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Effect of concentrated carbon dioxide exposure on the mycelial growth and fruit body initiation of *Ganoderma lucidum*.

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이산화탄소 농도가 영지버섯균의 균사생장과 자실체원기 유도에 미치는 영향

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ABSTRACT : The effect of CO_2 concentration (500, 3,000, 6,000㎕/ℓ) on the mycelial growth and fruit body primordium formation of *Ganoderma lucidum* on nutrient agar medium was examined. Optimum CO_2 concentration for vegetative growth was above 3,000㎕/ℓ. Fruit body initiation was accelerated at higher than 3,000㎕/ℓ CO_2 exposure but the maximum number and size of primordia, and primordium color were not influenced by CO_2 concentrations. Whereas each atypical fruiting structure forming stock culture showed different fruiting time under each concentration of CO_2 exposure.

KEYWORDS : *Ganoderma lucidum*, CO_2 concentration, mycelial growth, fruit body initiation

Carbon dioxide is one of important environmental factors that affect on growth and morphogenesis of Basidiomycetes. Excess CO_2 inhibits the fruiting; especially fruit body development, and sometimes generates abnormal fruit bodies in Basidiomycetes (Tschierpe, 1959; Niederpruem, 1963; Tschierpe and Sinden, 1964; Taber, 1966; Long and Jacobs, 1974; Kinugawa et. al., 1986, 1994). On the contrary, primordium formation of *Sphaerobolus stellatus* is inhibited when CO_2 is eliminated from air(Ingold and Nawarz, 1967). Hintikka and Korhonen(1970) found that vegetative growth of lignicolous basidiomycetes was markedly stimulated under higher CO_2 concentration while that of litter inhabiting basidiomycetes was suppressed severely in proportion to the increment of CO_2 concentration.

In vitro culture under the ventilation and light irradiation, the colonies of *Ganoderma lucidum* form atypical fruiting structure(AFS) bearing basidiospores without the basidiocarp formation and fruit body primordium(FBP)(Shin and Seo, 1988; Seo *et al.*, 1995). In AFS forming isolates, the colonies of *G. lucidum* formed AFS on the agar media. Basidia differentiated directly from generative hyphae on the outside of AFS. According to light response, *G. lucidum* isolates are divided into three groups; AFS-forming isolates, FBP forming isolates and chlamydospore forming isolates without fruiting on the agar media(Seo *et al.*, 1995). For the FBP formation, only light irradiation is essential, but for AFS formation both light irradiation and ventilation are required(Seo *et al.*, 1995). It has been well known in commercial cultivation that the fruit body formation of *G. lucidum* is inhibited even under weak semi-anaerobic condition. A major factor that reflects the inhibitive effect by bad ventilation has not been clarified in *G. lucidum* although excess CO_2 is speculated to be a major inhibitive factor.

Thus the present study is conducted to examine the effect of carbon dioxide on the mycelial expansion and AFS and FBP formation of *G. lucidum*, a white rot fungus, to elucidate the major inhibitive factor under bad ventilation.

Materials and Methods

Isolates

The dikaryon isolates of *G. lucidum* used in this study are listed in Table 1. Two isolates, Gl-009 and Gl-012, and two isolates, Gl-010 and Gl-020 were

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used as FBP and AFS forming isolate, respectively (Seo *et al.*, 1995). Isolate Gl-015 that forms AFS even under dark condition was isolated from white fruit body (Seo *et al.*, 1995).

Culture condition

All isolates were cultured and maintained on a nutritionally complete agar medium (CM) as reported previously (Seo *et al.*, 1995). Mycelial disks (6 mm in diameter) were placed in 90 mm plastic Petri dishes containing about 30ml of CM, and were incubated at 27 ±1℃ for 20 days under light and ventilated condition. As light source, white fluorescent lamp (FL 20 SD, Matsushita) was employed. The light intensity of fluorescent lamps was adjusted to about 500 to 1,000 lux by controlling the distance between lamps and the Petri dishes. The relative humidity was adjusted at 80 ±5% and CO_2 levels in the air of growth cabinets were maintained about 550, 3,000 and 6,000 ㎕/ℓ, respectively. Oxygen concentrations in all growth cabinets were maintained at about 20% until end of experiments. To examine the effect of concentrated CO_2 on mycelial growth, diameter of colony was measured by 10 days of inoculation. In each experiment, ten or fifteen replicates were applied for each CO_2 exposure.

Results and Discussion

The CO_2 optimum for vegetative growth of stock cultures G1-010, G1-012 and G1-020 was 3,000 ㎕/ℓ. Whereas, that for vegetative growth of stock cultures G1-009 and G1-015 was higher than 6,000 ㎕/ℓ (Fig. 1). Hintikka and Korhonen (1970) found that vegetative growth of lignicolous basidiomycetes was markedly stimulated at around 10% CO_2 but that of soil- and litter-inhabiting fungi was suppressed in proportion to the increment of CO_2 concentration of atmosphere (0.03%).

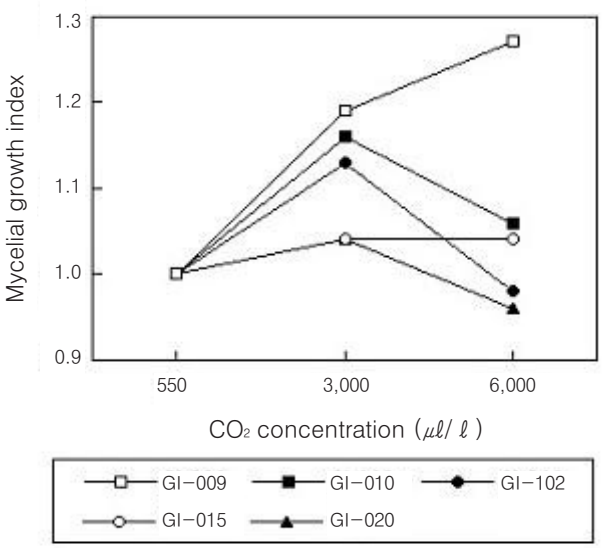

Fig. 1. Effect of different concentration of CO_2 exposure on mycelial growth of *G. lucidum*.
All cultures were incubated under light condition for 10 d on complete medium(CM; 2% glucose, 0.2% yeast extract, 0.2% peptone, 0.0046% KH_2PO_4, 0.01% KH_2PO_4, 0.005% $MgSO_4 \cdot 7H_2O$). All cultures were replicated 10 times. The light intensity was adjusted to about 500 to 1,000 lux. Mycelial growth index = mycelial growth rate of the control/mycelial growth rate on the CO_2 enriched conditions.

Mycelial expansion rates of *Flammulina velutipes, Pleurotus ostreatus, Pholiota nameko*, and *Hypsizygus marmoreus* have no significant difference between 600 ㎕/ℓ and 6,000 ㎕/ℓ CO_2 exposure (Kinugawa *et al.*, 1986). This suggests that the CO_2 optimum for vegetative growth *G. lucidum* is very low comparing with those of other wood rotting fungi. There may be an inverse relationship between vegetative growth and fruiting in fungi. The obtained data, however, indicate that this relationship cannot be always observed in *G. lucidum* (Fig. 1; Table 2).

Table 1. Isolates of *G. lucidum* used in this experiment

Isolate	Morphogenesis on agar media[1)]	Sources	Collected country
Gl-009	FBP-forming	Wild fruit body	Japan
Gl-010	AFS-forming	Cultivated fruit body	Korea
Gl-012	FBP-forming	Wild fruit body	Japan
Gl-015	AFS-forming	White fruit body	Korea
Gl-020	AFS-forming	Wild fruit body, TMI-50087	Japan

[1)] Mophogenesis on agar media were confirmed by Seo *et al.*, (1995), AFS; Atypical fruiting structure bearing the basidiospores, FBP; Fruit body primordium.

Table 2. Effect of different concentration of CO_2 ($\mu\ell$/ ℓ) exposure on the formation of AFS and FBP of *G. lucidum*[1)]

Isolate	AFS formation at different CO_2 concentration ($\mu\ell$/ ℓ)			FBP formation at different CO_2 concentration ($\mu\ell$/ ℓ)		
	550	3,000	6,000	550	3,000	6,000
Gl-009	-	-	-	-	++	++
Gl-012	-	-	-	+	++	+++
Gl-010	-	+	+	-	-	-
Gl-020	+++	++	+	-	-	-
Gl-015	+++	+++	+++	-	-	-

[1)] All cultures were incubated under light condition for 20 d. -; only vegetative growth, +; 10~39% forming (AFS- or FBP-forming Petri-dish / total replicate X 100), ++; 40~69% forming, +++; 70~100% forming.

When *G. lucidum* cultured under semi anaerobic condition, i. e., cultured in the Petri dish double sealed with Para film(American National Can), the mycelial growth was accelerated. This stimulation may base on the gaseous environments such as decrease of O_2 concentration, and increase of air humidity and various volatile substances, and not be caused merely by the increment of CO_2 concentration. FBP forming stock cultures formed FBP earlier under the higher CO_2 concentration than atmospheric condition(Table 2). No significant difference was observed on the number, size and color of FBP among different CO_2 concentrations(Table 2). AFS formation of stock culture G1-015 was not influenced under among different CO_2 concentrations (Table 2). AFS formation of stock culture G1-020 decreased with the increment of CO_2 concentration (Table 2). Stock culture G1-010 formed primordia at 3,000 and 6,000 $\mu\ell$/ ℓ CO_2 exposure, not at 550 $\mu\ell$/ ℓ CO_2 exposure(Table 2). In previous study(Seo *et al.*, 1995), the AFS formation of *G. lucidum* was inhibited but the mycelial growth was rather accelerated by preventing aeration, i. e., cultivation in the Petri dish double-sealed with Para film. The difference in the results between present research and previous one suggests that this may be derived from excess CO_2 accompanying other gaseous environments, and this can not be caused merely by the increment of CO_2 concentration.

Fruiting of *G. lucidum* also greatly affected by light and CO_2 level in cultivation house(Hemmi and Tanaka, 1936; Stamet, 1993). It has been observed that a long stipe and a small pileus, so called antler type fruit body were formed in aeration prevented artificial cultivation of *G. lucidum*, but has not been confirmed that whether those abnormal fruit bodies derived from higher CO_2 level or genetic backgrounds. In many basidiomycetes, stipe elongation and inhibition of pileus development by higher level of CO_2 exposure have been also reported(Taber, 1966; Kinugawa *et al.*, 1994). FBP forming isolates formed FBP earlier under the higher CO_2 levels than atmospheric condition (Table 2). However, no significant differences were observed on the number, size and color of FBP among different CO_2 levels. In previous study (Seo *et al.*, 1995), the AFS formation of *G. lucidum* was inhibited by preventing aeration, while the mycelial growth was rather accelerated. Whereas, the FBPs were formed regardless of aeration. These results might that higher concentration of CO_2 does not affect fruit body primordium initiation and stipe elongation. AFS-forming isolates, Gl-010 and Gl-015 did not show significant differences on the AFS formation among different CO_2 levels. However, the exposure of higher concentration of CO_2 inhibited AFS formation in isolate Gl-020. Although we have disregard for effect of oxygen concentration(in this experiment maintained about 20%) in the growth cabinet, high concentrated CO_2 is effective for induction of fruit body primodium, but not AFS formation including sporulation of *G. lucidum*.

In conclusion, *G. lucidum* is very sensitive to excess CO_2 than other wood rotting fungi that have been examined and it shows somewhat similar tendency in the reaction to CO_2 concentration with litter and soil inhabiting fungi.

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