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The Optimal Culture Conditions for the Mycelial Growth of *Oudemansiella radicata*

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***Oudemansiella radicata*, one of edible mushrooms belonging to Tricholomataceae of Basidiomycota, has been known to exhibit outstanding therapeutic effects on the hypertension caused by high blood pressure and inhibitory effects on the sarcoma 180 and Erhrlich carcinoma of mice. As one of preliminary experiments for producing fruiting-body of *O. radicata*, this study was carried out to obtain the basic information for culture conditions of mycelial growth of the fungus. The optimal temperature and pH for the mycelial growth were 25°C and pH 6, respectively. The medium for favorable mycelial growth of *O. radicata* was shown in the Lilly medium, whereas compact mycelial density was found in Hamada medium. The carbon and nitrogen sources promoting for mycelial growth of *O. radicata* were xylose and alanine, respectively. The optimum C/N ratio was about 20 : 1 in case that 3% glucose was supplimented to the basal medium as a carbon source.**

KEYWORDS: Cultural conditions, Edible mushroom, Medicinal mushroom, *Oudemansiella radicata*

Oudemansiella radicata (Relhan ex Fr.), one of edible and medicinal mushrooms belonging to Tricholomataceae of Agaricales has been known to be inhabited on the soil surface or rotted woods located in the broad-leaved forest, mixed forest of broad-leaved and needle-leaved trees for the duration of summer to autumn (Lee, 1988). Many mushroom researchers (Shim *et al*, 1997; Ha, 2001; Choi *et al*., 2003; Lee *et al*., 2004) have been worked to find substances to protect human health from intractable diseases such as a cancer, gastric ulcer and hypertension and so on. Oudenone, one of medicinal substance isolated from fruiting bodies of *O. radicata*, has been known to exhibit outstanding therapeutic effects on the hypertension caused by high blood pressure and inhibitory effect on *Pyricularia oryzae* which caused rice blast disease (Park and Lee, 1999; Ying *et al*., 1987). However, *O. radicata* has been collected occasionally in the remote regions of Mt. Seorak, Mt. Jiri, Mt. Dukyou and Mt. Sokri, the supply of the fruiting body could not meet the demand. Even though the demand for fruiting body of *O. radicata* has been increased, no attempt has been made to develop an artificial cultivation method of *O. radicata* in Korea. To obtain basic information for an artificial cultivation of *O. radicata*, this study was focused on culture conditions affecting the optimal mycelial growth of *O. radicata*.

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Materials and Methods

The collection and isolation of *O. radicata*. The fruiting bodies of *O. radicata* was collected at Donggureung, Guri City, Korea in August, 2003. To obtain the pure culture from fruiting bodies of *O. radicata*, surface sterilized small pieces of pileus and stipe were transferred to potato dextrose agar (PDA) supplemented with streptomycin (200 $\mu g/l$), incubated under the dark condition for 15 days at 25°C and used for an inoculum in this study. The pure culture of *O. radicata* was deposited to the "Culture Collection of Wild Mushroom Species" and acquired accession number "IUM00779". Unless otherwise stated, all the tests which the strain was used were performed with 4 replications.

Culture conditions for a mycelial growth of *O. radicata*.
pH: To screen pH value necessary for a favorable growth of *O. radicata*, a 5 mm diameter plug of an inoculum was removed with cork borer from 10 days old cultures of *O. radicata* grown on PDA, placed in the center of PDA adjusted to the range of pH 4~9 with 1 N NaOH or HCL and incubated under the dark condition for 10 days at 25°C. The measurement of mycelial growth was performed according to the method described by Shim *et al*. (1997).
Temperature: To screen pH value necessary for a favorable growth of *O. radicata*, the fungus was incu-

Table 1. Composition of the media for the mycelial growth of *Oudemansiella radicata*

	Media and composition (g/*l*)										
	Czapek dox	Ebiose	Hamada	Hennerberg	Glucose peptone	Glucose triptone	Lilly	Mushroom complete	PDA	PD(M)	YM
Asparagine							2				
Dextrose			10						20	20	10
Ebiose		5	5								
Hyponex			3								
Glucose				50	10	5					
Malt extract					15			20		5	3
Maltose							10				
Peptone					10			2			5
Potatoes									200	200	
Sucrose	30										
Triptone						10					
Yeast extract			3		10	3		2			3
$NaNO_3$	3			2							
K_2HPO_4	1							1			
$MgSO_4$	0.5			0.5			0.5	0.5			
KCl	0.5										
$FeSO_4$	0.01										
$CaCl_2$				0.1							
KH_2PO_4				1			1	0.5			
KNO_3				2							

bated for 10 days at 5 different temperatures. A 5 mm diameter plug of an inoculum was removed with cork borer from 10 days old cultures of *O. radicata* grown on PDA, placed in the center of PDA adjusted to pH 6, and incubated under the dark condition for 10 days at 15°C, 20°C, 25°C, 30°C and 35°C, respectively. The measurement of mycelial growth was also performed according to the method described by Shim *et al.* (1997).

Culture media: Eleven different culture media were prepared to screen favorable culture media to mycelial growth of *O. radicata* (Table 1). The culture media were adjusted to pH 6 before sterilization, sterilized for 15 minutes at 121°C and aseptically poured into a plate. A 5 mm diameter plug of an inoculum was removed from 10 days old cultures of *O. radicata* grown on PDA, placed in the center of each agar plate of 11 different culture media, and incubated under the dark condition for 10 days at 25°C. After 10 days of incubation, the mycelial growth and density of *O. radicata* were measured.

Effect of favorable nutrient sources.

Carbon sources: To screen carbon source favorable to the mycelial growth of *O. radicata*, the tests were performed on the basal medium supplemented with each of 11 carbon sources. The basal medium was composed of peptone 5 g, $MgSO_4$ 0.05 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, thiamine-HCl 120 μg, agar 20 g and distilled water 1000 *ml*. To screen carbon source favorable to the mycelial growth of *O. radicata*, each carbon source was added to the basal medium at the concentration of 0.1 M per 1000 *ml* and mixed throughly (Shim *et al.*, 1997). The basal medium was adjusted to pH 6 before sterilization, sterilized for 15 minutes at 121°C and aseptically poured into a plate. A 5 mm diameter plug of an inoculum was removed from 10 days old cultures of *O. radicata* grown on PDA, placed in the center of a basal medium containing each of 11 carbon sources and incubated under the dark condition for 10 days at 25°C. After 10 days of incubation, the mycelial growth and density of *O. radicata* were measured.

Nitrogen sources: To screen nitrogen source favorable to the mycelial growth of *O. radicata*, the basal medium was composed of $MgSO_4$ 0.05 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0g, thiamine-HCl 120 μg, agar 20 g and distilled water 1000 *ml* (Sung *et al.*, 1993) and then supplemented with each of 16 nitrogen sources. Also, D-glucose was supplemented to the basal medium at the concentration of 2% (w/v) and used as carbon source for expediting the mycelial growth of *O. radicata*. Each nitrogen source was added to the basal medium at the concentration of 0.02 M (Shim *et al.*, 1997). The basal medium containing each nitrogen source was adjusted to pH 6 before sterilization, sterilized for 15 minutes at 121°C and aseptically poured into a plate. A 5 mm diameter plug of an inoculum was placed in the center of a basal medium containing each nitrogen source and incubated under the dark condition for 10 days at 25°C. After 10 days of incubation, the mycelial growth and density of *O. radicata* were measured.

C/N ratio: On the basal media which were mixed with 1, 2, 3 and 4% glucose (w/v) as carbon source and then mixed continually with $NaNO_3$ as nitrogen source, the mycelial growth of *O. radicata* was measured. The C/N ratio ($NaNO_3$ versus D-glucose) was adjusted to 10 : 1, 20 :1 , 30 : 1 and 40 : 1 in each medium (Shim *et al.*, 1997). The basal medium which was adjusted to each C/N ratio (such as 10 : 1, 20 : 1, 30 : 1 and 40 : 1) was adjusted to pH 6 before sterilization, sterilized for 15 minutes at 121°C, aseptically poured into a plate and inoculated with an inoculum (such as a 5 cm diameter plug). After Inocula of *O. radicata* were incubated under the dark condition for 10 days at 25°C, its colony diameter was measured.

Results and Discussion

Culture conditions for *O. radicata.*

Effect of pH: Of 6 pH values, the mycelial growth of *O. radicata* was exceedingly favorable at pH 6 (Fig. 1). Shim *et al.* (1997) reported that the mycelial growth of *G. umbellata* was exceedingly favorable at pH 4 and suppressed in proportion to the rise of pH value. Shim *et al.* (2003) clarified that the pH value suitable for a favorable growth of *P. fumosoroseus* was obtained in the range of pH 6~9. Though the mycelial growth of *O. radicata* was exceedingly favorable at pH 6, the favorable growth of *O. radicata* was generally obtained in the range of pH 5~9. Since the result was similar to that of *P. fumosoroseus*, it is reasonable to clarify that there was no a wide difference of mycelial growth referring to *O. radicata* between pH 5 and 9. Presumably, *O. radicata* seems to show a favorable growth in the range of wide pH values.

Effect of the temperature. The temperature suitable for the mycelial growth of *O. radicata* was obtained at 25°C (Fig. 2). After the favorable growth of *O. radicata* peaked at 25°C, its mycelial growth was suppressed rapidly at the temperature higher than 25°C. Also, this case was similar to that of *P. fumosoroseus* (Shim *et al.*, 2003).

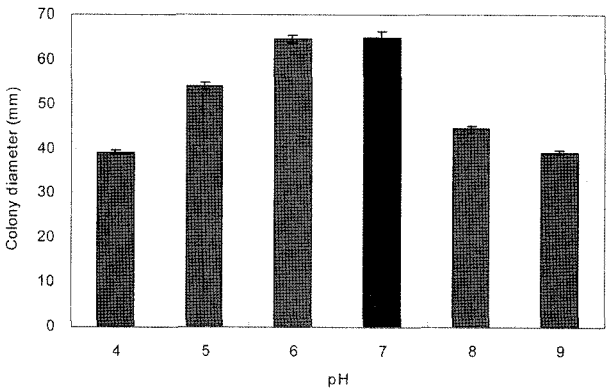

Fig. 1. Mycelial growth of *Oudemansiella radicata* on the PDA at different pHs for 10 days at 25°C.

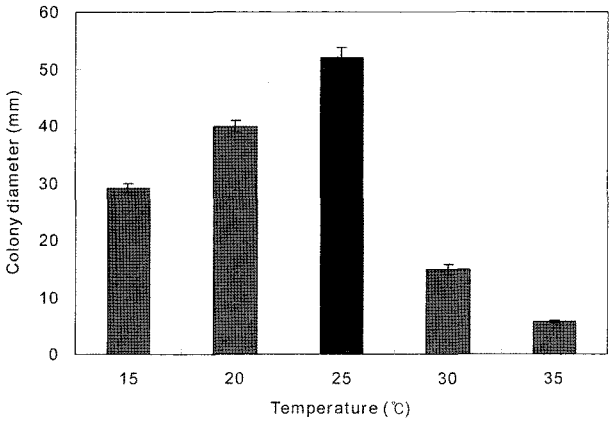

Fig. 2. Mycelial growth of *Oudemansiella radicata* on the PDA for 10 days at different temperatures.

Screening of the favorable culture media. Eleven different culture media were prepared to screen suitable culture media to mycelial growth of *O. radicata* (Table 1). The mycelial growth of *O. radicata* was most outstanding in the Lilly medium, whereas mycelial density Hamada medium (Table 2). Although the size of colony diameter was more large in the Lilly medium than Hamada medium, the difference of their sizes seemed to be scanty between Lilly and Hamada medium. The mycelial density of *O. radicata* was more compact in the Hamada medium than Lilly medium. Generally, it is reasonable to mention the fact that the mycelial density of filamentous fungi has been determined as the volumes of their accumulated mycelia on the culture media. In case of *O. radicata*, the criterion can be too obscure to decide if the meaning of suitable culture media should be focused on either the size of colony diameter or volume of accumulated mycelia. Though the application of criterion is some-

Table 2. Mycelial growth of *Oudemansiella radicata* on the various culture media

Culture media	Colony diameter[a] (mm)	Mycelial density[b]
Czapex dox	57.4±1.14[c]	T
Ebiose	43.8±2.14	T
Glucose peptone	69.9±1.13	CS
Triptone	53.5±1.29	T
Hamada	70.0±1.29	C
Hennerberg	37.5±0.82	T
Lilly	75.3±1.37	ST
Mushroom complete	61.4±0.88	SC
PDA	51.1±0.82	SC
PD(M)	69.3±0.83	C
YM	63.5±1.29	SC

[a]The colony diameter was measured after 10 days of incubation.
[b]Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.
[c]Values are average of 4 replicates and standard error.

what obscure in case of *O. radicata*, it may be unnecessary to worry the obscurity of criteria. It would be resonable to imply that the application of criteria can be flexible to coincide with various purposes of related researches in the future. Since the spectrum of criteria can be flexible for any purpose of related researches, it is desirable to emphasize that flexible criteria should be applied to induce various researches of *O. radicata* in the future

Effect of favorable nutrient sources.

Carbon and nitrogen sources: The carbon and nitrogen source suitable for promoting a mycelial growth of *O. radicata* were xylose and alanine, respectively (Table 3 and 4). Of 11 carbon sources, xylose showed colony diameter of 65.6 mm. The mycelial density of *O. radicata* was somewhat compact in xylose. Of 16 nitrogen sources, Alanine showed colony diameter of 66.8 mm. The mycelial density of *O. radicata* was compact in alanine. According to the above results, xylose and alanine seems to be effective to promoting a mycelial growth of *O. radicata.*

C/N ratio: On the culture media which were mixed with 3% glucose as carbon source and then adjusted to the C/N ratio of 20 : 1, *O. radicata* showed the most favorable mycelial growth (Table 5). Shim *et al.* (1997) clarified that an excessive concentration of glucose seemed to be attributable to suppress the mycelial growth of *Grifola umbellata* on the culture media. Also, our results were similar to those of Shim *et al.* (1997). Generally, the gradual rise of glucose concentration seemed to suppress the mycelial growth of *O. radicata.*

Table 3. Effect of carbon sources for the mycelial growth of *Oudemansiella radicata* in the basal medium[a]

Carbon source[b]	Colony diameter[c] (mm)	Mycelial density[d]
Dextrin	63.8±1.03[e]	SC
Fructose	53.9±1.76	ST
Galactose	58.6±0.64	T
Glucose	59.5±0.90	ST
Lactose	46.8±0.21	T
Maltose	59.6±2.24	T
Mannitol	59.9±0.78	T
Mannose	57.6±0.83	T
Ribose	62.8±0.54	SC
Sucrose	55.6±0.72	T
Xylose	65.6±1.14	SC

[a]The basal medium was composed of peptone 5 g, $MgSO_4$ 0.05 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, Thiamine-HCl 120 μg, agar 20 g and D.W. 1000 *ml*.
[b]Each carbon source was added to the basal medium at the concentration of 0.1 M.
[c]The colony diameter was measured after 10 days of incubation.
[d]Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.
[e]Values are average of 4 replicates and standard error.

Table 4. Effect of nitrogen sources for the mycelial growth of *Oudemansiella radicata* in the basal medium[b]

Nitrogen source[a]	Colony diameter[c] (mm)	Mycelial density[d]
Alanine	66.8±1.03[e]	C
Ammonium acetate	65.6±1.25	C
Ammonium oxalate	53.8±1.73	SC
Ammonium phosphate	61.1±0.66	SC
Arginine	52.3±0.66	C
Asparagine	61.6±1.09	C
Glutamic acid	46.1±0.79	SC
Glutamine	51.3±1.53	SC
Glycine	52.8±0.93	SC
Histidine	28.0±0.71	T
Methionine	62.4±0.90	ST
Phenylalanine	59.3±0.57	SC
Potassium nitrate	49.8±0.90	T
Sodium nitrate	37.8±0.50	T
Valine	32.1±0.59	ST
Urea	46.8±1.26	ST

[a]Each nitrogen source was added to the basal medium at the concentration of 0.02 M.
[b]The basal medium was composed of glucose 20 g, $MgSO_4$ 0.05 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, Thiamine-HCl 120 μg, agar 20 g and D.W. 1000 *ml*.
[c]The colony diameter was measured after 10 days of incubation.
[d]Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.
[e]Values are average of 4 replicates and standard error.

Table 5. Mycelial growth of *Oudemansiella radicata* at various C/N ratio in the basal medium[a]

C/N[c] ratio	Colony diameter[b] (mm) D-glucose concentration (%)			
C/N[c] ratio	1	2	3	4
10 : 1	53.6±0.79[d]	55.2±1.73	45.8±1.76	47.0±1.15
20 : 1	54.7±1.93	53.4±0.79	58.6±1.14	53.0±0.90
30 : 1	50.4±1.70	54.2±1.67	57.1±1.00	51.5±1.87
40 : 1	50.8±1.29	51.8±1.61	49.8±1.19	47.9±0.78

[a]Basal medium was composed of $MgSO_4$ 0.05 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, Thiamine-HCl 120 μg, agar 20 g and D.W. 1000 *ml*.
[b]The colony diameter was measured after 10 days of incubation.
[c]The ratio of $NaNO_3$ versus D-glucose were adjusted to the rate of 10 : 1, 20 : 1, 30 : 1, 40 : 1, respectively.
[d]Values are average of 4 replicates and standard error.

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References

Choi, K. D., Lee, K. T., Shim, J. O., Lee, Y. S., Lee, T. S., Lee,

S. S., Guo, S. X. and Lee, M. W. 2003. A new method for cultivation of sclerotium of *Grifola umbellata*. *Mycobiology* **31**: 105-112.

Ha, Y. D. 2001. Antitumoral , antioxidant, and antimicrobial activities of solvent fractions from *Grifola umbellatus*. *Kor. J. Postharvest Sci. Technol.* **8**: 481-487.

Lee, J. Y. 1988. Coloured Korean mushrooms. Academy Press, Seoul, Korea.

Lee, J. M., Kim, J. Y., Choi, K. D., Han, K. D., Hur, H., Kim, S. W., Shim, J. O., Lee, T. S. and Lee, M. W. 2004. Sawdust media affecting the mycelial growth and fruiting body formation of *Sparassis crispa*. *Mycobiology* **32**: 190-193.

Park, W. H. and Lee, H. D. 1999. Illustrated book of Korean Medicinal mushrooms. Kyo-Hak Publishing C. Ltd. 759pp.

Shim, J. O., Son, S. G., Kim, Y. H., Lee, Y. S., Lee, J. Y., Lee, T. S., Lee, S. S. and Lee, M. W. 1997. The cultural conditions affecting the mycelial growth of *Grifola umbellata*. *Kor. J. mycol.* **25**: 209-213.

Shim, S. M., Lee, K. R., Kim, S. H., Im, K. H., Kim, J. W., Lee, U. Y., Shim, J. O., Lee, M. W. and Lee, T. S. 2003. The optimal culture conditions affecting the mycelial growth and fruiting body formation of *Paecilomyces fumosoroseus*. *Mycobiology* **31**: 214-220.