± 1.24 T	17.7 ± 0.14 C	14.0 ± 1.63ST	9.5 ± 0.41 C	12.9 ± 0.08 C	10.7 ± 0.22 C	15.9 ± 0.36 C	14.5 ± 0.56 C	16.2 ± 0.64 C

[a]Three of 9 strains were selected randomly, inoculated on the sawdust media and cultured to check their mycelial growth.
[b]Each of 3 additives was mixed with oak sawdust (such as sawdust of *Quercus variabilis*) at the ratio of 5%, 10%, 15%, 20%, 25% and 30%, respectively and then put in the column (2 × 22 cm).
[c]Values are an average of 4 replications. and, within columns, are significantly different at p = 0.01.
[d]C, Compact; ST, Somewhat thin; T, thin.

Table 3. The fruiting body formation of *Oudemansiella radicata* on oak sawdust media[a]

[b]Strains of *O. radicata* / Content of rice bran (%)	Numbers of fruiting body									
	O. radicata IUM(2)762	*O. radicata* IUM(2)767	*O. radicata* IUM(3)781	*O. radicata* IUM(1)766	*O. radicata* IUM(1)779	*O. radicata* IUM(1)780	*O. radicata* IUM(2)770	*O. radicata* IUM 1259	*O. radicata* IUM 1260	Mean
0	–	3	4	6	–	2	3	–	8	2.9
5	16	12	14	21	20	24	3	28	30	16.4
10	3	34	8	41	10	30	26	20	35	23.0
15	10	26	17	24	12	19	6	20	3	15.2
20	6	49	12	4	15	25	14	11	20	17.3
25	2	25	16	39	12	26	9	4	4	15.2
30	2	7	10	14	2	20	16	23	12	11.8

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

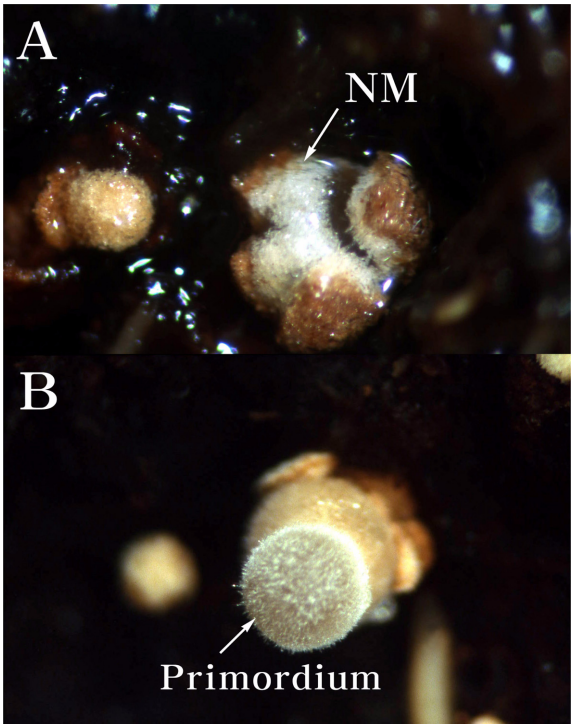

Fig. 1. The primordium of *Oudemansiella radicata* formed on sawdust media of *Quercus variabilis* mixed with 10% rice bran. A, The protrusion (18×) of new mycelium (NM) originated from a mycelial mass of *O. radicata* covering the surface of oak sawdust media. B, The primitive primordium (18×) of *O. radicata* on the surface of oak sawdust media.

duced on oak sawdust medium mixed with rice bran of 5 to 30% (Table 3). Of 9 strains, *O. radicata* IUM 1260 formed its mature fruiting body 6 days after an occurrence of primordia (Fig. 2).

Although fruiting bodies of 9 strains have been produced widely on oak sawdust media mixed with rice bran of 5 to 30%, the contamination of oak sawdust media seemed to be increased in proportion to the high mixture of rice bran. Generally, fruiting bodies of *O. radicata* were produced intensively on oak sawdust media mixed with rice bran of 10% (Table 3). According to Semerdzieva *et al.* (1988), the fruiting body of *O. radicata* has been known to be produced 10 weeks after the culture in case that both stationary and submerged cultures of *O. radicata* were cultured fully in the dark at 24°C, transferred to a lower temperature and periodically illuminated. In the further experiments, the more progressive method to produce fruiting body of *O. radicata* can be developed in the process of comparing and innovating our method with that of Semerdzieva *et al.* (1988). After all, it is a meaningful fact that this result is the first report associated with fruiting body formation of *O. radicata* in Korea.

Fig. 2. The fruiting bodies of *Oudemansiella radicata* on sawdust media of *Q. variabilis* mixed with 10% rice bran.

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