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 MgSO₄·7H₂O 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, α -D-glucan and β -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

Table 1. List of *Coriolus versicolor* strains used in this study

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

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Table 2. Composition of media used in this study

Nutritional reagents	Media and composition (g/L)											
	PDA	MEA	YEA	Czapek dox	Glucose peptone	YMA	Malt yeast extract	Leonian	MCM	Hennerberg	Lilly	Hopkins
Glucose			10		10		10	25	20	50		10
Sucrose				30								
Maltose											10	
Peptone		3			10	5			2			
Yeast extract			5		10	3	5		2			
Malt extract		30			15	3	3					
Potato extract	4											
DL-Asparagine											2	
Dextrose	20					10						
NaNO ₃				3						2		
MgSO ₄ ·7H ₂ O				0.5				0.5	0.5	0.5	0.5	0.5
KCl				0.5								
FeSO ₄ ·7H ₂ O				0.01				0.02				
CaCl ₂ ·2H ₂ O										0.1		
ZnSO ₄ ·7H ₂ O												
MnSO ₄ ·5H ₂ O								0.01				
K ₂ HPO ₄				1					1			
KH ₂ PO ₄				1				1	0.5	1	1	0.1
KNO ₃										2		2
Agar	15	15	20	20	20	20	20	20	20	20	20	20

PDA, potato dextrose agar; MEA, malt extract agar; YEA, yeast extract agar; MCM, mushroom complete medium.

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 concen-

tration 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 diameter 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

Fig. 1. Mycelial growth of *Coriolus versicolor* on for 5 days at different temperatures. Vertical bars show the standard errors (n = 3).

Fig. 2. Mycelial growth of *Coriolus versicolor* isolate ASI 16008 on different C/N ratios. A, 50; B, 40; C, 30; D, 20; E, 10; F, 5; G, 2; H, 1.

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	Colony diameter (mm/4 days) ^a						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
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	SC	T	ST	ST	ST	SC
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	SC	T	ST	ST	ST	SC
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	SC	T	ST	ST	ST	SC
7	69.0 ± 1.0d	18.3 ± 0.6a	61.0 ± 3.0b	65.0 ± 1.0b	54.3 ± 0.6bc	69.0 ± 1.0bcd	SC	T	ST	SC	ST	SC
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	SC	T	ST	SC	ST	SC
9	73.0 ± 1.0bc	18.0 ± 1.0a	60.3 ± 1.5b	60.3 ± 0.6c	53.3 ± 1.2c	68.0 ± 1.0cd	SC	T	ST	SC	ST	SC

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

^aValues 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) ^a						Mycelial density					
	ASI	ASI	ASI	ASI	ASI	GBCV	ASI	ASI	ASI	ASI	ASI	GBCV
	16002	16003	16006	16007	16008	-01	16002	16003	16006	16007	16008	-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 ± 0.8g	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 ± 1.7bcd	51.0 ± 0.8de	35.3 ± 1.7d	55.0 ± 1.0bc	C	T	C	C	C	C
YMA	67.0 ± 0.8ab	14.3 ± 0.5abc	57.7 ± 2.6ab	57.7 ± 1.2bc	45.0 ± 0.8ab	54.0 ± 1.0bc	SC	ST	ST	SC	SC	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	ST	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
Hopkins	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.

^aValues 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) ^a						Mycelial density					
	ASI	ASI	ASI	ASI	ASI	GBCV	ASI	ASI	ASI	ASI	ASI	GBCV
	16002	16003	16006	16007	16008	-01	16002	16003	16006	16007	16008	-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 ± 35.4a	61.0 ± 1.4bc	52.0 ± 2.8e	56.5 ± 2.1a	ST	SC	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 ± 3.5bcd	15.7 ± 0.6bc	59.0 ± 1.4a	63.0 ± 2.8bc	54.0 ± 2.8de	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	ST
Glucose	64.5 ± 2.1cd	16.0 ± 1.0abc	56.0 ± 1.4a	62.0 ± 1.4bc	55.0 ± 1.4cde	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 ± 1.4cde	53.0 ± 1.4ab	ST	SC	ST	T	T	ST

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

^aValues 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) ^a						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	T	ST	ST	ST
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	SC	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	T	ST	T	T	T	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.

^aValues 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) ^a						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 ± 1.2e	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	ST	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	T	ST	T

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

^aValues 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) ^a						Mycelial density					
	ASI	ASI	ASI	ASI	ASI	GBCV	ASI	ASI	ASI	ASI	ASI	GBCV
	16002	16003	16006	16007	16008	-01	16002	16003	16006	16007	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.

^aValues 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) ^a						Mycelial density					
	ASI	ASI	ASI	ASI	ASI	GBCV	ASI	ASI	ASI	ASI	ASI	GBCV
	16002	16003	16006	16007	16008	-01	16002	16003	16006	16007	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 ± 2.6c	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	T	T	T	T
Fumaric acid	34.3 ± 2.1b	12.7 ± 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.

^aValues 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) ^a						Mycelial density					
	ASI	ASI	ASI	ASI	ASI	GBCV	ASI	ASI	ASI	ASI	ASI	GBCV
	16002	16003	16006	16007	16008	-01	ASI 16002	ASI 16003	ASI 16006	ASI 16007	ASI 16008	-01
MgSO ₄ ·7H ₂ O	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	ST	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	ST	SC	SC	SC	SC
KH ₂ PO ₄	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	ST	SC	SC	SC	SC
K ₂ HPO ₄	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
ZnSO ₄ ·7H ₂ O	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
FeSO ₄ ·7H ₂ O	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	T	SC	SC	ST	SC
CuSO ₄ ·5H ₂ O	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	ST	SC	SC

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

^aValues 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 *Macrolepiota 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. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, KCl, and KH_2PO_4 were found to be excellent for the mycelial growth of *C. versicolor*, whereas $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ resulted in mostly negative growth (Table 10). Chi *et al.* [19] reported that the optimum growth of *Phellinus linteus* occurs when $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ is used as the mineral salt.

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