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Cultural Characteristics of Mycelial Growth by an Entomogenous Fungus, *Cordyceps pruinosa* Petch

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붉은자루동충하초의 균사생육에 관한 연구

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ABSTARCT

This study was carried out to obtain basic data on physiological characteristics for an artificial cultivation of fruiting body of *C. pruinosa*. *C. pruinosa* showed the most favorable growth on the MCM medium. The optimal condition for the mycelial growth was obtained at 25°C and pH 5.0, respectively. Carbon sources such as arabinose, mannose, xylose were favorable for stimulating a mycelial growth and fruiting bodies of *C. pruinosa*. Ammonium nitrate, ammonium citrate and ammonium tartrate of nitrogen sources also appeared to be good in the mycelial growth and fruiting body formation.

Key Words : Carbon source, *Cordyceps pruinosa*, Mycelial growth

Introduction

The caterpillar-shaped Chinese medicinal mushroom, *Cordyceps* species (DongChongXiaCao) looks like a worm in the winter and like a grass in the summer. Fruiting bodies have been regarded as popular folk or effective medicines used to treat human diseases such as asthma, bronchial and lung inflammation, and kidney disease (Zhu *et al.*, 1998b).

The fruiting body is derived from the pupa or larva of insects infected by the entomopathogenic fungus *Cordyceps* species. These fungi endophytically parasitize on dead or living caterpillars of the moth *Hepialus* spp. Spores of them germinate inside the caterpillars, filling the caterpillars with hyphae, and produce a stalked fruiting body (Li *et al.*, 1998). *C. sinensis* that grows on the larvae of *Lepidoptera* is one of the best known fungi used in traditional Chinese medicine for hundreds of years. It possesses many important pharmacological activities. It can modulate immune responses (Kuo *et al.* 1996), inhibit the growth of tumor cells (Bok *et al.*, 1999), enhance hepatic energy (Manabe *et al.* 1996), promote the secretion of adrenal hormones (Wang *et al.*, 1998) and possess hypotensive and vasorelaxant activities (Chiou *et al.*, 2000). Futhermore, reports illustrate that it can enhance reproductive activity and restore the impared reproductive function (Zhu *et al.*, 1998a; Huang *et al.*, 2001). Nevertheless the demand for *C. sinensis* has increased, the natural fruit bodies are expensive, scarce and partially difficulty in cultivation, so another *Cordyceps* sp. has been studied to substitute for *C. sinensis* fruiting body.

Various bioactive components were found in the genus *Cordyceps*. Cordycepin identified from *C. militaris* has several biological activities such as inhibition of RNA and DNA synthesis and suppression of viral replication (Kuo *et al.*, 1994). Galactomannan isolated from *C. cicadae* is shown to prevent the growth of sarcoma 180 mice (Huang *et al.* 1997). Polysaccharides purified from *C. ophioglossoides* have been reported as antitumor agents (Wu *et al.*, 2001).

C. pruinosa (Clavicipitaceae; Hypocreales; Ascomycotina) has received special attention for medicinal purpose due to its various physiological activites. The nucleoside derivative N^6-(2-hydroxyethyl) adenosine (HEA) isolated from it showed a Ca^{2+} antagonistic effect and negative inotropic response (Furuya *et al.* 1983).

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The artificial production of fruiting body of *C. pruinosa* has not yet been tried successfully by using living silkworm substrate. Therefore, the aim of this study was to obtain basic data on physiological characteristics for an artificial cultivation of fruiting body of *C. pruinosa*.

Materials and Methods

Isolates

The fruiting-bodies of *C. pruinosa* were collected at Mt. Halla in Jeju island and strain was isolated from conidiospores. This strain was cultured at 25°C on YMA (yeast-malt agar) medium (0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 1% dextrose, 2% agar, 1,000 ml of distilled water) slants. Subcultures were made routinely every 2 months.

Inoculation

The yeast-malt agar medium was autoclaved at 121°C for 15 minutes. After cooling, a piece of mycelia from the slant was inoculated on this agar medium plate to use as inoculum for the next step.

Selection of optimal media

Four different culture media were used to investigate a favorable growth of *C. pruinosa* (Table 1). The media were autoclaved for 15 minutes. at 121°C (15 psi pressure) before pouring into a Petri-dish. For inoculum, this fungus was grown for one month on the yeast-malt agar medium, and the center of each petri-dish was inoculated with one disk (5 mm dia.) of agar and mycelium obtained by using a sterile cork borer. Five inoculated Petri-dishes were incubated at 25°C. After one month of incubation, the mycelial growth was observed.

Effect of temperature on mycelial growth

A study on the effect of temperature on mycelial growth was carried out using the above selected medium, MCM medium. After autoclaving for 15 min at 121°C (15 psi pressure), 23 ml of the medium was septically poured into each Petri-dish. The inoculation were performed as the same method described above.

For incubation, each five inoculated Petri-dish was incubated at 15, 20, 25, and 30°C, respectively. After one month of incubation, the mycelial growth was observed.

Table 1. Composition of media used

Ingredient	Medium (g/l)*			
	MCM	MEA	PDA	YMA
$MgSO_4 \cdot 7H_2O$	0.5			
KH_2PO_4	0.46			
K_2HPO_4	1.0			
Peptone	1.0	5.0		5.0
Yeast extract	2.0			3.0
Malt extract		20.0		3.0
Dextrose	20.0			10.0
PDA (Difco)			39.0	
Agar	20.0	20.0		20.0

*MCM(Mushroom Complete Medium), MEA(Malt Extract Agar), PDA(Potato Dextrose Agar), YMA(Yeast Malt Agar)

Effect of pH on mycelial growth

MCM medium was used to screen pH value suitable for a favorable growth of *C. pruinosa*. A 5 mm diameter disc of an inoculum was isolated with cork borer from on MCM medium cultured under dark condition for one month, placed in the center of each agar plate of MCM medium adjusted to intervals of pH 1.0 in the range of pH 4~8 with 1 N NaOH or HCl, and incubated for one month under dark condition. Mycelial growth on agar was measured on the average of colony diameter of five plates, and each test was replicated two times.

Effect of Carbon source

Carbon source utilization by *C. pruinosa* was tested by the method described by Lilly & Barnett (1951). The medium contained : glucose 10 g, asparagine 2 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar 20 g and distilled water to make 1000 ml. Various carbon sources were substituted for glucose. Namely, based on each molecular weight of 13 different carbon sources, each carbon source was added to the basal medium at the concentration of 0.1 M per liter. The basal medium was adjusted to pH 5.0 and then autoclaved for 15 minutes at 121°C (15 psi pressure). Xylose was sterilized by millipore filtration to avoid their breakdown to glucose and furfural, respectively. The inoculum was obtained by growing the fungi for one month in Petri-dishes on the basal medium. Each Petri-dish was inoculated with 5 mm disk of the fungi. Five Petri-dishes of each carbon source were inoculated and five Petri-dishes of the basal medium lacking a carbon source were also inoculated for the control. The Petri-dishes were incubated at 25°C for one month after which the colony diameter of mycelia were measured as above.

Effect of nitrogen source

The ability of *C. pruinosa* to utilize differemt nitrogen sources for growth was also studied. Except for the substitution of 1% mannose as carbon source per 1000 ml, the basal medium was the same composition as the medium used for the carbon source requirements study. Various nitrogen sources were substituted for asparagine. Based on each molecular weight

of 9 different nitrogen sources, each nitrogen source was added to the basal medium at the concentration of 0.02 M per liter. The basal medium was adjusted to pH 5.0 and then autoclaved for 15 minutes at 121°C (15 psi pressure). All the other processes including the inoculation, incubation and measurement of mycelial growth were performed as the same method of carbon source study.

Results and Discussion

Selection of optimal culture media

Four different culture media which have been used for culture of mushrooms were tested to investigate a favorable growth of *C. pruinosa*. It was observed that mycelial growth of *C. pruinosa* was good on MCM medium and recorded colony diameter of 36.0 mm in one month incubation, but poor on YMA medium (Fig. 1). Lee *et al.* (2000) reported that a favorable growth of *C. scarabaeicola* could be obtained on the MCM culture medium. Consequently MCM medium was selected for the temperature and pH studies.

Effect of culture temperature

The mycelial growth of *C. pruinosa* cultured on MCM medium for one month at 4 different temperatures was favorable in the temperature of 20~25°C, and the optimal temperature was about 25°C. Mycelial growth of *C. pruinosa* was decreased rapidly above 30°C (Fig. 2).

Similar result was observed in *P. tenuipes*, *C. militaris* and *C. scarabaeicola* (Ban *et al.*, 1998; Sung *et al.*, 2002; Lee *et al.*, 2000).

Effect of pH

The mycelial growth of C. pruinosa was most favorable at pH 5.0, and unfavorable at pH 4.0 (Fig. 3). Ban *et al.* (1998) reported that pH value suitable for a favorable growth of *C. pruinosa* could be obtained in the range of pH 5.0~6.0. Lee

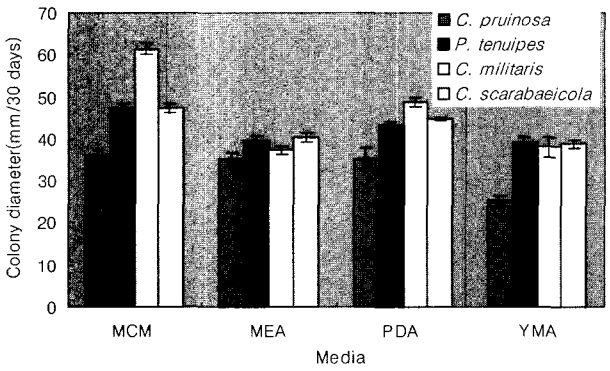

Fig. 1. Mycelial growth of *C. pruinosa* on different culture media.

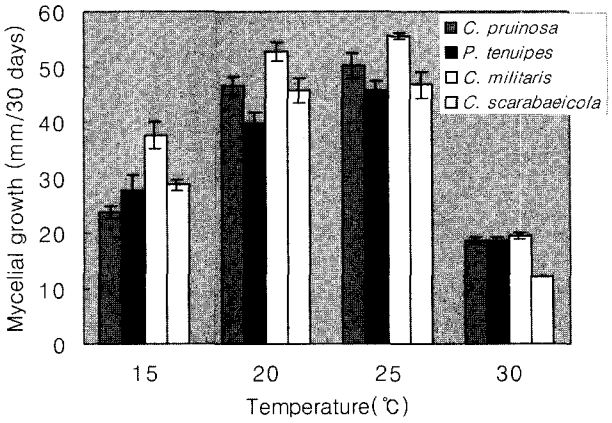

Fig. 2. Effect of temperature on mycelial growth of *C. pruinosa*.

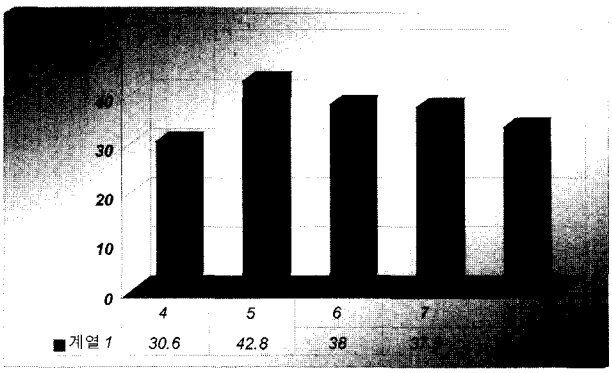

Fig. 3. Effect of initial pH on the mycelial growth of *C. pruinosa*.

Table 2. Effect of carbon source on the mycelial growth of *C. pruinosa*

Carbon source[a)]	Colony diameter[b)] (mm)	Mycelial density[c)]	Type of synemata
Arabinose	66.5±0.71	C	Individual
Dextrose	62.5±0.58	T	Bunch
Fructose	66.8±0.71	T	Bunch
Galactose	63.5±0.71	M	Bunch
Glucose	64.3±0.96	T	Bunch
Mannose	70.0±1.83	C	Bunch
Xylose	59.8±0.50	C	Bunch
Lactose	70.0±0.00	M	Individual
Maltose	61.5±0.71	T	Non-formation
Sucrose	20.0±0.00	T	Non-formation
Dextrin	63.0±0.00	T	Non-formation
Inuline	71.8±0.50	M	Individual
Soluble starch	55.0±0.00	T	Non-formation
Control	58.0±0.00	T	Individual

[a)]Each carbon source was added to the basal medium at the concentration of 0.1 M.

[b)]The colony diameter (Mean±SD) was measured after one month of incubation.

[c)]Mycelial density: T, thin; M, moderate; C, compact.

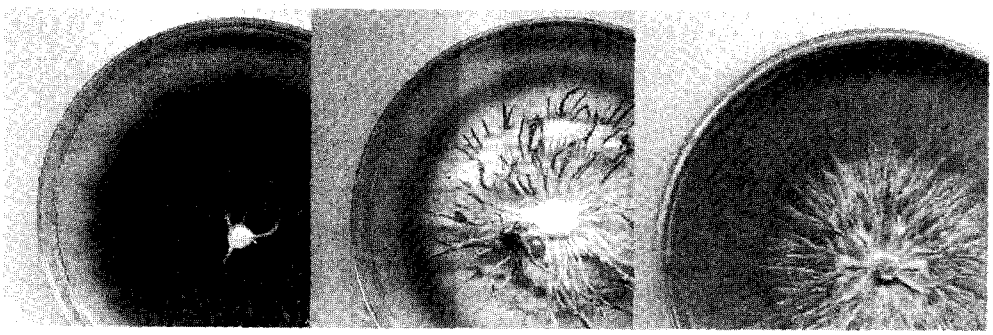

Fig. 4. Mycelia and synemata of *C. pruinosa* in culture media.

et al. (2000) suggested that pH range suitable for a favorable growth of *C. scarabaeicola* was from pH 7.5 to pH 9.0. Sung *et al.* (2002) obtained a favorable growth of *C. militaris* at pH 6.0~8.0 value.

Effect of carbon source

The mycelial growth of *C. pruinosa* was favorable on culture media contained each 13 carbon sources compared with the control (Table 2). The best growth of *C. pruinosa* was obtained on inuline, followed in order by lactose and mannose, but the mycelial density of *C. pruinosa* was best on culture media supplemented with arabinose, mannose and xylose. Synemata of bunch type were formed on media contained only monosaccharides such as dextrose, fructose, galactose, glucose, mannose and xylose. Therefore, the media contained carbon sources such as arabinose, mannose and xylose seem to be suitable in the mycelial growth, mycelial density and fruiting-body formation of bunch type (Fig. 4). The mycelial growth and mycelial density of *C. militaris* and *C. scarabaeicola* was good on the culture media supplemented with fructose or lactose as carbon source (Sung *et al.*, 2002; Lee *et al.*, 2000).

Effect of nitrogen source

Among the 9 nitrogen sources, the mycelial growth of *C. pruinosa* was best on the culture media which were supplemented with sodium nitrite and recorded colony diameter of 64.3 mm, and followed by calcium nitrate and urea except the control (Table 3). But the mycelial density of *C. pruinosa* was good on culture media contained ammonium citrate, ammonium nitrite and ammonium tartrate. Synemata of bunch type were formed on media supplemented with ammonium citrate, ammonium nitrate and ammonium tartrate. Therefore, it is considered that ammonium citrate was best for stimulating a mycelial growth and density, and synemata formation of bunch type. The mycelial growth and mycelial density of *C. militaris* and *C. scarabaeicola* was good on the culture media supplemented with potassium nitrate and ammonium phosphate, respectively (Sung *et al.*, 2002; Lee *et al.*, 2000).

Table 3. Effect of nitrogen source on the mycelial growth of *C. pruinosa*

Carbon source[a)]	Colony diameter[b)] (mm)	Mycelial density[c)]	Type of synemata
Ammonium chloride	31.0±1.07	M	Non-formation
Ammonium citrate	50.0±4.14	C	Bunch
Ammonium nitrate	39.1±2.75	C	Bunch
Ammonium sulfate	31.8±1.28	M	Non-formation
Ammonium tartrate	41.1±4.32	C	Bunch
Calcium nitrate	63.5±7.87	M	Non-formation
Potassium nitrate	61.8±2.19	M	Non-formation
Sodium nitrate	64.3±0.89	M	Non-formation
Urea	63.5±3.38	M	Non-formation
Control	65.8±1.39	T	Non-formation

[a)]Each nitrogen source was added to the basal medium at the concentration of 0.02 M.
[b)]The colony diameter (Mean±SD) was measured after one month of incubation.
[c)]Mycelial density: T, thin; M, moderate; C, compact.

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