Culture Conditions for Mycelial Growth of *Coriolus versicolor*

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*Coriolus versicolor* is one of the most popular medicinal mushrooms due its various biologically active components. This study was conducted to obtain basic information regarding the mycelial culture conditions of *C. versicolor*. Based on the culture, and MCM media were suitable for the mycelial growth of the mushroom. The optimum carbon and nitrogen sources were dextrin and yeast extract, respectively, and the optimum C/N ratio was 10 to 2 when 2% glucose was used. Other minor components required for optimal growth included thiamine-HCl and biotin as vitamins, succinic acid, lactic acid and citric acid as organic acids, as well as MgSO$_4$·7H$_2$O as mineral salts.

**KEYWORDS**: *Coriolus versicolor*, Culture condition, Medicinal mushroom

It is estimated that there are 140,000 species of mushrooms worldwide, yet only 10% have been identified to date [1]. Mushrooms have long been valued as edible and medicinal resources. *Coriolus versicolor*, which belongs to polyporaceae of basidiomycetes, is a wood-rotting fungi and can be easily found in the natural environment. It has been reported that around 10 species of *C. versicolor* grow naturally in Korea as well [2]. Morphologically, *C. versicolor* has a thin, solid and oval pileus, and it is characterized as an annual mushroom living in stock of old needleleaf trees or broadleaf trees. Locally, *C. versicolor* is called ‘Ungi’ and is often used as a home remedy or a dietary supplement.

*C. versicolor* reportedly contains a variety of enzymes, including lignin peroxidase, manganese peroxidase and laccase [3-5]. The mushroom reportedly contains a variety of biologically active components, including bitter triterpenoids, alnusenone, friedelin, $\alpha$-D-glucan and $\beta$-D-glucan [6-9]. Especially, *C. versicolor* has been actively researched, since it is known to have many pharmacological effects. Ever since Tsukagoshi and Ophashi [10] found that protein bound-polysaccharide has anti-tumor activity against sarcoma-180, the effort to apply to wider industry has continued. This study was conducted to determine the culture conditions for the optimal mycelial growth of *C. versicolor*.

**Materials and Methods**

**Fungal isolates.** The isolates of *C. versicolor* used in this study are listed in Table 1. *C. versicolor* ASI 16003, ASI 16006, ASI 16008, *C. pubescens* ASI 16002 and *C. brevis* ASI 16007 were obtained from the Rural Development Administration of Korea. *C. versicolor* GBCV-01 was collected in the wild. All isolates were maintained on potato dextrose agar (PDA).

**Effect of pH.** To determine the optimal pH value for growth of *C. versicolor*, 5 diameter plugs were removed from 5-day-old cultures of *C. versicolor* grown on PDA using a cork borer. The plugs were then placed on the center of PDA plates with an adjusted pH range from 4 to

| Scientific name   | Source of strains | Organization                                      |
|-------------------|-------------------|--------------------------------------------------|
| *C. pubescens*    | ASI 16002         | Rural Development Administration, Korea           |
| *C. versicolor*    | ASI 16003         | Rural Development Administration, Korea           |
| *C. versicolor*    | ASI 16006         | Rural Development Administration, Korea           |
| *C. brevis*        | ASI 16007         | Rural Development Administration, Korea           |
| *C. versicolor*    | ASI 16008         | Rural Development Administration, Korea           |
| *C. versicolor*    | GBCV-01           | Gyeongbuk Agricultural Technology Administration, Korea |

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9 using 1 N NaOH or HCl. Samples were then incubated in the dark for 4 days at 25°C. The mycelial growth was measured according to the method described by Shim et al. [11].

Temperature. Growth of the mushrooms was evaluated at temperatures ranging from 10–35°C. The fungi were cultured on PDA for 5 days, and mycelial growth was determined as described above.

Culture media. Twelve different culture media were screened to determine the optimal medium for the mycelial growth of C. versicolor (Table 2). All media were sterilized for 20 min at 121°C and then aseptically poured into plastic petri dishes. Inoculum was then removed from 5-day-old cultures of C. versicolor grown on PDA at 25°C, after which a mycelial disk (5 in diameter) was placed in the center of the prepared media. The fungi were then incubated in the dark for 4 days at 25°C, after which the mycelial growth and density of the colonies were examined.

Effect of favorable nutrient sources
Carbon sources. Suitable carbon sources were screened by culturing the mushroom on mushroom minimal media (MMM; 20 g of dextrose, 0.5 g of MgSO₄, 0.46 g of KH₂PO₄, 1 g of K₂HPO₄, 2 g of asparagine, 120 µg of thiamine-HCl, 20 g of agar, 1,000 mL of distilled water [DW]) supplemented with one-tenth carbon sources at a concentration of 2%. The fungi were incubated in the dark for 5 days at 25°C, after which the mycelial growth and density of the colonies were evaluated.

Nitrogen sources. To determine the optimal nitrogen source for the mycelial growth of C. versicolor, mushrooms were cultured on MMM supplemented with one of 12 nitrogen sources, each at a concentration of 0.2%. A 5 mm plug of C. versicolor was placed in the center of the petri dish, which was incubated in the dark for 5 days at 25°C. The mycelial growth and density of the colonies were then examined.

C/N ratio. To determine the optimal C/N ratio, MMM were prepared using 10, 8, 6, 4, 2, 1, 0.4 and 0.2% glucose as the carbon source and 0.2% NaNO₃ as the nitrogen source, giving C/N ratios of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 2:1 and 1:1, respectively. The petri dishes were then inoculated with C. versicolor and incubated in the dark for 6 days at 25°C, after which the mycelial growth and density of the colonies were examined.

Vitamins. To determine which vitamins are suitable for the mycelial growth of C. versicolor, mushrooms were cultured on sterilized MMM that had been amended with thiamine-HCl (0.1 mg/L), riboflavin (0.5 mg/L), biotin (0.005 mg/L), pyridoxine (0.5 mg/L) or nicotinamide (2.0 mg/L), followed by filtration through a metrical membrane.
filter with a pore size of 0.2 µm. The petri dishes were incubated in the dark for 5 days at 25°C, after which the mycelial growth and density of the colonies were examined.

**Organic acid.** To screen for mineral salts suitable for the mycelial growth of *C. versicolor*, MMM was prepared using acetic acid, citric acid, maleic acid, lactic acid, succinic acid or fumaric acid at a concentration of 0.1%. The petri dishes were then inoculated with *C. versicolor* and cultured in the dark for 5 days at 25°C, after which the mycelial growth and density of the colonies were examined.

**Mineral salt.** To screen for mineral salts suitable for the mycelial growth of *C. versicolor*, mushrooms were cultured on YM solid media (5 g of peptone, 3 g of yeast extract, 3 g of malt extract, 10 g of dextrose, 20 g of agar and 1,000 mL of DW. Consider specifying, also specify if Millipore water supplemented with one-ninth mineral salts at a concentration of 0.1%. The petri dishes were inoculated with *C. versicolor* and cultured in the dark for 5 days at 25°C. After which the mycelial growth and density of the colonies were examined.

**Results and Discussion**

**Effect of pH.** Favorable mycelial growth of *C. versicolor* was obtained within the pH range of 4~6. Among the six strains, *C. pubescens* isolate ASI 16002 showed the biggest colony with a diameter of 76.7 mm at pH 4 (Table 3). The optimum pH range for the growth of *C. versicolor* has been reported to be 5.0~5.8 [12]. The results of the present study suggest that the growth of *C. versicolor* mycelia can occur within a specific pH range.

**Effect of temperature.** Temperatures ranging from 25~30°C were found to be suitable for the mycelial growth of *C. versicolor* (Fig. 1). However, the mycelial growth of *C. versicolor* was suppressed rapidly at temperatures above 30°C and below 20°C. These findings are in agreement with the results of a study conducted by Park et al. [12], who reported that the optimum temperature for the growth of *C. versicolor* was 25~30°C.

**Screening for suitable culture media.** The mycelial growth of *C. versicolor* was favorable in PDA, MEA and malt yeast extract, whereas it was poor in Czapek Dox, glucose peptone and Hennerberg (Table 4). The mycelial

### Table 3. Effect of pH on the mycelial growth of *Coriolus versicolor* at 25°C

| pH | ASI 16002 (mm/4 days) | ASI 16003 (mm/4 days) | ASI 16006 (mm/4 days) | ASI 16007 (mm/4 days) | ASI 16008 (mm/4 days) | GBCV (mm/4 days) |
|----|---------------------|---------------------|---------------------|---------------------|---------------------|------------------|
| 4  | 76.7 ± 1.5a         | 13.7 ± 0.6b         | 71.7 ± 2.1a         | 68.7 ± 0.6a         | 58.3 ± 1.5a         | 73.7 ± 1.5a      |
| 5  | 76.3 ± 1.5ab        | 16.0 ± 1.0ab        | 71.0 ± 1.0a         | 70.0 ± 1.0a         | 56.3 ± 0.6ab        | 72.3 ± 1.5ab     |
| 6  | 72.7 ± 1.5c         | 18.0 ± 1.0a         | 68.3 ± 0.6a         | 69.0 ± 1.0a         | 56.3 ± 0.6ab        | 71.3 ± 1.5abc    |
| 7  | 69.0 ± 1.0d         | 18.3 ± 0.6a         | 61.0 ± 3.0b         | 65.0 ± 1.0b         | 54.3 ± 0.6bc        | 69.0 ± 1.0bd     |
| 8  | 69.7 ± 0.6cd        | 18.0 ± 1.0a         | 60.3 ± 3.1b         | 62.0 ± 1.0c         | 53.0 ± 1.0c         | 66.7 ± 1.5d      |
| 9  | 73.0 ± 1.0bc        | 18.0 ± 1.0a         | 60.3 ± 1.5b         | 60.3 ± 0.6e         | 53.3 ± 1.2c         | 68.0 ± 1.0cd     |

SC, somewhat compact; T, thin; ST, somewhat thin.

*a* Values in the same line with different letters differ significantly according to Duncan’s multiple range test (*p* < 0.05). Results shown are the mean ± SD of three replicates.
**Table 4.** Effect of culture medium on the mycelial growth of *Coriolus versicolor* at 25°C

| Culture media       | Colony diameter (mm/4 days)* | Mycelial density |
|---------------------|-----------------------------|------------------|
|                     | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 |
| PDA                 | 67.0 ± 0.8ab | 15.0 ± 0.8ab | 60.0 ± 0.8ab | 59.3 ± 2.1ab | 47.0 ± 0.8a | 55.7 ± 2.1bc | ST | ST | ST | ST | ST | ST |
| MEA                 | 64.0 ± 0.8b | 14.0 ± 0.8abc | 63.7 ± 1.2a | 58.0 ± 0.8abc | 45.3 ± 1.2ab | 58.0 ± 2.0b | C | ST | C | C | C | C |
| YEA                 | 54.3 ± 1.2c | 16.0 ± 0.8a | 56.0 ± 0.8bc | 54.0 ± 0.8cd | 44.0 ± 0.8ab | 50.3 ± 2.5c | ST | ST | SC | SC | ST | SC |
| Czapek cox          | 36.0 ± 0.8e | 16.0 ± 0.8a | 25.3 ± 4.0g | 32.0 ± 0.8e | 22.0 ± 0.8f | 33.7 ± 2.5e | T | T | T | T | T | T |
| Glucose peptone     | 58.0 ± 2.2c | 13.0 ± 0.8bcd | 53.3 ± 1.7bdc | 51.0 ± 0.8ede | 35.3 ± 1.7d | 55.0 ± 1.0b | C | C | C | C | C | C |
| YMA                 | 67.0 ± 0.8a | 14.3 ± 0.5abc | 57.7 ± 2.6ab | 57.7 ± 1.2bc | 45.0 ± 0.8ab | 54.0 ± 1.0b | SC | ST | SC | SC | ST | SC |
| Malt yeast extract  | 70.3 ± 1.2a | 15.3 ± 0.5a | 60.0 ± 0.8ab | 63.0 ± 0.8a  | 44.0 ± 0.8ab | 65.0 ± 1.0a | SC | ST | SC | SC | SC | SC |
| Leonian             | 48.0 ± 0.8d | 10.7 ± 0.5e | 44.0 ± 1.6ef | 47.7 ± 1.7ef | 38.3 ± 1.2cd | 44.0 ± 1.0d | T | T | T | T | T | T |
| MCM                 | 67.0 ± 0.8ab | 15.7 ± 0.5a | 50.0 ± 0.8cde | 51.0 ± 0.8de | 37.3 ± 1.7d | 55.0 ± 1.0bc | SC | T | ST | ST | ST | ST |
| Hennerberg          | 39.0 ± 0.8e | 12.3 ± 0.5cde | 40.3 ± 3.3f  | 43.7 ± 1.2f  | 30.0 ± 0.8e | 44.3 ± 3.1d | T | T | T | T | T | T |
| Lilly               | 56.0 ± 0.8c | 11.3 ± 0.5de | 46.7 ± 1.2def | 47.7 ± 2.1ef | 42.0 ± 0.8bc | 42.7 ± 2.1d | T | T | T | T | T | T |
| Hoppkins            | 55.3 ± 2.1c | 11.0 ± 0.8de | 45.0 ± 2.2ef | 50.7 ± 3.1de | 43.7 ± 1.2ab | 52.3 ± 3.2bc | T | T | T | T | T | T |

PDA, potato dextrose agar; MEA, malt extract agar; YEA, yeast extract agar; MCM, mushroom complete medium; ST, somewhat thin; C, compact; SC, somewhat compact; T, thin.

*Values in the same line with different letters differ significantly according to Duncan’s multiple range test (p < 0.05). Results shown are the mean ± SD of three replicates.

**Table 5.** Effect of carbon source on the mycelial growth of *Coriolus versicolor* at 25°C

| Carbon sources       | Colony diameter (mm/5 days)* | Mycelial density |
|----------------------|-----------------------------|------------------|
|                      | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 |
| Sucrose              | 70.5 ± 0.7abc | 15.7 ± 0.6bc | 55.0 ± 4.2a | 63.5 ± 2.1bc | 62.5 ± 0.7b | 50.0 ± 4.2abc | SC | SC | ST | ST | SC | ST |
| Lactose              | 66.0 ± 1.4bc | 17.0 ± 1.0abc | 30.0 ± 3.5a | 61.0 ± 1.4bc | 52.0 ± 2.8e | 56.5 ± 2.1a | ST | ST | ST | ST | ST | ST |
| Dextrin              | 71.0 ± 1.4ab | 16.7 ± 0.6abc | 66.0 ± 1.4a | 72.0 ± 1.4a | 70.5 ± 0.7a | 56.0 ± 1.4a | SC | SC | ST | ST | ST | ST |
| Mannitol             | 65.5 ± 3.5bcde | 15.7 ± 0.6bc | 59.0 ± 1.4a | 63.0 ± 2.8bc | 54.0 ± 2.8ede | 43.5 ± 0.7cd | SC | SC | ST | ST | SC | ST |
| Maltose              | 70.0 ± 1.4abc | 17.0 ± 1.0abc | 60.0 ± 1.4a | 63.5 ± 0.7bc | 59.5 ± 0.7bc | 46.0 ± 1.4bcd | ST | SC | ST | T | T | T |
| Glucose              | 64.5 ± 2.1cd | 16.0 ± 1.0abc | 56.0 ± 1.4a | 62.0 ± 1.4bc | 55.0 ± 1.4cd | 40.5 ± 0.7d | SC | SC | ST | ST | ST | ST |
| Fructose             | 72.5 ± 0.7a | 15.0 ± 1.0c | 62.5 ± 0.7a | 66.5 ± 0.7ab | 61.5 ± 0.7b | 46.5 ± 3.5bcd | SC | SC | ST | ST | ST | ST |
| Sorbitol             | 70.5 ± 2.1abc | 17.7 ± 0.6ab | 58.5 ± 0.7a | 63.5 ± 2.1bc | 57.5 ± 0.7bcd | 39.5 ± 2.1d | SC | SC | ST | ST | ST | ST |
| Mannose              | 72.5 ± 0.7a | 15.3 ± 0.6bc | 64.5 ± 0.7a | 65.5 ± 2.1b | 61.0 ± 1.4b | 53.5 ± 4.9ab | SC | SC | ST | ST | ST | ST |
| Starch               | 59.5 ± 2.1d | 18.7 ± 0.6a | 56.5 ± 2.1a | 58.5 ± 0.7c | 55.0 ± 1.4ede | 53.0 ± 1.4ab | SC | SC | ST | T | T | ST |

SC, somewhat compact; ST, somewhat thin; T, thin.

*Values in the same line with different letters differ significantly according to Duncan’s multiple range test (p < 0.05). Results shown are the mean ± SD of three replicates.
Table 6. Effect of nitrogen source on the mycelial growth of *Coriolus versicolor* at 25°C

| Nitrogen sources        | Colony diameter (mm/5 days)* | Mycelial density |
|-------------------------|------------------------------|------------------|
|                         | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 |
| Yeast extract           | 77.0 ± 1.4a | 15.3 ± 0.6a | 71.0 ± 1.4a | 71.5 ± 0.7a | 67.5 ± 3.5a | 71.0 ± 1.4a | C | C | C | C | C | C |
| Malt extract            | 70.5 ± 6.4ab | 15.0 ± 1.0ab | 63.5 ± 2.1a | 64.5 ± 2.1ab | 65.5 ± 4.9ab | 59.0 ± 1.4b | ST | C | ST | ST | ST | ST |
| Peptone                 | 62.5 ± 0.7bc | 15.0 ± 1.0ab | 55.5 ± 0.7b | 55.5 ± 3.5cd | 55.0 ± 0.7c | 56.5 ± 0.7bc | ST | C | T | T | ST | ST |
| Urea                    | 33.5 ± 0.7e | 12.3 ± 0.6c | 26.5 ± 0.7e | 26.5 ± 2.1g | 30.0 ± 0.7f | 23.5 ± 0.7h | ST | ST | ST | ST | SC | ST |
| Ammonium nitrate        | 61.0 ± 1.4bc | 12.7 ± 0.6bc | 53.5 ± 0.7b | 60.5 ± 0.7bc | 56.5 ± 4.9bc | 37.5 ± 2.1efg | ST | SC | T | ST | ST | ST |
| Ammonium chloride       | 54.5 ± 0.7cd | 12.7 ± 0.6bc | 54.5 ± 0.7b | 59.5 ± 2.1bc | 54.5 ± 0.7c | 38.5 ± 2.1efg | ST | C | ST | T | ST | T |
| Ammonium acetate        | 55.5 ± 2.1cd | 13.0 ± 1.0abc | 45.5 ± 6.4cd | 45.5 ± 0.7fg | 43.0 ± 1.4de | 39.0 ± 2.8efg | SC | C | ST | SC | ST | ST |
| Ammonium sulfate        | 62.0 ± 1.4bc | 13.0 ± 1.0abc | 49.5 ± 2.1bcd | 55.5 ± 0.7cd | 56.5 ± 0.7bc | 51.0 ± 4.2cd | ST | C | T | T | ST | ST |
| Potassium nitrate       | 57.5 ± 3.5cd | 13.7 ± 0.6abc | 44.5 ± 2.1cd | 51.0 ± 1.4def | 49.5 ± 2.1cd | 36.5 ± 0.7fg | T | SC | T | T | ST | T |
| Sodium nitrate          | 59.0 ± 1.4bcd | 14.0 ± 1.0abc | 45.5 ± 0.7cd | 48.0 ± 2.8efg | 51.5 ± 2.1cd | 36.0 ± 4.2g | ST | ST | T | T | ST | T |
| Calcium nitrate         | 52.5 ± 7.8cd | 12.0 ± 1.0c | 50.0 ± 2.8bcd | 50.0 ± 4.2def | 52.0 ± 0.7cd | 45.0 ± 0.7de | ST | ST | T | T | ST | T |
| L-glutamic acid         | 48.0 ± 2.8d | 13.7 ± 0.6abc | 43.5 ± 0.7d | 41.5 ± 0.7g | 35.5 ± 0.7ef | 34.0 ± 2.8g | SC | SC | T | T | T | T |
| L-arginine              | 59.5 ± 4.9bcd | 14.0 ± 1.0abc | 51.5 ± 0.7bc | 54.5 ± 0.7cde | 54.5 ± 3.5c | 44.0 ± 1.4def | ST | ST | T | T | ST | T |

C, compact; ST, somewhat thin; SC, somewhat compact; T, thin.

*Values in the same line with different letters differ significantly according to Duncan’s multiple range test (*p* < 0.05). Results shown are the mean ± SD of three replicates.

Table 7. Effect of C/N ratio on the mycelial growth of *Coriolus versicolor* at 25°C

| C/N ratio | Colony diameter (mm/6 days)* | Mycelial density |
|-----------|-----------------------------|------------------|
|           | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | GBCV -01 |
| 50 : 1    | 35.3 ± 1.2c | 19.0 ± 0.8d | 28.7 ± 1.2e | 33.0 ± 0.8e | 22.7 ± 1.7e | 37.0 ± 1.6e | SC | ST | ST | ST | ST | ST |
| 40 : 1    | 41.3 ± 1.2e | 19.7 ± 0.5cd | 38.7 ± 1.2d | 4.07 ± 1.7de | 26.3 ± 1.7e | 46.0 ± 0.8de | ST | ST | ST | ST | ST | ST |
| 30 : 1    | 53.0 ± 6.2d | 21.0 ± 0.8bcd | 49.7 ± 0.5c | 46.7 ± 2.1cd | 36.7 ± 1.2d | 49.0 ± 4.3cd | ST | ST | ST | ST | ST | ST |
| 20 : 1    | 70.0 ± 2.2c | 22.3 ± 1.2abc | 51.0 ± 0.8c | 56.0 ± 1.6b | 42.7 ± 1.2cd | 57.0 ± 0.8bc | ST | ST | T | ST | ST | ST |
| 10 : 1    | 84.0 ± 0.8a | 23.0 ± 0.8ab | 66.7 ± 2.6a | 68.3 ± 4.9a | 50.0 ± 0.8ab | 67.3 ± 3.1a | SC | ST | T | ST | ST | ST |
| 5 : 1     | 81.0 ± 2.2ab | 24.3 ± 0.5a | 69.0 ± 2.9a | 70.0 ± 1.6a | 54.3 ± 4.8a | 66.7 ± 1.2a | ST | ST | T | T | T | T |
| 2 : 1     | 81.3 ± 4.5ab | 25.0 ± 0.8a | 71.0 ± 2.2a | 72.7 ± 3.7a | 54.0 ± 0.8ab | 63.7 ± 3.4ab | ST | T | T | T | T | T |
| 1 : 1     | 72.0 ± 2.2bc | 23.0 ± 0.8ab | 57.7 ± 1.7b | 54.0 ± 2.2bc | 47.0 ± 0.8bc | 59.7 ± 3.7ab | ST | T | T | ST | T | ST |

SC, somewhat compact; ST, somewhat thin; T, thin.

*Values in the same line with different letters differ significantly according to Duncan’s multiple range test (*p* < 0.05). Results shown are the mean ± SD of three replicates.
Table 8. Effect of vitamins on the mycelial growth of *Coriolus versicolor* at 25°C

| Vitamins        | Colony diameter (mm/5 days)* | Mycelial density | GBCV |
|-----------------|-----------------------------|------------------|------|
|                 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | -01 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | -01 |
| Thiamine-HCl    | 68.0 ± 3.6a | 16.0 ± 1.0a | 61.0 ± 2.6a | 64.7 ± 1.5a | 60.7 ± 3.5a | 62.7 ± 3.1a | SC | SC | ST | ST | ST | SC |
| Riboflavin      | 52.0 ± 2.0c | 15.7 ± 0.6a | 55.0 ± 1.0b | 53.7 ± 0.6c | 48.3 ± 4.9b | 50.0 ± 1.0b | T | T | T | T | T | T |
| Biotin          | 58.0 ± 2.6bc | 17.3 ± 0.6a | 56.3 ± 1.5ab | 59.3 ± 2.1b | 43.3 ± 1.5b | 49.3 ± 0.6b | T | T | T | T | T | T |
| Pyridoxine      | 58.0 ± 3.0bc | 17.3 ± 1.5a | 53.0 ± 1.0b | 58.3 ± 2.5bc | 45.7 ± 2.1b | 48.7 ± 2.1b | T | T | T | T | T | T |
| Nicotinamide    | 63.7 ± 1.2ab | 16.3 ± 2.3a | 56.0 ± 3.0ab | 60.7 ± 0.6ab | 45.7 ± 3.2b | 46.0 ± 1.0b | T | T | T | T | T | T |

SC, somewhat compact; ST, somewhat thin; T, thin.

*Values in the same line with different letters differ significantly according to Duncan’s multiple range test (p < 0.05). Results shown are the mean ± SD of three replicates.

Table 9. Effect of organic acids on the mycelial growth of *Coriolus versicolor* at 25°C

| Organic acids | Colony diameter (mm/5 days)* | Mycelial density | GBCV |
|---------------|-----------------------------|------------------|------|
|               | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | -01 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | -01 |
| Acetic acid   | 34.3 ± 1.5b | 5.0 ± 0.0c | 42.0 ± 2.6c | 37.0 ± 2.0b | 39.3 ± 2.1b | 41.0 ± 1.0b | ST | T | ST | ST | ST | ST |
| Citric acid   | 66.0 ± 2.6a | 13.3 ± 0.6ab | 52.7 ± 1.5a | 51.3 ± 0.6a | 42.3 ± 0.6ab | 47.3 ± 1.5a | T | ST | ST | T | T | T |
| Maleic acid   | 33.7 ± 2.5b | 14.0 ± 1.0ab | 27.3 ± 0.6d | 29.0 ± 2.0c | 20.7 ± 1.2c | 24.0 ± 1.0c | ST | ST | T | T | T | T |
| Lactic acid   | 63.7 ± 1.5a | 14.0 ± 1.0ab | 52.3 ± 1.5ab | 48.7 ± 4.9a | 47.0 ± 2.6a | 48.3 ± 0.6a | ST | ST | T | T | T | T |
| Succinic acid | 62.3 ± 4.9a | 16.3 ± 2.5a | 46.7 ± 3.2bc | 54.0 ± 1.0a | 46.0 ± 4.0a | 43.0 ± 1.0b | T | ST | ST | T | T | T |
| Fumaric acid  | 34.3 ± 2.1b | 16.3 ± 0.6b | 25.0 ± 2.0d | 27.7 ± 1.5c | 23.3 ± 0.6c | 22.7 ± 1.2c | SC | SC | T | T | T | T |

ST, somewhat thin; T, thin; SC, somewhat compact.

*Values in the same line with different letters differ significantly according to Duncan’s multiple range test (p < 0.05). Results shown are the mean ± SD of three replicates.

Table 10. Effect of mineral salts on the mycelial growth of *Coriolus versicolor* at 25°C

| Mineral salts | Colony diameter (mm/5 days)* | Mycelial density | GBCV |
|---------------|-----------------------------|------------------|------|
|               | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | -01 | ASI 16002 | ASI 16003 | ASI 16006 | ASI 16007 | ASI 16008 | -01 |
| MgSO₄·7H₂O    | 73.7 ± 1.5a | 15.0 ± 1.0a | 71.0 ± 2.6a | 66.3 ± 1.5a | 61.0 ± 1.0a | 69.0 ± 1.0a | SC | SC | SC | SC | SC | SC |
| KCl           | 64.0 ± 5.3c | 15.0 ± 1.0a | 67.3 ± 1.5ab | 63.7 ± 1.2ab | 52.0 ± 4.4b | 64.0 ± 1.7ab | SC | SC | SC | SC | SC | SC |
| KH₂PO₄        | 64.0 ± 1.0c | 14.3 ± 0.6a | 68.7 ± 2.1ab | 61.3 ± 1.5b | 49.0 ± 1.0b | 58.0 ± 1.0c | SC | SC | SC | SC | SC | SC |
| K₂HPO₄        | 55.0 ± 2.0d | 16.0 ± 1.0a | 57.0 ± 1.0c | 53.7 ± 2.1c | 40.0 ± 1.0c | 51.0 ± 1.0d | C | ST | SC | SC | ST | SC |
| NaCl          | 67.0 ± 1.0bc | 15.0 ± 1.0a | 64.0 ± 1.0b | 63.0 ± 2.0ab | 50.0 ± 5.6b | 63.0 ± 2.0bc | SC | ST | ST | SC | SC | SC |
| ZnSO₄·7H₂O    | 8.7 ± 1.5g | 9.0 ± 1.0b | 16.0 ± 4.4e | 14.3 ± 3.1e | 14.7 ± 0.6d | 16.7 ± 4.0f | T | T | T | T | T | T |
| FeSO₄·7H₂O    | 39.0 ± 1.0e | 8.3 ± 2.1b | 41.0 ± 1.0d | 43.7 ± 0.6d | 31.3 ± 1.5c | 43.3 ± 1.2e | SC | SC | SC | SC | ST | SC |
| CuSO₄·5H₂O    | 16.0 ± 1.0f | 6.0 ± 1.0b | 13.3 ± 1.5e | 10.0 ± 1.0e | 10.0 ± 2.0d | 21.7 ± 0.6f | T | T | T | T | T | T |
| Control       | 70.3 ± 0.6ab | 15.0 ± 1.0a | 66.3 ± 3.2ab | 62.0 ± 1.0ab | 61.0 ± 5.3a | 64.0 ± 1.0ab | SC | ST | ST | SC | SC | SC |

SC, somewhat compact; ST, somewhat thin; T, thin.

*Values in the same line with different letters differ significantly according to Duncan’s multiple range test (p < 0.05). Results shown are the mean ± SD of three replicates.
growth of *C. versicolor* isolate ASI 16003 was lower than the other strains. The mycelial densities of *C. versicolor* were favorable in MEA but poor in Czapek Dox, Leonian, Hennerberg, Lily and Hopkins. Shim et al. [13] also reported that PDA, YMA, mushroom complete medium and Hamada were suitable for the growth of *Macroplethora procera*, whereas Czapek Dox and glucose peptone media were not.

**Effect of favorable nutrient sources**

**Carbon sources.** Dextrin, fructose and mannose were found to promote the mycelial growth of *C. versicolor* (Table 5). Of the 10 carbon sources evaluated, mannose led to the formation of *C. pubescens* isolate ASI 16002 colonies with the largest diameter (72.5 mm). The mycelial density of *C. versicolor* isolate ASI 16003 was somewhat compact for all carbon sources. Jeong et al. [14] reported that the optimum carbon source for the growth of *G. applanatum* is glucose while Jayasinghe et al. [15] reported that dextrin is the best carbon source for the mycelial growth of *G. lucidum*. Griffin [16] suggested that mannose and fructose are the most commonly utilized sugars after glucose.

**Nitrogen sources.** The nitrogen sources that promoted the best mycelial growth of *C. versicolor* were yeast extract and malt extract (Table 6). The mycelial densities of all *C. versicolor* strains were compact when grown in the presence of yeast extract. Among the 13 nitrogen sources evaluated, yeast extract resulted in the formation of *C. pubescens* isolate ASI 16002 colonies with a diameter of 77 mm. Jeong et al. [14] reported that the optimum nitrogen source for the culture of *G. applanatum* is corn steep powder (10%).

**C/N ratios.** The C/N ratios that promoted the mycelial growth of *C. versicolor* were 2:1, 5:1 and 10:1 (Table 7, Fig. 2). Generally, the mycelial density of *C. versicolor* is thin for all C/N ratios. Among the eight C/N ratios evaluated, a C/N ratio of 10:1 resulted in the growth of *C. pubescens* isolate ASI 16002 colonies with a diameter of 84.0 mm. Jo et al. [17] reported that the optimum C/N ratios for culture of *Phellinus* spp. are 10:1 and 5:1.

**Vitamins.** In order to evaluate the effect of vitamins, five varieties of vitamins were added to the MMM medium. The results show that thiamine-HCl produced excellent growth of *C. versicolor* mycelia (Table 8). After 5 days of cultivation, the diameter of the *C. pubescens* isolate ASI 16002 colonies grown in thiamine-HCl and nicotinamide were 68.0 mm and 63.7 mm, respectively. Cho et al. [18] reported that the optimum culture vitamins of *G. lucidum* are nicotinic acid and pantothenic acid.

**Organic acids.** Of the various organic acids were added to the MMM medium, succinic acid, citric acid and lactic acid were found to be excellent for the mycelial growth of *C. versicolor* (Table 9). After 5 days of cultivation, the diameter of *C. pubescens* isolate ASI 16002 colonies grown in the presence of citric acid and lactic acid were 66.0 mm and 63.7 mm, respectively.

**Mineral salts.** To evaluate the effect of various mineral salts on the mycelial growth of *C. versicolor*, 8 types of mineral salts were added to YM medium. MgSO₄·7H₂O, KCl, and KH₂PO₄ were found to be excellent for the mycelial growth of *C. versicolor*, whereas ZnSO₄·7H₂O resulted in mostly negative growth (Table 10). Chi et al. [19] reported that the optimum growth of *Phellinus linteus* occurs when MgSO₄·7H₂O is used as the mineral salt.

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