Culture Conditions for the Mycelial Growth of *Ganoderma applanatum*

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Ganoderma applanatum is one of the most popular medicinal mushrooms due to the various biologically active components it produces. This study was conducted to obtain basic information regarding the mycelial culture conditions of *Ganoderma applanatum*. Based on the colony diameter and mycelial density, PDA, YMA and MCM media were suitable for the mycelial growth of the mushroom. The optimum temperature for mycelial growth was found to be 25–30°C. The optimum carbon and nitrogen sources were mannose and dextrin, respectively, and the optimum C/N ratio was 2 to 10 when 2% glucose was used. Other minor components required for the optimal growth included thiamine-HCl and biotin as vitamins, succinic acid and lactic acid as organic acids, and MgSO₄·7H₂O, KH₂PO₄ and NaCl as mineral salts.

KEYWORDS : Culture condition, *Ganoderma applanatum*, Medicinal mushroom

It is estimated that there are 140,000 species of mushrooms worldwide, yet only 10% have been identified to date (Kirk *et al.*, 2001). Mushrooms have long been valued as edible and medicinal resources. *Ganoderma applanatum* (Pers.) Karst, which belongs to Polyporaceae of basidiomycetes, grows spontaneously on the branches of the broad leaf tree. Specifically, *G. applanatum* forms semicircular carpophores on the branch in parallel. *G. applanatum* is found worldwide, including in Korea (Park, 1991), where it has long been used as a medicine for the treatment of various human tumorigenic diseases (Kim *et al.*, 1980). The mushroom reportedly contains a variety of biologically active components, including bitter triterpenoids (Nishitoba *et al.*, 1989), alnusenone, friedelin (Protiya *et al.*, 1980), α -D-glucan and β -D-glucan (Mizuno *et al.*, 1981; Usui *et al.*, 1983). Moreover, *G. applanatum* has been reported to exert biological effects such as modulation of the humoral immune response and antibacterial and antiviral activities against pathogenic microorganisms via FDP (Fibrinogen Degradation Product) and its aqueous extract, respectively, without any acute toxicity (Kim *et al.*, 1994a, b, c; Rym *et al.*, 1999). This study was conducted to evaluate the culture conditions for the optimal mycelial growth of *G. applanatum*.

Materials and Methods

Fungal isolates. The isolates of *G. applanatum* used in this study are listed in Table 1. *G. applanatum* ASI 50167,

ASI 52821, ASI 52822, ASI 52823, ASI 53399 and *G. lucidum* ASI 7125 were obtained from the Rural Development Administration in Korea. *G. applanatum* GBGA-01 and GBGA-02 were collected in the wild. All isolates were maintained on Potato Dextrose Agar (PDA).

Effect of pH. To screen for the optimal pH value for the favorable growth of *G. applanatum* species, 5 mm diameter plugs were removed from 5 day old cultures of *G. applanatum* grown on PDA using a cork borer. The plugs were then placed in the center of PDA plates with pHs that had been adjusted to 4–9 using 1 N NaOH or HCl. The samples were then incubated in the dark for 5 days at 25°C, after which the mycelial growth was measured according to the method described by Shim *et al.* (1997).

Temperature. The growth of the mushrooms was evaluated at temperatures ranging from 10–35°C. The fungi were cultured on PDA for 6 days and the mycelial growth was determined as described above.

Culture media. Twelve different culture media were screened to determine if they were suitable for the mycelial growth of *G. applanatum* and *G. lucidum* (Table 2). All media were sterilized for 20 minutes at 121°C and then aseptically poured into plastic Petri dishes. An inoculum was then removed from five day old cultures of *G. applanatum* grown on PDA at 25°C and a mycelial disk (5 mm in diameter) was placed in the center of the prepared media. The fungi were then incubated in

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Table 1. List of *G. applanatum* strains used in this study

Scientific name	Strain name	Origin culture	Organization
<i>Ganoderma applanatum</i>	Samjung	GBGA-01	Gyeongbuk Agricultural Technology Administration, Korea
<i>Ganoderma applanatum</i>	–	GBGA-02	Gyeongbuk Agricultural Technology Administration, Korea
<i>Ganoderma applanatum</i>	–	ASI 50167	Rural Development Administration, Korea
<i>Ganoderma applanatum</i>	–	ASI 52821	Rural Development Administration, Korea
<i>Ganoderma applanatum</i>	–	ASI 52822	Rural Development Administration, Korea
<i>Ganoderma applanatum</i>	–	ASI 52823	Rural Development Administration, Korea
<i>Ganoderma applanatum</i>	–	ASI 53399	Rural Development Administration, Korea
<i>Ganoderma lucidum</i>	–	ASI 7125	Rural Development Administration, Korea

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

the dark for 5 days at 25°C, after which the mycelial growth, density and color 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 dextrose, 0.5 g MgSO₄, 0.46 g KH₂PO₄, 1 g K₂HPO₄, 2 g asparagine, 120 µg thiamine-HCl 120, 20 g agar, 1,000 ml distilled water) supplemented with one of 10 carbon sources at a concentration of 2%. The fungi were incubated in the dark for 7 days at 25°C, after which the mycelial growth, density and color of the colonies were evaluated.

Nitrogen sources. To screen for nitrogen source suitable for the mycelial growth of *G. applanatum* mushrooms were cultured on the mushroom minimal media supplemented with each of 12 nitrogen sources at a concentration of 0.2%. A 5 mm diameter plug an inoculum of *G. applanatum* was

placed in the center of the Petri dish, which was then incubated in the dark for 6 days at 25°C. The mycelial growth, density and color of the colonies were then examined.

C/N ratio. To screen for the optimal C/N ratio, mushroom minimal media 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 to give 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 *G. applanatum* and incubated in the dark for 6 days at 25°C, after which the mycelial growth, density and color of the colonies were examined.

Vitamins. To determine which vitamins were suitable for the mycelial growth of *G. applanatum*, mushrooms were cultured on sterilized mushroom minimal media 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)

Fig. 1. Mycelial growth of *G. applanatum* on PDA for 6 days at different temperatures. Vertical bars show the standard errors (n = 3). 1: 10°C, 2: 15°C, 3: 20°C, 4: 25°C, 5: 30°C, 6: 35°C.

or nicotinamide 2.0 (mg/l) and then filtered through a metrical membrane filter with a pore size of 0.2 μ m. The Petri dishes were incubated in the dark for 5 days at 25°C, after which the mycelial growth, density and color of the colonies were examined.

Organic acid. To screen for mineral salts suitable for the mycelial growth of *G. applanatum*, mushroom minimal media 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 *G. applanatum* and cultured in the dark for 5 days at 25°C, after which the mycelial growth, density and color of the colonies were examined.

Mineral salt. To screen for mineral salts suitable for the mycelial growth of *G. applanatum*, the mushrooms were cultured on YM solid media (5 g peptone, 3 g yeast extract, 3 g malt extract, 10 g dextrose, 20 g agar and 1,000 ml DW (distilled water). Consider specifying, also specify if Millipore water supplemented with one of 9 mineral salts at a concentration of 0.1%. The Petri dishes were inoculated with *G. applanatum* and then cultured in the dark for 7 days at 25°C, after which the mycelial growth, density and color of the colonies were examined.

Results and Discussion

Effect of pH. A pH of 6–9 was found to be suitable for the growth of *G. applanatum*. However, the mycelial growth

Fig. 2. Colonies of *G. applanatum* grown on PDA for 6 days at 30°C. A, GBGA-01; B, GBGA-02; C, ASI 50167; D, ASI 52821; E, ASI 52822; F, ASI 52823; G, ASI 53399; H, ASI 7125.

and density of *G. applanatum* was almost identical at all pHs within this range (Table 3). The optimum pH range for the growth of *Ganoderma* spp. has been reported to be 5–9 and 5.5–6.0 (Jayasinghe *et al.*, 2008; Cho *et al.*, 1993). The results of the present study suggest that the growth of *G. applanatum* mycelia can occur at a broad pH range.

Effect of temperature. Temperatures ranging from 25–30°C were found to be suitable for the mycelial growth of *G. applanatum* (Fig. 1, 2). However, the mycelial growth of *G. applanatum* was suppressed rapidly at temperatures above 30°C and below 20°C. These findings are similar to those of a study conducted by Jeong *et al.* (2005), who reported that the optimum temperature for the growth of

Table 3. Effect of pH on the mycelial growth of *G. applanatum* at 25°C

pH	GBGA-01	GBGA-02	Colony diameter (mm/5 days) ^a of ASI No.						GBGA-01	GBGA-02	Mycelial density ^b of ASI No.					
			50167	52821	52822	52823	53399	7125			50167	52821	52822	52823	53399	7125
4	67.7 ± 2.5a	54.0 ± 7.2b	28.0 ± 6.1c	24.3 ± 1.5c	31.7 ± 3.5c	39.0 ± 5.6b	27.0 ± 5.3b	72.0 ± 1.0d	C	SC	ST	ST	SC	SC	C	SC
5	69.7 ± 1.5a	53.7 ± 1.5b	44.7 ± 6.8b	25.3 ± 0.6c	44.7 ± 4.2ab	55.7 ± 4.5a	28.3 ± 5.7ab	78.7 ± 1.2bc	C	SC	C	ST	SC	C	SC	SC
6	71.0 ± 1.0a	59.7 ± 4.9ab	41.3 ± 6.0bc	26.3 ± 2.1bc	39.0 ± 2.6bc	59.0 ± 2.0a	35.7 ± 6.0ab	78.3 ± 0.6c	C	SC	C	SC	SC	C	SC	SC
7	73.3 ± 3.1a	62.0 ± 2.6ab	61.3 ± 4.7a	27.7 ± 0.6abc	38.0 ± 4.0bc	47.7 ± 2.1ab	27.3 ± 3.1ab	80.0 ± 2.0bc	C	SC	C	ST	SC	C	C	SC
8	71.0 ± 2.6a	61.0 ± 2.0ab	65.3 ± 4.5a	33.7 ± 4.5a	40.3 ± 3.5bc	53.0 ± 5.3a	32.7 ± 3.1ab	82.0 ± 1.0ab	SC	SC	SC	SC	SC	C	C	SC
9	67.7 ± 6.8a	66.7 ± 0.6a	71.3 ± 1.5a	32.0 ± 1.7ab	50.3 ± 2.5a	53.7 ± 3.1a	39.7 ± 1.5a	84.0 ± 1.0a	SC	SC	SC	SC	SC	SC	C	SC

^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 ± standard deviation of three replicates.

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

Table 4. Effect of culture medium on the mycelial growth of *G. applanatum* at 25°C

Culture media	GBGA-01	GBGA-02	Colony diameter (mm/5 days) ^a of ASI No.						GBGA-01	GBGA-02	Mycelial density ^b of ASI No.					
			50167	52821	52822	52823	53399	7125			50167	52821	52822	52823	53399	7125
PDA	64.3 ± 2.1ab	59.3 ± 4.2a	46.7 ± 0.6bc	21.7 ± 2.3ef	54.7 ± 6.8ab	43.7 ± 6.7a	40.3 ± 3.1bc	63.7 ± 2.3a	C	C	C	C	C	C	C	SC
MEA	52.3 ± 1.5cd	49.0 ± 0.0cd	48.0 ± 0.0b	31.7 ± 5.8cd	49.7 ± 1.5b	47.0 ± 1.7a	42.3 ± 0.6bc	60.7 ± 0.6ab	C	C	C	C	SC	C	C	C
YEA	62.3 ± 3.5abc	52.0 ± 0.0bc	46.0 ± 1.7bc	28.7 ± 1.2d	54.7 ± 5.7ab	46.3 ± 1.2a	41.7 ± 1.5bc	57.7 ± 1.5bc	C	C	C	C	C	C	C	C
Czapek dox	33.7 ± 3.1fg	32.3 ± 0.6f	24.3 ± 1.5d	13.3 ± 0.6gh	33.3 ± 2.1c	34.3 ± 0.6b	27.0 ± 0.0d	50.0 ± 0.0ef	T	T	T	T	ST	ST	ST	T
Glucose peptone	55.3 ± 4.5bcd	52.3 ± 0.6bc	50.0 ± 2.7ab	35.7 ± 0.6bc	59.7 ± 1.2ab	45.0 ± 1.7a	44.3 ± 1.2ab	55.0 ± 2.0cde	C	C	C	C	C	C	C	C
YMA	66.0 ± 7.2a	57.0 ± 2.0ab	46.0 ± 1.7bc	28.3 ± 3.2de	56.3 ± 3.2ab	49.7 ± 0.6a	37.3 ± 0.6c	49.7 ± 1.5f	C	C	C	SC	C	C	C	SC
Malt yeast extract	68.3 ± 0.6a	59.0 ± 1.7a	53.0 ± 1.0a	43.0 ± 2.7a	60.0 ± 0.0ab	51.7 ± 2.3a	48.3 ± 3.1a	56.0 ± 2.7bcd	C	C	C	C	C	C	C	SC
Leonian	33.7 ± 3.2fg	38.0 ± 1.7e	22.0 ± 1.7d	17.0 ± 0.0fgh	15.7 ± 6.8de	19.7 ± 1.2c	19.0 ± 1.7e	52.0 ± 1.7def	T	T	T	T	T	T	T	T
MCM	60.3 ± 2.1abc	56.3 ± 2.1ab	43.0 ± 2.7c	40.0 ± 2.7ab	62.7 ± 2.5a	51.7 ± 6.5a	44.0 ± 1.7ab	55.7 ± 2.5bcd	C	C	C	C	C	C	C	SC
Hennerberg	26.7 ± 2.3g	31.7 ± 1.2f	12.0 ± 0.0e	10.7 ± 0.6h	13.7 ± 1.2e	20.3 ± 0.6c	15.3 ± 3.79e	31.3 ± 0.6g	T	T	T	T	T	T	T	T
Lilly	47.0 ± 5.0de	44.3 ± 3.1d	20.7 ± 0.6d	20.0 ± 1.7fg	35.3 ± 2.5c	32.0 ± 1.0b	28.0 ± 2.0d	57.3 ± 1.2bc	SC	SC	SC	T	SC	ST	SC	ST
Hopkins	40.7 ± 2.1ef	35.3 ± 1.5ef	21.0 ± 0.0d	17.3 ± 0.6fgh	25.0 ± 5.0cd	21.3 ± 2.3c	19.0 ± 1.7e	54.3 ± 2.5c-f	ST	T	T	T	ST	ST	ST	T

^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 ± standard deviation of three replicates.

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

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

Culture media	GBGA-01	GBGA-02	Colony diameter (mm/7 days) ^a of ASI No.						GBGA-01	GBGA-02	Mycelial density ^b of ASI No.					
			50167	52821	52822	52823	53399	7125			50167	52821	52822	52823	53399	7125
Sucrose	50.7 ± 3.1bc	52.0 ± 1.0bc	43.0 ± 3.5bc	17.0 ± 1.0cd	44.3 ± 0.6d	44.3 ± 4.6bc	44.7 ± 1.2bc	70.7 ± 1.2bcd	SC	SC	ST	T	SC	SC	SC	ST
Lactose	32.0 ± 1.0d	24.0 ± 1.0d	16.7 ± 0.6d	15.3 ± 0.6d	19.0 ± 2.0f	18.7 ± 1.5e	24.0 ± 1.0d	72.3 ± 1.5abc	C	ST	C	T	ST	T	ST	ST
Dextrin	57.7 ± 1.5ab	56.3 ± 1.5b	50.3 ± 4.0b	19.7 ± 0.6bc	50.0 ± 1.0ab	54.0 ± 1.0a	53.3 ± 2.1a	76.3 ± 1.5a	SC	SC	ST	T	SC	ST	ST	ST
Mannitol	49.7 ± 4.5bc	51.3 ± 2.1c	47.3 ± 3.1bc	18.3 ± 0.6bcd	45.7 ± 2.5bcd	50.0 ± 3.5ab	48.0 ± 3.0abc	74.0 ± 1.0ab	ST	SC	SC	T	SC	SC	ST	ST
Maltose	56.7 ± 1.5ab	53.7 ± 1.2bc	41.3 ± 3.1c	20.7 ± 0.6abc	45.0 ± 1.0cd	47.3 ± 4.9abc	44.3 ± 2.5c	65.7 ± 2.1de	ST	SC	ST	T	ST	SC	SC	ST
Glucose	45.0 ± 4.4c	49.3 ± 2.1c	42.3 ± 2.1bc	18.3 ± 0.6bcd	42.0 ± 1.7de	39.7 ± 1.5c	44.3 ± 1.5c	59.3 ± 0.6fg	ST	ST	ST	T	ST	SC	ST	ST
Fructose	50.0 ± 2.6bc	52.7 ± 1.5bc	45.3 ± 2.1bc	18.7 ± 0.6bcd	49.7 ± 0.6abc	47.3 ± 0.6abc	51.0 ± 1.0ab	70.3 ± 0.6bcd	ST	ST	ST	T	ST	SC	SC	ST
Sorbitol	48.0 ± 6.0bc	52.3 ± 1.5bc	44.3 ± 4.0bc	17.7 ± 1.2cd	41.7 ± 1.5de	39.7 ± 1.5c	42.0 ± 3.6c	56.7 ± 2.3g	ST	ST	SC	T	ST	ST	ST	ST
Mannose	64.3 ± 8.1a	61.7 ± 1.5a	49.3 ± 1.2bc	22.3 ± 1.2ab	52.7 ± 2.1a	44.7 ± 4.7bc	48.0 ± 3.5abc	63.3 ± 4.2ef	SC	C	SC	T	SC	SC	C	ST
Starch	66.0 ± 2.0a	62.7 ± 2.5a	62.7 ± 3.5a	24.3 ± 3.8a	39.0 ± 2.6e	29.7 ± 2.5d	41.7 ± 1.2c	67.7 ± 2.1cde	SC	SC	SC	T	ST	ST	SC	ST

^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 ± standard deviation of three replicates.

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

Table 6. Effect of nitrogen source on the mycelial growth of *G. applanatum* at 25°C

Culture media	GBGA-01	GBGA-02	Colony diameter (mm/6 days) ^a of ASI No.						GBGA-01	GBGA-02	Mycelial density ^b of ASI No.					
			50167	52821	52822	52823	53399	7125			50167	52821	52822	52823	53399	7125
Yeast extract	85.0 ± 0.0a	71.0 ± 2.6a	78.0 ± 4.0a	50.0 ± 6.0a	69.3 ± 4.2a	70.0 ± 3.6a	60.0 ± 5.3a	83.3 ± 1.5a	C	C	SC	C	C	C	C	SC
Malt extract	67.0 ± 3.6b	66.7 ± 2.1ab	73.3 ± 1.5ab	25.0 ± 2.0b	56.3 ± 1.2b	59.3 ± 1.2b	57.3 ± 2.5a	84.3 ± 1.2a	SC	ST	SC	T	ST	ST	ST	SC
Peptone	53.3 ± 1.2c	60.3 ± 0.6bcd	58.3 ± 5.0cd	19.7 ± 1.2bc	46.0 ± 4.0cd	46.0 ± 3.6cd	38.7 ± 2.5cd	82.7 ± 0.6a	SC	SC	SC	T	SC	SC	ST	SC
Urea	41.7 ± 1.5d	47.7 ± 1.5f	44.7 ± 1.5e	12.7 ± 1.2d	35.3 ± 2.9f	37.7 ± 2.1ef	40.0 ± 1.7bcd	56.7 ± 0.6d	ST	ST	SC	T	ST	ST	ST	SC
Ammonium nitrate	54.0 ± 0.0c	62.0 ± 1.7bc	56.7 ± 3.2cde	19.3 ± 0.6bc	44.0 ± 1.0cde	48.7 ± 2.3c	39.7 ± 6.7bcd	74.3 ± 2.5b	ST	ST	C	T	ST	SC	ST	SC
Ammonium chloride	52.7 ± 2.1c	61.0 ± 2.0bcd	58.3 ± 2.1cd	19.3 ± 0.6bc	45.3 ± 4.2cde	46.3 ± 3.2cd	46.3 ± 2.9bc	83.7 ± 1.2a	ST	ST	SC	T	ST	ST	ST	SC
Ammonium acetate	51.3 ± 11.1cd	51.3 ± 2.1ef	47.7 ± 2.1de	19.7 ± 2.3bc	42.0 ± 1.0def	45.3 ± 2.5cde	44.0 ± 2.0bcd	67.3 ± 1.2c	SC	ST	C	T	ST	ST	ST	C
Ammonium sulfate	57.3 ± 2.1bc	61.3 ± 2.1bcd	61.7 ± 3.5bc	20.3 ± 0.6bc	51.7 ± 3.5bc	49.7 ± 1.2c	48.0 ± 3.0b	82.3 ± 0.6a	ST	ST	SC	T	SC	ST	ST	SC
Potassium nitrate	52.7 ± 2.5c	57.3 ± 0.6cde	54.0 ± 5.3cde	18.7 ± 0.6c	43.7 ± 4.0c-f	42.3 ± 1.2cde	39.3 ± 2.1bcd	78.0 ± 1.0b	ST	ST	C	T	ST	ST	ST	SC
Sodium nitrate	52.7 ± 0.6c	54.7 ± 2.3def	56.0 ± 2.0cde	17.3 ± 0.6cd	40.0 ± 4.4def	40.3 ± 1.5def	36.7 ± 1.5d	77.0 ± 2.6b	ST	ST	C	T	ST	ST	ST	SC
Calcium nitrate	53.7 ± 1.2c	61.3 ± 3.2bcd	57.0 ± 2.6cde	19.7 ± 0.6bc	37.3 ± 1.5ef	33.7 ± 1.5f	26.3 ± 3.8e	85.0 ± 0.0a	ST	ST	C	T	ST	ST	T	SC
L-glutamic acid	48.7 ± 3.2cd	25.7 ± 5.5g	32.0 ± 11.8f	15.0 ± 0.0cd	17.7 ± 1.5g	18.0 ± 5.6g	25.7 ± 3.1e	67.3 ± 1.2c	ST	ST	C	T	T	T	T	SC
L-arginine	58.0 ± 1.0bc	56.0 ± 2.6cde	58.3 ± 2.1cd	19.7 ± 1.5bc	40.3 ± 0.6def	39.7 ± 2.5def	46.0 ± 1.0bc	77.0 ± 1.0b	ST	ST	C	T	ST	ST	ST	SC

^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 ± standard deviation of three replicates.

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

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

Culture media	GBGA-01	GBGA-02	Colony diameter (mm/6 days) ^a of ASI No.						GBGA-01	GBGA-02	Mycelial density ^a of ASI No.					
			50167	52821	52822	52823	53399	7125			50167	52821	52822	52823	53399	7125
50 : 1	12.7 ± 0.6d	10.7 ± 0.6f	6.3 ± 1.2d	6.0 ± 0.0b	14.7 ± 2.5c	9.7 ± 4.6d	7.7 ± 0.6d	17.7 ± 0.6g	ST	ST	T	T	ST	ST	ST	SC
40 : 1	16.0 ± 1.0d	19.0 ± 0.0e	11.7 ± 1.2cd	8.7 ± 1.5b	18.7 ± 2.1bc	18.7 ± 0.6cd	10.7 ± 1.2cd	22.7 ± 1.2f	SC	ST	SC	T	SC	SC	ST	SC
30 : 1	28.3 ± 1.5c	32.0 ± 1.0d	17.7 ± 1.2bc	16.0 ± 2.6a	32.0 ± 1.0a	20.7 ± 16.5bc	18.0 ± 3.0c	32.0 ± 1.0e	SC	SC	SC	T	SC	SC	ST	SC
20 : 1	37.3 ± 0.6b	40.0 ± 2.0c	26.0 ± 1.7ab	19.0 ± 1.0a	31.3 ± 9.8ab	32.3 ± 2.5bcd	30.7 ± 1.5ab	40.0 ± 0.0d	SC	SC	SC	T	SC	SC	ST	SC
10 : 1	43.0 ± 3.0ab	49.7 ± 1.5a	34.7 ± 1.5a	19.3 ± 1.2a	36.7 ± 2.5a	38.0 ± 3.0a	29.3 ± 5.1b	53.7 ± 0.6c	C	C	SC	T	SC	SC	SC	C
5 : 1	46.0 ± 3.5ab	52.3 ± 1.5a	31.3 ± 2.3a	19.0 ± 1.0a	31.7 ± 4.0a	35.7 ± 0.6ab	31.3 ± 5.8ab	55.7 ± 4.0bc	C	C	SC	T	SC	SC	SC	C
2 : 1	49.3 ± 1.2a	51.0 ± 1.0a	31.3 ± 5.5a	20.0 ± 2.0a	37.7 ± 5.7a	36.3 ± 2.3a	37.0 ± 1.7ab	60.0 ± 0.0ab	C	C	SC	T	SC	SC	SC	C
1 : 1	48.3 ± 7.2a	44.7 ± 0.6b	31.7 ± 5.8a	19.3 ± 1.2a	33.7 ± 2.1a	33.3 ± 2.1abc	38.3 ± 0.6a	61.0 ± 1.0a	SC	SC	SC	T	ST	SC	SC	C

^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 ± standard deviation of three replicates.

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

Table 8. Effect of vitamins on the mycelial growth of *G. applanatum* at 25°C

Culture media	GBGA-01	GBGA-02	Colony diameter (mm/5 days) ^a of ASI No.						GBGA-01	GBGA-02	Mycelial density ^b of ASI No.					
			50167	52821	52822	52823	53399	7125			50167	52821	52822	52823	53399	7125
Thiamine-HCl	75.0 ± 7.2a	67.7 ± 2.5a	49.7 ± 15.6a	24.0 ± 2.0a	62.7 ± 6.7a	62.7 ± 0.6a	45.0 ± 1.7ab	85.0 ± 0.0a	SC	C	C	C	C	C	C	SC
Riboflavin	77.0 ± 9.5a	62.7 ± 8.4ab	63.7 ± 3.2a	24.3 ± 1.5a	58.7 ± 9.1a	64.3 ± 6.0a	54.0 ± 2.6a	82.0 ± 1.0ab	SC	C	SC	ST	C	C	C	SC
Biotin	76.7 ± 5.9a	55.0 ± 9.8ab	59.3 ± 6.1a	23.7 ± 0.6a	58.3 ± 9.3a	61.3 ± 3.5a	52.3 ± 3.8a	83.3 ± 0.6ab	SC	C	SC	C	C	C	C	SC
Pyridoxine	79.3 ± 1.2a	51.0 ± 3.0ab	63.3 ± 3.1a	25.0 ± 0.0a	44.3 ± 6.1a	62.0 ± 1.7a	33.3 ± 2.3b	82.0 ± 2.0ab	SC	SC	SC	ST	C	C	SC	SC
Nicotinamide	74.0 ± 2.6a	48.0 ± 2.0b	53.3 ± 8.4a	24.3 ± 1.5a	42.0 ± 7.0a	63.3 ± 8.3a	36.3 ± 8.5b	78.7 ± 3.8b	SC	SC	C	ST	C	C	SC	SC

^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 ± standard deviation of three replicates.

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

Table 9. Effect of organic acid on the mycelial growth of *G. applanatum* at 25°C

Culture media	GBGA-01	GBGA-02	Colony diameter (mm/5 days) ^a of ASI No.						GBGA-01	GBGA-02	Mycelial density ^b of ASI No.					
			50167	52821	52822	52823	53399	7125			50167	52821	52822	52823	53399	7125
Acetic acid	50.0±4.4ab	57.0±1.0a	57.3±5.5a	31.0±7.5a	35.3±9.0ab	35.3±5.5a	28.3±2.3ab	72.0±0.0a	C	C	C	C	SC	SC	C	ST
Citric acid	46.3±3.8ab	52.0±1.7a	53.3±3.8a	20.0±0.0bc	27.3±4.5b	24.7±1.5a	20.3±5.5b	47.7±17.6b	SC	C	C	T	C	ST	C	ST
Maleic acid	59.7±2.1a	6.3±5.5b	14.7±3.2b	12.7±0.6c	31.0±11.5ab	30.3±11.5a	32.3±5.9a	78.0±1.0a	SC	SC	SC	T	SC	SC	SC	ST
Lactic acid	45.0±2.0ab	55.0±0.0a	48.7±4.0a	23.7±0.6ab	31.7±3.8ab	25.0±6.1a	26.3±1.2ab	72.3±0.6a	SC	SC	C	T	SC	ST	SC	ST
Succinic acid	47.3±6.4ab	53.7±0.6a	50.0±3.5a	22.7±0.6ab	48.0±1.0a	34.3±3.1a	24.7±1.2ab	67.3±0.6ab	C	SC	C	T	C	SC	SC	ST
Fumaric acid	42.7±9.3b	56.3±2.3a	58.0±4.4a	22.7±0.6ab	41.0±1.7ab	36.3±7.6a	25.0±1.7ab	67.7±0.6ab	SC	SC	SC	T	SC	SC	SC	ST

^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 ± standard deviation of three replicates.

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

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

Culture media	GBGA-01	GBGA-02	Colony diameter (mm/7 days) ^a of ASI No.							GBGA-01	GBGA-02	Mycelial density ^b of ASI No.					
			50167	52821	52822	52823	53399	7125	50167			52821	52822	52823	53399	7125	
MgSO ₄ ·7H ₂ O	84.0±0.0a	81.7±2.1a	84.0±1.7a	27.7±1.5abc	74.0±2.0ab	83.7±0.6a	79.3±7.2a	83.0±0.0a	C	C	C	ST	SC	SC	SC	SC	
KCl	75.3±5.7ab	82.0±2.6a	85.0±0.0a	42.0±3.0a	71.3±11.0ab	84.7±0.6a	81.7±2.3a	83.0±0.0a	SC	SC	SC	ST	SC	SC	SC	SC	
KH ₂ PO ₄	74.7±4.9ab	71.7±6.1ab	85.0±0.0a	33.3±9.5ab	68.7±12.5ab	84.0±0.0a	69.0±13.0ab	82.3±0.6a	SC	SC	SC	ST	SC	SC	SC	SC	
K ₂ HPO ₄	62.0±2.6bc	53.0±10.4cd	83.3±1.5a	40.0±1.7a	76.7±3.2ab	69.0±1.7c	68.3±8.0ab	77.0±0.0b	SC	SC	SC	ST	SC	SC	SC	SC	
NaCl	77.3±8.1a	74.7±7.6ab	85.0±0.0a	39.0±9.6a	81.0±1.7a	82.7±1.5a	73.7±4.2a	77.3±2.1b	SC	SC	C	ST	SC	SC	SC	SC	
ZnSO ₄ ·7H ₂ O	12.7±4.7d	42.0±3.6d	55.0±5.2c	8.7±2.5d	5.0±0.0c	10.3±2.3e	40.0±6.1c	60.7±0.6d	ST	ST	SC	T	T	ST	ST	SC	
FeSO ₄ ·7H ₂ O	52.0±5.3c	60.3±3.8bc	38.0±1.7d	16.7±0.6cd	56.3±4.5b	57.7±2.9d	53.0±4.4bc	75.0±1.7b	ST	ST	ST	ST	ST	ST	ST	ST	
CuSO ₄ ·5H ₂ O	70.3±4.0ab	59.7±5.8bc	75.7±3.5b	19.7±2.1bcd	55.7±11.1b	73.7±2.1bc	71.7±0.6a	67.0±1.0c	SC	SC	SC	ST	SC	SC	SC	SC	
Control	69.7±6.7ab	70.7±7.5ab	85.0±0.0a	29.3±7.6abc	72.0±14.9ab	79.0±6.1ab	69.7±4.2ab	75.7±1.5b	SC	SC	SC	ST	SC	SC	SC	SC	

^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 ± standard deviation of three replicates.

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

G. applanatum was 25°C.

Screening for suitable culture media. The mycelial growth of *G. applanatum* was favorable in PDA, YMA, and MCM, whereas it was poor in Czapek Dox, Leonian, Hennerberg and Hopkins medium (Table 4). The mycelial growth of *G. applanatum* isolate ASI 52821 was lower than that of GBGA-01, GBGA-02, ASI 50167, 52821, 52822, 52823, 53399, and 7125. The mycelial densities of *G. applanatum* isolates GBGA-01, GBGA-02, ASI 50167, 52821, 52822, 52823, and 53399 were greater than that of *G. lucidum* isolate ASI 7125. The colonies formed by the seven *G. applanatum* isolates and *G. lucidum* isolate ASI 7125 were white. Shim *et al.* (2005) also reported that PDA, YM, Mushroom Complete Medium and Hamada were the suitable for the growth of *Macrolepiota procera*, whereas Czapek Dox and Glucose Peptone media were not.

Effect of favorable nutrient sources.

Carbon sources: Dextrin and mannose were found to promote mycelial growth and increased mycelial density of *G. applanatum* (Table 5). Of the 10 carbon sources evaluated, mannose led to the formation of *G. applanatum* isolate GBGA-01 colonies with the greatest diameter (64.3 mm). The mycelial density of *G. applanatum* isolate GBGA-02 was compact in mannose. Jeong *et al.* (2005) reported that the optimum carbon source for the growth of *G. applanatum* was glucose. Jayasinghe *et al.* (2008) reported that dextrin was the best carbon source for the mycelial growth of *G. lucidum*. Griffin (1994) suggested that mannose and fructose were the most commonly utilized sugars after glucose.

Nitrogen sources: The nitrogen sources that promoted the mycelial growth of *G. applanatum* were malt extract and yeast extract (Table 6). The mycelial density of *G. applanatum* isolate GBGA-01 was compact when grown in the presence of yeast extract. Among the 13 nitrogen sources evaluated, yeast extract resulted in the formation of *G. applanatum* isolate GBGA-01 colonies with a diameter of 85 mm. Jeong *et al.* (2005) reported that the optimum nitrogen source for the culture of *G. applanatum* was corn steep power (10%).

C/N ratios: The C/N ratios promoting the mycelial growth of *G. applanatum* were 1 : 1, 2 : 1, 5 : 1 and 10 : 1 (Table 7, Fig. 3). The mycelial density of *G. applanatum* isolate GBGA-01 was compact when it was cultured in the presence of C/N ratios of 10 : 1, 5 : 1 and 2 : 1. Among the 8 C/N ratios evaluated, a C/N ratio of 5 : 1 resulted in the growth of *G. applanatum* isolate GBGA-02 colonies with a diameter of 52.3 mm. Jo *et al.* (2006) reported that the optimum culture C/N ratios for *Phellinus* spp. were 10 : 1 and 5 : 1.

Vitamins: When various vitamins were added to the

Fig. 3. Mycelial growth of *G. applanatum* GBGA-01 on PDA containing different C/N ratios. A: 50, B: 40, C: 30, D: 20, E: 10, F: 5, G: 2, H: 1.

MMM medium, thiamine-HCl and biotin produced excellent growth of the mycelium of *G. applanatum* (Table 8). After 5 days of cultivation, the diameter of colonies of *G. applanatum* isolate GBGA-01 grown in thiamine-HCl and biotin were 77.0 mm and 76.7 mm, respectively. Cho *et al.* (1993) reported that the optimum culture vitamins of *G. lucidum* were nicotinic acid and pantothenic acid.

Organic acids: When various organic acids were added to the MMM medium, succinic acid and lactic acid were found to be excellent for the mycelial growth of *G. applanatum* (Table 9). After 5 days of cultivation, the colony diameters of *G. applanatum* isolate GBGA-01 grown in the presence of succinic acid and lactic acid were 47.3 mm and 45.0 mm, respectively.

Mineral salts: When various mineral salts were added to the YM medium, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, KH_2PO_4 and NaCl were found to be excellent for the mycelial growth of *G. applanatum*, whereas $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ resulted in almost no growth of *G. applanatum* isolates GBGA-01, ASI 52821, ASI 52822 and ASI 52823. (Table 10). Chi *et al.* (1996) reported that the optimum growth of *P. linteus* occurred when $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was provided as the mineral salt.

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