Exploration on Filamentous Phenotype of Coprinus comatus Collected from Different Ecological Origins

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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 °C 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 |
|------------|---------------------|-----------------|
| RUM 0004   | Seaside, Korea      | September 3, 2005|
| RUM 0707   | Bupyeong–dong, Korea| July 2, 2003    |
| RUM 0756   | Incheon, Korea      | May 7, 2002     |
| RUM 1544   | Beijing, China      | February 24, 2005|
| RUM 1573   | Sanghia, China      | April 11, 2005  |
| RUM 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...
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$_2$PO$_4$ 0.46 g, K$_2$HPO$_4$ 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.

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**Table 2. Media and their compositions used in this study**

| Composition     | 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  |     |     |     |    |    |     |    |     |     |
| Ebiose          | 5   |     |     |     |    |    |     |    |     |     |
| Hypoxene        | 3   |     |     |     |    |    |     |    |     |     |
| Glucose         | 50  | 10  | 10  | 5   |    |    |     |    |     |     |
| Malt–extract    | 15  |     |     |     |    |    |     |    |     |     |
| Maltose         |     |     |     |     | 10 |    |     |    |     |     |
| Peptone         |     |     |     |     |    | 2  |     |    |     |     |
| Potatoes        |     |     |     |     |    |    |     |    |     | 200 |
| Sucrose         | 30  |     |     |     |    |    |     |    |     |     |
| Triptone        |     |     |     |     | 10 |    |     |    |     |     |
| Yeast–extract   | 3   |     |     |     |    |    |     |    | 2   | 3   |
| NaNO$_3$        | 3   | 2   |     |     |    |    |     |    |     |     |
| K$_2$HPO$_4$    | 1   |     |     |     |    |    |     |    |     |     |
| MgSO$_4$        | 0.5 | 0.5 | 0.5 | 0.5 |    |    |     |    | 0.5 | 0.5 |
| KCl             | 0.5 |     |     |     |    |    |     |    |     |     |
| FeSO$_4$        | 0.01|     |     |     |    |    |     |    |     |     |
| CaCl$_2$        | 0.1 |     |     |     |    |    |     |    |     |     |
| KH$_2$PO$_4$    | 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
also reported that the most favorable and most unfavorable 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).

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 IUM0004 and IUM0707, the highest growth was also achieved and 28.7 and 32.3 mm at pH 7, respectively. Rest of the temperatures was found to be compact (Table 3). Shim et al. (2005) and Jo et al. (2006) stated that the favorable mycelial growth of Macrolepiota procera and Phellinus spp. was at 30°C. Therefore, these results are corresponded with that of our findings.

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 IUM0707 was 30.3 and 31.7 mm on Hamada medium, respectively. Rests of the strains were showed the best mycelial growth in YM and Czapek'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. (2006) also reported that PDA, YM, Mushroom complete and Hamada were the most suitable, where Czapek 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

| Strain   | Mycelial growth (mm) and density | pH  | 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±9.0c 58.3±4.5c 45.3±4.5c |
| IUM 1573 | 46.7±2.8c 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±2.0c 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 |

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

| Strain | 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 | 77.0±0.0c |
| IUM 0756 | 33.3±3.1c | 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 | 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±3.6c | 87.0±0.0c | 79.3±3.5c | 61.0±6.0c |

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

| Strain | 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±4.0c | 34.0±6.2c | 31.0±1.0c | 5.7±4.9c | 23.0±2.0c | 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±0.0c | 85.3±1.5t | 29.3±6.0st |
| IUM 0756 | 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 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 |

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

**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*.

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