Mycelial growth and fruiting body production of *Cordyceps militaris* in different culture chambers

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ABSTRACT

Cordyceps militaris is one of the most edible mushrooms which has been widely used in medicinal and therapeutic applications in China and South-East Asia, especially Thailand. More recently, its fruiting body production can be artificially cultivated using growth chambers. The aim of the present study was to determine efficiency of mycelial growth rate and fruiting body production of *C. militaris* (strain *Cm*1) under differentially modified artificial chambers. The experiment was performed by culturing the *Cm*1 on three different chambers, commercial growth chamber (T1), modified beverage cooler chamber (T2), and culture room (T3), with 10 biological replicates. The result revealed that its mycelial growth rate was significantly the highest and fastest growth on the fruiting body culture (FBC) medium surface incubated in the T3 chamber (90%) and followed by T2 chamber (70%) at the 1st week (in dark condition), compared to T1 (40%). At the 2nd week of cultivation (in light condition), aerial mycelia incubated in T1 and T2 chambers was able to produce yellowish or yellow pigments, but aerial mycelia incubated in T3 did not developed pigment as shown in white. At the 7th week of cultivation, both its fresh (17.78±1.26-19.05±1.04 g/bottle) and dry (1.77±0.40-1.90±0.32 g/bottle) weights harvested from all chambers were not statistically significant differences at p-value < 0.05. Our findings concluded that all studied growth chambers obtained similar results of both mycelial growth and fruiting body production (fresh

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and dry weight) of *C. militaris* strain *Cm*1 after two and seven weeks of cultivation on the FBC medium, respectively. This indicated that the modified beverage cooler chamber (T2) can be beneficially used as culturing chamber for *Cordyceps*, and can substitute the expensive commercial growth chamber.

Keywords: *Cordyceps militaris, mycelial growth rate, fruiting body production, culture chamber*

INTRODUCTION

Cordyceps militaris, entomopathogenic fungus belonging to Ascomycota, is one of the most edible mushrooms which has been widely used in medicinal and therapeutic applications in China and South-East Asia (Mehra et al., 2017). Its stroma (fruiting body) contains abundant bioactive compounds such as adenosine (Liu et al., 2015; Tatani et al., 2016), ergosterol, mannitol, and exopolysaccharide (Raethong et al., 2018), including cordycepin (Yuan et al., 2016). Of these compounds, cordycepin (nucleoside analog) is the most important biologically active metabolites, gained significant importance in immunological regulation (Ma et al., 2015), bacteriostasis (Chiu et al., 2016), anticancer (Luo et al., 2017), antiasthmatic effect (Tianzhu et al., 2015), antihyperuricemic (Yuan et al., 2016) and antihypoglycemic (Ma et al., 2015). The cordycepin can be chemically synthesized, but the processes are complicated with low-yield and high-cost, additionally, the natural fruiting bodies of *C. militar*is are very rare and costly to harvest (Jian et al., 2017; Lee et al., 2017). More recently, artificial cultivation methods for fruiting body production of *C. militaris* has been developed using synthetic media (Lee et al., 2013; Jian et al., 2017), and various parameters such as temperature (Adnan et al., 2017), humidity (Yang et al., 2014), and light intensity (Dang et al., 2018).

In general, these artificial cultivation methods for fruiting body production of *C. militaris* required three steps; 1) mycelial growth required the temperature at 22-25°C, moderate humidity of 65-70% for 7-10 days in dark condition; 2) induction of stroma formation required the temperature at 18-22°C, high humidity of 70-80% under a 18 hours light intensity of 400-500 lux, and 3) the growth of fruiting body required

the temperature at 10-25°C, high humidity of 80-90% under a 12-16 hours high light intensity of 800-1,000 lux (Kim et al., 2010; Hong et al., 2010). To exactly control the light intensities and temperatures in culture conditions, the growth chamber is most suitable application to produce high quality and quantity of fruiting body of *C. militaris* than the culture room, because it is less expensive and easier management. Since the commercial growth chambers are more expensive approximately two folds higher than the modified beverage cooler chamber, which has been modified by setting the light-emitting diode (LED), and the temperature controller, resulting that it was able to stably and consistently control and adjust light intensity and temperature respectively (Pradechboon et al., 2019). Therefore, the aim of the present study was to determine efficiency of mycelial growth rate and fruiting body production of *C. militaris* under differentially modified chambers.

METHODOLOGY

1) Fungal sample

Thai commercial *C. militaris* strain *Cm*1 used in the present experiments was obtained from Noparut et al (2018). The culture was stored at -80°C in tubes containing in a sterilized 15% glycerol and potato dextrose broth (PDB) medium (Sigma-Aldrich, USA).

2) Mycelial culture

Mycelium of *C. militaris* was activated growth on a sterilized PDA medium plate and incubated at 25°C in the darkness for 7 days. From the PDA plate, the regions of mycelial tip were punched out approximately 6 mm of PDA discs, and transferred into tube containing a sterilized PDB medium (3 mL). This mixture was cultured on a rotary shaker incubator (Daihan Scientific, Korea) under conditions of 19-21°C and darkness with shaking at 150 rpm for 3 days.

3) Fruiting body culture (FBC) medium

The FBC medium, containing round glass bottle with plastic cap (24 oz with diameter 8 cm), was prepared by mixing of 30 g brown-rice seed (Sungyod cultivar) and added liquid medium (25 mL), consisting of the boiled mixture (1 L) of 5 g peptone, 5 g yeast extract, 200 g potato, 20 g glucose, 20 g baby corn and 10 g silkworms. Meanwhile, the plastic lid of its bottle was holed by punching approximately 1.5 cm in diameter, and its hole was sealed by cotton. This brow-rice medium was sterilized by autoclaving at 120^{o}C for 20 minutes.

Subsequently, the mycelial culture medium (3 mL) was inoculated into the bottle containing the FBC medium. The bottle lid was sealed with two layers of polypropylene film before incubation with three different culture chambers.

4) Experimental design and statistical analysis

The experiment for determining efficiency of mycelial growth rate and fruiting body formation of *C. militaris* on three different culture chambers was performed by using CRD consisting of three treatments, commercial growth chamber (Treatment 1; T1) (MLR351 Sanyo, Japan), modified beverage cooler chamber (Treatment 2; T2) (Pradechboon et al., 2019), and culture room (Treatment 3; T3), with 10 biological replicates.

The mycelial growth rate (%) on FBC medium was measured every week of 4 weeks after culturing in different treatments. Its growth rate (%) was calculated by using the equation of [colony diameter (cm) / bottle diameter (8 cm)] x 100%. At the end of treatment (the 7th week), the mycelial pigmentation characteristic was accessed. Additionally, the fresh weight and dry weight of fruiting body was measured.

The data were statistically assessed using one-way analysis of variance (ANOVA), and mean comparisons between treatments were assessed by Duncan's Multiple Range Test (DMRT) at p-value less than 0.05 statistical significance using Statistical Product and Service Solution version 17.0 software (SPSS Inc., Chicago, USA). All values were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

In this present experiment, three different culture chambers were a commercial growth chamber (Treatment 1; T1), a modified beverage cooler chamber (Treatment 2; T2) (Pradechboon et al., 2019), and a culture room (Treatment 3; T3), were used to evaluate mycelial growth rate of *C. militaris*. The result revealed that its mycelium was significantly the highest and fastest growth on the FBC medium surface incubated in the T3 chamber (90%) and followed by T2 chamber (70%) at the 1st week (in dark condition) (Table 1), compared to T1 (40%). The reason of this might be that the T3 chamber obtained more sufficient air exchanges, which had affected to significantly increase the mycelial growth (Lin et al., 2018). Additionally, the result found that its growth rate of mycelia was similar from the 2nd until the 4th weeks after inoculation (corresponding to 100%) in all culture chambers (Table 1 and Figure 1). These indicated that three different culture chambers were suitable for mycelial growth of *C. militaris*.

At the 2nd week of cultivation (light condition), aerial mycelia on FBC incubated in T1 and T2 chambers produced yellowish or yellow pigments, but aerial mycelia in T3 did not developed pigment as shown in white (Figure 1). This was possible that the light intensity in T1 and T2 chambers was consistent (approximately 500 lux) around chamber because the side mounted LED lights were vertically arranged in both chambers, and easily maintained at light intensity during the day. This result indicated that consistent light intensity might significantly effect on diverse development of mycelial pigmentation of studied *Cordyceps* strain. A similar study was conducted by Chao et al (2019), who reported that high light intensity (1,000 lux) increased pigment production and accumulation of *C. militaris*.

At the 3rd and 4th weeks, the mycelia had started to form the fruiting body, which was subsequently developed to mature fruiting body (mushroom) at the 7th week (Figure 1). At the end of cultivation, the efficiency of different-culture chambers on fruiting body production of *Cm*1 was determined by comparing fresh weight, dry weights, and stromal-strip size. The result revealed that both its fresh weight and dry weight harvested from all different-culture chambers were not statistically significant differences at p-value < 0.05 (Table 2). However, the T3 chamber trended to obtain

slightly higher fresh weight and dry weight of fruiting body more than the T1 and T2 chambers. However, the T3 chamber obtained the longest stromal strip (approximately 7.11±0.47 cm length), which was significantly different from T1 and T2 chambers at p-value < 0.05 (Table 2). Moreover, the T3 chamber trended to have slightly higher both fresh weight (19.05±1.04 g/bottle) and dry weight (1.90±0.32 g/bottle) than T1 and T2 chambers.

This was possible that the humidity in T3 chamber was higher and more consistent (approximately 80-90%) than T1 and T2 by spraying water twice a day using a cool-mist humidifier (Vicks, USA). Similarly, high humidity (90%) was necessary to promote stromal development of *C. militaris* strain from Korean (Kang et al., 2017), and Thailand (Tapingkae, 2012). Therefore, the further study should be evaluate the humidity effect on fruiting body production of *C. militaris* strain *Cm*1 and other strains by setting the spray system (such as cool-mist humidifier) inside the commercial growth chamber (T1), the modified beverage cooler chamber (T2).

Table 1. Mycelial growth rate of *C. militaris* (strain *Cm*1) on FBC medium incubated at different-culture chambers

culture incubator	**mycelium growth rate (%) at**			
	1st wk.	**2nd wk.**	**3rd wk.**	**4th wk.**
T1	40%	100%	100%	100%
T2	70%	100%	100%	100%
T3	90%	100%	100%	100%

Note: T1, T2, and T3 represent commercial growth chamber, modified beverage cooler chamber, and culture room, respectively.

Table 2. Fresh weight, dry weight and size of fruiting body of *C. militaris* (strain *Cm*1) cultured on FBC medium for 7 weeks

Culture chamber	Fresh weight (g/bottle)	Dry weight (g/bottle)	Size of stromal strip (width/length) (cm)
T1	17.78±1.26[a]	1.77±0.40[a]	0.37±0.02[b] / 2.85±0.21[b]
T2	18.15±1.85[a]	1.81±0.58[a]	0.41±0.02[b] / 3.77±0.33[b]
T3	19.05±1.04[a]	1.90±0.32[a]	0.56±0.02[a] / 7.11±0.47[a]

Note: Values were expressed by mean ± standard error (SE), measured from fruiting body of *C. militaris* with ten biological replicates. The same letters in each column indicates non-significant difference of mean values at p-value < 0.05, calculated by using DMRT method. Whereas,T1, T2, and T3 represent commercial growth chamber, modified beverage cooler chamber, and culture room, respectively.

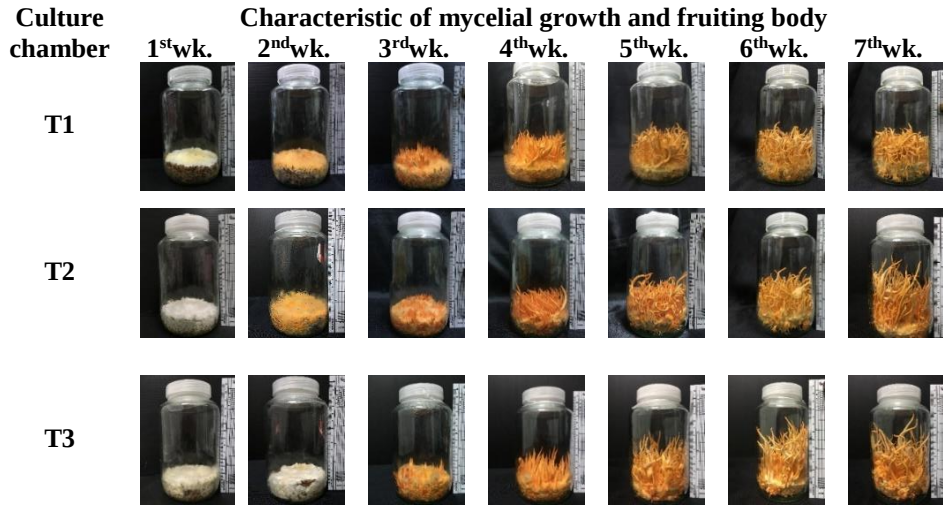

Figure1. Mycelial growth and fruiting body characteristic of *C militaris* (strain *Cm*1) cultured on FBC medium at different time points

Note: One representative sample of deca-replicates were randomly photographed to show in this figure.

CONCLUSIONS

These findings revealed that mycelia of *Cordyceps militaris* (strain *Cm*1) were completely grown (100%) around all surfaces of the fruiting body culture (FBC) after 2 weeks incubation in three different culture chambers (a commercial growth chamber; T1), a modified beverage cooler chamber; T2, and a culture room; T3). In addition, at the 7th week of cultivation, both its fresh (17.78±1.26-19.05±1.04 g/bottle) and dry (1.77±0.40-1.90±0.32 g/bottle) weights harvested from all chambers were not statistically significant differences at p-value < 0.05.

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