Cultural characteristics of *Cordyceps militaris* strain ‘Yedang 3’ on various media and nutritional conditions

Byung-Joo Lee^{1*}, Mi-Ae Lee¹, Yong-Gyun Kim¹, Kwang-Won Lee¹,
Young-Sang Choi², Byung-Eui Lee³ and Ho-Yeon Song⁴

¹Crop Research Division, Chungcheongnam-do Agricultural Research & Extension Services, Yesan 340-861, Korea

²Yedang Mushroom Institute, Yesan 305-764, Korea

³Industry Academy Cooperation Foundation, Soonchunghyang University, Asan 336-745, Korea

⁴College of Medicine, Soonchunghyang University, Chunan 330-930, Korea

(Received September 13, 2013 / Revised September 21, 2013 / Accepted September 27, 2013)

ABSTRACT – The effects of environmental and nutritional requirement such as temperature, pH, different media, carbon, nitrogen, and carbon-to-nitrogen ratio on the mycelial growth of *Cordyceps militaris* strain ‘Yedang 3’ were studied. The optimum temperature and pH for the growth of mycelium were 20-25°C and pH 6-7, respectively. Out of ten media tested, mushroom complete media (MCM) was the best medium for fast mycelial growth, and Sabouraud’s dextrose agar yeast extract (SDAY), malt extract yeast extract agar (YMA) also were favored. The color and shape of colonies varied in different media. The best carbon sources for mycelial growth were fructose, mannitol, and sucrose, whereas the best nitrogen sources were tryptone and peptone. However, mycelia grew slowly in inorganic nitrogen compounds such as NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , and NaNO_3 . The optimum C:N ratio observed on the culture media was 30-40 range. These results provided basic information on cultural characteristics of vegetative growth and might be useful for spawn production in *Cordyceps militaris*.

KEYWORDS – Carbon-to-nitrogen ratio, *Cordyceps militaris*, Culture medium, Mycelial growth, Temperature

Introduction

Mushrooms have been sought and cultivated for the production of food consumption due to its nutritive and medicinal values (Fan *et al.*, 2006). *Cordyceps* species are important mushrooms traditionally used for healths and vitality. *C. sinensis* (syn. *Ophiocordyceps sinensis*) is the most highly sought-after species among them and its main bioactive metabolite is cordycepin (3'-deoxyadenosine). Cordycepin has been found to be pharmacologically functional such as anti-tumor, immunomodulation, anti-inflammatory, anti-virus, anti-leukemic, anti-cancer, anti-diabetic, preventing and treating of obesity (De Julian-Ortiz *et al.*, 1999; Masuda *et al.*, 2007; Wong *et al.*, 2010; Wu *et al.*, 2011; Yoo *et al.*, 2004). However, artificial production of fruiting bodies in *C. sinensis* has not yet to be achieved (Guo and Yang, 1999; Jiang and Yao, 2003; Liu *et al.*, 1999).

It was found that most of active constituent of *C. militaris* was similar to those of *C. sinensis* including cordycepin and even would be better in some medicinal compounds (Das *et al.*, 2010; Oh *et al.*, 2003; Patel and Ingallhalli, 2013; Yu *et al.*, 2006; Zhou *et al.*, 2009). *C. militaris* can be cultivated artificially and its natural compounds are similar to artificially cultured ones (Jiang and Sun, 1999; Tong *et al.*, 1997). Therefore, it has been considered as an alternative to *C. sinensis* for a wide variety of purposes (Dong *et al.*, 2012; Gao, 2008; Gui and Zhu, 2008).

C. militaris is an entomopathogenic fungus infecting mainly on Lepidoptera larvae. It belongs to the genus *Cordyceps* in family *Clavicipitaceae* of order *Hypocreales*, class *Ascomycetes*. The genus *Cordyceps* has a worldwide distribution from China, Japan, Britain, Norway, Nepal and Korea comprising of approximately 400 species (Liang, 2001; Liu, 1999; Ma *et al.*, 2007; Stensrud *et al.*, 2005; Sung *et al.*,

*Corresponding author: byungjoo@korea.kr

2007; Wang *et al.*, 2008). They are particularly abundant and diverse in humid temperate and tropical forests.

The total genome of *C. militaris* was 32.2 Mb, which is composed of seven chromosomes ranging between 2.0 and 5.7 Mb (Wang *et al.*, 2010; Zheng *et al.*, 2011). In the sexual reproduction, *C. militaris* is dominantly heterothallic having a single locus with two alternate allelic mating system mediated by mating-type (MAT) genes (Wen *et al.*, 2012; Yokoyama *et al.*, 2003; 2004; Zheng *et al.*, 2011). There are two types of MAT gene: MAT1-1-1 which is characterized by the presence of DNA sequence encoding an alpha-domain protein and MAT1-2-1 which is characterized by the presence of sequence encoding a high mobility group domain protein (Tan *et al.*, 2011; Turgeon and Yoder 2000). When two different mating-types are present, it can be compatible to generate perithecial fruiting bodies which have both MAT1-1-1 and MAT1-2-1 (Li, 2007).

New strains were bred by crossing single spore strains (Choi *et al.*, 2009; Du *et al.*, 2010; Sun *et al.*, 2009) and UV induced mutagenesis (Che *et al.*, 2004). Cultivation of *C. militaris* mycelium using artificial media has been developed by different methods especially for high cordycepin production (Hung *et al.*, 2009; Mao and Zhong, 2006) and the fruiting body has also been produced artificially on various media (Choi *et al.*, 1999; Das *et al.*, 2010; Dong *et al.*, 2012; Sato and Shimazu, 2002; Sung *et al.*, 2002). However, the fruiting body production is unstable and variable in serial subcultures of the same strain compared to the initial cultivation, which was mainly caused by degeneration of the strains (Sung *et al.*, 2006; Wen *et al.*, 2012).

This study was carried out to investigate the effect of basic environment and nutritional conditions such as temperature, pH, carbon source and nitrogen source affecting on the mycelia growth of *C. militaris* strain 'Yedang 3'. This could help to understand the cultural characteristic of mycelial growth and further cultivation of fruiting bodies in commercial production.

Materials and Methods

Fungal strain

C. militaris 'Yedang 3' was originally bred by crossing single ascospore isolates C0601S05 and C0601S33 in Yesan, Chungnam, Korea in 2006. It produced more fruiting bodies and contained more cordycepin content (Choi *et al.*, 2009). Its colonies were maintained and subcultured individually onto fresh PDA plates. Then each mycelium was determined in the colony diameter and morphology type of each treatment.

pH, temperature and light

For pH experiment of *C. militaris* 'Yedang 3' was studied by growing the mycelial cultures on PDA with a pH range of 3, 4, 5, 6, 7, 8, 9 adjusted by 1 mol/l hydrochloric acid or 1mol/l sodium hydroxide solution. The effect of temperature on mycelial growth was studied at 5, 10, 15, 20, 25 and 30°C in a growth chamber. The experiment was performed in triplicate. Mycelial growth on each plate was measured at 3 directions and average value was calculated with those 3 measurements. All of the inoculated culture media were tested either in the dark or light at 24°C. Fluorescent lamps were used for light source at 14.3 Wm⁻² in an illumination incubator.

Mycelial culture and media

Mycelia of *C. militaris* strains were cultured vegetatively at 24°C on PDA medium. The medium was sterilized at 121°C for 20 minutes. Agar blocks of 5 mm in diameter were taken from actively growing colonies on PDA plates and inoculated into each culture media for mycelial growth (Table 1). The experiment was placed by completely randomized design with 3 replications and performed twice.

Carbon and nitrogen sources

As the basal medium 2% water agar was used and it was supplemented with twelve different carbon and twelve different nitrogen sources at 2%(w/v) concentration. Carbon sources used were

Table 1. Culture media and their constituents used for mycelial growth

Composition	Media ^a (g/l)									
	GT	HAM	HEN	HOP	MC	MCM	MYA	PDA	SDAY	YMA
Agar	20	20	20	20	20	20	20	20	15	20
Asparagine										
Dextrose		10				20	4	20	40	
Ebiose		5								
Hypoxex		3								
Glucose	5		50	10						4
Malt-extract					20		10			10
Maltose										
Peptone					2	2			10	
Potato								200		
Sucrose										
Tryptone	10									
Yeast-extract	3	3			2	2	4		10	4
NaNO ₃			2							
K ₂ HPO ₄					1	1				
MgSO ₄			0.5	0.5	0.5	0.5				
KCl										
FeSO ₄										
CaCl ₂			0.1							
KH ₂ PO ₄			1	0.1	0.5	0.46				
KNO ₃			2	2						

^aGT, glucose trypton; HAM, Hamada; HEN, Hennerberg; HOP, Hopppkins; MC, mushroom complete; MCM, mushroom complete media; MYA, malt extract yeast extract agar; PDA, potato dextrose agar; SDAY, Sabouraud's dextrose agar yeast extract; YM, yeast malt; YMA, yeast extract malt extract agar.

fructose (C1), galactose (C2), glucose (C3), mannitol (C4), dextrose (C5), lactose (C6), maltose (C7), sucrose (C8), sorbitol (C9), dextrin (C10), xylose (C11), starch (C12). Nitrogen sources were ammonium phosphate (N1), calcium nitrate (N2), glycine (N3), potassium nitrate (N4), yeast extract (N5), malt extract (N6), peptone (N7), typtone (N8), ammonium chloride (N9), ammonium sulfate (N10), sodium nitrate (N11), ammonium nitrate (N12). The basal medium was adjusted to pH 6 and sterilized for 20 min at 121°C, and aseptically added into a plate at 2%(w/v). A 5 mm diameter plug of inoculum was taken from PDA plate, placed in the center of basal media containing one of carbon and nitrogen sources and incubated at 24°C. The carbon-to-nitrogen (C:N) ratio was adjusted to 10:1, 20:1, 30:1, and 40:1 with varying quantities of sucrose as

carbon source and 5 g/l of peptone as sole nitrogen source. The carbon content of sucrose was calculated based on the molecular weight of C₁₂H₂₂O₁₁ as 42% and the nitrogen content of the peptone was determined based on the analysis of Kjeldahl method as 12%.

Result and Discussion

The pH value for a favorable mycelial growth of *C. militaris* was in the range of pH 6~7 (Fig. 1). With regard to the low pH at 3 to 4, the fungus grew very slowly with an average growth rate of less than 1.5 mm/day. Sung *et al.* (2002) and Park *et al.* (2001) reported that mycelia growth of *C. militaris* was optimal at pH 6 in agreement with this study but the mycelia grew slowly in 'Yedang

Fig. 1. Mycelial growth of *C. militaris* 'Yedang 3' on the PDA at different pH value. Bars indicate standard errors.

Fig. 2. Mycelial growth of *C. militaris* 'Yedang 3' on the PDA at different temperature. Bars indicate standard errors.

3' in the range of 8-9 contradicting other report (Sung *et al.*, 2002). These results suggest that *C. militaris* may have optimal pH range at 6~7 in general but mycelial growth out of this range could be different with other strains.

The temperature favourable for the mycelial growth of *C. militaris* 'Yedang 3' was obtained at 20~25°C (Fig. 2). Similarly Sung *et al.* (2002) reported that the mycelial growth of *C. militaris* strains was optimal at 25°C. Although the mycelial growth was favourable at the range of 20-25°C, the fungus appeared to be suppressed at the high temperature of 35°C. However, in the static liquid culture in Erlenmeyer flasks, optimum temperature for mycelial growth was 15-20°C and cordycepin production was the highest at 25°C (Hung *et al.*, 2009). In the submerged culture, the optimal temperature for mycelial growth were found to be at

Fig. 3. Mycelial growth of *C. militaris* 'Yedang 3' on various culture media under light and dark conditions.

20°C (Park *et al.*, 2001). Therefore, the mycelial growth in *C. militaris* might be affected differently by strain and culture method.

Ten different culture media were used to test mycelial growth of *C. militaris* 'Yedang 3' under light and dark conditions (Fig. 3). Of ten culture media, MCM was the most favorable for mycelia growth followed by SDAY and YMA. When assessed twenty two different types of media and categorized into poor and rich media in nutritional sources, Shrestha *et al.* (2006) reported a similar result that SDAY, YMA and MCM developed abundant mycelial density. He suggested that nutritionally rich media produced abundant mycelial growth and this may be because of the sufficiency of all the requirements for the vegetative growth in *C. militaris*. After 15 days of mycelial culture under light condition, seven media from 10 culture media tested were found to be favorable for mycelial growth which were GT, HOP, MC, MYA, PDA, SDAY and YMA media. Mycelial growth in MC showed the most difference with 1.1 mm between light and dark conditions. Although the mycelial growth was greatest in MCM under dark condition, the fungus grew fastest in YMA under light condition. Light was found to be the most critical factor in determining mycelial growth characteristics such as the density, texture, and pigmentation (Shrestha *et al.*, 2006). Similarly, light was regarded as one of most important environmental factors affecting fruiting body production because no fruiting body was formed in darkness in *C. militaris* (Sato and Shimazu, 2002). In general, optimum light intensity was in the range of 500-1,000 lx and the upper limit was 1,400 lx (Hong *et al.*, 2010,

Fig. 4. Mycelial colonies of *C. militaris* 'Yedang 3' on various carbon sources.

Fig. 5. Mycelial colonies of *C. militaris* 'Yedang 3' on various nitrogen sources.

Sato and Shimazu, 2002). It is apparent that light plays important role in mycelial growth and fruiting body formation but further studies are required in the subject of mycelial growth conditions in relation to fruiting body formation.

The mycelial colonies of *C. militaris* 'Yedang 3' were tested on various carbon and nitrogen sources (Fig. 4 and 5). The morphological characteristics such as the mycelial growth, colonial color and shape were varied on different carbon and nitrogen sources. The colonial color by various carbon sources were white on C1, C2, C7, C9, yellowish on C5 and C10 and orange on C3, C4, C6, C11, C12. The colonial color by various nitrogen sources were white on N1, N3, N9, N10, N11, yellowish on N4, N5, N12, orange on N7 and N8. Also

Fig. 6. Mycelial growth of *C. militaris* 'Yedang 3' on various carbon sources.

Fig. 7. Mycelial growth of *C. militaris* 'Yedang 3' on various nitrogen sources.

transparent colonies were examined on N2, and N6. Colonial shapes were different depending on carbon and nitrogen sources from cottony, streak, concentric to radial. Such morphological characteristics on different media by various carbon and nitrogen sources may be useful to characterize or give characteristic features in different strain.

The mycelial growth of *C. militaris* 'Yedang 3' varied significantly on various carbon sources but it was not strict on the requirement of carbon sources compared with nitrogen sources. C1, C4, C8 and C10 were the most suitable carbon sources for the mycelial growth followed by C2, C5, C9 and C12. The fungus grew slowly on C3 and C11 as carbon sources (Fig. 6).

After 15 days of culture, it was found that mycelial growth was affected significantly on various nitrogen sources. N8, N7, N5 and N5 was favorable for the mycelial growth of *C. militaris* 'Yedang 3' (Fig. 7). These were followed by N3, N6 and N11. The least mycelial growth was recorded on N9 nitrogen source. In the results, organic nitrogen sources such as N5, N7, N8

Fig. 8. Mycelial growth of *C. militaris* 'Yedang 3' on various C:N ratio.

showed favorable mycelial growth compared with inorganic sources such as N1, N9, N10. Such results indicated that the organic nitrogen sources supported the mycelial growth of *C. militaris* 'Yedang 3' better than the inorganic sources, because the inorganic nitrogen sources are not easily utilized than organic sources.

Sucrose and peptone were suitable as carbon and nitrogen sources for the mycelial growth of *C. militaris* 'Yedang 3'. The C:N ratio was adjusted to 10, 20, 30, and 40 with varying quantities of sucrose as carbon source and peptone as nitrogen source. With increasing C:N ratio the mycelial growth was increased from 10:1 to 40:1 and the greatest mycelial growth was resulted at 40:1 (Fig. 8). At the high carbon ratio in the range of 50:1 to 60:1, the mycelial growth decreased from 7.19 cm to 6.79 cm. Therefore it was observed that the optimum C:N ratio for mycelial growth was 30~40 range in *C. militaris* 'Yedang 3' as compared with other C:N ratios. Different carbon, nitrogen and C:N ratio with different strains have been investigated (Mao and Zhong, 2004; Mao *et al.*, 2005; Shih *et al.*, 2006) on mycelial growth of *C. militaris* but the direct comparison of the various studies is very difficult because the culture conditions, carbon and nitrogen sources used are different.

References

- Che, Z. M., Wang, Y., Zhou, L. L., and Tang, C. L. 2004. Study on the breeding of a new variety of *Cordyceps militaris* by mutated with ultraviolet radiation. Food Ferment. Ind. **30** : 35-38.
- Choi, I. Y., Choi, J. S., Lee, W. H., Yu, Y. J., Joung, G. T., Ju, I. O. and Choi, Y. K. 1999. The condition of production of artificial fruiting body of *Cordyceps militaris*. Kor. J. Mycol. **27** : 243-248.
- Choi, Y. S., Kim, H. K., Lee, B. J., and Kim, Y. G. 2009. Characteristics and breeding of a new variety *Cordyceps militaris* 'Yedang 3'. J. Mushroom Sci. Production **7** : 182-186.
- Das, S. K., Masuda, M., Sakurai, A., and Sakakibara, M. 2010. Medicinal uses of the mushroom *Cordyceps militaris*: Current state and prospects. Fitoterapia **81** : 961-968.
- De Julian-Ortiz, J. V., Galvez, J., Munoz-Collado, C., Garcia-Domenech, R., and Gimeno-Cardona, C. 1999. Virtual combinatorial syntheses and computational screening of new potential anti-herpes compounds. J. Med. Chem. **42** : 3308-3314.
- Dong, J. Z., Lei, C., Ai, X. R., and Wang, Y. 2012. Selenium enrichment on *Cordyceps militaris* Link and analysis on its main active components. Appl. Biochem. Biotechnol. **166** : 1215-1224.
- Du, A. L., Zhang, X., and Zhang, H. Z. 2010. A new high cordycepin *Cordyceps militaris* cultivar 'Haizhou 1'. Acta Hortic. Sin. **37** : 1373-1374.
- Fan, L., Pan, H., Scoccol, A. T., Pandey, A., and Soccol, C. R. 2006. Advances in mushroom research in the last decade. Food Technol. Biotechnol. **44** : 303-311.
- Gao, X. H. 2008. Mating system of *Cordyceps militaris*. Acta Edulis Fungi **15** : 6-10.
- Gui, Z. Z. and Zhu, Y. H. 2008. Advance on cultivation, bioactive compound and pharmacological mechanism of *Cordyceps militaris*. Sci. Seri **34** : 178-184.
- Guo, H. P. and Yang, Z. M. 1999. Progress in research of pharmacological of *Cordyceps sinensis*. Traditional Herbal Drugs **30** : 231-233.
- Hung, L. T., Keawsompong, S., Hanh, V. T., Sivichai, S., and Hywel-Jones, N. L. 2009. Effect of temperature on cordycepin production in *Cordyceps militaris*. Thai J. Agric. Sci. **42** : 219-225.
- Jiang, X. L. and Sun, Y. 1999. The determination of active components in various *Cordyceps militaris* strains. Acta Edulia Fungi **6** : 47-50.
- Jiang, Y. and Yao, Y. J. 2003. Anamorphic fungi related to *Cordyceps sinensis*. Mycosystema **22** : 161-176.
- Li, M. F. 2007. Molecular biology studies on the different phenotypes of fruiting-body forming of *Cordyceps militaris*. Master Thesis. Guizhou Univ., Guiyang, China.
- Liang, Z. Q. 2001. current situation and ponderation of *Cordyceps* Fr. research and exploitation in China. Acta Edulis Fungi **8** : 53-62.
- Liu, X. M., Cheng, Y. H., and Tian, D. 1999. Progress in the research of pharmacological *Cordyceps* in China. Natl. Product Res. Development **11** : 87-91.
- Liu Z. Y. 1999. Studies on relationship between *Cordyceps spp.* and their anamorphs. PhD dissertation. Huazhong Agric. Univ., China.
- Ma, T. Feng, Y., Wu, X. P., Zhang, Y. H., Ma, Y., and Wang, Z. L. 2007. Primary investigation of a host insect of *Cordyceps militaris* and analysis of its main ingredients. For. Res. **20** : 63-67.

- Mao, X. B., Eksriwong, T., Chauvatcharin, S., and Zhong, J. J. 2005. Optimization of carbon source and C:N ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. *Process Biochem.* **40** : 1667-1672.
- Mao, X. B. and Zhong, J. J. 2004. Hyperproduction of cordycepin by two-stage dissolved oxygen control in submerged cultivation of medicinal mushroom *Cordyceps militaris* in bioreactors. *Biotechnol. Prog.* **20** : 1408-1413.
- Mao, X. B. and Zhong, J. J. 2006. Significant effect of NH_4^+ on cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. *Enzyme Microbiol. Technol.* **38** : 343-350.
- Masuda, M., Urabe, E., Honda, H., Sakurai, A., and Sakakibara, M. 2007. Enhanced production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. *Enzyme Microbiol. Technol.* **40** : 1199-1205.
- Oh, S. W., Kim, S. H., Song, H. N., and Han, D. S. 2003. Comparative chemical compositions of four kinds of Tochuso. *Kor. J. Food Sci. Technol.* **35** : 15-22.
- Park, J. P., Kim, S. W., Hwang, H. J., and Yun, J. W. 2001. Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by *Cordyceps militaris*. *Lett. Appl. Microbiol.* **33** : 76-81.
- Patel, K. J. and Ingallhalli, R. S. 2013. *Cordyceps militaris* (L.: Fr.) Link An important medicinal mushroom. *J. Pharm. Phytochem.* **2** : 315-319.
- Sato, H. and Shimazu, M. 2002. Stroma production for *Cordyceps militaris* (Clavicipitales: Clavicipitaceae) by injection of hyphal bodies to alternative host insects. *Appl. Entomol. Zool.* **37** : 85-92.
- Shih, I. L., Tsai, K. L., and Hsieh, C. Y. 2006. Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of *Cordyceps militaris*. *Biochem. Eng. J.* **33** : 193-201.
- Shrestha, B., Lee, W. H., Han, S. K., and Sung, J. M. 2006. Observations on some of the mycelial growth and pigmentation characteristics of *Cordyceps militaris* isolates. *Mycobiol.* **34** : 83-91.
- Stensrud, Ø, Hywel-Jones, N. L., and Schumacher, T. 2005. Towards a phylogenetic classification of Cordyceps: ITS nrDNA sequence data confirm divergent lineages and paraphyly. *Mycol. Res.* **109** : 41-56.
- Sun, J. D., Xiong, S. T., and Wang, P. 2009. Study on biological and cultivated characters of *Cordyceps militaris* SN3. *J. Fungal Res.* **7** : 148-152.
- Sung, G. H., Hywel-Jones, N. L., Sung, J. M., Luangsa-Ard, J. J., Shrestha, B., and Spatafora, J. W. 2007. Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. *Stud. Mycol.* **57** : 50-59.
- Sung, J. M., Choi, Y. S., Shrestha, B., and Park, Y. J. 2002. Investigation on artificial fruiting of *Cordyceps militaris*. *Kor. J. Mycol.* **30** : 6-10.
- Sung, J. M., Park, Y. J., Lee, J. O., Han, S. K., Lee, W. H., Choi, S. K., and Shrestha, B. 2006. Effect of preservation periods and subcultures on fruiting body formation of *Cordyceps militaris* in vitro. *Mycobiol.* **34** : 196-199.
- Tan, Q., Cai, T., Wei, J., Feng, A. P., Mao, W. J., and Bao, D. P. 2011. Molecular identification of mating type genes in asexual spores of *Cordyceps militaris*. *ICMBMP7 2011* : 52-56.
- Tong, Y. K., Kuang, T., Wu, Y. X., Zhang, Q. Y., and Ren, J. 1997. Comparison of components of Cordyceps mycelium and natural *Cordyceps sinensis*. *Shi Pin Yan Jiu Yu Kai Fa* **18** : 40-42.
- Turgeon, B. G., and Yoder, O. C. 2000. Proposed nomenclature for mating type genes of filamentous ascomycetes. *Fungal Genet. Biol.* **31** : 1-5.
- Wang, L., Zhang, W. M., Hu, B., Chen, Y. Q., and Qu, L. H. 2008. Genetic variation of *Cordyceps militaris* and its allies based on phylogenetic analysis of rDNA ITS sequence data. *Fungal Diversity.* **31** : 147-155.
- Wang, X. Y., Rong, Y. W., Xu, L., Zhang, N. S., Li, C. R., and Fan, M. Z. 2010. Electrophoretic karyotype analysis of *Paecilomyces militaris*, the anamorph of *Cordyceps militaris*. *J. Anhui Agric. Univ.* **37** : 716-719.
- Wen, T. C., Li, M. F., Kang, J. C., and He, J. 2012. A molecular genetic study on fruiting-body formation of *Cordyceps militaris*. *Afr. J. Microbiol. Res.* **6** : 5215-5221.
- Wong, Y. Y., Moon, A., Duffin, R., Barthet-Barateig, A., Meijer, H. A., Clemens, M. J., and De Moor, C. H. 2010. Cordycepin inhibits protein synthesis and cell adhesion through effects on signal transduction. *J. Biol. Chem.* **285** : 2610-2621.
- Wu, F. Y., Yan, H., Ma, X. A., Jia, J. Q., Zhang, G. Z., Guo, X. J., and Gui, Z. Z. 2011. Structural characterization and antioxidant activity of purified polysaccharide from cultured *Cordyceps militaris*. *Afr. J. Microbiol. Res.* **5** : 2743-2751.
- Yokoyama, E., Yamagishi, K., and Hara, A. 2004. Development of a PCR-based mating-type assay for *Clavicipitaceae*. *FEMS Microbiol. Lett.* **237** : 205-212.
- Yokoyama, E., Yamagishi, K., and Hara, A. 2003. Structures of the mating-type loci of *Cordyceps takaomontana*. *Appl. Environ. Microbiol.* **69** : 5019-5022.
- Yoo, H. S., Shin, J. W., Cho, J. H., Son, C. G., Lee, Y. W., Park, S. Y., and Cho, C. K. 2004. Effects of *Cordyceps militaris* extract on angiogenesis and tumor growth. *Acta Pharm. Sinica* **25** : 657-665.
- Yu, H. M., Wang, B. S., Huang, S. C., and Duh, P. D. 2006. Comparison of preventive effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. *J. Agric. Food Chem.* **54** : 3132-3138.
- Zheng, P., Xia, Y. L., Xiao, G. H., Xiong, C. H., Hu, X., Zhang, S. W., Zheng, H. J., Huang, Y., Zhou, Y., Wang, S. Y., Zhao, G. P., Liu, X. Z., St Leger, R. J., and Wang, C. S. 2011. Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. *Genome Biol.* **12** : R116.
- Zhou, X. W., Gong, Z. H., Su, Y., Lin, J., and Tang, K. X. 2009. Cordycepin fungi: natural products, pharmacological functions and developmental products. *J. Pharm. Pharmacol.* **61** : 279-291.