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# The Optimal Culture Conditions Affecting the Mycelial Growth and Fruiting Body Formation of *Paecilomyces fumosoroseus*

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**The fruiting body of *Paecilomyces fumosoroseus* was collected at Mt. Mani, Ganghwa Island, Korea in September, 2001. This study was carried out to obtain the basic informations for the mycelial growth and fruiting body production of *P. fumosoroseus* in artificial media. The optimal conditions for the mycelial growth were obtained at 25°C and in the range of pH 6~9, respectively. *P. fumosoroseus* showed the favorable growth on Hamada medium. The carbon and nitrogen source favorable for mycelial growth were dextrin and histidine, respectively. Optimum C/N ratio suitable for optimal growth of *P. fumosoroseus* was observed on the culture media adjusted to the ratio of 40:1. The mycelial growth of *P. fumosoroseus* was optimal on corn meal agar supplemented with 30% of silkworm pupae. The most favorable fruiting body formation of *P. fumosoroseus* was obtained in the medium containing unpolished rice supplemented with 20% (w/w) silk worm pupae at 25°C under 100 lux.**

**KEYWARDS: Cultural conditions, Fruting body, Mycelial growth, *Paecilomyces fumosoroseus***

*Paecilomyces fumosoroseus*, a common insect-borne filamentous fungus belongs to Hyphomycetes of Deuteromycota and have been reported to cause diseases in a wide species of insects, occasionally resulting in natural epizootics (Altre *et al.*, 1999; Cantone and Vandenberg, 1999; Lacey *et al*., 1999; Nam *et al*, 2000). Some of entomopathogenic fungi have been known to exhibit outstanding effects for curing diseases of human being (Furuya *et al*., 1983; Manabe *et al*., 1996; Choi *et al*., 1999). Therefore, these entomopathogenic properties have led to the development (Jackson *et al*., 1997; Lee *et al*., 1999) and commercialization (Bolckmans *et al*., 1995; Furuya *et al*., 1983; Manabe *et al*., 1996) of mycoinsecticide or medicine. However, only a few species of *Paecilomyces* have been artificially cultured in Korea (Sung *et al*., 1997; Lee *et al*., 1999; Kim *et al*., 2002; Cho *et al*., 2002; Shim *et al*., 2003). To develop *P. fumosoroseus* as a mycoinsecticide for biological control or medicine for human beings, it is necessary to develop method for mass production of fruiting bodies in *in vitro* condition. But, there has been no report associated with the mycelial growth and fruting body formation of *P. fumosoroseus* in Korea. Therefore, pure culture was obtained from wild fruiting body of *P. fumosoroseus* in September, 2001. This study was carried out to obtain the basic informations affecting the mycelial growth and fruiting body formation of *P. fumosoroseus* on artificial media for the biological control of insects and medicinal purposes of human beings. This is the first report associated with a optimal mycelial growth and artificial fruiting body formation of *P. fumosoroseus* on artificial medium in Korea.

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## Materials and Methods

**The collection and isolation of *P. fumosoroseus*.** The fruiting body of *Paecilomyces fumosoroseus* was collected at Mt. Mani, Ganghwa island, Korea in September, 2001. To obtain the pure culture from *P. fumosoroseus*, single spore was isolated from fruiting bodies of *P. fumosoroseus* and placed on 2% water agar and incubated for 2 days at 25°C. The mycelia germinated from single spores were transferred to potato dextrose agar (PDA) supplemented with streptomycin (200 $\mu$g/l), incubated for 15 days at 25°C and used for an inoculum in the study. The pure culture of *P. fumosoroseus* was deposited in "Culture Collection of Wild Mushroom Species" and acquired accession number "IUM00078". Unless otherwise stated, all the tests which the strain was used were performed with 3 replications.

**Culture conditions for mycelial growth of *P. fumosoroseus*.**

**Effect of pH:** A 5 mm diameter plug of an inoculum was removed with cork borer from 15 days old cultures of *P. fumosoroseus* 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 17 days at 25°C (Fig. 2). The measurement of mycelial growth was per-

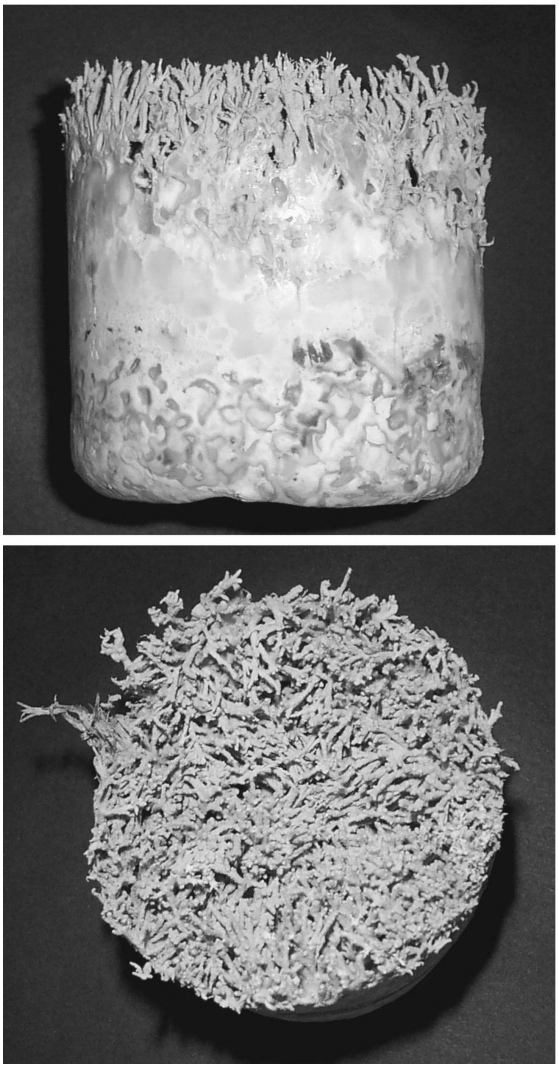

**Fig. 1.** Fruiting body of *Paecilomyces fumosoroseus* formed on unpolished rice medium supplemented with 20% (w/w) silkworm pupae after 40 days of incubation at 25°C under 100 lux.

formed according to the method described by Shim *et al.* (1997).

**Effect of the temperature:** To screen the temperature favorable for the mycelial growth of *P. fumosoroseus*, the fungus was incubated for 17 days at 5 different temperatures. A 5 mm diameter plug removed from 15 days old cultures of *P. fumosoroseus* grown on PDA was placed in the center of each plate filled with PDA. The PDA was adjusted to pH 6 and incubated for 17 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:** Ten different culture media were prepared to investigate a mycelial growth of *P. fumosoroseus*. The media were adjusted to pH 6 before autoclave (Table 1). 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 15 days old culture of *P. fumosoroseus* grown on PDA and placed in the center of each agar plate of 10 different culture media. After 14 days of incubation at 25°C, the mycelial growth and density of *P. fumosoroseus* were measured.

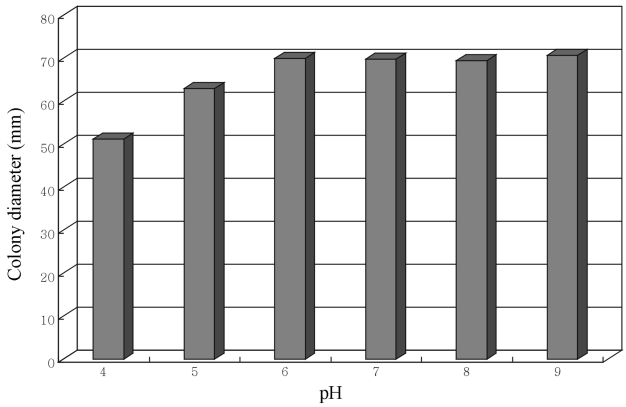


**Fig. 2.** Mycelial growth of *Paecilomyces fumosoroseus* on the PDA at different pHs for 17 days of incubation at 25°C.

**Effect of carbon and nitrogen sources:** To screen carbon and nitrogen source favorable to the mycelial growth of *P. fumosoroseus*, the tests were performed on the basal medium (Sung *et al*., 1993) supplemented with each of 11 carbon and 17 nitrogen sources. The 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 $\mu$g, Agar 20 g and distilled water 1,000 *ml*, To screen carbon source favorable to the mycelial growth of *P. fumosoroseus*, each carbon source was added to the basal medium at the concentration of 0.1 M per 1,000 *ml* and mixed throughly (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 *P. fumosoroseus*. The basal medium which was used for screening a favorable nitrogen source was made of 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, *P. fumosoroseus* was incubated for 17 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 *P. fumosoroseus*, D-glucose and $NaNO_3$ have been added

**Table 1.** Composition of the media used for the growth of *Paecilomyces fumosoroseus*

| | Media and composition (g/*l*) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Czapek dox | Hamada | Hennerberg | Hoppkins | Glucose peptone | Glucose tryptone | Lilly | Mushroom complete | PDA | YM |
| Asparagine | | | | | | | 2 | | | |
| Dextrose | | 10 | | | | | | | 20 | 10 |
| Ebiose | | 5 | | | | | | | | |
| Hyponex | | 3 | | | | | | | | |
| Glucose | | | 50 | 10 | 10 | 5 | | 20 | | |
| Malt extract | | | | | 15 | | | | | 3 |
| Maltose | | | | | | | 10 | | | |
| Peptone | | | | | 10 | | | 2 | | 5 |
| Potato | | | | | | | | | 200 | |
| Sucorose | 30 | | | | | 10 | | | | |
| Tryptone | | 3 | | | | 3 | | | | |
| Yeast extract | | | 2 | | 10 | | | 2 | | 3 |
| $NaNO_3$ | 3 | | | | | | | | | |
| $K_2HPO_4$ | 1 | | 0.5 | 0.5 | | | | 1 | | |
| $MgSO_4$ | 0.5 | | | | | | 0.5 | 0.5 | | |
| KCl | 0.5 | | | | | | | | | |
| $FeSO_4$ | 0.01 | | 0.1 | | | | | | | |
| $CaCl_2$ | | | 1 | 0.1 | | | | | | |
| $KH_2PO_4$ | | | 2 | 2 | | | 1 | 0.5 | | |
| $KNO_3$ | | | | | | | | | | |

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 $NaNO_3$. 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 17 days at 25°C, the colony diameter was measured.

**Effect of cereal extract media:** Each of 4 different cereal extract media was prepared by mixing 20 g of agar with 1000 *ml* of extract solution which extracted from 40 g of each cereal powder. To add nutrient source to each of 4 different cereal extract media, each of 3 nutrient sources (such as silkworm pupae, milk solution and rice bran) was mixed with each cereal extract medium at the rate of 10, 20 and 30%. After then, cereal extract medium was adjusted to pH 6, autoclaved for 15 minutes at 121°C, aseptically poured into a plate and used for inoculating *P. fumosoroseus*. To measure colony diameter on the cereal extract media, *P. fumosoroseus* was incubated for 10 days at 25°C.

**Formation of fruiting body:** To produce the fruiting body of *P. fumosoroseus*, an inoculum was prepared by removing a 5 mm diameter plug of *P. fumosoroseus* from PDA, transferring it to 500 *ml* of erlenmeyer flask containing 100 *ml* of potato dextrose broth and incubated for 5 days at 25°C in shaking incubator (150 rpm). Six different culture media were used to investigate the favorable media for fruiting body formation of *P. fumosoroseus*. The culture media were prepared by mixing 50g of cereal or silkworm pupae with 100 *ml* of tap water (1:2, w/v) in polypropylene bottle (550 *ml*) and autoclaved for 1 hour at 121°C. An unpolished rice medium was also supplemented with 20% (w/w) silkworm pupae, mixed with 100 *ml* of tap water (1:2, w/v) in polypropylene bottle (550 *ml*) and autoclaved for 1 hour at 121°C. Each of six different culture media was inoculated with 10 *ml* of an inoculum and incubated for 40 days at 20°C, 25°C, and 30°C under 100 lux and 500 lux conditions, respectively.

## Results and Discussions

**Cultural conditions of *P. fumosoroseus.***

**Effect of pH:** The pH value suitable for a favorable growth of *P. fumosoroseus* was obtained in the range of pH 6~9. Choi *et al.* (1999) reported that mycelial growth of *P. japonica* was optimal at pH 7. Shim *et al*. (2003) also reported that *P. sinclairii* showed maximal mycelial growth at pH 8. However, the mycelial growth and density of *P. fumosoroseus* was almost identical in the range of pH 6~9 (Fig. 2). These results suggest that *P. fumosoroseus* may have a broad pH range for its favorable mycelial growth in nature.

**Effect of temperature:** The temperature suitable for the mycelial growth of *P. fumosoroseus* was obtained at 25°C (Fig. 3) and the result was similar to that of Lee *et al.* (1999). Lee *et al.* (1999) reported that the mycelial growth

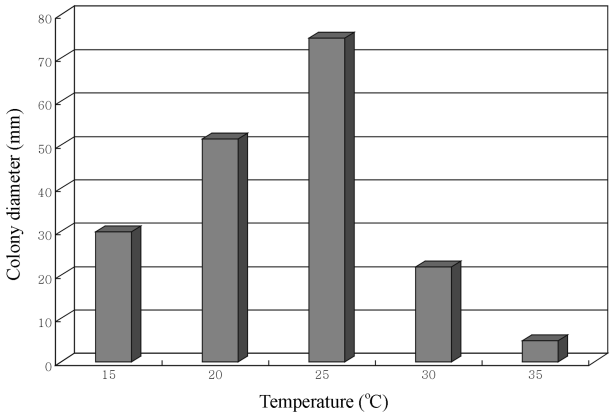


**Fig. 3.** Mycelial growth of *Paecilomyces fumosoroseus* on the PDA for 17 days of incubation at different temperatures.

of *P. fumosoroseus* had been expedited gradually in proportion to the rise of temperature and was the most suitable at 25°C. Even though the mycelial growth of *P. fumosoroseus* was favorable at the range of 20 to 25°C and had been expedited in proportion to the rise of temperature, the mycelial growth appeared to be suppressed at the temperature higher than 30°C (Fig. 3). Since most of entomopathogenic fungi has been known to complete their life cycle under the humid, cool deposits covered with fallen leaves (Sung *et al*., 1997), it is reasonable to predict that the mycelial growth of *P. fumosoroseus* was good in the temperature range of 20~25°C.

**Screening of favorable culture media:** Ten different culture media were used to screen the optimal mycelial growth of *P. fumosoroseus*. Of 10 culture media, Hamada medium was the most suitable for a favorable growth of *P. fumosoroseus* (Table 2). This result is corresponded with that of *P. sinclairii* which had been reported by Shim *et al.* (2003). Even though the mycelial growth of *P. fumosoroseus* and *P. sinclairii* was optimal on Hamada medium, the mycelial growth of *Grifola umbellata* was very poor on Hamada medium. This contradictory result indicates that taxonomically distinct fungal groups may have different nutritional requirement. Even though the mycelial growth in 10 different media showed wide range of variations (55.5~77.0 mm), the mycelial density were compact in 9 of 10 culture media tested (Table 2).

**Table 2.** Mycelial growth of *Paecilomyces fumosoroseus* on various culture media

| Culture medium | Colony diameter[a] (mm) | Mycelial density[b] |
|---|---|---|
| Czapex dox | 62.5 | C |
| Glucose peptone | 68.8 | C |
| Glucose triptone | 62.5 | C |
| Hamada | 77.0 | C |
| Hennerberg | 66.3 | C |
| Hoppkins | 59.5 | C |
| Lilly | 57.5 | C |
| Mushroom complete | 67.3 | SC |
| PDA | 67.3 | C |
| YM | 55.5 | C |

[a]The colony diameter was measured at 14 days after incubation.
[b]Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.

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

| Carbon source[b] | Colony diameter[c] (mm) | Mycelial density[d] |
|---|---|---|
| Dextrin | 78.0 | T |
| Fructose | 66.3 | T |
| Galactose | 59.0 | T |
| Glucose | 70.3 | T |
| Lactose | 69.8 | T |
| Maltose | 73.8 | T |
| Mannitol | 68.0 | T |
| Mannose | 64.8 | T |
| Sorbitol | 65.6 | T |
| Sucrose | 69.3 | T |
| Xylose | 50.8 | T |

[a]The basal medium was composed of $MgSO_4$ 0.05 g, $KH_2PO_4$ 0.46 g $K_2HPO_4$ 1.0 g, peptone 5 g Thiamine-HCl 120 $\mu$g, 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 at 17 days after incubation.
[b]Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.

**Effect of carbon and nitrogen sources:** Dextrin and histidine were screened as suitable carbon and nitrogen source for the mycelial growth of *P. fumosoroseus* (Table 3 and Table 4). The colony diameter of *P. fumosoroseus* after 14 days of incubation were recorded 78.0 mm in dextrin and 74.5 mm in histidine containing basal media, respectively. The mycelial density of *P. fumosoroseus* showed thin appearances in all carbon source, whereas compact appearances were observed in 16 nitrogen sources. Shim *et al*. (2003) also reported that *P. sinclairii* grew very well in basal medium which contained dextrin and glutamine, respectively. Since *P. fumosoroseus* and *P. sinclairii* belong to genus *Paecilomyces* of Hyphomycetes, Deuteromycota, they are taxonomically considered as very close species (Lacey *et al*., 1999). However, the nutritional requirement for the mycelial growth of *P. fumosoroseus* and *P. sinclairii* seems to be different.

**Effect of C/N ratio:** Optimum C/N ratio suitable for a favorable growth of *P. fumosoroseus* was observed on the culture media which were adjusted to C/N ratio of 40:1. On the culture media which were mixed with 1% glucose as carbon source and then adjusted to the ratio of 30:1, *P. sinclairii* showed the most favorable mycelial growth (Shim *et al*., 2003). Despite a gradual rise of 1%, 2%, 3%

**Table 4.** Effect of nitrogen sources for the mycelial growth of *Paecilomyces fumosoroseus* in the basal medium[a]

| Nitrogen source[b] | Colony diameter[c] (mm) | Mycelial density[d] |
|---|---|---|
| Alanine | 58.1 | C |
| Ammonium acetate | 68.1 | C |
| Ammonium oxalate | 58.8 | C |
| Ammonium phosphate | 54.3 | C |
| Arginine | 62.0 | C |
| Asparagine | 66.6 | C |
| Calcium nitrate | 68.3 | C |
| Glutamic acid | 64.1 | C |
| Glutamine | 58.5 | C |
| Glycine | 64.3 | C |
| Histidine | 74.5 | C |
| Methionine | 44.0 | SC |
| Phenylalanine | 72.8 | C |
| Potassium nitrate | 69.0 | C |
| Sodium nitrate | 69.3 | C |
| Valine | 67.6 | C |
| Urea | 68.6 | C |

[a]The 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 $\mu$g, glucose 20 g, agar 20 g and D. W. 1000 *ml*.
[b]Each nitrogen source was added to the basal medium at the concentration of 0.02 M.
[c]The colony diameter was measured at 17 days after incubation.
[b]Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.

**Table 5.** Mycelial growth of *Paecilomyces fumosoroseus* at 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 | 70.6 | 69.5 | 69.4 | 68.0 |
| 20:1 | 70.8 | 72.0 | 71.6 | 72.2 |
| 30:1 | 71.4 | 72.0 | 71.5 | 72.9 |
| 40:1 | 72.1 | 73.0 | 72.4 | 75.4 |

[a]The 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 $\mu$g, agar 20 g and D. W. 1000 *ml*.
[b]The ratio of $NaNO_3$ versus D-glucose was adjusted to the ratio of 10:1, 20:1, 30:1 and 40:1, respectively.
[c]The colony diameter was measured at 17 days after incubation.

and 4% glucose, C/N ratio of 40:1 showed the most favorable mycelial growth of *P. fumosoroseus* as compared with other C/N ratios (Table 5).

**Effect of cereal extract media:** Sung *et al.* (1993) reported that the mycelial growth of *Cordyceps militaris* was favorable on the artificial medium supplemented with silkworm pupae, rice powder or wheat powder. Shim *et al.* (2003) also reported that silkworm pupae supplemented to the cereal extract medium showed favorable mycelial growth of *P. sinclairii*. The mycelial growth of *P. fumosoroseus* was optimal on corn meal agar supplemented with 10~30% of silkworm pupae (Table 6). Particularly, the mycelial growth of *P. fumosoroseus* recorded 71.9 mm in PDA plate with maximal value on corn meal agar supplemented with 30% of silkworm pupae. Based on these results, silkworm pupae may contain good nutritional sources to promote mycelial growth of *P. fumosoroseus*.

**Table 6.** Mycelial growth of *Paecilomyces fumosoroseus* on 4 different cereal extract media[a] mixed with 3 different nutrient sources

| Nutrition source(%)[b] | | Colony diameter(mm)[c] | | | |
|---|---|---|---|---|---|
| | | Polished rice | Wheat | Corn meal | Unpolished rice |
| Silkworm pupae | 10 | 68.2 | 67.7 | 71.6 | 67.0 |
| | 20 | 65.5 | 66.2 | 68.8 | 66.4 |
| | 30 | 62.4 | 65.0 | 71.9 | 57.6 |
| Milk solution | 10 | 62.6 | 64.6 | 60.9 | 62.6 |
| | 20 | 64.8 | 64.9 | 64.9 | 64.2 |
| | 30 | 66.3 | 66.8 | 64.3 | 66.9 |
| Rice bran | 10 | 59.8 | 61.8 | 62.6 | 56.3 |
| | 20 | 50.0 | 52.9 | 60.1 | 49.7 |
| | 30 | 43.3 | 44.5 | 50.5 | 43.8 |

[a]These media made by mixing 20 g of agar with 1000 *ml* of stock solution was extracted from 40 g of cereal powder.
[b]Each nutrient source was added to the cereal extract medium in the range of 10~30% (w/v).
[c]The colony diameter was measured at 10 days after incubation.

**Formation of fruiting body:** The fruiting body of *P. fumosoroseus* was formed abundantly on an unpolished rice medium supplemented with 20% (w/w) silkworm pupae at 25°C under 100 lux (Fig. 1, Table 7). Shim *et al.* (2003) reported that fruiting body of *P. sinclairii* was formed after 10 days of incubation at 25°C under 500 lux. The fruiting body of *P. fumosoroseus* was formed after 12 days of incubation at 25°C under light condition. But, unlike the *P. sinclairii*, the fruiting body of *P. fumosoroseus* was formed more abundantly under 100 lux than 500 lux. The fruiting body of *P. fumosoroseus* was not formed at 30°C. This result also coincide with mycelial growth of the fungi which was suppressed at 30°C (Fig. 3). Even though *P. fumosoroseus* have been considered as a good candidate for biocontrol of insect pests, there has been no report that *P. fumosoroseus* was used commercially for the biocontrol agent in Korea (Altre *et al.*, 1999; Cantone and Vandenberg, 1999; Lacey *et al.*, 1999; Lee *et al.*, 1999; Nam *et al.*, 2000). To develop *P. fumosoroseus* as a mycoinsecticide, it is necessary to develop culture method to produce fruiting bodies in *in vitro* condition. In this study, the conditions for optimal mycelial growth and fruiting body formation of *P. fumosoroseus* was developed for the first time in Korea. Thus, the basic information obtained from this study can be used for the mass production of fruiting bodies of *P. fumosoroseus*.

**Table 7.** Artificial fruiting body formation of *Paecilomyces fumosoroseus* under various culture conditions after 40 days of incubation

| Light intensity (lux) | Media | Temperature (°C) | Pinheading days | Total yield[b] (g) | Fruiting body | | |
|---|---|---|---|---|---|---|---|
| | | | | | Length (mm) | Number (no.) | Yield (g) |
| 100 | Unpolished rice | 20 | –[a] | 85.8 | – | – | – |
| | | 25 | 12 | 83.2 | 34.0 | 151 | 7.6c[c] |
| | Wheat | 20 | – | 87.6 | – | – | – |
| | | 25 | 12 | 83.8 | 38.5 | 173 | 10.5b |
| | Silkworm pupae | 20 | – | 84.8 | – | – | – |
| | | 25 | 12 | 83.5 | 13.0 | 36 | 4.9c |
| | Unpolished rice + 20% silkworm pupae (w/w) | 20 | 21 | 93.0 | 5.0 | 11 | 5.2c |
| | | 25 | 12 | 89.4 | 37.0 | 160 | 15.0a |
| | Corn | 20 | – | 75.7 | – | – | – |
| | | 25 | 12 | 75.0 | 40.0 | 106 | 9.2b |
| | Barley | 20 | – | 88.7 | – | – | – |
| | | 25 | 12 | 84.2 | 36.0 | 196 | 10.8b |
| 500 | Unpolished rice | 20 | 10 | 90.1 | 38.5 | 110 | 7.5c |
| | | 25 | 10 | 84.3 | 33.0 | 39 | 5.9c |
| | Wheat | 20 | 12 | 89.3 | 34.5 | 192 | 9.3b |
| | | 25 | 11 | 87.7 | 39.0 | 118 | 9.3b |
| | Silkworm pupa | 20 | 10 | 77.7 | 26.0 | 91 | 6.0c |
| | | 25 | 8 | 73.7 | 27.5 | 46 | 3.2c |
| | Unpolished rice + 20% silkworm pupae (w/w) | 20 | 12 | 96.3 | 37.5 | 217 | 12.2b |
| | | 25 | 10 | 91.2 | 31.5 | 175 | 11.2b |
| | Corn | 20 | 10 | 68.4 | 35.0 | 72 | 7.2c |
| | | 25 | 10 | 64.9 | 29.0 | 76 | 6.3c |
| | Barley | 20 | 12 | 90.4 | 33.5 | 261 | 11.0b |
| | | 25 | 11 | 87.1 | 35.5 | 158 | 7.6c |

[a]Values are the means of 3 replications. Averages followed by the same letters within a column are not significantly different according to Duncan's multiple range test ($P<0.05$).
[b]Data were based on fresh weights.
[c]Number could not be counted.

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