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

Sung Mi Shim, Yun Hee Oh, Kyung Rim Lee, Seong Hwan Kim, Kyung Hoan Im, Jung Wan Kim, U Young Lee, Jae Ouk Shim, Mi Ja Shim, Min Woong Lee, Hyeon Su Ro, Hyun Sook Lee and Tae Soo Lee

Department of Biology, University of Incheon, Incheon 402-749, Korea
1Department of Life Science, University of Seoul, Seoul 130-743, Korea
2Department of Biology, Dongguk University, Seoul 100-715, Korea
3Department of Microbiology, Gyeongsang National University, Jinju 660-701, Korea

(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, mannotol, 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, 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 Youngkeonreung, 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 Shin et al. (1997).
Effect of the temperature: To investigated 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 MgSO$_4$·7H$_2$O 0.05 g, KH$_2$PO$_4$ 0.46 g, K$_2$HPO$_4$ 1.0 g, Thiamine-HCl 120 µg, agar 20 g and distilled water 1,000 ml. D-glucose 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 NaNO$_3$ have been added to the basal medium. The basal medium which D-glucose was mixed at the rate of 1, 2, 3 and 4% (w/v) were continually added with NaNO$_3$. Finally, the C/N ratio (D-glucose versus NaNO$_3$) 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.

| Table 1. Composition of the media used for the growth of *Macrolepiota procera* |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                               | Czapek-dox      | Hamada          | Henner-berg     | Glucose         | Glucose         | Lilly           | Mushroom        | PDA             | YMA             |
| Asparagine                    |                 |                 |                 | 2               |                 |                 |                 |                 |                 |
| Dextrose                      | 10              |                 |                 |                 |                 |                 |                 |                 |                 |
| Ebiose                        | 5               |                 |                 |                 |                 |                 |                 |                 |                 |
| Hyponex                       | 3               |                 |                 |                 |                 |                 |                 |                 |                 |
| Glucose                       | 50              | 10              | 5               | 20              |                 |                 |                 |                 |                 |
| Malt extract                  | 15              |                 |                 |                 |                 |                 |                 |                 |                 |
| Maltose                       | 10              |                 |                 |                 |                 |                 |                 |                 |                 |
| Peptone                       | 10              |                 |                 |                 |                 |                 |                 |                 |                 |
| Potato                        |                 |                 |                 |                 |                 |                 |                 | 200             |                 |
| Sucrose                       | 30              |                 |                 |                 |                 |                 |                 |                 |                 |
| Tryptone                      | 3               | 2               | 10              |                 |                 |                 | 2               |                 | 3               |
| Yeast extract                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| NaNO$_3$                      | 3               |                 |                 |                 |                 |                 |                 |                 |                 |
| K$_2$HPO$_4$                  | 1               |                 | 0.5             | 1               | 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$_3$                       |                 |                 |                 |                 |                 |                 |                 |                 |                 |
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. 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.

![Fig. 1. Mycelial growth of *Macrolepiota procera* on the PDA at different pHs for 10 days of incubation at 25°C.](image1)

![Fig. 2. Mycelial growth of *Macrolepiota procera* on the PDA for 10 days of incubation at different temperatures.](image2)

| Culture medium       | Colony diameter (mm) | Mycelial density |
|----------------------|----------------------|-----------------|
| 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               |

The colony diameter was measured at 10 days after incubation.

| Carbon source  | Colony diameter (mm) | Mycelial density |
|----------------|----------------------|-----------------|
| 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              |

The 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.

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

The colony diameter was measured at 10 days after incubation.

Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.
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).

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

| Nitrogen source | Colony diameter (mm) | Mycelial density* |
|-----------------|----------------------|-------------------|
| Alamine         | 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                |

*The 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. Each carbon source was added to the basal medium at the concentration of 0.1 M. The colony diameter was measured at 10 days after incubation.*

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

| C/N ratio | Colony diameter (mm) at different D-glucose concentrations (%) | Mycelial density* |
|-----------|---------------------------------------------------------------|-------------------|
|           | 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                 |

*The 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. The ratio of NaN₃ versus D-glucose was adjusted to the ratio of 10 : 1, 20 : 1, 30 : 1 and 40 : 1, respectively. The colony diameter was measured at 10 days after incubation.*

### 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.

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