Exploration on Filamentous Phenotype of Coprinus comatus Colleted form Different Ecological Origins

Imtiaj, Ahmed

Lee, Tae-Soo
Department of Biology, University of Incheon

Ohga, Shoji
Laboratory of Forest Resources Management, Division of Forest Ecosphere Management, Department of Forest and Forest Products Sciences, Kyushu University

https://doi.org/10.5109/18830

出版情報：九州大学大学院農学研究院紀要. 55 (2), pp.203-207, 2010-10-29. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **55** (2), 203–207 (2010)

Exploration on Filamentous Phenotype of *Coprinus comatus* Colleted form Different Ecological Origins

Ahmed IMTIAJ, Tae–Soo LEE[1] and Shoji OHGA*

Laboratory of Forest Resources Management, Division of Forest Environmental Sciences,
Department of Agro–environmental Sciences, Faculty of Agricultare,
Kyushu University, Fukuoka 811–2415, Japan
(*Received May 20, 2010 and accepted July 9, 2010*)

Coprinus comatus is an edible and nematophagous basidiomycete fungus. The optimal culture conditions for the filamentous growth and density of 6 strains of this mushroom were investigated. The temperature suitable for the filamentous growth and density was obtained at 25 ºC and optimal range of temperature was 20~30 ºC. This mushroom showed a broad pH range (5~9) for its mycelial growth and density. The mostly favorable growth was found at pH 7. According to filamentous growth Czapek's, PDA, YM, and Hamada were the most suitable, and Hennerberg and Hoppkins were the most unfavorable for this mushroom. Among 10 different carbon sources, sucrose and sorbitol were the best but lactose and xylose were the most unfavorable carbon sources. In all carbon sources, mycelial density was found to be compact. The most suitable nitrogen source was arginine and glycine but the most unsuitable was histidine for the mycelial growth. The mycelial density in nitrogen source containing medium was found to be different on the culture media.

INTRODUCTION

Coprinus comatus is a nematophagous basidiomycete mushroom. It is often seen growing on lawns, along gravel roads and waste areas. This fungus is called 'shaggy ink cap' or 'shaggy mane' and cultivated in China as food. The genus '*Coprinus*' was formerly considered to be a large one with well over 100 species and its specific name derives from coma or hair, hence comatus, 'haired' or 'shaggy'. The young mushroom, before the gills start to turn black, is edible. The young fruiting bodies first appear as white cylinders emerging from the ground and then the bell–shaped caps open out. The caps are covered with scales that are the origin of the common names of the fungus. The gills secrete a black liquid filled with spores which considered as nematode killing device (Tzean and Liou, 1993). A recent study has found the shaggy ink cap kills nematode species *Panagrellus redivivus* and *Meloidogyne arenaria*. *C. comatus* is shown to be a nematode destroying fungus, producing a new structure designated spiny ball. The infection process of *P. redivivus* by the fungus is already studied (Luo *et al*., 2004). Similar secretory appendages were found on lawn mushroom *Conocybe lacteal*, in which they are more likely to be defense apparatus. After paralyzing and killing nematodes, *C. lacteal* does not use them as food (Hutchison *et al*., 1995). The wood–decay fungi obtain nitrogen supplement from prey, including nematodes, to survive in such nitrogen–restricted habitat as rotting wood and forest soil (Thorn and Barron, 1984 & 1986). Along the hyphae of these nematophagous basidiomycete fungi, some appendages were found to be attack or defense weapons.

Based on these significances, a study has been conducted on the mycelial growth and density of 6 strains of *C. comatus*. The different environmental and nutritional factors were used to assess the optimal culture conditions for the mycelial growth and density of this fungus and presented in this paper.

MATERIALS AND METHODS

Collection, identification and isolation

The fruiting bodies of 6 strains of *Coprinus comatus* were collected from different regions of Korea and China shown in Table 1. After identification mycelia were isolated, cultured on potato dextrose agar (PDA) medium and incubated at 25 ºC for further study. The pure cultures of mushroom were deposited in 'Culture Collection of Wild Mushroom (CCWM)' and acquired accession number from University of Incheon Mushroom (IUM). All of the strains used in different experiments were performed with 3 replications.

Table 1. List of *C. comatus* strains used in this study

Strain No.	Geographical origin	Collection date
IUM 0004	Seaside, Korea	September 3, 2005
IUM 0707	Bupyeong–dong, Korea	July 2, 2003
IUM 0756	Incheon, Korea	May 7, 2002
IUM 1544	Beijing, China	February 24, 2005
IUM 1573	SangHai, China	April 11, 2005
IUM 1820	Beijing, China	August 14,2005

Effect of temperature

To detect the suitable temperature for the mycelial growth of the mushroom, 5 different temperatures were studied. A 5 mm diameter agar plug removed from 10 days old culture grown on PDA and placed in the centre

[1] Department of Biology, University of Incheon, Incheon 402–749, Korea
* Corresponding author (E–mail: ohga@forest.kyushu–u.ac.jp)

of each plate filled with 20 *ml* of PDA. The medium was adjusted to pH 6 and incubated for 10 days at 15 °C, 20 °C, 25 °C, 30 °C and 35 °C separately. The measurement of mycelial growth was performed according to the method described by Shim *et al*. (1997).

Effect of pH

A agar plug of 5 mm diameter of an inoculum was removed with cork borer from 10 days old culture grown on PDA was placed in the centre of each agar plate. The medium was adjusted to pH of 5, 6, 7, 8 and 9 with the addition of 1 N NaOH or HCl and incubated at 25 °C for 10 days. The mycelial growth was also measured according to the method described by Shim *et al*. (1997).

Screening of favorable culture media

Ten different culture media were prepared to investigate the mycelial growth of used mushroom strains (Table 2). 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 grown on PDA and placed in the centre of each plate of 10 different culture media. After 10 days of incubation at 25 °C, mycelial growth and density was measured.

Effect of carbon and nitrogen

To test out carbon and nitrogen source favorable for the mycelial growth of mushroom strains, the tests were conducted on the basal medium supplemented with each of 10 carbon and 10 nitrogen sources separately. 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 μg, agar 20 g and 1000 *ml* of distilled water. To screen carbon source favorable to the mycelial growth, each carbon source with 5 g of peptone was added to the basal medium separately at the concentration of 0.1 M per 1000 *ml* and mixed thoroughly (Shim *et al*., 1997). 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 with 20 g of glucose was added to the basal medium at the concentration of 0.02 M (Shim *et al*., 1997). In both cases the basal medium was adjusted to pH 6 before autoclave for 15 minutes at 121 °C and poured into a plate. To measure colony diameter of mycelia on the media, all plates were incubated for 10 days at 25 °C. After incubation, mycelial radial growth and density was measured following same manner.

RESULTS AND DISCUSSION

Effect of temperature

Temperature suitable for the mycelial growth and density of tested fungal strains was obtained at 25 °C. The strain IUM0756 showed an exceptional mycelial growth where the highest was counted at 15 °C. No mycelial growth (except IUM0707) was found at 35 °C and the lowest mycelial growth was recorded at 30 °C. The optimal range of temperature was 20~30 °C for mycelial growth and density of *C. comatus*. In every case of temperature effect, mycelial density was found to be compact (Table 3). Lee *et al*. (1999) and Shim *et al*.

Table 2. Media and their compositions used in this study

Composition	Media (g/l)									
	Cza	Ham	Hen	Hop	GP	GT	Lil	MC	PDA	YM
Agar	20	20	20	20	20	20	20	20	20	20
Asparagine							2			
Dextrose		10							20	10
Ebiose		5								
Hyponex		3								
Glucose			50	10	10	5				
Malt–extract					15			20		3
Maltose							10			
Peptone					10			2		5
Potatoes									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	0.5		
KCl	0.5									
$FeSO_4$	0.01									
$CaCl_2$			0.1							
KH_2PO_4			1	0.1			1	0.5		
KNO_3			2	2						

Cza: czapek's, Ham: hamada, Hen; hennerberg, Hop: hoppkins, GP: glucose peptone, GT: glucose tryptone, Lil: lilly. MC: mushroom complete, PDA: potato dextrose agar and YM: yeast–malt extract

Table 3. Effect of temperatures and pH on the mycelial growth and density of different strains of *C. comatus*

Strain	Mycelial growth (mm)[a] and density									
	Temperature					pH				
	15 ℃	20 ℃	25 ℃	30 ℃	35 ℃	5	6	7	8	9
IuM 0004	13.7±1.5c	15.0±2.6c	19.3±2.3c	10.7±1.2c	–	35.0±4.4c	54.3±5.5c	59.7±8.6c	52.0±7.1c	48.0±9.3c
IUM 0707	72.0±1.7c	83.0±1.0c	87.0±0.0c	87.0±0.0c	11.3±0.6c	37.7±2.5c	87.0±0.0c	87.0±0.0c	86.0±1.7c	83.3±3.5c
IUM 0756	28.0±2.0c	24.3±4.5c	24.0±1.0c	14.7±1.5c	–	16.7±2.5c	22.7±0.6c	22.7±1.5c	22.0±1.7c	20.7±2.3c
IUM 1544	43.7±1.2c	51.7±8.0c	80.0±0.0c	66.0±7.9c	–	29.0±2.6c	69.0±9.0c	75.0±0.0c	58.3±4.5c	45.3±4.5c
IUM 1573	46.7±5.8c	54.3±1.2c	57.3±8.1c	31.7±4.7c	–	34.7±4.6c	53.3±8.0c	58.3±8.5c	51.3±8.2c	47.7±9.5c
IUM 1820	33.0±3.0c	36.3±2.3c	58.3±9.8c	15.3±6.7c	–	42.3±1.2c	79.7±4.2c	79.7±9.0c	79.3±3.2c	63.0±1.0c

[a]Mean of three replications. Temperature and pH effects were conducted in potato dextrose agar medium (PDA). c: Compact. sc: Somewhat compact. st: Somewhat thin and t: Thin

(2003) reported that the mycelial growth of *Paecilomyces fumosoroseus* had been expedited gradually in proportion to the rise of temperature and the most suitable was at 25 ℃. Even though the mycelial growth of *P. fumosoroseus* was favorable at the range of 20~25 ℃ and had been expedited in proportion to the rise of temperature, the mycelial growth appeared to be suppressed at the temperature higher than 30 ℃. Shim *et al*. (2005) and Jo *et al*. (2006) stated that the favorable mycelial growth of *Macrolepiota procera* and *Phellinus* spp. was at 30 ℃. Therefore, these results are corresponded with that of our findings.

Effect of pH

To monitor pH value suitable for the favorable growth and density of *C. comatus*, it was observed at the range of 5~9 and the best was pH 7. In case of IUM0707 and IUM0756, the highest growth was also appeared 87.0 and 22.7 mm at pH 6, respectively. Rest of the temperatures was also showed good mycelial growth of different strains of *C. comatus*. In every case of used pH, mycelial density was found to be compact (Table 3). Shim *et al*. (2005) revealed that pH 7 is the most suitable for the optimal growth of *M. procera*. Choi *et al*. (1999) and Chi *et al*. (1996) reported that mycelial growth of *Phellinus japonica* and *P. linteus* was optimal at pH 7 and 6~7, respectively. Shim *et al*. (2003) shown that optimal pH of *Paecilomyces sinclairii* was 8. Shim *et al*. (1997) also reported that the most favorable and most unfavorable pH of *Grifola umbellata* was 4 and 9, respectively. This result suggested that mushrooms may have a broad pH range for their optimal mycelial growth in nature. The results of this study is completely similar to Shim *et al*. (2005), Choi *et al*. (1999) and Chi *et al*. (1996) but not similar to Shim *et al*. (2003 & 1997).

Screening of favorable culture media

Ten different culture media were used to display the optimal mycelial growth of different strains of *C. comatus*. The highest mycelial growth of IUM0004 and IUM0756 was 30.3 and 31.7 mm on Hamada medium, respectively. Rests of the strains were showed the best mycelial growth in YM and Czapeck's media. According to mycelial growth Czapek's, PDA, YM, and Hamada were the most suitable, and Hennerberg and Hoppkins were the most unfavorable for mycelial growth of *C. comatus* (Table 4). Besides of slow growth, mycelial density was also somewhat thin to thin on Czapek's, and Hoppkins media. This result is corresponded with that of *P. sinclairii* and *P. fumosoroseus* which had been reported by Shim *et al*. (2003) where mycelial growth was optimal on Hamada medium. Shim *et al*. (2005) also reported that PDA, YM, Mushroom complete and Hamada were the most suitable, where Czapex dox and Glucose peptone were unfavorable to mycelial growth of *M. procera*.

Effect of carbon sources

Ten different carbon sources were used to find out the optimal culture condition. The best carbon sources for the suitable mycelial growth were sucrose and sorbitol but unfavorable were lactose and xylose. All of the carbon sources showed compact mycelial density of *C. comatus* (Table 5). This result is completely similar to

Table 4. Effect of media on the mycelial growth and density of different strains of *C. comatus*

Strain	Mycelial growth (mm)[a] and density									
	Cza	Ham	Hen	Hop	GP	GT	Lil	MC	PDA	YM
IUM 0004	28.7±1.2st	30.3±2.5c	15.7±1.2c	23.0±1.0t	25.7±1.2c	24.7±3.5c	23.3±4.2c	23.3±1.5c	27.1±2.1c	22.0±1.0c
IUM 0707	87.0±0.0t	87.0±0.0c	59.0±1.0c	87.0±0.0t	87.0±0.0c	87.0±0.0c	87.0±0.0c	87.0±0.0c	87.0±0.0c	87.0±0.0c
IUM 0756	29.7±1.2st	31.7±2.5c	15.8±1.1c	23.2±1.0t	24.7±3.2c	25.7±3.5c	23.3±4.2c	24.3±2.5c	25.0±2.0c	23.0±3.0c
IUM 1544	82.7±4.0t	47.3±2.5c	39.7±8.7c	45.0±2.6t	58.3±7.0c	54.3±4.0c	44.7±9.6c	77.0±2.6c	80.0±0.0c	82.3±0.6c
IUM 1573	71.3±8.5st	44.7±0.6c	40.7±2.1c	40.7±3.5t	47.0±2.6c	56.3±8.0c	43.7±1.5c	53.3±9.1c	57.3±8.1c	74.0±7.9c
IUM 1820	84.3±3.1st	47.7±4.6c	74.3±7.4c	37.3±3.2st	78.3±9.6c	85.3±2.9c	47.0±6.1c	79.7±3.5c	58.3±6.8c	85.7±1.5c

[a]Mean of three replications. Cza: czapek's. Ham: hamada. Hen: hennerberg. Hop: hoppkins. GP: glucose peptone. GT: glucose tryptone. Lil: lilly. MC: mushroom complete. PDA: potato dextrose agar and YM: yeast–malt extract. c: Compact. sc: Somewhat compact. st: Somewhat thin and t: Thin

Table 5. Effect of carbon sources on the mycelial growth and density of different strains of *C. comatus*

Strain	Mycelial growth (mm)[a] and density									
	Dex	Fr	Ga	Gl	Lac	Mal	Man	Sor	Sur	Xy
IUM 0004	31.0±1.7c	32.0±1.0c	24.0±1.0c	31.0±1.0c	21.3±0.6c	32.0±2.6c	30.3±1.5c	30.0±1.0c	37.7±2.9c	20.3±1.2c
IUM 0707	76.0±1.7c	86.3±1.2c	74.0±0.0c	87.0±0.0c	48.0±0.0c	87.0±0.0c	87.0±0.0c	87.0±0.0c	87.0±0.0c	77.0±0.0c
IUM 0756	33.3±1.5c	33.7±1.2c	23.3±1.2c	30.7±1.2c	21.0±1.7c	31.7±2.9c	30.3±0.6c	29.0±1.0c	38.3±3.5c	19.0±1.0c
IUM 1544	64.3±3.1c	79.7±5.8c	54.0±8.7c	87.0±0.0c	32.0±5.3c	87.0±0.0c	86.0±1.7c	87.0±0.0c	87.0±0.0c	33.3±4.2c
IUM 1573	51.3±4.0c	75.0±9.0c	29.7±8.5c	77.7±4.6c	18.0±3.6c	84.0±2.6c	69.7±4.7c	87.0±0.0c	87.0±0.0c	24.3±1.2c
IUM 1820	37.3±4.6c	42.7±7.5c	52.7±5.4c	52.0±9.9c	19.7±3.8c	70.7±4.0c	51.3±4.2c	87.0±0.0c	79.3±3.5c	61.0±6.0c

[a]Mean of three replications. Dex: dextrin. Fr: fructose. Ga: galactose. Gl: glucose. Lac: lactose. Mal: maltose. Man: mannose. Sor: sorbitol. Suc: sucrose and Xy: xylose. Each carbon source was added to the basal medium at the concentration of 0.1 M. c: Compact. sc: Somewhat compact. st: Somewhat thin and t: Thin

Table 6. Effect of nitrogen sources on the mycelial growth and density of different strains of *C. comatus*

Strain	Mycelial growth (mm)[a] and density									
	Ala	AA	AP	Arg	CN	Gly	His	Met	PN	Ur
IUM 0004	17.3±4.0c	26.0±7.8c	26.3±5.5c	29.3±4.9c	34.0±6.2c	31.0±1.0c	5.7±4.9sc	23.0±2.0sc	19.0±6.6c	20.7±9.3c
IUM 0707	70.0±8.0st	73.7±6.0sc	39.7±2.5st	44.7±8.1sc	69.7±4.9c	87.0±0.0c	–	87.0±0.0t	85.3±1.5t	29.3±6.0st
IUM 0756	18.7±1.2c	22.7±3.1c	22.7±3.8c	24.0±1.7c	27.3±4.2c	31.3±1.2c	–	23.7±1.2sc	24.3±4.0c	12.3±0.6c
IUM 1544	23.0±3.0c	54.7±0.6c	46.3±7.6c	72.7±7.9c	40.7±9.3c	70.7±7.1c	14.7±2.9sc	38.7±1.2sc	62.7±4.6c	87.0±0.0c
IUM 1573	15.0±2.0c	33.7±1.2c	30.7±3.1c	47.3±4.6c	38.3±2.5c	34.7±3.2c	8.3±0.6sc	20.0±2.6sc	15.7±4.0c	39.0±8.9c
IUM 1820	17.3±2.5c	31.3±4.7c	23.0±2.6c	48.0±2.0c	27.3±4.5c	45.7±9.9sc	–	25.3±0.6sc	26.3±2.3sc	24.0±1.7c

[a]Mean of three replications. Ala: alanine. AA: ammonium acetate. AP: ammonium phosphate. Arg: arginine. CN: calcium nitrate. Gly: glycine. His: histidine. Met: methionine. PN: potassium nitrate and Ur: urea. Each nitrogen source was added to the basal medium at the concentration of 0.02 M. c: Compact. sc: Somewhat compact. st: Somewhat thin and t: Thin

Shim *et al*. (2005 & 1997) but partially similar to Shim *et al*. (2003). Shim *et al*. (2005) proved that maltose, dextrin, sucrose and mannose were effective where lactose was highly negative. Shim *et al*. (1997) reported that *G. umbellata* was favorable to maximum carbon sources except salicin, cellobiose and lactose. Shim *et al*. (2003) revealed that dextrin was suitable for mycelial growth of *P. fumosoroseus* which is not similar to our findings and they showed that in all carbon sources, mycelial density is thin where our result is opposite.

Effect of nitrogen sources

It was observed that the most suitable nitrogen sources were arginine (IUM1573 and IUM1820) and glycine (IUM0707 and IUM0756) and the most unsuitable (sometime no growth) was histidine for mycelial growth of *C. comatus* on the culture media. The highest mycelial growth of IUM0004 and IUM1544 were found 34.0 (calcium nitrate) and 87.0 mm (urea), respectively. On nitrogen supplemented medium, compact to thin (all kinds) mycelial density was found (Table 6). Shim *et al*. (2005) clarified that glycine was the most favorable and histidine, arginine and ammonium oxalate were the most unfavorable for the mycelial growth of *M. procera* on the culture media. Lee *et al*. (2005) showed that soytone, malt extract, yeast extract and bacto–peptone were the most favorable but NaNO3 and urea were the most unfavorable for the mycelial growth of *Ramaria botrytis*.

CONCLUSION

This study was conducted for the best promising filamentous growth and density of 6 strains of *C. comatus*. To acquire factors affecting mycelial growth and density, numerous strains of *C. comatus* were experimented. The obligation was different for the mycelial growth and density of ecologically diverse strains. Thus the basic information obtained from this study can be used for the heap manufacture of *C. comatus*.

REFERENCES

Chi, J. H., T. M. Ha, Y. H. Kim and Y. D. Rho 1996 Studies on the main factors affecting the mycelial growth of *Phellinus linteus*. Korean J. Mycol., **24**(3): 214–222

Choi, Y. J., H. K. Hwang and W. H. Lee 1999 The production of artificial fruiting body of *Paecilomyces japonica*. Korean J. Mycol., **27**(2): 87–93

Hutchison, L. J., S. E. Madzia and G. L. Barron 1995 The presence and antifeedent function of toxin–producing secretory cells on hyphae of the lawn–inhabiting agaric *Conocybe lactea*. Can. J. Bot., **74**: 431–434

Jo, W. S., Y. H. Rew, S. G. Choi, G. K. Seo, J. M. Sung and J. Y. Uhm 2006 The culture conditions for the mycelial growth of *Phellinus* spp. Mycobiol., **34**(4): 200–2005

Lee, I. K., H. J. Shim, S. D. Woo, Y. H. Je, Z. Yang and S. K. Kang 1999 Variation in growth and pathogenicity of *Beauveria brassiana* and *Paecilomyces fumosoroseus* pathogenic to the pine gall midge, *Thecodiplosis japonensis*. Korean J. Appl. Microb. Biotech., **27**(5): 415–418

Lee, T. H. and H. H. Han 2005 Cultural characteristics for the enhanced mycelial growth of *Ramaria botrytis*. Mycobiol., **33**(1): 12–14

Luo, H., M. Mo, X. Huang, X. Li and K. Zhang 2004 *Coprinus comatus*: A basidiomycete fungus forms novel spiny structures and infects nematode. Mycologia, **96**(6): 1218–1225

Shim, J. O., S. G. Son, Y. H. Kim, Y. S. Lee, J. Y. Lee, T. S. Lee, S. S. Lee and M. W. Lee 1997 The culture conditions affecting the

mycelial growth of *Grifola umbellate*. Korean J. Mycol., **25**(3): 209–218

Shim, S. M., K. R. Lee, S. H. Kim, K H. Im, J. W. Kim, U. Y. Lee, J. O. Shim, M. W. Lee and T. S. Lee 2003 The optimal culture conditions affecting the mycelial growth and fruiting body formation of *Paecilomyces fumosoroseus*. Mycobiol., **31**(4): 214–220

Shim, S. M., Y. H. Oh, K. R. Lee, S. H. Kim, K. H. Im, J. W. Kim, U. Y. Lee, J. O. Shim, M. J. Shim, M. W. Lee, H. S. Ro, H. S. Lee and T. S. Lee 2005 The characteristics of culture conditions for the mycelial growth of *Macrolepiota procera*. Mycobiol., **33**(1): 15–18

Sung, J. M., C. H. Kim, K. J. Yang, H. K. Lee and Y. S. Kim 1993 Studies on distribution and utilization of *Cordyceps militaris* and *C. nutans*. Korean J. Mycol., **21**(2): 94–105

Thorn, R. G. and G. L. Barron 1984 Carnivorous mushrooms. Science, **224**: 76–78

Thorn, R. G. and G. L. Barron 1986 Nematoctonus and the tribe resupinateae in Ontario, Canada. Mycotaxon, **25**: 321–453

Tzean, S. S. and J. Y. Liou 1993 Nematophagous resupinate basidiomycetous fungi. Phytopathol., **83**: 1015–1020