Mycobiology 39(2) : 85-91 (2011)
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DOI:10.4489/MYCO.2011.39.2.085
pISSN 1229-8093
eISSN 2092-9323

Growth and Cultural Characteristics of *Ophiocordyceps longissima* Collected in Korea

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(Received March 2, 2011. Accepted May 10, 2011)

We investigated the effect of nutritional and environmental factors on *Ophiocordyceps longissima* mycelial growth. The longest colony diameter was observed on *Schizophyllum* (mushroom) genetics complete medium plus yeast extract, *Schizophyllum* (mushroom) genetics minimal medium, and Sabouraud dextrose agar (SDA); however, malt-extract yeast-extract agar, SDA plus yeast extract, yeast-extract malt-extract peptone dextrose agar, SDA, oatmeal agar, and potato dextrose agar showed higher mycelia density. A temperature of 25°C was optimum and 7.0 was the optimum pH for mycelial growth. Colony diameter was similar under light and dark conditions. Maltose and yeast extract showed the highest mycelial growth among carbon and nitrogen sources respectively. The effect of mineral salts was less obvious; however, K_3PO_4 showed slightly better growth than that of the other mineral salts tested. Among all nutrition sources tested, complex organic nitrogen sources such as yeast extract, peptone, and tryptone were best for mycelial growth of *O. longissima*. *Ophiocordyceps longissima* composite medium, formulated by adding maltose (2% w/v), yeast extract (1% w/v), and K_3PO_4 (0.05% w/v) resulted in slightly longer colony diameter. *In vitro* mycelial *O. longissima* growth was sustainable and the production of fruiting bodies could be used for commercial purposes in the future.

KEYWORDS : Carbon source, Growth characteristics, Mineral salt, Nitrogen source, *Ophiocordyceps longissima*

Many *Cordyceps* species grow on nymphs (larvae) of cicada (Cicadidae, Homoptera) [1-3]. One is *C. longissima* Kobayasi, which was first reported from Japan on *Tanna japonensis* nymphs [2]. This species was later confirmed in Korea in 1998 [4]. One year later, it was reported in China together with its *Hirsutella* anamorph [5]. Li *et al*. confirmed the anamorph as *H. longissima* Li *et al*. [6]. Recently, Sung *et al*. transferred *C. longissima* to a new genus *Ophiocordyceps* based on a phylogenetic classification and renamed it *O. longissima* (Kobayasi) Sung *et al*. [7].

The stromata of *O. longissima* are 5~20 cm long, sometimes much longer (Fig. 1). The stroma of *O. longissima* is characterized by a long stalk with a terminal clavate fertile part without any clear demarcation between the two parts. Perithecia are ovoid to long ovoid, with a short neck, 440~590 × 130~300 μm; asci and secondary spores measure 190~350 × 5~6 μm and 8~11 × 1~1.2 μm respectively (Figs. 2 and 3).

Cordyceps species, including cicadicolous fungi such as *O. sobolifera*, are regarded as medicinal mushrooms in oriental society [2, 8-11]. In this context, many researchers have begun to study cultivation characteristics of *Cordyceps* and allied species [5, 12-23]. Within the past few years, *O. longissima* specimens have been collected by the Cordyceps Research Institute (CRI), Mushtech, Korea on Mt. Halla at Jeju-do and on Mt. Duryun at Jeollanam-do Korea. In this study, we provide detailed information on mycelial growth characteristics of *O. longissima* collected in Korea for the first time.

Materials and Methods

Fungal isolates. Multi-ascospore isolates were derived from fresh *O. longissima* specimens CRI C-6764, CRI C-7080, and CRI C-8587 following the method of Sung *et al*. [24]. Specimen CRI C-6764 was collected on Mt. Duryun at Jeollanam-do on July 8, 2001. Similarly, two other specimens, CRI C-7080 and CRI C-8587, were collected on Mt. Halla at Jeju-do on July 10 and 12, 2001 respectively. The specimens have been preserved at CRI, Mushtech, Korea. The multi-ascospore isolates, after growing on Sabouraud dextrose agar plus yeast extract (SDAY;

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Fig. 1. Various natural specimens of *Ophiocordyceps longissima* collected in Korea.

Fig. 2. Morphological characteristics of *Ophiocordyceps longissima*. A~C, Apical fertile part of stromata; D~F, Cross-section of stromata showing perithecia; G, H, Ascus heads; I, Threadlike fragmented ascospores.

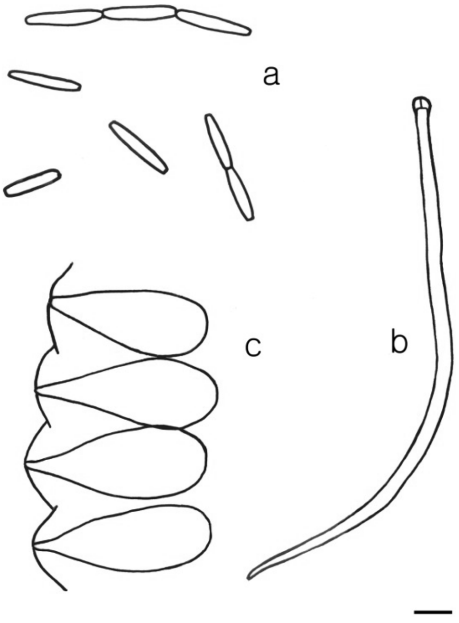

Fig. 3. Microscopic structures of *Ophiocordyceps longissima*. a, fragmented ascospores; b, ascus; c, perithecia (scale bar = 10 mm [a], 25 mm [b], 200 mm [c]).

dextrose 20 g, yeast extract 5 g, peptone 5 g and agar 15 g per 1,000 mL; pH 5.6) agar plates at 24 ± 1°C for 30 days, were used in the experiment.

Effect of medium, temperature, light, and pH on *O. longissima* mycelial growth. Nine different types of agar media were used to observe the growth characteristics of *O. longissima* isolates (Table 1). Mycelial discs (5 mm) of all three isolates were inoculated in the center of the agar media and incubated at 25°C for 30 days. Water agar (2%, WA) was used as the control. Colony diameter (CD) was measured in mm and mycelial density (MD) was qualitatively graded as thin (+), moderate (++), or compact (+++) after the incubation.

Schizophyllum (mushroom) genetics minimal medium (MM) and malt-extract yeast-extract agar (MYA) showed better mycelial growth and, hence, were used for selecting the optimum temperature for growth of the *O. longissima* isolates. Mycelial discs were inoculated on MM and MYA agar plates and incubated at various temperatures ranging from 15~35°C at regular intervals of 5°C for 30 days. Similarly, to observe the effect of light on growth, mycelial discs were inoculated on MM agar plates and incubated under continuous light and dark conditions for 30 days at 25°C. CD and MD were recorded after the incubation, as described above.

Liquid MM (100 mL MM without agar) was prepared in 250 mL Erlenmeyer flasks. The pH of the liquid medium was adjusted from 4.0~10.0 at intervals of 1.0 before sterilization. Five mycelial discs were inoculated in the liquid medium with different pH levels and incubated on a rotary shaker at 120 rpm for 30 days at 25°C. The liquid cultures were then filtered through Whatman no. 2 filter paper, the residual mycelia were dried at 60°C for 24 hr, and the dry weight (DW) of the mycelium was measured in g.

Selection of the optimum carbon source, nitrogen source, mineral salts, and carbon/nitrogen (C/N) ratio. *O. longissima* isolates were grown on WA supplemented with carbon sources (2% w/v) only. Additionally, 100 mL of MM liquid medium prepared with the carbon sources

Table 1. Synthetic media composition

Nutritional reagents	Medium (g/L)									
	WA	OA	MYA	MM	PDA	MCM	YMA	CDA	SDAY	SDA
Dextrose			4	20	20	20	10		20	20
Malt extract			10				3			
Sucrose								30		
Oatmeal flake		30								
Potato					200					
Peptone						2	5		5	5
Yeast extract			4			2	3		5	
$NaNO_3$								3		
DL-asparagine				2						
$MgSO_4 \cdot 7H_2O$				0.5		0.5		0.5		
KCl								0.5		
$FeSO_4 \cdot 7H_2O$								0.01		
KH_2PO_4				0.46		0.46				
K_2HPO_4				1		1		1		
Agar	20	20	15	20	20	20	20	20	15	15

WA, water agar; OA, oatmeal agar; MYA, malt-extract yeast-extract agar; MM, *Schizophyllum* (mushroom) genetics minimal medium; PDA, potato dextrose agar; MCM, *Schizophyllum* (mushroom) genetics complete medium plus yeast extract; YMA, yeast-extract malt-extract peptone dextrose agar; CDA, Czapek-dox agar; SDAY, Sabouraud dextrose agar plus yeast extract; SDA, Sabouraud dextrose agar.

(2% w/v) in 250 mL Erlenmeyer flasks were inoculated with the isolates. Similarly, isolates were inoculated in WA and MM liquid media supplemented with nitrogen sources (2% w/v) only. Different mineral salts (0.05% w/v) were also tested for their effect on mycelial growth of *O. longissima* isolates in WA and MM liquid media. Agar cultures and liquid cultures were incubated as described above.

Maltose and yeast extract were the best carbon and nitrogen sources respectively for the mycelial growth of *O. longissima* isolates. Hence, they were added together in the WA and MM liquid media at different ratios of 20 : 1, 10 : 1, 5 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 5, and 1 : 10 and inoculated with the isolates. Maltose concentration was fixed at 0.5%, 1.0%, and 2.0% (w/v) for all ratios. Agar and liquid cultures were incubated as described above, and WA medium was used as the control. CD and MD were recorded on agar cultures, and DW was measured in liquid cultures, as described above.

Comparison of *O. longissima* composite medium (OLCM) with MYA. OLCM was prepared by adding maltose, yeast extract, and K_3PO_4 at concentrations of 2% (w/v), 1% (w/v), and 0.05% (w/v), respectively, on WA. The *O. longissima* isolates were inoculated on OLCM and MYA agar plates and observed for CD and MD. WA was used as the control.

Results and Discussion

Selection of optimum medium, temperature, and pH. The CD of *O. longissima* isolates was relatively longer on *Schizophyllum* (mushroom) genetics complete medium plus yeast extract, MM, and Sabouraud dextrose agar (SDA); however, compact or moderate density was observed on MYA, SDAY, yeast-extract malt-extract peptone dextrose agar, SDA, potato dextrose agar (PDA), and oatmeal agar (Table 2). CD differed among the isolates; CRI C-7080 and CRI C-8587 grew faster than CRI C-6764. It was clear that media that induced a compact density produced a shorter CD compared to media that produced moderate density (Table 2), as shown in *C. militaris* isolates [25]. This result indicates that MD and CD of the isolates do not correlate with each other on agar culture. Czapek-dox agar (CDA) produced a thin density and the shortest CD in *O. longissima* isolates, as reported in other *Cordyceps* species [26, 27]. CDA does not contain an organic nitrogen source, which may be why it could not support rich growth of the *O. longissima* isolates. Li *et al*. [5, 6] showed longer CD of *O. longissima* isolates on Czapek agar than that on PDA, but did not report the MD. CD of *O. longissima* isolates in our study was generally longer than that of Li *et al*. [5, 6].

The longest CD was observed at 25°C (Fig. 4), agree-

Table 2. Effect of medium on *Ophiocordyceps longissima* mycelial growth

Medium	Isolate No.					
	CRI C-6764		CRI C-7080		CRI C-8587	
	CD	MD	CD	MD	CD	MD
MCM	31	++	50	++	34	++
MM	30	++	46	++	44	++
SDA	24	+++	28	+++	43	++
MYA	30	+++	37	+++	30	+++
OA	27	++	25	+++	36	++
PDA	32	+++	25	+++	36	++
SDAY	32	+++	18	+++	36	+++
YMA	25	+++	34	+++	26	+++
CDA	11	+	20	+	15	+
WA	11	+	20	+	11	+

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; MCM, *Schizophyllum* (mushroom) genetics complete medium plus yeast extract; MM, *Schizophyllum* (mushroom) genetics minimal medium; SDA, Sabouraud dextrose agar; MYA, malt-extract yeast-extract agar; OA, oatmeal agar; PDA, potato dextrose agar; SDAY, Sabouraud dextrose agar plus yeast extract; YMA, yeast-extract malt-extract peptone dextrose agar; CDA, Czapek-dox agar; WA, water agar.

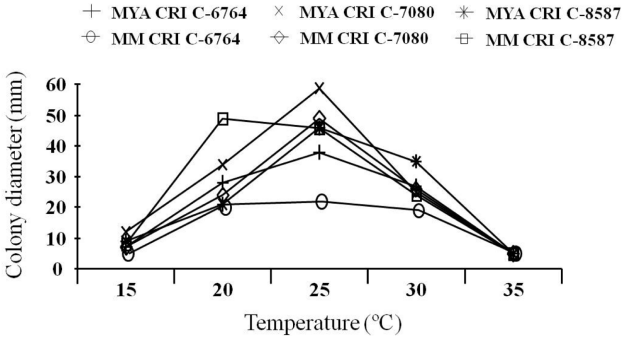

Fig. 4. Effect of temperature on mycelial growth of *Ophiocordyceps longissima* isolates cultured on malt-extract yeast-extract agar (MYA) and *Schizophyllum* (mushroom) genetics minimal medium (MM). CRI, Cordyceps Research Institute.

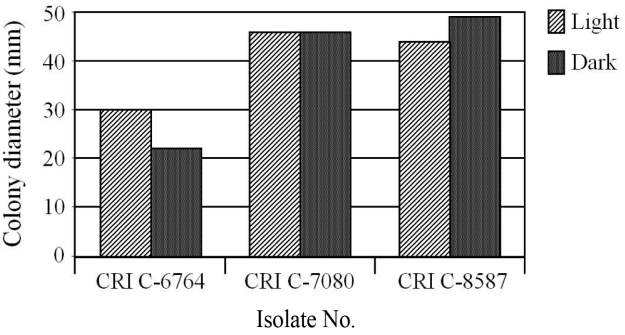

Fig. 5. Effect of light on colony diameter of *Ophiocordyceps longissima* isolates cultured on *Schizophyllum* (mushroom) genetics minimal medium. CRI, Cordyceps Research Institute.

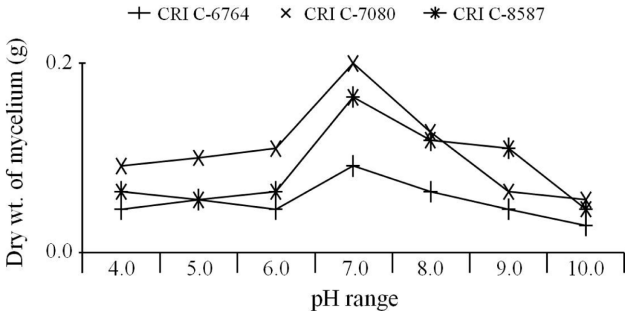

Fig. 6. Effect of pH on mycelial dry wt. of *Ophiocordyceps longissima* isolates cultured in liquid *Schizophyllum* (mushroom) genetics minimal medium. CRI, Cordyceps Research Institute.

ing with previous studies [5, 14, 24, 26, 28, 29]. *O. longissima* isolates had a similar CD at 20°C and 30°C, which was similar to *Metacordyceps yongmunensis* and *O. heteropoda* [27, 29]. However, this was in contrast to *C. cardinalis* that showed no growth at 30°C and above [26]. Almost no mycelial growth of *O. longissima* isolates occurred at 15°C and 35ºC (Fig. 4).

No obvious difference was observed in the CD of *O. longissima* between light and dark conditions (Fig. 5). The prime effect of light is the induction of pigmentation [25]. In our study, the *O. longissima* isolates produced reddish white pigmentation under light, similar to that observed by Li *et al*. [5, 6]. In addition to pigmentation, light also controls fruiting morphology, such as elongation and branching in culture [14]. Cycles of dark/light periods may be critical in some species to induce fruiting bodies [16]. A pH of 7.0 produced the highest DW, followed by pH 8.0, which was similar to previous studies (Fig. 6) [14, 26, 28].

Selection of optimum carbon source, nitrogen source, mineral salts, and C/N ratio. It was very difficult to observe the effect of carbon source on mycelial growth of *O. longissima* in agar culture because the CD was too short in all carbon sources tested, particularly for the CRI C-6764 isolate (Table 3). Furthermore, all carbon sources produced only a thin density of mycelia, showing that carbon sources do not sustain rich mycelial growth (Table 3). Among the carbon sources tested, maltose produced the highest DW in liquid culture, whereas the remaining carbon sources produced less than half of that of maltose. Based on DW, maltose was the best carbon source among those tested.

Among the nitrogen sources tested, yeast extract produced the highest mycelial growth, followed by peptone

Table 3. Effect of carbon source on *Ophiocordyceps longissima* mycelial growth

Carbon source	Isolate No.								
	CRI C-6764			CRI C-7080			CRI C-8587		
	CD	MD	DW	CD	MD	DW	CD	MD	DW
Maltose	6	+	0.1200	14	+	0.1083	10	+	0.0983
Dextrin	7	+	0.0513	10	+	0.0333	7	+	0.0360
Fructose	6	+	0.0487	8	+	0.0417	6	+	0.0530
Mannose	6	+	0.0467	15	+	0.0337	5	+	0.0427
Saccharose	6	+	0.0467	17	+	0.0330	11	+	0.0417
Glucose	6	+	0.0467	16	+	0.0397	9	+	0.0403
WA	7	+	0.0110	10	+	0.0113	7	+	0.0113

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; DW, dry wt. of mycelium; WA, water agar.

Table 4. Effect of nitrogen source on *Ophiocordyceps longissima* mycelial growth

Nitrogen source	Isolate No.								
	CRI C-6764			CRI C-7080			CRI C-8587		
	CD	MD	DW	CD	MD	DW	CD	MD	DW
Yeast extract	26	+++	0.1249	33	+++	0.2556	28	+++	0.2196
Peptone	16	++	0.0739	13	+++	0.0592	15	+++	0.0916
Tryptone	19	+++	0.0736	17	++	0.0732	18	+++	0.0598
$NaNO_3$	10	+	0.0434	7	+	0.0514	7	+	0.0542
KNO_3	14	+	0.0305	8	+	0.0603	9	+	0.0291
Glycine	7	+	0.0374	6	+	0.0459	6	+	0.0427
L-asparagine	8	++	0.0366	9	++	0.0393	10	++	0.0392
Ammonium tartrate	14	+	0.0305	9	+	0.0328	8	+	0.0244
WA	7	+	0.0114	10	+	0.0112	7	+	0.0113

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; DW, dry wt. of mycelium; WA, water agar.

and tryptone in both agar and liquid cultures (Table 4). Yeast extract, peptone, and tryptone also produced much better mycelial growth than the carbon sources. $NaNO_3$, KNO_3, glycine, and ammonium tartrate all produced short, thin densities, as with WA (Table 4). However, L-asparagine produced moderate density and a similar CD as $NaNO_3$, KNO_3, glycine, and ammonium tartrate. These results showed that complex organic nitrogen sources such as yeast extract, peptone, and tryptone sustain favorable growth of *O. longissima*, as in other *Cordyceps* and allied species [25-27, 29]. Yeast extract was selected as the best nitrogen source for mycelial growth of *O. longissima* isolates and was used for further tests. Mineral salts showed the poorest mycelial growth among all nutritional sources tested (Table 5). All mineral salts tested produced thin MD and CD, similar to WA. K_3PO_4 showed slightly better mycelial growth than that of other mineral salts. Notably, $CuSO_4 \cdot 5H_2O$ showed a rather shorter CD than that of WA in all isolates (Table 5).

O. longissima isolates produced compact MD at all C/N ratios (Table 6). However, C/N ratios of 2 : 1 and 1 : 1 showed the longest CD (Table 6). Nitrogen favors mycelial growth but higher nitrogen ratios slowed CD, probably due to higher nutrient concentrations; thus, increasing the water potential of the medium and consequently decreasing the amount of water available to the isolates. A 2 : 1 C/N ratio was selected with maltose and yeast extract at concentrations of 2% and 1%, respectively, to formulate the OLCM.

Table 5. Effect of mineral salt on *Ophiocordyceps longissima* mycelial growth

Mineral salt	Isolate No.					
	CRI C-6764		CRI C-7080		CRI C-8587	
	CD	MD	CD	MD	CD	MD
K_3PO_4	5	+	5	+	6	+
$MgSO_4 \cdot 7H_2O$	3	+	5	+	6	+
$CaCO_3$	3	+	6	+	4	+
K_2HPO_4	4	+	5	+	5	+
KH_2PO_4	4	+	5	+	5	+
$FeSO_4 \cdot 7H_2O$	2	+	4	+	3	+
$CuSO_4 \cdot 5H_2O$	2	+	2	+	2	+
WA	3	+	4	+	3	+

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; WA, water agar.

Table 6. Effect of carbon/nitrogen (C/N) ratio on *Ophiocordyceps longissima* isolate CRI C-7080 mycelial growth

C/N ratio	Maltose concentration					
	0.5%		1.0%		2.0%	
	CD	MD	CD	MD	CD	MD
20 : 1	32	+++	29	+++	28	+++
10 : 1	31	+++	31	+++	27	+++
5 : 1	30	+++	33	+++	31	+++
2 : 1	36	+++	40	+++	47	+++
1 : 1	35	+++	38	+++	43	+++
1 : 2	19	+++	20	+++	22	+++
1 : 5	19	+++	20	+++	19	+++
1 : 10	23	+++	21	+++	18	+++

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density.

Table 7. Comparison between *Ophiocordyceps longissima* composite medium (OLCM) and malt-extract yeast-extract agar (MYA)

Medium	Isolate No.					
	CRI C-6764		CRI C-7080		CRI C-8587	
	CD	MD	CD	MD	CD	MD
OLCM	39	+++	41	+++	40	+++
MYA	34	+++	38	+++	36	+++
WA	17	+	14	+	12	+

CRI, Cordyceps Research Institute; CD, colony diameter; MD, mycelial density; WA, water agar.

Comparison of OLCM with MYA. Both OLCM and MYA produced compact MD (Table 7). However, OLCM produced slightly longer CD than that of MYA. A higher concentration of yeast extract is likely favorable for *O. longissima* mycelial growth, as in *C. militaris* [25]. The medium is the most important factor for mycelial growth and fruiting body formation in *Cordyceps* species. Commonly used mycological media can support profuse mycelial growth in *Cordyceps* species. In contrast, cereals such as rice, supplemented with pupal power, sawdust, peptone, and yeast extract are used for fruiting body production [5, 14]. Fruiting medium is much more complex than mycelial growth medium. Obviously, fruiting body formation is a much more complex physio- and morpho-genetic process. As with mycelial growth, nitrogen sources are likely the most important nutritional factor for fruiting body formation. Fruiting body formation of *O. longissima* has been reported by Li *et al*. [5, 6] in rice medium. It is concluded that various nutritional and environmental factors should be tested to induce profuse fruiting bodies in *O. longissima*, so that it can be commercially utilized as a health and medicinal food.

Acknowledgements

We acknowledge the Cordyceps Research Institute (CRI), Mushtech, Korea for providing facilities to conduct this study.

References

1. Kobayasi Y. On the genus *Cordyceps* and its allies on Cica-

didae from Japan. Bull Biogeogr Soc Jpn 1939;9:145-76.
2. Kobayasi Y, Shimizu D. Monographic studies of *Cordyceps* 2: Group parasitic on Cicadidae. Bull Natl Sci Mus Tokyo 1963;6:286-314.
3. Sung JM. The insects-born fungus of Korea in color. Seoul: Kyohak Publishing Co., Ltd.; 1996.
4. Lee JB, Oh DC. Higher fungi of Cheju-do (1): unrecorded mushrooms. Kor J Mycol 1998;26:538-50.
5. Li CR, Huang B, Fan MZ, Li ZZ. *Cordyceps longissima* and its *Hirsutella anamorph*. J Anhui Agric Univ 1999;26:374-7.
6. Li CR, Fan MZ, Huang B, Li ZZ. *Hirsutella longissima* sp. nov., the anamorph of *Cordyceps longissima*. Mycosystema 2001;20:29-34.
7. Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B, Spatafora JW. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Stud Mycol 2007; 57:5-59.
8. Kiho T, Ukai S. Tochukaso (Semitake and others), *Cordyceps* species. Food Rev Int 1995;11:231-4.
9. Mizuno T. Medicinal effects and utilization of *Cordyceps* (Fr.) Link (Ascomycetes) and *Isaria* Fr. (Mitosporic fungi) Chinese caterpillar fungi, "Tochukaso" (Review). Int J Med Mushrooms 1999;1:251-61.
10. Kinjo N, Zang M. Morphological and phylogenetic studies on *Cordyceps sinensis* distributed in southwestern China. Mycoscience 2001;42:567-74.
11. Shrestha B, Zhang W, Zhang Y, Liu X. What is the Chinese caterpillar fungus *Ophiocordyceps sinensis* (Ophiocordycipitaceae)? Mycology 2010;1:228-36.
12. Yahagi N. Cultivation of *Isaria japonica* and inoculation to silkworm. Touchu-Kasou 1985;5:27-30.
13. Harada Y, Akiyama N, Yamamoto K, Shirota Y. Production of *Cordyceps militaris* fruit body on artificially inoculated pupae of *Mamestra brassicae* in the laboratory. Trans Mycol Soc Jpn 1995;36:67-72.
14. Yamanaka K, Inatomi S. Cultivation of *Isaria japonica* fruit bodies on mixed plant/insect media. Food Rev Int 1997; 13:455-60.
15. Yamanaka K, Inatomi S, Hanaoka M. Cultivation characteristics of *Isaria japonica.* Mycoscience 1998;39:43-8.
16. Kana-uchi A, Fukatsu T. Light-induced fruit body formation of an entomogenous fungus *Paecilomyces tenuipes*. Mycoscience 1999;40:349-51.
17. Chen R, Ichida M. Infection of the silkworm, *Bombyx mori*, with *Cordyceps militaris*. J Insect Biotechnol Sericology 2002;71:61-3.
18. Sato H, Shimazu M. Stromata production for *Cordyceps militaris* (Clavicipitales: Clavicipitaceae) by injection of hyphal bodies to alternative host insects. Appl Entomol Zool 2002; 37:85-92.
19. Yahagi N, Yahagi R, Takano F, Fushiya S, Tanaka T, Murakami K, Ohta T. Growth of ascocarps from cultured *Cordyceps militaris* (L.:Fr.) Fr. and *Cordyceps formicarum* Kobayasi in an agar medium. Nippon Kingakkai Kaiho 2004;45:15-9.
20. Li CR, Nam SH, Geng DG, Fan MZ, Li ZZ. Artificial culture of seventeen *Cordyceps* spp. Mycosystema 2006;25:639-45.
21. Lee JO, Shrestha B, Kim TW, Sung GH, Sung JM. Stable formation of fruiting body in *Cordyceps bassiana*. Mycobiology 2007;35:230-4.
22. Lee JO, Shrestha B, Sung GH, Han SK, Kim TW, Sung JM. Cultural characteristics and fruiting body production in *Cordyceps bassiana*. Mycobiology 2010;38:118-21.
23. Kim SY, Shrestha B, Sung GH, Han SK, Sung JM. Optimum conditions for artificial fruiting body formation of *Cordyceps cardinalis*. Mycobiology 2010;38:133-6.
24. Sung GH, Shrestha B, Park KB, Sung JM. Cultural characteristics of *Shimizuomyces paradoxus* collected from Korea. Mycobiology 2010;38:189-94.
25. Shrestha B, Lee WH, Han SK, Sung JM. Observations on some of the mycelial growth and pigmentation characteristics of *Cordyceps militaris* isolates. Mycobiology 2006;34:83-91.
26. Sung GH, Shrestha B, Han SK, Kim SY, Sung JM. Growth and cultural characteristics of *Cordyceps cardinalis* collected from Korea. Mycobiology 2010;38:274-81.
27. Sung GH, Shrestha B, Han SK, Sung JM. Cultural characteristics of *Ophiocordyceps heteropoda* collected from Korea. Mycobiology 2011;39:1-6.
28. Sasaki F, Miyamoto T, Tamai Y, Yajima T. Optimum temperature and pH for mycelial growth of *Cordyceps nutans* Pat. (Ascomycetes). Int J Med Mushrooms 2005;7:301-4.
29. Sung GH, Shrestha B, Sung JM. Characteristics of *Metacordyceps yongmunensis*, a new species from Korea. Mycobiology 2010;38:171-5.