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Effect of light on fruit body primordium formation of *Ganoderma lucidum* on nutrient agar medium

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광(光)이 *Ganoderma lucidum*의 자실체 원기 형성에 미치는 영향

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ABSTRACT: The objective of this research was to determine the effect of light quality on formation of fruit body primordia (FBPs) of *Ganoderma lucidum*. To achieve this 5 isolates of the fungus that develops fruit body primordia on nutrient agar media were incubated with or without continuous irradiation. The fluorescent lamps used different colors such as black light blue (BLB), pure blue (P-B), pure green (P-G), pure yellow (P-Y) and pure red (P-R). Effect of periodic light and dark exposures on FBP formation of isolate GI-009 was investigated. The FBP formation in *G. lucidum* isolates was also tested under monochromatic light produced by the combination of interference filters and colored glass filters. Three isolates produced FBPs under all kinds of fluorescent lamps, whereas two induced FBPs only under visible light except for BLB fluorescent lamp. However, these isolate did not form FBPs in the dark. The FBP was formed at light intensity from 0.05 to 10.0 μmol m^{-2} s^{-1}, and begun to reduce its number as light intensity increase over 0.5 μmol m^{-2} s^{-1}. When the isolate was incubated under periodic light and dark exposures, the number and weight of FBP increased as compared with those under continuous light. Initiation of FBP requires at least 4 days of light illumination. Although isolate GI-003 produced FBPs in a wide range of 400 to 800 nm, other four isolates had two effective regions 400 to 500 nm and 700 to 750 nm in FBP formation.

KEYWORDS: Colored fluorescent lamp, Fruit body primordium, *Ganoderma lucidum*, Monochromatic light

A stalked mushroom with porous hymenium, *Ganoderma lucidum* (Fr.) Karst decays hard wood such as oak, maple, sycamore and ash (Hepting, 1971; Blanchette, 1984). Although species of *Ganoderma* are important pathogens causing wood rot of forest trees, their fruit bodies are also popular and have long been used as a traditional medicine material in oriental countries such as China, Japan and Korea. Traditional identification of *Ganoderma* was mainly based on host specificity, geographical distribution, and macromorphological features of the fruit body including context color, the shape of the margin of the pileus, and whether the fruit bodies are stipitate or sessile as primary tax-

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onomic characters (Atkinson, 1908; Haddow, 1931; Steyaert, 1972 and 1980). However, the basidiocarp of *Ganoderma* species has very similar morphological characteristics that have caused confusion in identification (Lyvarden, 1994). Furthermore, morphology of fruit bodies in *G. lucidum* varies according to their host or habitat (Shin *et al.*, 1986). According to different colors and shapes, it has been called red-, black-, blue-, white-, yellow- and purple-types, as well as antler- and kidney-shapes in oriental countries. The taxonomic differences among them, however, are not clear. Moreover, the fruit bodies obtained from artificial cultivation of *G. lucidum* were different in both color and morphological feature among isolates (Shin and Seo, 1988b).

Fruit body development of *G. lucidum* was also affected by light during the *in vitro* culture or artificial cultivation using the sawdust and wood logs. Fruit bodies obtained from artificial cultivation under light illumination show polymorphism such as appearance of kidney- and antler-type fruit bodies and change in their color (Hemmi and Tanaka, 1936; Shin and Seo, 1988b). *In vitro* culture under the ventilation and light irradiation, *G. lucidum* form atypical fruiting structure (AFS) bearing basidiospores without the basidiocarp formation and fruit-body primordium (FBP) (Shin and Seo, 1988a; Seo *et al*, 1995a). According to light response, *G. lucidum* isolates are divided into three groups; AFS-forming isolates, FBP-forming isolates and only vegetative growing isolates including chlamydospore-forming isolates on the agar media (Seo *et al*, 1995a).

Seo *et al* (1995b) recently reported that effective light quality and intensity for AFS formation vary depending on isolate indicating that *G. lucidum* is heterogeneous in the photo-response with regard to AFS formation. On the other hand, some isolates of *G. lucidum* induce normal FBP which differ remarkably from AFS under light illumination. FBP induction was absolutely affected by light, whereas aeration was not (Seo *et al*, 1995a). However, light condition for FBP formation in *G. lucidum* has not yet been determined. Therefore, the objective of this research was to determine the light quality using colored fluorescent lamps and monochromatic light which is effective for FBP induction in *G. lucidum*.

Materials and Methods

Isolates

The dikaryon isolates of *G. lucidum* which were confirmed to form FBPs in a previous report (Seo *et al*, 1995a) were used in this study. Isolate Gl-003 was obtained from the context tissue of wild-type fruit bodies in Korea (Mt. Songri). Isolate Gl-009 (ASI 7018), Gl-012 (ASI 7024) and Gl-024 (ASI 7016) were kindly presented by the Agriculture Science Institute, Korea and isolate Gl-028 from the Mushroom Research Institute, University of Pennsylvania, U.S.A.

Culture condition

Complete agar medium (CM; Seo *et al*, 1995a) was employed for culture and maintenance in all isolates. Mycelial disks (6 mm in diameter) from plate cultures were placed in 90 mm plastic Petri dishes containing about 30 m*l* of CM and were incubated at 27±1°C for 30 days in dark or light condition with continuous ventilation. Then, the formation of FBPs was confirmed visually.

Light illumination

Various colored fluorescent lamps (FL 20 SD, Matsushita, Japan) and a 250 W halogen lamp were employed as light sources. The colored fluorescent lamps used in this ex-

periment were as follows; black light blue lamp which has peak wavelength at 352 nm (FL20S BLB, abbreviated as BLB): pure blue lamp which has peak wavelength at 452 nm (FL20S B-F, abbreviated as P-B): pure green lamp which has peak wavelength at 530 nm (FL20S G-F, abbreviated as P-G): pure yellow lamp which has peak wavelength at 585 nm (FL20S Y-F, abbreviated as P-Y) and pure red lamp which has peak wavelength at 656 nm (FL20S R-F, abbreviated as P-R). The spectral energy distributions and transmittance of these colored fluorescent lamps were shown in previous paper (Seo *et al*, 1995b). The light intensity of colored fluorescent lamps were adjusted to about 0.3 to 0.5 μmol m^{-2} s^{-1} by controlling the distance between lamps and the Petri dishes. To examine the effect of light intensity on formation of FBPs, spectral energies were adjusted from 0.05 to 10.0 μmol m^{-2} s^{-1} under daylight fluorescent lamp which has peak wavelength at 480 and 570 nm (FL20SD, abbreviated as D-L). Light intensity was determined with an LI-189 Quantum-meter (LI-COR, Inc.). Monochromatic light was obtained from the halogen lamp by the combinations with nine pieces of the interference filters (KL-40, KL-45, KL-50, KL-55, KL-60, KL-65, KL-70, KL-75 and KL-80) and seven pieces of the colored glass filters (Y-43, Y-48, O-53, O-57, R-63, R-68 and R-69, Toshiba Machine Co., Ltd.). The spectral energy distributions and transmittance of interference filters were shown in previous paper (Seo *et al*, 1995b).

To examine the effect of periodic light and dark exposures on FBP formation, light and dark periods during the incubation were set under time schedule as follows; In the first experiment, cultures were incubated under light-dark cycles of 8~16 hrs and 16~8 hrs for 30 days. As a control, cultures were incubated for 30 days with or without continuous light. In the second experiment, either cultures were grown in dark for 2 to 14 days, then transferred to light condition or grown under light for 2 to 14 days, then transferred to dark condition. FBP formation of the cultures was determined after incubation for 16 days. As a control, cultures were incubated for 16 days with or without continuous light. In these experiments, P-B fluorescent lamp which has 3.5 μmol m^{-2} s^{-1} in light intensity was used as a light source.

Statistical analysis

Table 1. Formation of fruit body primordia (FBP) by *G. lucidum* isolates under different coloured fluorescent lamps

Light source[1)]	No. of FBP[2)]				
	Gl-003	Gl-009	Gl-012	Gl-024	Gl-028
Dark	0	0	0	0	0
BLB	0	2.4±1.4	0	2.3±0.5	1.0±0.0
P-B	3.3±0.5	3.6±1.7	2.3±0.5	1.6±0.5	1.0±0.0
P-G	2.0±0.0	1.2±0.4	2.0±0.0	1.6±0.5	1.6±0.9
P-Y	1.0±0.0	1.8±0.7	2.3±0.5	2.0±0.8	2.3±1.3
P-R	1.0±0.0	1.6±1.2	3.0±0.0	5.0±0.8	2.0±0.0

[1)]The light intensity of each lamps were adjusted to about 0.3 to 0.5 μmol $m^{-2}s^{-1}$.

[2)]Isolates were incubated for 30 days under continuous irradiation or in the dark. All cultures were replicated five times and each value shows the mean of the numbers of FBP per Petri dish with standard deviation.

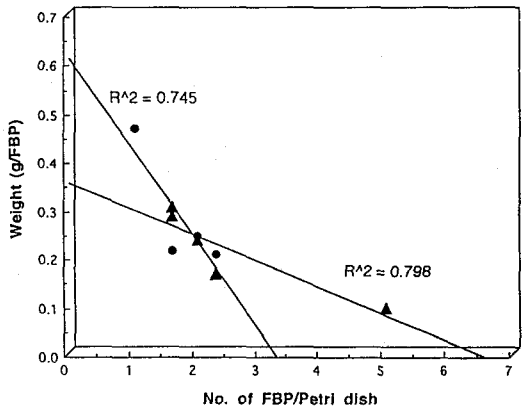


Fig. 1. Relationship between number and weight of fruit body primordia (FBPs) of *G. lucidum*, isolate Gl-024 (▲) and Gl-028 (●) formed on one Petri dish. Weight of a FBP measured at 30th days from inoculation.

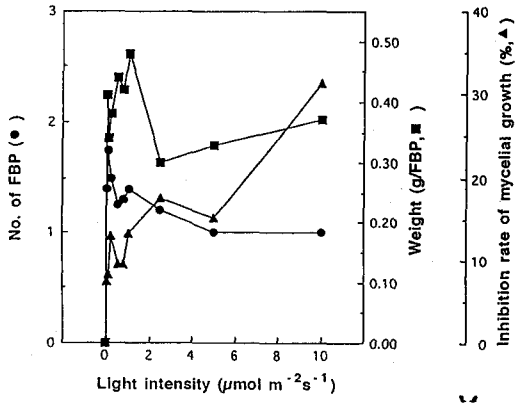


Fig. 2. Effect of light intensity on fruit body primordia (FBP) development and mycelial growth of *G. lucidum*, isolate Gl-009. D-L lamp was used as light source. Inhibition rates of mycelial growth were calculated according to the following formula:

Inhibition rate(%) =

$$\left(1-\frac{\text{Mycelial growth under light condition}}{\text{Mycelial growth under dark condition}}\right)\times 100$$

Four or five replications were made in all experiments. Each value in all data expressed by the mean with standard deviation.

Results

Effect of irradiation with colored fluorescent lamps on FBP formation

The effect of the light quality on FBPs formation of *G. lucidum* isolates were grown on CM for 30 days under the different colored fluorescent lamps or in the dark are shown in Table 1. All isolates did not induced FBPs in dark but induced those under visible light from P-B to P-R fluorescent lamps. However, the isolates were shown different response to light quality. Isolate Gl-009, Gl-024 and Gl-028 induced FBPs under BLB light. Isolate Gl-003 and Gl-009 induced many FBPs under P-B fluorescent light, while the other two isolate Gl-024 and Gl-028 induced more FBPs under P-Y or P-R fluorescent lamps as compared with P-B fluorescent lamp. Isolate Gl-012 was not shown any difference on FBP number. As the number of FBPs in a colony increased, the weight of the FBP reduced (Fig. 1).

Relationship between light intensity and FBP formation

Isolate Gl-009 was incubated under continuous light with various intensities for 30 days (Fig. 2). Mycelial growth was inhibited gradually with increase in light intensity. The FBP was observed at light intensity from 0.05 to 10.0 μmol m^{-2} s^{-1}, but the number and weight of FBP reduced when light intensity was higher than 0.5 and 1.0 μmol m^{-2} s^{-1}, respectively. The maximum number and weight of FBP were recorded in the dim light ranged from 0.1 to 0.25 and 0.1 to 1.0 μmol m^{-2} s^{-1}, respectively.

Effect of periodic light and dark exposures on FBP formation

The effect of periodic photo-illumination on FBPs formation of isolate Gl-009 was shown

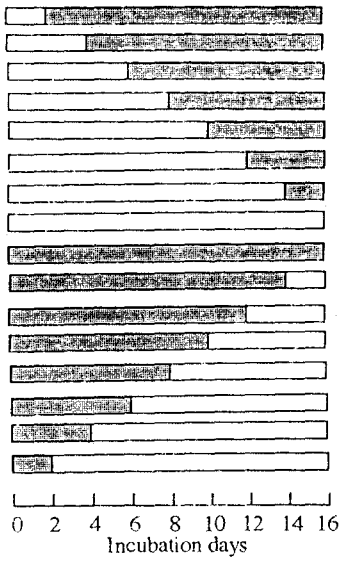
Table 2. Effect of periodic photo exposures on mycelial growth and fruit body primordia (FBP) development of *G. lucidum*, isolate Gl-009

Light-Dark (hr)[1]	Inhibition rate[2]	FBP[3]		
		Initial day	Number	Weight (g)
0~24	0	-	0	0
8~16	31.2±5.8	10	1.8±0.4	0.66±0.11
16~8	42.4±5.9	9	1.6±0.5	0.70±0.08
24~0	41.6±3.5	10	1.4±0.5	0.47±0.05

[1]Period of light and dark per day. P-B fluorescent lamp (light intensity; 3.5 μmol^{-2}s^{-2}) was used as a light source.
[2]Inhibition rates of mycelial growth were calculated by the formula given in Fig. 2. Average diameter of colony in the dark was 64.3 mm.
[3]The initial day that FBP was formed. Weight of a FBP measured at 30th days from inoculation. The parameters of number of FBP are the same as that in Table 1.

in Table 2. Mycelial growth was strongly inhibited by periodic photo-exposures for 8 or 16 hrs. The number and weight of FBP under periodic photo-illumination increased a little as compared with continuous light. The relationship between light illumination period and FBP formation of isolate Gl-009 was shown in Table 3. When the isolate was incubated in light after inoculation followed by transferring to dark, the period of light illumination for at least 4 days was necessary in FBP formation. The formation of FBPs when the isolate was transferred to light illumination after incubation in darkness for 2 to 8 days was less than those under the light illumination without pre incubation in darkness. But it was faster in cultures pre incubated under darkness than those in illumination after inoculation. FBPs occurred at 3 to 6 days after light illumination after dark pre-incubation though the FBPs were initiated at about 10 days from light incubation without pre-incubation in darkness. The

Table 3. Relationship between light illuminating periods and formation of fruit body primordia (FBP) of *G. lucidum*, isolate Gl-009

Light and dark treatment[1]	FBPs[2]	
	Initial day	Number
	-	0
	*	2.7±0.5
	*	2.3±0.5
	*	2.0±1.4
	*	1.7±0.5
	10	1.7±0.5
	10	1.7±0.5
	10	1.0±0.0
	-	0
	-	0
	-	0
	-	0
	11	1.0±0.0
	10	0.3±0.5
	9	1.0±0.8
	8	1.0±0.0

[1]P-B fluorescent lamp (light intensity; 3.5 μmol^{-2}s^{-1}) was used as a light source.
▒: Dark, □: Light
[2]Parameter used is the same as that in Table 1 and 3.
*Initial day was not determined because FBPs was formed in the period of dark.

FBPs could induced even the dark when transferred to darkness after light illumination for 4 to 10 days.

Effect of monochromatic light irradiation on FBP formation

To determine more precisely the light quality effective for the formation of FBP in *G. lucidum*, five isolates were incubated under continuous monochromatic light with different wavelength for 30 days (Table 4). All isolates produced FBPs in blue region of the wavelength from 400 to 500 nm. Isolate Gl-

Table 4. Effect of monochromatic irradiation on formation of fruit body primordia (FBP) by *G. lucidum* isolates

Wave length[1)]	Formation of FBP[2)]				
	Gl-003	Gl-009	Gl-012	Gl-024	Gl-028
Dark	−	−	−	−	−
400	+	+	−	+	+
450(430)	+	+	+	+	+
500(480)	+	+	−	+	+
550(530)	+	−	−	−	−
600(570)	+	−	−	−	−
650(630)	+	−	−	−	−
700(680)	+	+	−	+	+
750(690)	+	+	+	−	−
800(690)	+	−	−	−	−

[1)]Numbers indicate the peak wavelength (nm) of each interference filter and the lower cut wavelength (nm) is given in parenthesis, respectively.

[2)]All cultures were grown under light in combination with interference and colored glass filters for 30 days. Intensity of the monochromatic radiation was 0.3 μmol $m^{-2}s^{-1}$.

−: Only vegetative growth, +: Formation of FBPs

003 that formed FBPs under P-B and P-G lamps produced FBPs in a wide range of wavelength from 400 to 800 nm (Table 4). However, isolate Gl-009, Gl-024 and Gl-028 that formed FBPs under almost all fluorescent lamps had two effective regions in FBP formation. The two regions are short (400 to 500 nm) and long (700 to 750 nm) wavelength bands. Isolate Gl-012 which did not form FBPs under BLB lamp had also two effective regions in FBP formation such as short (450 nm) and long (750 nm) wavelength bands.

Discussion

Many basidiomycetes show a positively phototrophic reaction for fruiting, which causes the fruit bodies to grow toward light. Of course, light acts as an inducer/stimulator or an inhibitor on the morphogenesis (Durand and Furuya, 1985). With a some exception of a few basidiomycetes including *Agaricus bisporus* which do not require light for fruiting, most basidiomycetes such as *Coprinus* spp. (Tsusue, 1969; Manachère, 1980), *Favolus arcularius* (Kitamoto *et al*, 1968 and 1972), and *Schizophyllum commune* (Raudaskoski and Viitanen, 1982; Raudaskoski and Yli-Mattila, 1985) show light-dependent morphogenesis during the their life cycle. In *G. lucidum*the development of fruit body is very sensitive to light. The pileus of *G. lucidum* has phototropism during the artificial cultivation (Hemmi and Tanaka, 1936). Under dim light or in the dark, the pileus is unexpanded and often form abnormal pileus called stag-horn or antler-type fruitbody (Hemmi and Tanaka, 1936). On the other hand, *G. lucidum* also induced FBPs or AFSs with basidiospores as well as inhibited mycelial growth under light and ventilation in vitro culture, (Shin and Seo, 1988a; Seo *et al*, 1995a).

Although *G. lucidum* isolates formed FBPs in blue to red regions under fluorescent lamp, the results of monochromatic irradiation showed that the light quality is effective for FBP initiation in short (400-500) and long (700-750) wavelength regions. But this had exception in a isolate (Gl-003) which formed FBPs in broad region from 400 to 800 nm. On the other hand, the number of FBP of *G. lucidum* increased remarkably under long wavelength region. Because of, however, restricted nutrient condition in media, FBPs were not progressed to more differentiation. Furthermore, the weight of FBP that formed under long wavelength region was lighter than those of under short wavelength region such as P-B light. Fruit bodies for magnificent commercial goods of *G. lucidum* re-

quired largest and heavier than number in artificial cultivation. Therefore, the short wavelength lights such as blue region may be recommendable for fruit body production in *G. lucidum* as effective lights.

Fruit body development in most basidiomycetes is described as one of the photomorphogenetic responses to blue region and near ultraviolet light (Aschan-Åberg, 1960; Tsusue, 1969; Eger-Hummel, 1980; Durand and Furuya, 1985; Yli-Mattila, 1985 and 1990). However, in a few fungi such as *Melanotus* sp. (Newman, 1968) and *Sphaerobolus stellatus* (Ingold and Nawaz, 1967), blue, yellow and red regions were shown to be effective wavelength for fruit body development. Leatham and Stahmann (1987) reported that red light stimulated and blue light inhibited fruiting of *Lentinula edodes* on low calcium media, whereas blue light stimulated the fruiting on high calcium media. These results suggest that action spectra for fruiting in basidiomycetes vary according to species and environmental conditions such as nutritional and ventilation condition.

Optimum light intensity for the initiation and development of fruit bodies of basidiomycetes also vary according to isolate, species, and a kind of light (Suzuki, 1979). In most basidiomycetes, formation of fruit body primordia was induced and stimulated by higher irradiation of light. However, extremely strong irradiation inhibited and delayed the formation of fruit body primordium (Kitamoto *et al*, 1968; Kitamoto *et al*, 1972 and 1985). On the other hand, fruit body primordium initiation of *Coprinus congregatus* was also stimulated by higher irradiation, but the developmental stage such as sporulation was inhibited by the same light intensity as that for induction of fruit body primordium (Durand and Furuya, 1985). In this study, induction of FBP formation in *G. lucidum* show significant difference by increasing to light intensity. However, number of FBP was decreased with increasing of light intensity.

Flavins (Briggs, 1976) and the pigment pterin (containing the pteridine ring; Ninnemann, 1987; Yli-Mattila, 1990) have been proposed as photoreceptor candidates for UV-A and blue light response in fungi. On the other hand, yellow and/or red lights have an effect on fruit body development in some basidiomycetes (Ingold and Nawaz, 1967; Leatham and Stahmann, 1987). In addition, a semiquinon form of the photochromic flavoprotein and reduced cytochromes (Galland *et al*, 1989) has been documented as photoreceptor candidates for yellow or red light response in fruiting of some fungi. Although isolates of *G. lucidum* formed FBPs under blue and red lights, whether these photoreceptor candidates are associated with FBP formation is unclear.

Recently, Seo *et al* (1995a) reported that *G. lucidum* isolates could be divided into three groups by their photomorphogenetic responses. The first group is those which induced well-developed AFS under light and ventilation condition. The second group are those which induced FBP under light condition. The third group are those which progressed only to vegetative mycelial growth even in light condition. Moreover, the capacity and action spectra for AFS formation in first group were clearly different according to the isolates examined (Seo *et al*, 1995b). Isolates which is classified into each group were confirmed as same species by dikaryotization with di-mon test (unpublished data). These result suggests that *G. lucidume* are heterogeneous in light response.

적 요

Ganoderma lucidum 균주는 인공 배지 상에서

광에 의하여 자실체 원기가 형성되었으며, 광질이 자실체 원기 형성에 미치는 영향을 조사한 결과, 자실체 원기 형성능을 가지고 있는 5균주중 3균주는 공시한 모든 형광등의 아래에서 즉, BLB, 순청색, 순녹색, 순황색, 순적색 형광등의 아래에서 자실체 원기가 형성되었고, 2균주는 BLB 형광등을 제외한 가시광선 영역에서 형성되었다. 그러나 암상태에서는 공시한 모든 균주가 자실체를 형성하지 않았다. 자실체 원기는 광도 0.05에서 10.0 μmol m^{-2} s^{-1}의 범위에서 형성되었고, 광도가 0.5 μmol m^{-2} s^{-1} 이상에서는 자실체 원기의 수가 감소하였다. 주기적인 광조사가 자실체 원기의 형성에 미치는 영향을 조사한 결과, GI-009 균주는 자실체 원기의 수와 무게가 연속광을 조사한 경우보다 증가하였다. 자실체 원기를 형성하기 위해서는 최소 4일간의 광조사가 필요하였다. 단색광의 조사가 자실체 원기의 형성에 미치는 영향을 조사한 결과 GI-003 균주는 400에서 800 nm의 모든 단색광 처리구에서 자실체 원기가 형성되었고, 그 외의 4균주는 400에서 500 nm, 그리고 700에서 750 nm 범위에서 형성되었다.

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