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Comparison of Characteristics of *Ganoderma lucidum* According to Geographical Origins : Consideration of Growth Characteristics (I)

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Nine species of genus *Ganoderma* collected in Korea and abroad including *Ganoderma lucidum* complex and *G. lucidum* were compared by investigating growth characteristics. In the bottle culture, the mycelial growth periods of *G. lucidum* from Taiwan and North America was 26 to 30 days compared to that of Korean *G. lucidum*, which was 30 to 32 days. Cultivation period of Taiwan and North American isolates was 30 to 32 days which were 11 to 17 days shorter than those of Korean isolates. Biological efficiency of Taiwan and North American isolates were ranged from 3.3 to 5.5%, which were apparently lower than that of Korean isolates which ranged from 6.2 to 9.4%. Korean isolates had longer stipes (15~40 mm) and more number of pileus (4~6/bottle) than those of Taiwan and North American isolates. The *G. lucidum* isolates collected from Korea will be regarded as the independent species from the *G. lucidum* collected from Taiwan and North America since, the *G. lucidum* from Korea showed much different growth characteristics in various aspects compared to the *G. lucidum* from Taiwan and North America.

KEYWORDS: Basidiocarp, *Ganoderma lucidum* complex, *Ganoderma lucidum*, Growth characteristics, Geographical origin

Ganoderma lucidum, first known as single species in 1881 by Karst, was reported as many different species by many researchers based on the shape, color, and incidence of basidiocarp. Many researchers had different point of view in classification of the genus *Ganoderma*, because there were no confirmed type species (Mims, 1989; Steyaert, 1980; Kim, 1998; Sung *et al.*, 1996). Some researchers including Zhao from China presumed more than 400 species in the genus *Ganoderma* (Zhao, 1979, 1989; Hseu, 1991).

G. lucidum isolates collected from Far Eastern area including Korea and Japan were quite different from those collected from North America and Europe. Also Korean *G. lucidum* isolates have different characteristics from Taiwan isolates. There were many previous studies on *G. lucidum* (Shin and Seo, 1986; 1988). However, there were no reports on the differences between Korean *G. lucidum* isolates and the other *Ganoderma* species or foreign isolates. Until now, many different opinions were arisen among researchers on the classification and cultivation of *G. lucidum*.

Therefore, the objectives of this study were as follows; (1) to analyze the fundamental characteristics such as incubation periods, growth days, and growth characteristics by collecting and classifying the 9 species of *G. lucidum* from Korea and other countries, and (2) to obtain the basic data for establishing the classification of *G. lucidum* from Korea by investigating the various cultural characteristics of Korean cultivating or wild type isolates and foreign isolates of *G. lucidum* under the same cultivation conditions.

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Materials and Methods

Collection of isolates. Twenty-one isolates of 9 species, *G. oerstedii*, *G. applanatum*, *G. meredithae*, *G. microsporum*, *G. pfeifferi*, *G. subamboinense* as well as *Ganoderma lucidum* complex such as *G. lucidum*, *G. oregonensa*, *G. resinaceum* were used in this study (Table 1). Four isolates were collected in Chungnam and Chungbuk area and 17 other isolates were obtained from Institute of Agricultural Science and Technology at Suwon, Kangwon National University at Chunchon, and Korean Research Institute of Bioscience and Biotechnology at Daejon City. All of the isolates were cultured on potato dextrose agar (PDA) and stored at 4°C until use.

Preparation of sawdust medium. The medium was prepared by mixing oak sawdust and wheat bran (8:2, v/v), and the water was added to 65% of the total volume. Mixed medium was put into 250 m*l* flask or 2,000 m*l* plastic bottle for bottle cultivation. The medium contained in the flask or bottle was sterilized at 121°C for 60 minutes and cooled to 20°C afterward. The medium was inoculated with each isolate and cultured in the incubator at 21 to 22°C.

Bottle cultivation. Bottles were moved to the cultivation room, and the caps were removed after the mycelia covered the medium. The cultivation room was maintained at 28 to 31°C and 85% of relative humidity until pinheading. The cultivation room was ventilated for 10 to

Table 1. Isolates of *Ganoderma* species used in the study

Isolate	Species	Source of isolate[a]	Origin
G 1	*G. applanatum*	ATCC 44053	Japan
G 3	*G. lucidum*	ATCC 64251	Taiwan
G 6	*G. lucidum*	ASI 7002	Wild *Ganoderma*, Korea
G 8	*G. lucidum*	ASI 7004	Cultivated *Ganoderma*, Korea
G 9	*G. lucidum*	ASI 7071	Cultivated *Ganoderma*, Korea
G14	*G. lucidum*	MRI 5005	U.S.A.
G15	*G. lucidum*	MRI 5008	U.S.A.
G16	*G. lucidum*	MRI 5002	U.S.A.
G19	*G. meredithae*	ATCC 64490	U.S.A.
G20	*G. microsporum*	ATCC 76024	Taiwan
G23	*G. oerstedii*	ATCC 52411	Argentina
G24	*G. oregonense*	ATCC 64487	U.S.A.
G26	*G. pfeifferi*	CBS 747.84	Netherlands
G28	*G. resinaceum*	ATCC 52413	U.S.A.
G29	*G. subamboinense*	ATCC 52420	Argentina
G36	*Ganoderma* sp.	CNRDA 19	Cultivated *Ganoderma*, Korea
G39	*Ganoderma* sp.	CNRDA 24	Wild *Ganoderma*, Korea
G41	*Ganoderma* sp.	CNRDA 32	Wild *Ganoderma*, Korea
G43	*Ganoderma* sp.	CNRDA 34	Wild *Ganoderma*, Korea
G45	*Ganoderma* sp.	KNU 87	Wild *Ganoderma*, Korea
G46	*Ganoderma* sp.	KNU 89	Wild *Ganoderma*, Korea

[a]ATCC : American Type Culture Collection, CBS : Centraalbureau voor Schimmelcultures, Netherlands, MRI : Mushroom Research Institute, University of Pennsylvania, U.S.A. CNRDA : Chungnam Rural Developmemt Association. ASI : Institute of Agriculture Science, Korea. KNU : Kangwon National University.

20 minutes, once or twice a day after pinheading. When stipes were grown, the relative humidity was controlled as 80 to 85% and the ventilation was performed twice to 3 times. The relative humidity was controlled at 75 to 80%, and the maximum ventilation was done to form the pileus, as stipes were grown to 3~4 cm. After the pileus were formed, ventilations were made 5 to 6 times a day, and the relative humidity was controlled at 80 to 85%. As the pileus were thickened and the yellow color of the margin of pileus turned to a brown color, the relative humidity was lowered to 60 to 70%. The standard cultivating method (Cha *et al.*, 1989; Kim, 1995) was used thereafter.

Characteristics investigation. Maximum mycelial growth was decided as the date when mycelia were covered the medium completely. Period of primodium initiation was the period from the date when the bottle cap was removed at cultivation room to pinheading date. The cultivation period was lasted until the harvesting date of fruiting body. The thickness of pileus was measured by calipers after cutting the crossing point of the width and length of fruiting body. Biological efficiency (biological efficiency = dry weight of fruiting body/dry weight of medium ×100) was decided with 6 replicates.

Results and Discussion

Period of mycelial growth. Approximately 30 days were required for mycelial growth completion, however, it took 40 days for isolate G26 (*G. pfeifferi*) (Table 2). The mycelial growth period of North American *G. lucidum* (G15, G16) was 26 days. On the other hand, the mycelial growth period of Korean *G. lucidum* was 32 to 33 days (G8, G9) and showed much differences according to the geographical origin. The mycelial growth periods of other isolates such as *G. oregonens*, *G. resinaceum*, *G. oerstedii*, and *G. subamboinense* were similar to those of Taiwan and North American isolates rather than those of Korean isolates. The mycelial growth period of *G. meredithae* (G19) was 33 days, which is similar to those of Korean cultivating isolates G8 or G9. Meanwhile Nobles (1948) introduced the cultural characteristics as a standard of classification for *G. applanatum*, *G. lobatum*, *G. lucidum*, *G. oregonens*, *G. tsugae*, and *G. sessile* since he noticed that the cultural characteristics were different among *G. lucidum*, *G. tsugae*, and *G. oregonens*.

Period of cultivation. Periods of pinhead initiation were ranged from 6 to 15 days, and differed from each species as shown in Table 2. Particularly, pinhead initiation period of North American *G. lucidum* isolates (G14, G15, and G16) were 6 to 7 days, which were 4 to 5 days faster than Korean cultivating isolates (G8 and G9) or wild type isolates (G39, G41, G43, and G45).

During the bottle cultivation, the isolates used in this study required 10 to 26 days for the pileus induction, and showed much differences in the periods according to the species. The periods of pileus induction of *G. micro-*

Table 2. Growth characteristics of fruiting body of *Ganoderma* species cultivated on oak sawdust media

Group[a] (range)	Isolate	Species	Days for basidiocarps growth	Days of complet mycelial growth[b]	Days for initiation of primodium	Period required for initiation of pileus
I	G1	*G. applanatum*	32	31	10	14
(30~40 days)	G3	*G. lucidum*	32	30	10	16
	G14	*G. lucidum*	32	30	7	10
	G15	*G. lucidum*	30	26	6	10
	G16	*G. lucidum*	30	26	6	10
	G23	*G. oerstedii*	33	29	9	13
	G24	*G. oregonense*	32	29	8	15
	G28	*G. resinaceum*	33	30	9	12
II	G6	*G. lucidum*	48	33	10	16
(41~50 days)	G8	*G. lucidum*	44	32	10	16
	G9	*G. lucidum*	42	33	10	17
	G19	*G. meredithae*	48	33	10	18
	G26	*G. pfeifferi*	42	40	11	19
	G39	*Ganoderma* sp.	44	30	10	18
	G41	*Ganoderma* sp.	47	31	10	16
	G43	*Ganoderma* sp.	45	30	10	13
	G45	*Ganoderma* sp.	44	28	10	14
	G46	*Ganoderma* sp.	45	28	10	15
III	G20	*G. microsporum*	59	30	15	26
(51 days~)	G29	*G. subamboinense*	57	31	15	26
	G36	*Ganoderma* sp.	54	32	11	18

[a]Groups seperated by days for basidiocarp growth.
[b]Temperature: 22~25°C, Medium: Oak sawdust 80% + Wheat bran 20%, Bottle: 2,000 ml P.P.

sporum (G20), and *G. subamboinnense* (G29) were 26 days, which were the longest periods among the used isolates. The isolates of *G. meredithae* and *G. pfeifferi* took 18 to 19 days for the pileus induction. On the other hand, the pileus induction of North American *G. lucidum* isolates (G14, G15, and G16) took about 10 days, which was the shortest one. Korean cultivating isolate (G8, G9) and wild type isolates (G6, G45, and G46), however, took 13 to 18 days, which required 3 to 8 more days than those of North American *G. lucidum* isolates.

As the *Ganoderma* species were divided into 3 groups based on the growth days, *G. lucidum* from Taiwan and North America, *G. applanatum*, *G. oerstedii*, *G. oregonensa*, and *G. resinaceum* were belonged to Group I, which were about 30~40 days. Group II included *G. pfeifferi*, *G. meredithae*, and Korean cultivating isolate and wild-type isolates, which were about 41~50 days (Fig. 1). Group III, which had over 51 days of growth, included *G. microsporum* and *G. subamboinense*. Therefore, the growth period of fruiting body could presumably be used as an important basic criteria for classification.

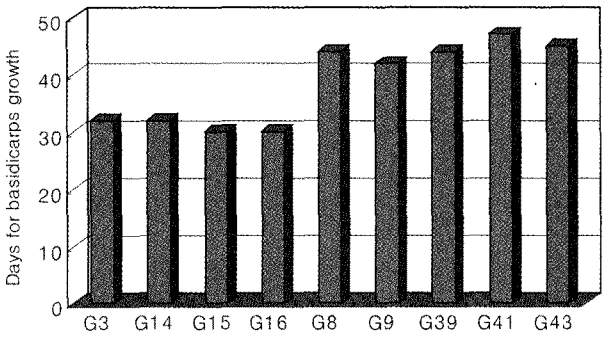

Fig. 1. Growing days for basidiocarps of *Ganoderma lucidum* according to geographical orgins. G3 (Taiwan); G14, G15, G16 (U.S.A.); G8, G9, G39, G41, G43 (Korea).

Morphological characteristics of pileus. Shapes of pileus of *Ganoderma* spp. were morphologically divided into kidney type, antler type, fabelliform type, and circular type (Fig. 2). Korean cultivating isolate G8 and wild-type isolates G6, G45, and G46 were kidney type, and obviously showed concentric rings on the surface. G9 and G39 isolates of Korea gave irregular shapes of kidney or fabelliform types. G36 and G41 isolates revealed antler types and *G. meredithae* (G19) showed fabelliform type. On the other hand, Taiwan isolate (G3) and North American isolates (G14, G15) of *G. lucidum*, *G oregonensis* (G24), *G. preifferi* (G26), *G. resinaceum* (G28), *G. applanatum* (G1), *G. microsporum* (G20), and *G. suamboinense* (G29) were mostly circular or kidney types. Wrinkles on the surface of pileus were not formed on Korean isolates, while they were formed on the surface of North American isolates. Korean isolates become glossy before late maturing stage. However, North American and Taiwan isolates were glossy, during all the growth stages.

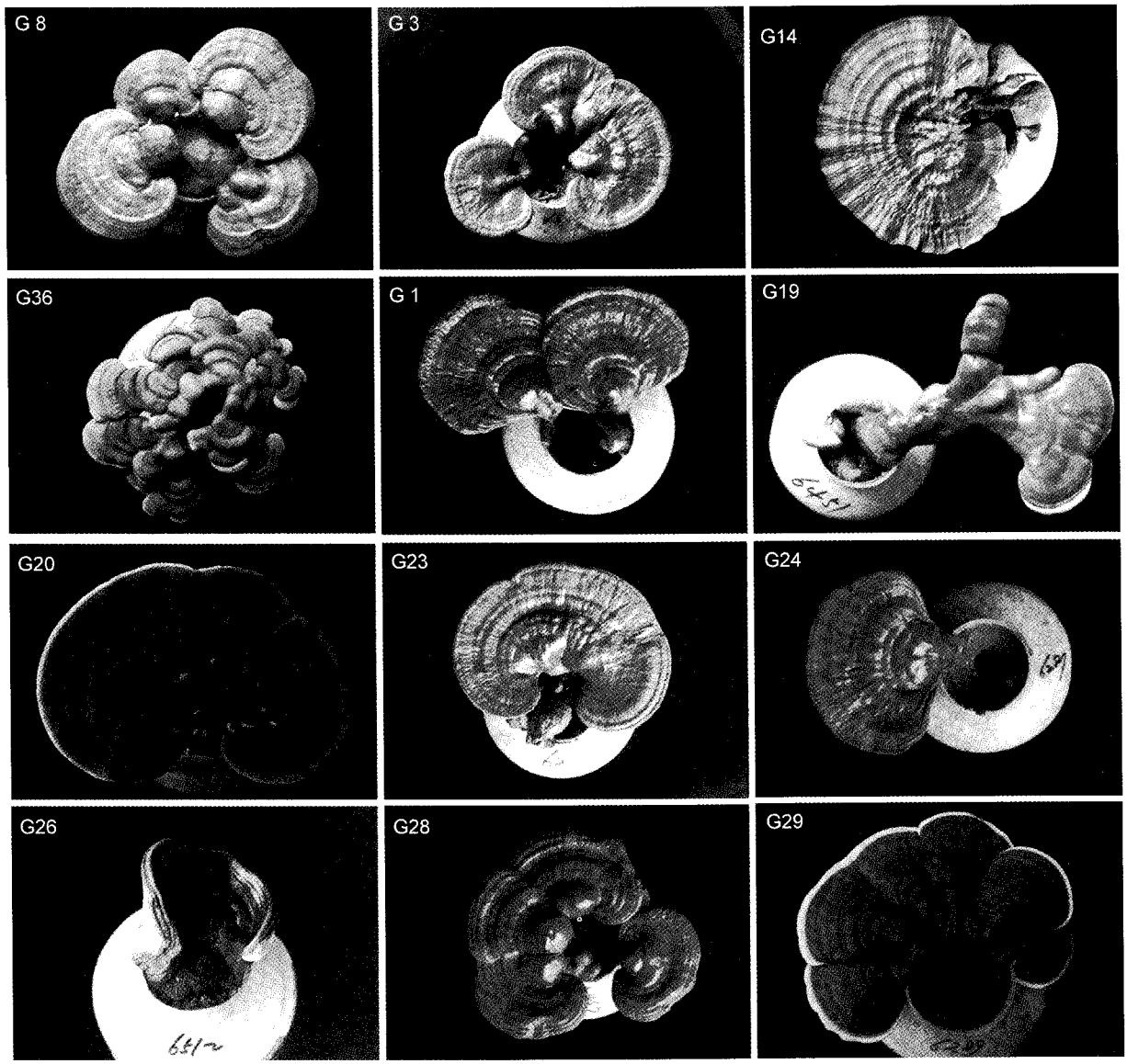

Fig. 2. Pileus types of *Ganoderma* species collected from various regions. Isolates are as following: G8: *G. lucidum* (Korea), G3: *G. lucidum* (Taiwan), G14: *G. lucidum* (U.S.A.), G36: *G. lucidum* (Korea), G1: *G. applanatum* (Japan), G19: *G. meredithae* (U.S.A.), G20: *G. microsporum* (Taiwan), G23: *G. orestedii* (Argentina), G24: *G. oregonense* (U.S.A.), G26: *G.pfeifferi* (Netherlands), G28: *G.resinaceum* (U.S.A.), G29: *G. subamboinense* (Argentina).

The size of Korean cultivating isolates and wild-type isolates were 52~92×29~41 mm, and that of North American isolates were 105~129×46~52 mm (Table 3). The numbers of pileus Taiwan and North American isolates were two, while those of Korean isolates were three to six. Therefore, Korean isolates were varied in shapes and sizes. However, Taiwan or North American isolates were less varied in shapes and sizes.

Growth characteristics of stipe. Stipe lengths were separated into four groups (Table 3). Taiwan and North American *G. lucidum*, *G. oregonense*, *G. pfeifferi*, *G. resinaceum* and *G. applanatum*, which was Group I, had almost no stipes. Korean cultivating (G8, G9) or wild type (G43, G45, G46) isolates and *G. oerstedii* had 10 to 20mm stipes and belonged to Group II. *G. microsporum* (G20) and *G. subamboinense* (G29) had 20 to 30 mm stipes in Group III. *Ganoderma* species belonging to group IV had stipes longer than 30 mm, and included mostly the antler type isolates (G19, G36, and G41). *G. meredithae* (G19) had the longest stipe of 64 mm. This criterion is also considered to be a very useful key for species classification of genus *Ganoderma*.

Hseu (1990) reported that, as the concentration of carbon dioxide was increased, stipe length was also increased. Also, when the concentration of carbon dioxide was higher than 0.1%, the growth of stipe was stimulated and branched stipes were produced. However, in this study,

Table 3. Size, number and dry weight of basidiocarps of *Ganoderma* species cultivated on oak sawdust media

Group[a] (range)	Isolate	Species	Size of pileus (mm)	Number of basidiocarps per bottle	Stipe length (mm)	Dry weight of fruiting bodies (g)[b]	Biological efficiency (%)[c]
I	G1	*G. applanatum*	79×52	1.9	0	18.6	3.8
(< 10)	G3	*G. lucidum*	74×46	2.2	0	21.8	4.5
	G14	*G. lucidum*	129×48	2.0	0	16.1	3.3
	G15	*G. lucidum*	105×46	2.2	0	26.6	5.5
	G16	*G. lucidum*	105×52	2.4	0	24.8	5.1
	G26	*G. pfeifferi*	66×37	1.3	0	18.4	3.8
	G28	*G. resinaceum*	85×42	3.9	0	28.4	5.8
II	G6	*G. lucidum*	91×41	3.3	12	34.4	7.1
(10~20)	G8	*G. lucidum*	92×38	4.9	18	42.4	8.7
	G9	*G. lucidum*	60×36	4.2	13	32.9	6.8
	G23	*G. oerstedii*	110×53	1.9	18	28.3	5.8
	G43	*Ganoderma* sp.	84×40	4.7	17	36.1	7.4
	G45	*Ganoderma* sp.	86×40	4.9	15	40.7	8.3
	G46	*Ganoderma* sp.	72×41	3.3	14	37.6	7.7
III	G20	*G. microsporum*	79×42	3.6	23	24.7	5.1
(20~30)	G29	*G. subamboinense*	82×46	5.3	29	25.2	5.2
	G39	*Ganoderma* sp.	49×29	5.9	23	30.4	6.2
IV	G36	*Ganoderma* sp.	52×30	4.8	38	46.0	9.4
(> 30)	G41	*Ganoderma* sp.	57×36	6.3	40	36.4	7.5
	G19	*G. meredithae*	63×46	2.5	64	26.4	5.4

[a]Groups seperated by the stipe length.
[b]Sawdust medium 1,390 g, water content 65%.
[c]Biological efficiency (%) = dry weight of fruitbody/dry weight of medium ×100.

since all of the cultivating conditions were the same, it was presumably considered that there were no environmental effects on the growth of stipe, and observed morphological differences might be caused by genetic factors (Shin and Seo, 1988).

Biological efficiency. Recently, the biological efficiency is often used as one of the major investigating items to distinguish the characteristics and differences among isolates. Therefore, the basidiocarp of *Ganoderma* spp. was dried for 24 hours at 60°C after harvesting from the bottle cultivation. The biological efficiency of *G. pfeifferi* and *G. applanatum* was below 4.0% and showed low rates compared to those of other species (Table 3). The biological efficiencies of *G. resinaceum*, *G. oerstedii*, *G. subamboinense*, *G. meredithae*, and *G. subamboinense* were similar to those of *G. lucidum* from Taiwan and North America. On the other hand, the biological efficiency of *G. lucidum* from Taiwan or North America was ranged from 3.3 to 5.5%. The biological efficiency of Korean isolates wewe ranged from 6.2 to 9.4%. Therefore, it was considerably conformed that *G. lucidum* isolates in Korea was not the same as those from Taiwan or North America.

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