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Characteristics of artficially cultivated *Ganoderma applanatum* fruitingbody

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ABSTRACT: *Ganoderma applanatum* is one of the most popular medicinal mushrooms due to the various biologically active components it produces. In order to study for the possibility of artificially cultivating *G. applanatum*, we investigated the growth status of *G. applanatum* mycelium using oak sawdust bottle(850 ml). It took approximately 17~27 days for *G. applanatum* cultivation and 15~16 days for *G. lucidum* cultivation with sawdust until the mycelial growth was complete. The weight of dried fruiting bodies showed that *G. applanatum* GBGA-01 weighed 16.0±7.4 g, and *G. lucidum* ASI 7125 weighed 7.9±2.7g. The thickness of the pileus was measured as 10.6±2.6 mm for *G. applanatum* GBGA-01, 16.3±8.7 mm for *G. applanatum* ASI 52823 and 8.6±3.5 mm for *G. lucidum* ASI 7125. The color of *Ganoderma* spp. showed that L degree was 55.5±1.1 for the contex of *G. applanatum* GBGA-01, 47.7±2.2 for the contex of *G. applanatum* ASI 52823 and 39.6±2.9 for the contex of *G. lucidum* ASI 7125.

KEYWORDS: Artificial Cultivation, Fruiting bodies, *Ganoderma applanatum*

Introduction

Ganoderma lucidum (Fr.) Karst, stalked mushroom with porous hymenium causes white rot of wood by decomposing lignin cellulose and related polysaccharides. The fungus primarily decays hard woods such as oak, maple sycamore and ash(Hepting, 1971; Blanchette, 1984). It is estimated that there are 140,000 species of mushrooms worldwide, with only 10% of them having been identified(Kirk *et al*., 2001). Mushrooms have long been valued as edible and medicinal resources. *Ganoderma applanatum* (Pers.) Karst, belonging to the Polyporaceae family of basidiomycetes, spontaneously grows on the branches of broad leaf tree. Specifically, *G. applanatum* forms semicircular carpophores in parallel on the branch. It is found worldwide, including in Korea(Park, 1991), where it has been used as a tradional medicine for treating various tumorigenic diseases in humans(Kim *et al*., 1980). The mushroom is known to contains a variety of biologically active components, including bitter triterpenoids(Nishitoba *et al*., 1988, 1989), alnusenone, friedelin(Protiva *et al*., 1980), α-D-glucan and β-D-glucan(Mizuno *et al*., 1981, 1985; Usui *et al*., 1983). The objective of the study was to evaluate the culture conditions and artificially cultivate fruiting bodies of *G. applanatum*.

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Materials and Methods

Fungal isolates

The fungal isolates used in this study are listed in Table 1. *G. applanatum* ASI 50167, ASI 52821, ASI 52822, ASI 52823, ASI 53399 and *G. lucidum* isolate ASI 7125 were obtained from the Rural Development Administration in Korea. *G. applanatum* isolate GBGA-01 and GBGA-02 were collected from the apartment garden in Nam-gu, Daegu and Mt. Palgong. All isolates were maintained on Potato Dextrose Agar(PDA).

Table 1. List of *Ganoderma* spp. strains used in this study

Scientific name	Origin culture	Origin
Ganoderma applanatum	GBEA-01	Gyeongbuk Agricultural Technology Administration, Korea
Ganoderma applanatum	GBEA-02	Gyeongbuk Agricultural Technology Administration, Korea
Ganoderma applanatum	ASI 50167	Rural Development Administration, Korea
Ganoderma applanatum	ASI 52821	Rural Development Administration, Korea
Ganoderma applanatum	ASI 52822	Rural Development Administration, Korea
Ganoderma applanatum	ASI 52823	Rural Development Administration, Korea
Ganoderma applanatum	ASI 53399	Rural Development Administration, Korea
Ganoderma lucidum	ASI 7125	Rural Development Administration, Korea

Substrates Analysis

The chemical compositions of substrates were analyzed using the corresponding RDA Soil Physico-Chemistry Analysis methods(Han, 1988). CaO, MgO and K_2O analyzed using an Atomic Absorption Spectrometer(Perkin Elmer 2380). Carbohydrate, nitrogen, P_2O_5 and pH were analyzed using the Tyurin assay, Kjeldahl assay, Colorimetric assay and pH-Meter(Fisher model 50), respectively.

Inoculation

The PDA medium was sterilized for 20 minutes at 121°C and then aseptically poured into a petri-dish. After it cooled down, a piece of mycelia was inoculated on the PDA medium plate to be used as an inoculum for the next step.

Mother spawn

The sawdust of oak tree(*Quercus* spp.) was mixed with rice bran at a ratio of 9:1(v/v) and the moisture content was adjusted to approximately 65% by adding water. The mixed medium was then placed into a 250 ml flask and sterilized at 121°C for 90 minutes. After cooling to 20°C, a piece of mycelia from the PDA plate was inoculated on the sawdust medium as an inoculum for the planting spawn.

Planting spawn

The planting spawn medium was prepared using the same method as the mother spawn. The medium consisting of sawdust and rice bran, was placed into 850 ml polyethylene bottle and sterilized at 121°C for 90 minutes. After cooling to 20°C, the mother spawn was inoculated into the sawdust culture medium in 850 ml polyprophylene bottle. The inoculated sawdust media were incubated at 25°C for approximately 35 days, until the mycelia spread throughout the medium and then used as an inoculum for cultivation.

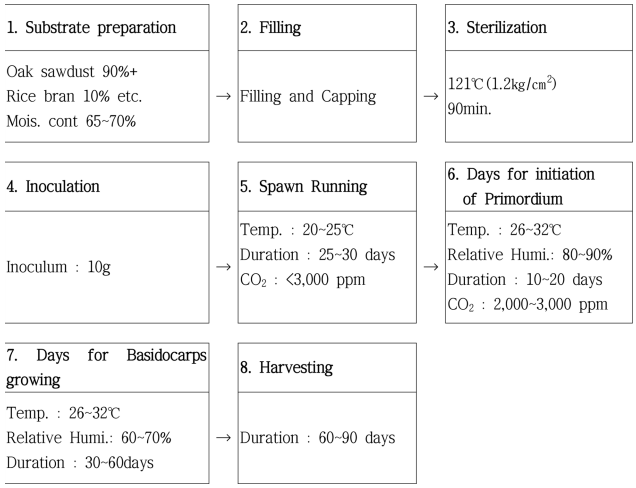

Fig. 1. The cultivation process of *Ganoderma* spp.

Sawdust cultivation process

The cultivation method for *Ganoderma* spp. was modified in the following order; substrate preparation, transfer substrates to polyprophylene bottle(850 ml), sterilization, inoculation, spawn run, initiation of primordium, and growing of basidiocarps(Fig. 1).

Substrates and preparation

A variety of sawdusts were collected from a local sawmill in Namwon City, Jeollabuk-Do, Province. The sawdust was stored in an enclosed warehouse until it was used. The media were prepared using sawdust from *Quercus acutissima*(oak). The moisture content of each sawdust medium was adjusted to approximately 65% by adding water. Each medium weighing 550 g was placed into a polypropylene bottle(850 ml) and sterilized at 121°C for 90 minutes. After cooling to 20°C, approximately 10g of inoculum was added to each sawdust medium. The inoculated media were incubated in a dark room at 22±2°C for 30 days, and the duration of mycelial growth, mycelial density were examined.

Table 2. Chemical compositions of substrate for artificial cultivation of *Ganoderma* spp.

Substrate	pH	T-C[a)]	T-N[b)]	C/N	P_2O_5	K_2O	CaO	MgO
	(1:5)				(%)			
Oak sawdust	6.0	46.6	0.28	166	0.07	0.22	0.92	0.11
Rice bran	6.6	38.9	2.20	18	4.75	2.28	0.08	1.39

a) : total carbon, b) : total nitrogen

Table 3. Trace element compositions of substrates for artificial cultivation of *Ganoderma* spp.

Substrate	Fe	Mn	Cu	Zn	Pb	Cd	Cr	As
	ppm							
Oak sawdust	92.0	140.5	2.6	2.67	0.92	-	-	1.53
Rice bran	80.5	246.6	5.3	57.25	1.17	-	-	3.20

Experimental conditions

After the completion of spawn run(15~27 days), the caps were removed and the inoculated media were transferred to a cultivation house. The relative humidity was maintained at 80~90% during the initiation of primordium and at 60~70% during fruiting body development. The temperature was maintained at 26~32°C throughout the experiments. The fruiting body characteristics of *Ganoderma* spp. cultivated on sawdust media were evaluated based on the weight of dried individual fruiting bodies(g), the major axis of pileus(mm), the minor axis of pileus(mm), the thickness of the pileus(mm), and the weight of dried fruiting bodies(g).

Color degree and Hardness

Color degrees were analyzed using a chroma meter(CR-200, Minolta, Japan) and the results were indicated by L, a and b values. Hardness degrees were analyzed using a hardness tester(TA-HD, Stable Micro System, Haslemere, England).

Results and Discussion

Physico-chemical analysis

The physico-chemical analysis was conducted on the medium resources to investigate their natural properties. The chemical analysis revealed similar values for the percentage of total carbon(T-C) with oak sawdust showing a value of 46.6% and rice bran showing a value of 38.9%. The pH value was measured as 6.0 for oak sawdust and 6.6 for rice bran. Oak sawdust exhibited weak acidity while rice bran was neutral(Table 2). A heavy metal examination showed that value of Fe was 92.9ppm of oak sawdust and their Mn value was 140.5ppm of oak sawdust, 246.5ppm of rice bran, and Cu value was 2.6 ppm of oak sawdust, 5.3 ppm of rice bran, and Zn value was 2.67ppm of oak sawdust, 57.2 ppm of rice bran, and Pb value was 0.92 ppm of oak sawdust, 1.17 ppm of rice bran. As value was 1.53 ppm of oak sawdust, 3.2 ppm of rice bran. Cd and Cr were not detected in all substrates(Table 3).

Growth of mycelium in sawdust substrate

To study for the possibility of artificial cultivation of *G. applanatum*, we investigated its mycelium growing status with oak sawdust bottle(850 ml). It took about 17~27 days on cultivation of *G. applanatum* and 15~16 days on cultivation of *G. lucidum* with sawdust until completing mycelial growth. The days for primordium formation showed us almost similar as 11~12 days of *G. applanatum* GBGA-01 and 14~15 days of *G. lucidum* ASI 7125(Table 4). This is a photograph taken in 2007 of *G. lucidum* and *G. applanatum* growing naturally on the old tree of broadleaf trees at the same time in the garden of the apartment Nam-gu, Daegu(Fig. 2).

Character of fruiting body in sawdust substrate

Weight of dried fruitingbody showed that 16±7.4g of *G. applanatum* GBGA-01 and 7.9±2.7g of *G. lucidum* ASI 7125. Thickness of pileus showed that 10.6±2.6 mm on *G. applanatum* GBGA-01, 16.3±8.7 mm on *G. applanatum* ASI 52823 and 8.6±3.5 mm on *G. lucidum* ASI 7125. These results showed that the thickness of pileus on *G. applanatum* was thicker than *G. lucidum* ASI 7125. The major axis of pileus(mm) showed that 70.2±12.9 mm on *G. applanatum* GBGA-01, 51.7±6.1 mm on *G. applanatum* ASI 52823 and 83.2±15.9 mm on *G. lucidum* ASI 7125. These results showed that the major axis of pileus on *G. applanatum* was

Fig. 2. Wild fruitingbody of *G. applanatum*(A) and *G. lucidum*(B)

Table 4. Mycelial growth and fruitbody yields of *Ganoderma* spp. on oak sawdust bottle

Scientific name	Origin culture	Duration of mycelial growth(days)	Days for pinhead formation	Wt. of dried fruitingbody(g)[a)]
Ganoderma applanatum	GBGA-01	17~18	11~12	16.0±7.4
Ganoderma applanatum	GBGA-02	18~19	12~13	10.8±3.3
Ganoderma applanatum	ASI 50167	20~21	14~15	10.5±2.8
Ganoderma applanatum	ASI 52821	25~27	15~16	10.7±3.5
Ganoderma applanatum	ASI 52822	22~24	14~15	10.5±1.2
Ganoderma applanatum	ASI 52823	24~26	14~15	9.6±2.6
Ganoderma applanatum	ASI 53399	18~19	13~14	10.5±4.8
Ganoderma lucidum	ASI 7125	15~16	14~15	7.9±2.7

The sawdust bottle was 550 g
[a)]Results are mean±standard deviation of three replicates.

Table 5. Morphological characteristics of basidiocarps of *Ganoderma* spp. on oak sawdust bottle

Scientific name	Origin culture	Major axis of Pileus (mm)	Minor axis of Pileus (mm)	Thickness of Pileus (mm)
Ganoderma applanatum	GBGA-01	70.2±12.9	42.7±5.4	10.6±2.6
Ganoderma applanatum	GBGA-02	65.1±3.9	49.1±9.2	11.0±3.4
Ganoderma applanatum	ASI 50167	52.0±8.4	45.3±7.1	12.2±5.5
Ganoderma applanatum	ASI 52821	57.8±14.0	43.5±8.4	10.7±1.3
Ganoderma applanatum	ASI 52822	46.8±4.9	37.2±3.9	14.4±4.6
Ganoderma applanatum	ASI 52823	51.7±6.1	31.5±4.0	16.3±8.7
Ganoderma applanatum	ASI 53399	59.5±4.0	40.9±9.2	12.2±2.1
Ganoderma lucidum	ASI 7125	83.2±15.9	51.2±12.1	8.6±3.5

The sawdust bottle was 550 g
[a)]Results are mean±standard deviation of three replicates.

shorter than *G. lucidum* ASI 7125(Table 4, 5, Fig. 3). These results were similar to previous report in which the cultivation characteristic in *Ganoderma* spp.(Jo, 2021).

Color degree and Hardness

Color of *Ganoderma* spp. showed that L was 55.5±1.1 on context of *G. applanatum* GBGA-01, 47.7±1.9 on context of *G. applanatum* ASI 52823 and 39.6±2.9 on context of *G. lucidum* ASI 7125, a was 8.7±1.1 on context of *G. applanatum* GBGA-01, 9.0±0.5 on context of *G. applanatum* ASI 52823 and 13.1±0.8 on context of *G. lucidum* ASI 7125 and b was 20.5±1.2 on context of *G. applanatum* GBGA-01, 20.2±0.5 on context of *G. applanatum* ASI 52823 and 24.5±1.8 on context of *G. lucidum* ASI 7125(Table 6). The L value showed *G. applanatum* higher than *G. lucidum*. Hardness of *Ganoderma* spp. showed that 2,300 kg/cm^2 on

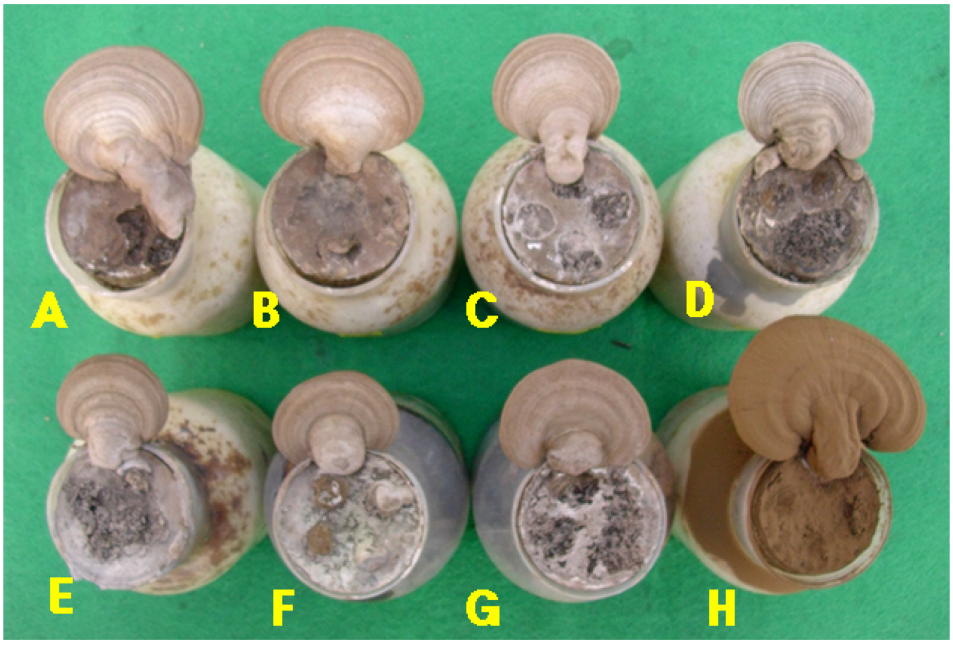

Fig. 3. Fruitingbodies of *G. applanatum*(A~G) and *G. lucidum*(H) A: GBGA-01, B: GBGA-02, C: ASI 50167, D: ASI 52821, E: ASI 52822, F: ASI 52823, G: ASI 53399, H: ASI 7125

Table 6. Color of pileus of *Ganoderma* spp. basidiocarps on oak sawdust bottle

Scientific name	Origin culture	L		a		b	
		context[a)]	tubes	context	tubes	context	tubes
Ganoderma applanatum	GBGA-01	55.5±1.1	64.8±3.0	8.7±1.1	2.7±0.8	20.5±1.2	10.9±0.2
Ganoderma applanatum	GBGA-02	49.2±5.3	69.1±1.4	9.7±1.3	1.5±0.2	21.6±1.6	13.7±0.5
Ganoderma applanatum	ASI 50167	55.7±0.6	64.2±2.3	7.8±0.3	1.1±1.1	21.9±1.4	12.9±1.6
Ganoderma applanatum	ASI 52821	58.5±4.9	71.7±2.1	4.6±0.3	1.2±2.1	18.7±1.1	15.4±1.9
Ganoderma applanatum	ASI 52822	52.5±0.1	63.5±2.2	9.2±0.6	3.1±0.6	21.8±1.0	15.9±0.7
Ganoderma applanatum	ASI 52823	47.7±1.9	60.7±0.8	9.0±0.5	4.2±0.2	20.2±0.5	14.4±0.4
Ganoderma applanatum	ASI 53399	47.0±2.2	68.1±1.1	9.7±0.4	2.8±0.1	19.1±0.9	15.1±0.3
Ganoderma lucidum	ASI 7125	39.6±2.9	48.5±10.7	13.1±0.8	3.4±0.4	24.5±1.8	13.2±4.6

L; lightness, a; redness, b; yellowness
[a)]Results are mean±standard deviation of three replicates.

context of *G. applanatum* GBGA-01, 2,840 kg/cm^2 on context of *G. applanatum* ASI 52823 and 5,680 kg/cm^2 on context of *G. lucidum* ASI 7125. The hardness of raw materials revealed that the context parts were 1,460 ~5,680 kg/cm^2, the tubes part were 2,470~8,460 kg/cm^2 (Table 7). These results were similar to previous report in which the tubes' hardness of *Phellinus gilvus* mushroom was found higher than the context parts(Jo *et al*., 2009).

Conclusion

To study for the possibility of artificial cultivation of *G. applanatum*, we investigated *G. applanatum* mycelium growing status with oak sawdust media in PP bottle(850 ml). It took about 17~27 days on cultivation of *G. applanatum* and 15~16 days for *G. lucidum* for completing mycelial growth running. Weight of dried fruiting body showed that 16±7.4g of *G. applanatum* GBGA-01 and 7.9±2.7g of *G. lucidum* ASI 7125. Thickness of pileus showed that 10.6±2.6 mm on *G. applanatum* GBGA-01, 16.3±8.7 mm on *G. applanatum* ASI 52823 and 8.6±3.5 mm on *G. lucidum* ASI 7125. These results showed that the thickness of pileus on *G. applanatum* was thicker than *G. lucidum* ASI 7125. Color of *Ganoderma* spp. showed that L degree was

Table 7. Hardness of pileus of *Ganoderma* spp. basidiocarps on oak sawdust bottle

Scientific name	Origin culture	context (kg/cm^2)	tubes (kg/cm^2)
Ganoderma applanatum	GBGA-01	2,300	2,470
Ganoderma applanatum	GBGA-02	1,460	2,670
Ganoderma applanatum	ASI 50167	3,870	2,750
Ganoderma applanatum	ASI 52821	2,950	2,630
Ganoderma applanatum	ASI 52822	3,110	3,140
Ganoderma applanatum	ASI 52823	2,840	3,690
Ganoderma applanatum	ASI 53399	3,400	3,350
Ganoderma lucidum	ASI 7125	5,680	8,460

55.5±1.1 on contex of *G. applanatum* GBGA-01, 47.7±1.9 on contex of *G. applanatum* ASI 52823 and 39.6±2.9 on contex of *G. lucidum* ASI 7125, a degree was 8.7±1.1 on contex of *G. applanatum* GBGA-01, 9.0±0.5 on contex of *G. applanatum* ASI 52823 and 13.1±0.8 on contex of *G. lucidum* ASI 7125 and b degree was 20.5±1.2 on contex of *G. applanatum* GBGA-01, 20.2±0.5 on contex of *G. applanatum* ASI 52823 and 24.5±1.8 on contex of *G. lucidum* ASI 7125. Hardness of *Ganoderma* spp. showed that 2,300 kg/cm^2 on contex of *G. applanatum* GBGA-01, 2,840 kg/cm^2 on contex of *G. applanatum* ASI 52823 and 5,680 kg/cm^2 on contex of *G. lucidum* ASI 7125.

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