

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

Sung Mi Shim, Kyung Rim Lee, Seong Hwan Kim, Kyung Hoan Im, Jung Wan Kim, U Youn Lee, Jae Ouk Shim¹, Min Woong Lee¹ and Tae Soo Lee*

Department of Biology, University of Incheon, Incheon 402-749, Korea

¹Department of Biology, Dongguk University, Seoul 100-715, Korea

(Received November 25, 2003)

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.

KEYWORDS: Cultural conditions, Fruiting 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 (Aitre *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 fruiting 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.

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 µg/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-

*Corresponding author <E-mail: tslee@incheon.ac.kr>

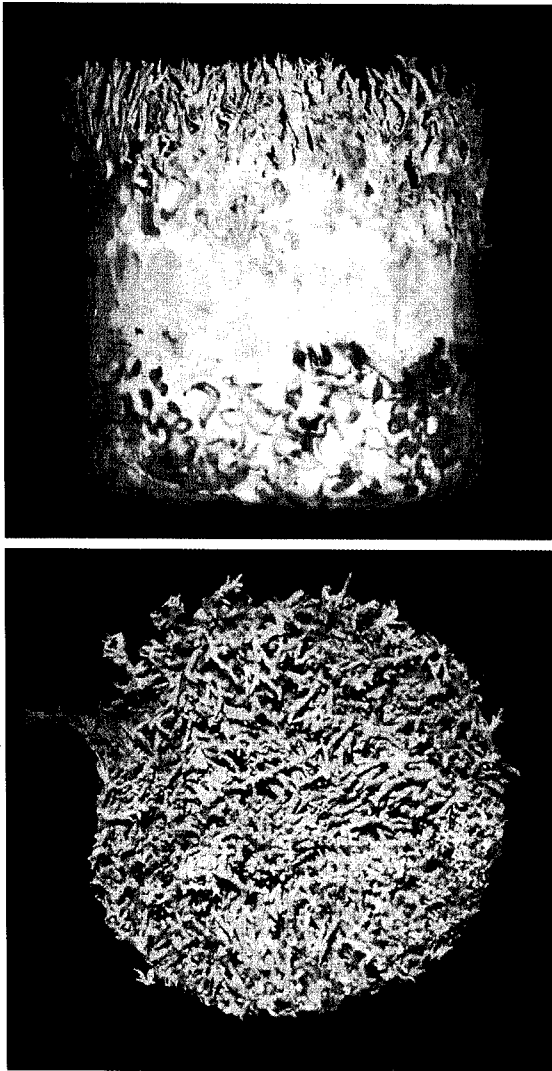


Fig. 1. Fruiting body of *Paecilomyces fumosoroseus* formed on unpolished rice medium supplemented with 20% (w/w) silkworm pupae after 40 days of incubation at 25°C under 100 lux.

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

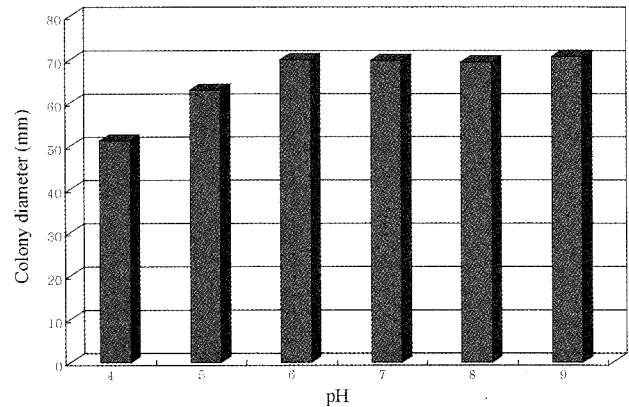


Fig. 2. Mycelial growth of *Paecilomyces fumosoroseus* on the PDA at different pHs for 17 days of incubation at 25°C.

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.

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 MgSO₄ 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 *P. fumosoroseus*, 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 *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 NaNO₃ 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	
Sucrose	30					10				
Tryptone		3				3				
Yeast extract			2		10			2		3
NaNO ₃	3									
K ₂ HPO 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 ₂ PO 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 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 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

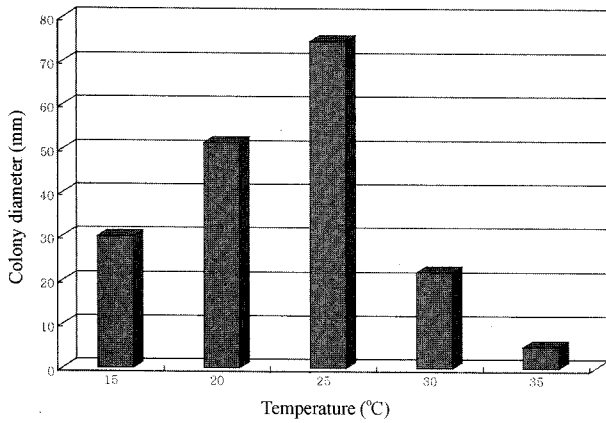


Fig. 3. Mycelial growth of *Paecilomyces fumosoroseus* on the PDA for 17 days of incubation at different temperatures.

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.*

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

^aThe colony diameter was measured at 14 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 *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

^aThe basal medium was composed of MgSO₄ 0.05 g, KH₂PO₄ 0.46 g, K₂HPO₄ 1.0 g, peptone 5 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 17 days after incubation.

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

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).

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

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

^bEach nitrogen source was added to the basal medium at the concentration of 0.02 M.

^cThe colony diameter was measured at 17 days after incubation.

^dMycelial 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
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

^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 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 supple-

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
	20	65.5	66.2	68.8
	30	62.4	65.0	71.9
Milk solution	10	62.6	64.6	60.9
	20	64.8	64.9	64.9
	30	66.3	66.8	64.3
Rice bran	10	59.8	61.8	62.6
	20	50.0	52.9	60.1
	30	43.3	44.5	50.5

^aThese media made by mixing 20 g of agar with 1000 ml of stock solution was extracted from 40 g of cereal powder.

^bEach nutrient source was added to the cereal extract medium in the range of 10~30% (w/v).

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

mented 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*.

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	

^aValues 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).

^bData were based on fresh weights.

^cNumber could not be counted.

Acknowledgement

This work was supported by research grant from Kosef through "Culture Collection of Wild Mushroom Species" in the University of Incheon.

References

- Altre, J. A., Vandenberg, J. D. and Cantone, F. A. 1999. Pathogenicity of *Paecilomyces fumosoroseus* isolates to Deamondback moth, *Plutella xylostella*: correlation with spore size, germination speed, and attachment to cuticle. *J. Invertebr. Pathol.* **73**: 332-338.
- Bolckmans, K., Steck, G., Eyal, J., Seles, B. and Stepman, W. 1995. PreFeRal, (*Paecilomyces fumosoroseus* strain Apopka 97), A new microbial insecticide for the biological control of whiteflies in greenhouses. *Med. Fac. Land. Toeg. Biol. Weten. Univ. Gent.* **60**: 707-711.
- Cantone, F. A. and Vandenberg, J. D. 1999. Use of the green fluorescent protein for investigations of *Paecilomyces fumosoroseus* in insect hosts. *J. Invertebr. Pathol.* **74**: 193-197.
- _____ and Vandenberg, J. D. 1999. Genetic transformation and mutagenesis of the entomopathogenic fungus *Paecilomyces fumosoroseus*. *J. Invertebr. Pathol.* **74**: 281-288.
- Choi, Y. J., Hwang, H. K. and Lee, W. H. 1999. The production of artificial fruiting body of *Paecilomyces japonica*. *Kor. J. Mycol.* **27**(2): 87-93.
- _____, Hwang, H. J., Kim, S. W., Song, C. H. and Yun, J. W. 2002. Effect of carbon source and aeration rate on broth rheology and fungal morphology during red pigment production by *Paecilomyces sinclairii* in a batch bioreactor. *J. Biotechnol.* **95**(1): 13-23.
- Furuya, T., Hirofani, M. and Matsuzawa, M. 1983. N⁶-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelia of *Cordyceps* and *Isaria* species. *Phytochemistry* **22**(11): 2509-2512.
- Jackson, M. A., MacGuire, M. R., Lacey, L. A. and Wraight, S. P. 1997. Liquid culture production of desiccation tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. *Mycol. Res.* **101**: 35-41.
- 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*. *Letters in Applied Microbiology* **34**(60): 389-393.
- Lacey, L. A., Kirk, A. A., Millar, L., Mercadier, G. and Vidal, C. 1999. Ovicidal and larvicidal activity of conidia and blastospores of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay system allowing prolonged survival of control insects. *Biocontrol Science and Technology* **9**: 9-18.

- Lee, I. K., Shim, H. J., Woo, S. D., Je, Y. H., Yang, Z. and Kang, S. K. 1999. Variation in growth and pathogenicity of *Beauveria bassiana* and *Paecilomyces fumosoroseus* pathogenic to the pine gall midge, *Thecodiplosis japonensis*. *Kor. J. Appl. Microbiol. Biotechnol.* **27**(5): 415-418.
- Manabe, M., Sugimoto, M., Azuma, Y., Takemoto, N., Yamashita, A., Tsuboi, H., Tsunoo, A., Kinjo, N., Huang, N. L. and Miyamoto, H. 1996. Effects of the mycelial extract of cultured *Cordyceps sinensis* on *in vitro* hepatic energy metabolism in the mouse. *Jpn. J. Pharmacol.* **70**: 85-88.
- Nam, S. H., Yoon, S. S., Kim, K. Y., Cho, S. Y., Han, M. S. 2000. Report on the muscardine (*Paecilomyces fumosoroseus*) of silkworm (*Bombyx mori*) in Korea. *Korean J. Seric. Sci.* **42**(1): 28-30.
- 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.
- Sung, J. M., Kim, C. H., Yang, K. J., Lee, H. K. and Kim, Y. S. 1993. Studies on distribution and utilization of *Cordyceps militaris* and *C. nutans*. *Kor. J. Mycol.* **21**(2): 94-105.
- _____, Lee, H. K., Choi, Y. S., Kim, Y. Y., Kim, S. H. and Sung, K. H. 1997. Distribution and taxonomy of entomopathogenic fungal species from Korea. *Kor. J. Mycol.* **25**(4): 239-252.