

The Characteristics of Cultural Conditions for the Mycelial Growth of *Macrolepiota procera*

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(Received December 13, 2004)

Macrolepiota procera, one of edible mushrooms belongs to Agaricaceae of Basidiomycota, has a good taste and good medicinal value. As a preliminary study for the development of artificial cultivation method of edible mushroom, cultural characteristics of *M. procera* was investigated on various culture media under different environmental conditions. Mycelial growth was compared on culture media composed of various carbon and nitrogen sources, and C/N ratios. The optimal conditions for the mycelial growth were 30°C and pH 7. *M. procera* showed the rapid mycelial growth in the PDA media. The optimal carbon and nitrogen sources were maltose and glycine, respectively. The optimum C/N ratio was about 10 : 1 in case that 1% glucose was supplemented to the basal media as carbon source.

KEYWORDS: Cultural conditions, Edible mushroom, *Macrolepiota procera*, Mycelial growth

Macrolepiota procera (Scop. ex Fr.) Sing., an edible mushroom belongs to Agaricaceae of Agaricales, has been known to be distributed in the northern areas of Asia such as Korea, China and Japan (Park and Lee, 1996). The pileus is 7~20 cm in diameter, oval or spherical in the early stage of its development and gradually opens to a umbonate plane when it matures. The surface color of the pileus ranges from light grayish brown to grayish brown, and becomes reddish-brown and rough scales appears when its epithelial tissues are cleaved. The lamella is white in color and free from the stipe. The stipe is 15~30 × 0.6~1.5 μm in size and empty in its pith. The surface of stipe ranges from brown to grayish brown in its color and is similar to skin of snake in its morphology when its epithelial tissues are cleaved. The spore is oval or ellipsoidal, 15~20 × 10~13 μm in size, and spore print is white. For the duration of summer to fall, *M. procera* occurs as a fairy ring of a gregarious style on soil surface of the field with rich organic matter such as roadside, pasture, lawn and flower garden. *M. procera* has been known to contain glycerol, mannitol, glucose, trehalose, lepiotan and about 20 amino acids. Since *M. procera* has been reported to demonstrate anti-tumor activity to human body and exhibit an antibiotic activity against gram negative bacteria, the fruiting bodies have been extensively used for manufacturing traditional foods and medicines. When *M. procera* is used for edible purposes, the fruiting body of *M. procera* which contains the proteins, iron, zinc, chitin,

chitosan, fiber, vitamins and minerals have been known to support the health and sustain physiological homeostasis of human body. Therefore, in order to obtain basic data for an artificial cultivation of *M. procera*, the culture conditions affecting the optimal mycelial growth of *M. procera* were investigated.

Materials and Methods

The collection and isolation of *M. procera*. The fruiting body of *M. procera* was collected at Yungkeonreung, Hwaseong city, Korea in August, 2001. To obtain the pure culture from *M. procera*, surface sterilized small pieces of fruiting body was transferred to potato dextrose agar (PDA) supplemented with streptomycin (200 μg/l), incubated for 15 days at 25°C and used for an inoculum in the study. The pure culture of *M. procera* was deposited to the "Culture Collection of Wild Mushroom Species" and acquired accession number, "IUM00103". Unless otherwise stated, all the tests which the strain was used were performed with 4 replications.

Culture conditions for mycelial growth of *M. procera*.

Effect of pH: A 5 mm diameter plug of an inoculum was removed with cork borer from 10 days old cultures of *M. procera* grown on PDA, placed in the center of each agar plate of PDA adjusted to the range of pH 4~9 with 1 N NaOH or HCl and incubated for 10 days at 25°C. The measurement of mycelial growth was performed according to the method described by Shim *et al.* (1997).

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Table 1. Composition of the media used for the growth of *Macrolepiota procera*

	Media and composition (g/l)								
	Czapek -dox	Hamada	Henner -berg	Glucose peptone	Glucose tryptone	Lilly	Mushroom complete	PDA	YMA
Asparagine						2			
Dextrose		10						20	10
Ebiose		5							
Hyponex		3							
Glucose			50	10	5		20		
Malt extract				15					3
Maltose						10			
Peptone				10			2		5
Potato								200	
Sucrose	30								
Tryptone		3							
Yeast extract			2	10			2		3
NaNO ₃	3								
K ₂ HPO ₄	1		0.5				1		
MgSO ₄	0.5					0.5	0.5		
KCl	0.5								
FeSO ₄	0.01		0.1						
CaCl ₂			1						
KH ₂ PO ₄			2			1	0.5		
KNO ₃									

Effect of the temperature: To investigate the temperature favorable for the mycelial growth of *M. procera*, the fungus was incubated for 10 days at 5 different temperatures. A 5 mm diameter plug removed from 10 days old cultures of *M. procera* grown on PDA was placed in the center of each plate filled with PDA. The PDA was adjusted to pH 6 and incubated 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).

Screening of favorable culture media: Nine different culture media were prepared to investigate favorable culture media to mycelial growth of *M. procera* (Table 1). The media were adjusted to pH 6 before autoclave. After autoclave for 15 minutes at 121°C, 20 ml of each medium was aseptically poured into a plate. A 5 mm diameter plug of an inoculum was removed from 10 days old culture of *M. procera* grown on PDA and placed in the center of each agar plate of 9 different culture media. After 10 days of incubation at 25°C, the mycelial growth and density of *M. procera* were measured.

Effect of carbon and nitrogen sources: To screen carbon and nitrogen sources favorable to the mycelial growth of *M. procera*, the basal medium (Sung *et al.*, 1993) supplemented with each of 10 carbon and 17 nitrogen sources was prepared. The basal medium was composed of MgSO₄·7H₂O 0.05 g, KH₂PO₄ 0.46 g, K₂HPO₄ 1.0 g, Thiamine-HCl 120 µg, agar 20 g and distilled water 1,000 ml. To screen carbon source favorable to the mycelial growth of *M. procera*, each carbon source was added to the basal

medium at the concentration of 0.1 M per 1,000 ml and mixed thoroughly (Shim *et al.*, 1997). The basal medium was adjusted to pH 6 before autoclave for 15 minutes at 121°C and aseptically poured into a plate. D-glucose was added to the basal medium at the concentration of 2% (w/v) and used as carbon source for expediting the mycelial growth of *M. procera*. The basal medium which was used for screening a favorable nitrogen source was made of the same additives as those described by Sung *et al.* (1993). Each nitrogen source was added to the basal medium at the concentration of 0.02 M (Shim *et al.*, 1997). The basal medium was also adjusted to pH 6 before autoclave for 15 minutes at 121°C and aseptically poured into a plate. To measure colony diameter on the media, *M. procera* was incubated for 10 days at 25°C. Most of the procedures including the inoculation, incubation and measurement of a mycelial density were carried out according to the method described by Shim *et al.* (1997).

Effect of C/N ratio: To expedite the mycelial growth of *M. procera*, D-glucose and NaNO₃ have been added to the basal medium. The basal media which D-glucose was mixed at the rate of 1, 2, 3 and 4% (w/v) were continually added with NaNO₃. Finally, the C/N ratio (D-glucose versus NaNO₃) were adjusted to 10 : 1, 20 : 1, 30 : 1 and 40 : 1 in each medium. The basal medium was also adjusted to pH 6 before autoclave for 15 minutes at 121°C and aseptically poured into a plate. After incubation on the media for 10 days at 25°C, the colony diameter was measured.

Results and Discussions

Culture conditions of *M. procera*.

Effect of pH: The pH value suitable for a favorable growth of *M. procera* was obtained in a broad range of pH 5~8 (Fig. 1). Of 6 pH values, the mycelial growth of *M. procera* was exceedingly favorable at pH 7 (Fig. 1). Shim *et al.* (1997) reported that the mycelial growth of *Grifola umbellata* was exceedingly favorable at pH 4. Chi *et al.* (1996) suggested that the mycelial growth of *Phellinus linteus* was favorable in the range of pH 6~7. In case of *M. procera*, the favorable mycelial growth of seems to be exceedingly suitable at pH 7 in nature and similar to that of *Phellinus linteus* (Chi *et al.*, 1996).

Effect of temperature: Sung *et al.* (1999) reported that *Pleurotus ostreatus* showed the most favorable mycelial growth at 30°C. The mycelial growth of *M. procera* was most favorable at 30°C. Even though the mycelial growth of *M. procera* was gradually increased in proportion to the rise of temperature and then exceedingly favorable at 30°C, the mycelial growth was suppressed at 35°C (Fig. 2). Since most of *M. procera* occurs in hot and humid summer months of the year, the mycelial growth of *M.*

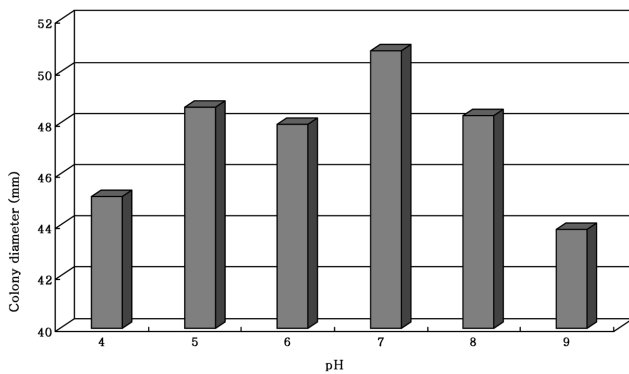


Fig. 1. Mycelial growth of *Macrolepiota procera* on the PDA at different pHs for 10 days of incubation at 25°C.

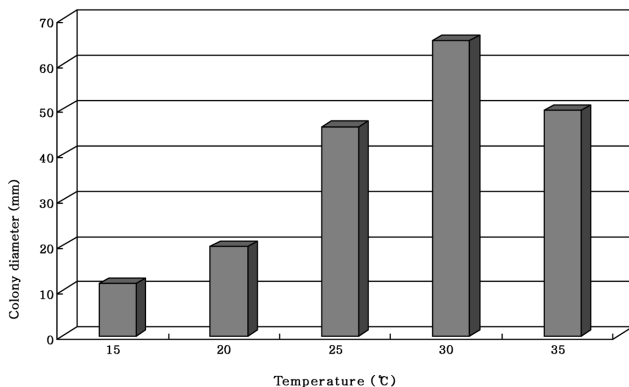


Fig. 2. Mycelial growth of *Macrolepiota procera* on the PDA for 10 days of incubation at different temperatures.

procera might be favorable around 30°C.

Screening of favorable culture media: Nine different culture media were used to screen the optimal mycelial growth of *M. procera*. PDA medium showed colony diameter of 67.8 mm, which indicated the rapid growth of *M. procera* (Table 2). Even though the mycelia of *M. procera* were spread rapidly on the PDA medium in a short period of time, the mycelial density was poor on the PDA medium. The compact mycelia of filamentous fungi on the medium contained more volume of accumulated hyphae than that of thin hyphae. Since the criterion for screening of suitable culture medium for fungi is based on the size of colony diameter on a given period of time. Therefore, we must have reasonable assaying method to find the media for suitable mycelial growth of *M. procera* in the future.

Table 2. Mycelial growth of *Macrolepiota procera* on various culture media

Culture medium	Colony diameter ^a (mm)	Mycelial density ^b
Czapex dox	20.3	SC
Glucose peptone	20.3	C
Glucose triptone	27.6	T
Hamada	41.8	SC
Hennerberg	23.2	SC
Lilly	26.2	SC
Mushroom complete	43.5	C
PDA	67.8	T
YM	50.9	T

^aThe colony diameter was measured at 10 days after incubation.

^bMycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.

Table 3. Effect of carbon sources for the mycelial growth of *Macrolepiota procera* in the basal medium^a

Carbon source ^b	Colony diameter ^c (mm)	Mycelial density ^d
Dextrin	25.2	ST
Fructose	14.3	T
Galactose	14.6	ST
Glucose	21.6	T
Lactose	8.9	T
Maltose	33.1	ST
Mannitol	17.6	ST
Mannose	23.6	T
Sorbitol	21.6	ST
Sucrose	24.7	ST

^aThe basal medium was composed of peptone 5 g, MgSO₄ 0.05 g, KH₂PO₄ 0.46 g, K₂HPO₄ 1.0 g, Thiamine-HCl 120 µg, agar 20 g and D. W. 1000 ml.

^bEach carbon source was added to the basal medium at the concentration of 0.1 M.

^cThe colony diameter was measured at 10 days after incubation.

^dMycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.

Table 4. Effect of nitrogen sources for the mycelial growth of *Macrolepiota procera* in the basal medium^a

Nitrogen source ^b	Colony diameter ^c (mm)		Mycelial density ^d
Alanine	17.2		ST
Ammonium acetate	20.6		C
Ammonium oxalate	5.6		ST
Ammonium phosphate	30.6		C
Arginine	6.7		SC
Asparagine	27.7		SC
Calcium nitrate	27.0		ST
Glutamic acid	29.2		C
Glutamine	28.4		C
Glycine	32.6		SC
Histidine	5.2		ST
Methionine	16.2		ST
Phenylalanine	20.4		SC
Potassium nitrate	21.7		T
Sodium nitrate	23.7		SC
Valine	17.3		SC
Urea	17.9		ST

^aThe basal medium was composed of MgSO₄ 0.05 g, KH₂PO₄ 0.46 g, K₂HPO₄ 1.0 g, Thiamine-HCl 120 µg, agar 20 g and D. W. 1000 ml.

^bEach carbon source was added to the basal medium at the concentration of 0.1 M.

^cThe colony diameter was measured at 10 days after incubation.

^dMycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.

Table 5. Mycelial growth of *Macrolepiota procera* on various C/N ratio in the basal medium^a

C/N ^b ratio	Colony diameter ^c (mm) at different D-glucose concentrations (%)			
	1	2	3	4
10 : 1	22.0	20.0	19.4	17.7
20 : 1	22.0	18.8	17.0	16.5
30 : 1	20.4	18.3	15.4	15.5
40 : 1	19.3	19.2	14.5	14.2

^aThe basal medium was composed of MgSO₄ 0.05 g, KH₂PO₄ 0.46 g, K₂HPO₄ 1.0 g, Thiamine-HCl 120 µg, agar 20 g and D. W. 1000 ml.

^bThe ratio of NaNO₃ versus D-glucose was adjusted to the ratio of 10 : 1, 20 : 1, 30 : 1 and 40 : 1, respectively.

^cThe colony diameter was measured at 10 days after incubation.

Effect of carbon and nitrogen sources: Maltose and glycine were screened as carbon and nitrogen sources suitable for the mycelial growth of *M. procera* (Table 3 and Table 4). After 10 days of incubation, the colony diameters of *M. procera* recorded 33.1 mm in maltose and 32.6 mm in glycine, respectively. Shim *et al.* (1998) reported that the mycelial growth of *Sparassis crispa* was exceedingly favorable on the basal medium supplemented with glycine. Though mycelia of *M. procera* were spread

rapidly on the basal medium supplemented with glycine, the mycelial density of *M. procera* was higher on the basal medium supplemented with ammonium phosphate than on the basal medium supplemented with glycine (Table 4).

Effect of C/N ratio: Chi *et al.* (1996) suggested that optimum C/N ratio suitable for favorable growth of *P. lin-teus* was 30 : 1. However, optimum C/N ratio suitable for a favorable growth of *M. procera* was 10 : 1 (Table 5). The mycelial growth of *M. procera* was suppressed in proportion to the gradual rise of C/N ratio. Since the suitable C/N ratio for mycelial growth of edible fungi seems to be dissimilar according to their indigenous characteristics, the mycelial growth of *M. procera* was favorable in the high nitrogen content of the medium.

Acknowledgement

This work was supported in part by a 2004 research grant from University of Incheon and KOSEF through Culture Collection of Wild Mushroom Species in the University of Incheon.

References

- Chi, J. H., Ha, T. M., Kim, Y. H. and Rho, Y. D. 1996. Studies on the main factors affecting the mycelial growth of *Phellinus lin-teus*. *Kor. J. Mycol.* **24**(3): 214-222.
- Kim, S. W., Hwang, H. J., Xu, C., Na, Y. S., Song, S. K. and Yun, J. W. 2002. Influence of nutritional conditions on the mycelial growth and exo-biopolymer production in *Paecilomyces sinclairii*. *Lett. Appl. Microbiol.* **34**(6): 389-393.
- Park, W. H. and Lee, H. D. 1996. Wild fungi of Korea. Published (6th edition) in Kyo-Hak Publishing Co., Ltd. pp. 1-508.
- 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**(3): 209-218.
- Shim, S. M., Lee, K. L., Im, K. H., Lee, U. Y., Lee, M. W. and Lee, T. S. 2003. The characteristics of cultural conditions for the mycelial growth and fruiting body formation of *Paecilomyces sinclairii*. *Kor. J. Mycol.* **31**(1): 8-13.
- _____, _____, 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**(4): 214-220.
- Song, C. H. and Cho, K. Y. 1987. A synthetic medium for the production of submerged cultures of *Lentinula edodes*. *Mycologia* **79**(6): 866-876.
- Sung, J. M., Kim, C. H., Yang, K. J., Lee, H. K. and Klim, Y. S. 1993. Studies on distribution and utilization of *Cordyceps militaris* and *C. nutans*. *Kor. J. Mycol.* **21**(2): 94-105.
- _____, Moon, H. W. and Park, D. S. 1999. Growth condition of liquid culture by *Pleurotus ostreatus*. *Kor. J. Mycol.* **27**(1): 1-9.