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Review

Authentication and quality control of *Cordyceps sinensis*, a traditional Chinese medicine known as winter-worm summer-grass

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SUMMARY

Cordyceps, one of the most valued traditional Chinese medicines, consists of the dried fungus *Cordyceps sinensis* growing on the larva of caterpillar. It is also known as "winter-worm and summer-grass" because of its appearance during different seasons. The parasitic complex of the fungus and the caterpillar is found in soil of a prairie at an elevation of 3,500 to 5,000 meters in northwestern part of China. According to Chinese medicinal theory, *Cordyceps* is used to replenish the kidney and soothe the lung, and indeed many clinical applications have been reported. The natural *Cordyceps* is rare and expensive on the local market, and therefore, several mycelial strains have been isolated from natural *Cordyceps* and manufactured in large quantities by fermentation technology, and they are commonly sold as health food products in Orient. The adulterants of *Cordyceps* are commonly found on the market, and therefore the authentication of these products has to be defined. Having the urgent need from current market, different chemical markers such as nucleoside, ergosterol, mannitol and polysaccharide are being used for quality control of *Cordyceps*. Unfortunately, these markers are far from optimization, and therefore extensive works are needed to define the pharmacological efficiency of these markers.

Key words: *Cordyceps* ; Quality control; Chemical marker

INTRODUCTION

Cordyceps sinensis is the complex of fungus *Cordyceps sinensis* (Berk.) Sacc. (Clavicipitaceae) growing on the larva of *Hepialus armoricanus* Oberthur (caterpillar) that lives few inches underground. It is also commonly known as *Cordyceps*, or "Dong Chong Xia Cao" (winter-worm and summer-grass) in Chinese, because of its appearance during different seasons. *Cordyceps* has been used in China for medication over few hundred years. *Cordyceps* is first recorded in "Ben Cao Cong Xin" by Wu Yiluo in 1757 AD. Later on it was revealed that the original description of *Cordyceps* should be in "Ben Cao Bei Yao" by Wang Ang in 1694 AD, which wrote: "*Cordyceps* is sweet in taste and neutral in nature, and replenishing the kidney and soothing the lung, arresting bleeding, resolving phlegm, and killing the cough".

Cordyceps was known to Europe in 17th century. In 1723 AD, *Cordyceps* was brought from China to France as Materia Medica, and which was presented at the Conference of Paris Science Academic Institute.

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Then, *Cordyceps* was considered as a precious medical material and recorded at the memo of the Conference in 1727 AD. Hundred years later, Italian scholar Saccardo in 1878 AD named *Cordyceps* derived from China officially as *C. sinensis*; this nomenclature was adopted until today. Functionally, *Cordyceps* is well known to ensure the normal functioning of various parts of the body, to strengthen the immune system and to promote overall vitality and longevity (Zhu *et al.*, 1998a, b). Today, *Cordyceps* is commonly used in many hospitals in China and as a household remedy. However, more than hundred different types of *Cordyceps* or its substitutes have been found worldwide today. Thus, the authentication of *Cordyceps* is a serious problem on the market. Here, we discuss the chemical composition of natural and cultured *Cordyceps* and their problems in authentication and quality control.

Natural and cultured *Cordyceps*

Cordyceps composes of fungus fruiting body and larva of the host, and its distribution is closely related to distribution of the host. At present, possible hosts of *C. sinensis* have been identified (Li and Tsim, 2004). Many *Cordyceps*-related species could be found, which are based on different fungus growing on different insect hosts; however, most of them are not considered as *Cordyceps* for clinical usage, except *C. sinensis* that is listed officially in Chinese Pharmacopoeia (Zheng, 2005). China is the major producer of *Cordyceps*. In China, the parasitic complex is found in soil of a prairie at an elevation of 3,500 to 5,000 meters, mainly in the provinces of Qinghai, Tibet, Sichuan, Yunnan and Gansu (Fig. 1A).

Formation of *Cordyceps* can be divided into 3 stages (Fig. 1B): infection, parasitism (development of fungus before the insect death) and saprophyte (growth of fungus after the insect death). After the infection, *Cordyceps* fungus makes use of the bowels of the host as nutrient and starts to grow. The mycelia creep over the insect body while the host is still alive. Subsequently, the color of the host surface (shell) will fade in few days from dark brown-yellow turn into light yellow, and then the entire body is covered by gray mycelia. After the host has died, the coarsely mycelia will form a hard

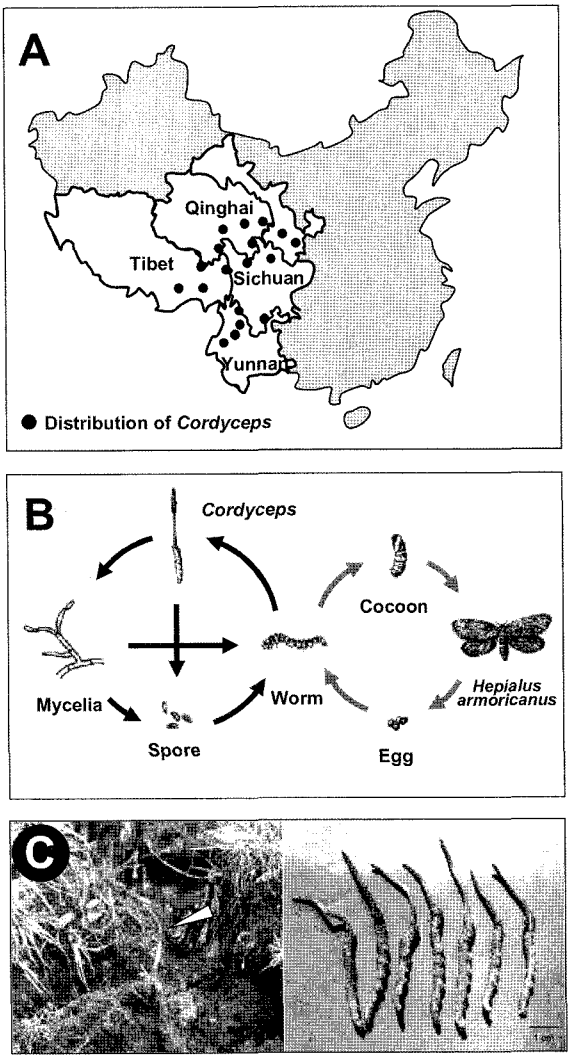

Fig. 1. (A) A map shows the distribution of *Cordyceps* in China. **(B)** Host (*H. armoricanus*) can be invaded at the state of larva by *C. sinensis*. After the infection, *Cordyceps* fungus makes use of the bowels of the host as nutrient and starts to grow. After the host has died, the coarsely mycelia will form a hard tissue. If the condition is suitable, the mycelia in the host will grow out through the oral cavity, and form the fruiting body; therefore, this is the formation of *Cordyceps*. **(C)** Freshly collected *Cordyceps* is shown. Arrowhead in (left panel) indicates a living *Cordyceps* before the collection.

tissue. If the condition is suitable, the mycelia within the host will grow out through the oral cavity, and form the fruiting body; therefore, this is the formation of *Cordyceps*. The host loses its biological and chemical characteristic, and eventually occurred by *C. sinensis* mycelia. The collected *Cordyceps* have to be dried before they are sold on the market (Fig. 1C).

The growth of *C. sinensis* has a very restricted habitat, and the yield is decreasing every year. In 2001, a total of few thousands kg of *Cordyceps* were collected in China; this represents a decrease of over 70% as compared to 1978. Because of the environmental concerns, the Ordinance of Resources Protection on Wild Herbal Medicine was issued in 1987 by the Chinese Government, and therefore the collection of *Cordyceps* is being restricted. The price of *Cordyceps* is US$ 5,000 per kg in 2005, about 100 folds higher than that in 1980's.

Scientists in Orient have extensively developed substitutes by using mycelial fermentation that is deriving from natural *Cordyceps*. Up to date, more than 9 genera including 31 species have been isolated from natural *C. sinensis*. Mycelia, or fruiting bodies, of 16 species have been produced in large quantities by culture. More than 20 fermented products are commonly sold as health food products in China, and the annual production value is more than US$ 100 million.

Among all the fermented *Cordyceps*, CS-4 is the most common one and claimed to be isolated from *C. sinensis*; CS-4 is known to be *Paecilomyces hepialid.* Fermentation methodology, chemical composition, therapeutic function, basic biology and toxicity of CS-4 have been investigated extensively. JinShuiBao capsule, the commercial product that derived from CS-4, has been sold and used in clinics throughout China. This product generates over several million US dollars of sales per year. Besides CS-4, several mycelial strains have been isolated from natural *Cordyceps*, and some of them are manufactured in large scale by fermentation (Yin and Tang, 1995). For instance, *Synnematum sinensis*, *Cephalosporium sinensis*, *Gliocladium roseum*, and *Mortierella hepiali* are the nonsexual phase strains of *Cordyceps*; their commercial names in China are known as BaiLing, NingXinBao, XinGanBao and ZhiLing, respectively. In addition, *Paecilomyces sinensis*, *Scytalidium hepiali*, *Tolypocladimn sinensis*, *Hirsutella sinensis*, *Chrysosporium sinensis* and others have also been isolated from natural *Cordyceps* and manufactured in large quantities by fermentation (Wang *et al.*, 1995b; Zhu *et al.*, 1998a, b). In addition, *Cordyceps militaris* is often used as a substitute of *C. sinensis* (Wang, 1995) in health food market of Orient, which is also known as *Cordyceps* from the north (Bei Chong Cao). Thus, the cultivated products of *Cordyceps* as health food products are very popular, but confusing, in Asia, and their marketable values are extremely high. However, the genuineness of these products is still doubtful, and on the other hand the adulterants of *Cordyceps* are commonly found on the market.

Chemical and biological properties of *Cordyceps*

The chemical composition of *C. sinensis* was first described in 1947 by Tang (Wang, 1995). In average, natural *Cordyceps* contains 25% protein, 8.4% fat, 18.5% fiber, 29% carbohydrate and 4.1% ash. In 1957, cordycepic acid, which was subsequently identified as D-mannitol, was isolated from *C. sinensis*, and which therefore has been used as a quality control marker of *Cordyceps* for a number of years (Wang and Pang, 1995; Yue *et al.*, 1995). In 1964, 3'-deoxyadenosine, namely cordycepin, was isolated from *C. militaris* (Wang, 1995), a related species of *C. sinensis* commonly used as a substitute of *Cordyceps*; however, the existence of cordycepin in *C. sinensis* is controversial. Up to date, cordycepin has never been identified from *C. sinensis*. At present, *C. sinensis* is known to contain steroids, nucleosides, carbohydrates and amino acids. Subsequently, uracil, adenine, adenosine, trehalose, mannitol, ergosterol and stearic acid were identified from *C. sinensis* (Yu *et al.*, 1981) (Fig. 2).

A very common question is being asked for

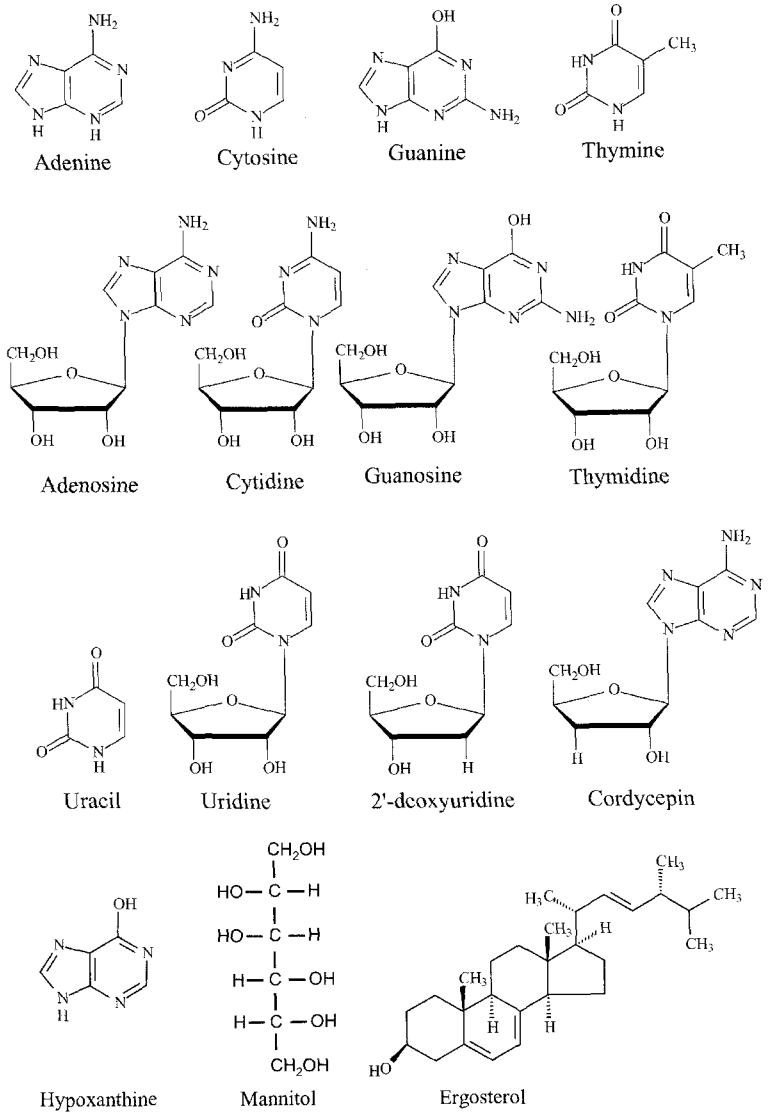

Fig. 2. Structures of chemical markers isolated from *Cordyceps*.

many years regarding the values of *Cordyceps*. Is the worm or the fungus more important? In order to answer this question, the biological activity and chemical composition of the fruiting body and the worm were investigated (Li *et al.*, 2002). The water extracts of the individual parts were analyzed by capillary electrophoresis, and the content of nucleosides was determined. The fruiting body and the worm showed a close resemblance in their nucleoside peaks and overall profiles, while the dry naïve worm with no *Cordyceps* mycelia showed a very distinct chemical profile (Fig. 3). In addition, similar amounts of polysaccharides were found in fruiting body and worm. Biologically, the anti-oxidating activity of *Cordyceps*, from either the fruiting body or worm, was determined; the water extracts of fruiting body and worm showed similar IC_{50} values in their inhibition of free radical formation (Li *et al.*, 2002). On the other hand, the naïve worm did not show any anti-oxidating

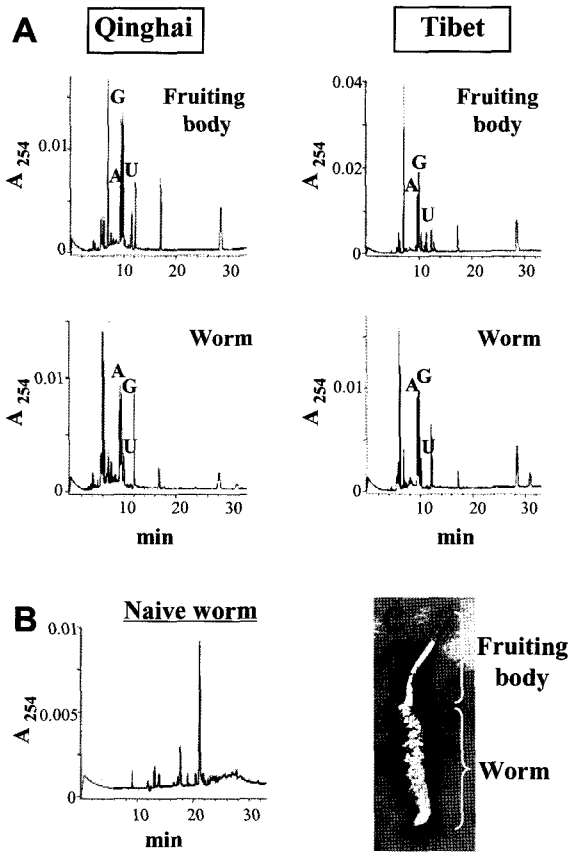

Fig. 3. Capillary electrophoresis profiles of water-soluble constituents from fruiting body and worm of natural *Cordyceps*. Condition: pressure injection 586 kPa for 5 seconds, 57 × 75 cm column, running buffer 200 mM boric acid-sodium hydroxide (pH 8.5). The profile was monitored on-line at 254 nm, 0.100 AU at a data collection rate of 5 Hz for 40 minutes. Adenosine (A), guanosine (G) and uridine (U) are labeled. Data are modified from Li *et al.* (2002). **(A)** *Cordyceps* from Qinghai and Tibet. **(B)** extract from naive worm. **(C)** separation of fruiting and worm from nature *Cordyceps*.

activity at the range of mg/ml. These results suggest that the function of the worm in *Cordyceps* is to provide a growth medium for the fruiting body, and eventually, the worm is totally invaded by *C. sinensis* mycelia.

In Chinese medicinal theory, *Cordyceps* processes both "Yin-nourishing" and "Yang-invigorating" activities (Siu *et al.*, 2004). Indeed, numerous reports have shown the pharmacological properties of either natural or cultured *Cordyceps* (reviews in Zhu *et al.*, 1998a, b; Li and Tsim, 2004). Different therapeutic purposes of *Cordyceps* were reported to: stimulate immune response; inhibit cancer growth; protect kidney and liver; stimulate cardiovascular circulation; lower blood glucose; and against free-radical formation. Although several functions of *Cordyceps* have been described, the comparison among different types, both natural and cultured, of *Cordyceps* in a defined biological activity has not been described. Several years ago, our laboratory had compared different types of *Cordyceps* by their anti-oxidating activities. The anti-oxidating activity of *Cordyceps* was compared by using different methods including the inhibition on xanthine oxidase, the induction of hemolysis in erythrocytes and the prevention of lipid peroxidation in liver (Li *et al.*, 2001a). Water extract from natural or cultured *Cordyceps* significantly scavenged the formation of free radical. In the same study, the anti-oxidating activities of different cultured or natural products of *Cordyceps* were compared (Table 1). The natural *Cordyceps* from Tibet showed the strongest scavenging activity of free radical with an IC_{50} of 0.08 mg/ml, while *Cordyceps* from Yunnan had an IC_{50} of 0.24 mg/ml; the difference was ~3 folds between the two sources of *Cordyceps*. Similar difference could also be observed in cultured *Cordyceps* that was fermented from various producers. In contrast, different sources of *Cordyceps*, either natural or cultured *Cordyceps*, showed a close inhibition on the free radical-induced hemolysis of erythrocytes; their IC_{50} varied from 1.5 to 2.0 mg/ml. Furthermore, the anti-oxidating activity of *Cordyceps* could be enriched by ~15 folds in polysaccharide-enriched fraction after ion exchange column. By using the partial purified polysaccharide in the anti-oxidation assays, the increment of inhibition activity was ~25 folds in xanthine oxidase assay, ~11 folds in hemolysis assay and ~32 folds in lipid peroxidation assay. The result indicated that polysaccharide could be one of the active constituents in *Cordyceps* of having anti-oxidating activity (Li *et al.*, 2001a).

Table 1. The inhibition of peroxide anion formation, hemolysis and lipid peroxidation by water extracts from different types of natural and cultured *Cordyceps*

Sample	peroxide anion formation[a]	hemolysis[b]	lipid peroxidation[c]
Natural *Cordyceps*			
Qinghai	0.20[d]	0.20	0.66
Xizang	0.08	0.23	0.52
Sichuan	0.08	0.17	0.39
Yunnan	0.24	0.30	0.71
Cultured *Cordyceps*			
Jiangxi	0.09	0.15	0.53
Huadong	0.21	0.18	0.68
Wanfong	0.34	0.18	0.57
Hebei	0.91	0.19	0.63

[a,b,c]The IC_{50} in the inhibition and refer to reference (Li *et al.*, 2001a). [d]The mean values of five determinations are presented. The SEM is less than 5% of the mean, which is not shown for clarity.

Quality control of *Cordyceps*

Natural *Cordyceps* are mainly found in the provinces of Qinghai, Tibet, Sichuan, Yunnan and Gansu of China; however, Qinghai and Tibet are believed to produce the best quality of *Cordyceps*, and their prices are much higher that those from other areas. In addition, different types of cultured *Cordyceps* are being sold on the current markets, and their prices are markedly lower than that of the natural one. According to the sources of *Cordyceps*, the market price of *Cordyceps* varies greatly, and unfortunately an absolute chemical marker for better quality or even the markers for authenticity of *Cordyceps* is missing. At present, the quality of these products is an emerging question concerned by the consumers. Under many circumstances, adenosine has been used as a marker for quality control of natural *Cordyceps* and cultured *Cordyceps* mycelia (Zheng, 2005). While, mannitol is used as a marker for quality control of *Cordyceps* mycelia because of its certain pharmacological activity (Chen *et al.*, 1992; Wang and Pang, 1995). But all of these chemical markers are far from perfect.

Adenosine is not a good marker for *Cordyceps*

Nucleoside is believed to be the active component in *Cordyceps*. Indeed, *Cordyceps* contains a high concentration of adenosine, guanosine and uridine (Li *et al.*, 2001b, c; Gong *et al.*, 2004). Among these nucleosides, adenosine is considered to play a key role in many pharmacological effects of *Cordyceps*, which include the widespread effects on coronary and cerebral circulation, the prevention of cardiac arrhythmias and the functions in nervous system e.g. the inhibition of neurotransmitter release and the modulation of adenylate cyclase activity. The amount of nucleoside within *Cordyceps* changes according to different environmental conditions. Fresh natural *Cordyceps* contains very little amount of nucleoside, as compared to dry and processed *Cordyceps* (Li *et al.*, 2001b), and more interestingly cultured *Cordyceps* mycelium contains high level of nucleosides (Table 2). Furthermore, humidity and heat significantly increased the amount of nucleoside in natural *Cordyceps*. Storage of *Cordyceps* at 75% relative humidity and 40°C for 10 days, the nucleoside content in natural *Cordyceps* markedly increased to about 4 folds. However, the effect of humidity and heat in altering the content of nucleotide could not be revealed in cultured *Cordyceps* mycelia (Li *et al.*, 2001c). Therefore, it is believed that nucleosides in natural *Cordyceps* may be derived from the degradation of nucleic acids. In addition, recent studies from our laboratory indicated that the content of adenosine in *Cordyceps* has no obvious relationship with its anti-oxidating activity (Li *et al.*, 2002). In addition,

Table 2. The contents of ergosterol, nucleosides and their bases in *Cordyceps*

Marker	Natural *C. sinensis*			Cultured *C. sinensis*				Cultured *C. militaris*	
	Qinghai	Tibet 1	Tibet 2	Jiangxi	Huadong	Wanfong	Boding	Jining	Oli
Ergosterol	1.43[a]	1.00	0.97	2.71	2.68	3.23	4.14	4.19	1.00
Adenosine (+Adenine)	0.45	0.42	0.36	3.07	1.97	5.13	2.66	1.42	0.64
Cytosine	-[b]	+[c]	+	0.10	0.24	0.09	+	-	+
Cytidine	0.29	0.19	0.32	0.58	0.25	0.04	0.71	0.47	0.59
Cordycepin	+	+	+	+	+	+	+	4.96	4.79
Guanosine	0.20	0.18	0.32	2.80	1.82	4.45	2.55	0.69	0.17
Thymine	-	+	-	-	0.11	0.15	0.19	0.05	0.13
Thymidine	-	+	+	0.29	+	0.28	0.15	-	+
Uracil	+	0.05	0.05	0.36	0.10	0.26	0.08	0.11	+
Uridine	0.66	0.83	0.83	3.11	1.54	8.14	1.93	1.96	0.51
2′-deoxyuridine	-	+	+	0.16	-	0.24	0.09	-	0.17
Hypoxanthine	0.03	0.06	0.13	0.06	-	0.07	0.10	-	+

[a]The amount of marker is in mg/g of dry weight (Li *et al.*, 2004a). The mean values of five determinations are presented. The SEM is less than 5% of the mean, which is not shown for clarity. [b]Undetectable. [c]Beyond lower limit of linear range of detection.

the hypolipidemic activity of adenosine has never been reported. Therefore, having adenosine as a marker for good quality of *Cordyceps* may not be indicative.

The level of ergosterol shows the characteristic of *Cordyceps*

Ergosterol is an unique component in fungi, and it is required for vitamin D_2 synthesis. Thus, ergosterol could be another choice of chemical marker for quality control of *Cordyceps*. Sterols and their derivatives have been isolated from natural and cultured *Cordyceps*; they are ergosterol, Δ^3 ergosterol, ergosterol peroxide, ergosteryl-3-O-β-D-glucopyranoside, 22,23-dihydroergosteryl-3-O-β-D-glucopyranoside, β-sitosterol, daucosterol, cholesterol, cholesteryl palmitate, campesterol and dihydrobrassicasterol. Ergosterol exists as free and combined forms in *Cordyceps*. Li *et al.* (1991) determined the amount of total (free and combined forms) ergosterol in *Cordyceps* by using HPLC, where the pre-treatment of sample was performed. However, the requirement for pre-treatment is time consuming and with poor reproducibility. The determination of free ergosterol in *Cordyceps* by using HPLC is easier to perform.

The content of ergosterol is higher in cultured *Cordyceps* mycelia than that in natural *Cordyceps* (Table 2), and the level of ergosterol could reflect the amount of *Cordyceps* mycelia (Li *et al.*, 1991, 2004a). During the fermentation of *Cordyceps*, the level of ergosterol changed according to the time of culture; a steady level of ergosterol was revealed when the maturation of *Cordyceps* mycelia was reached (Fig. 4).

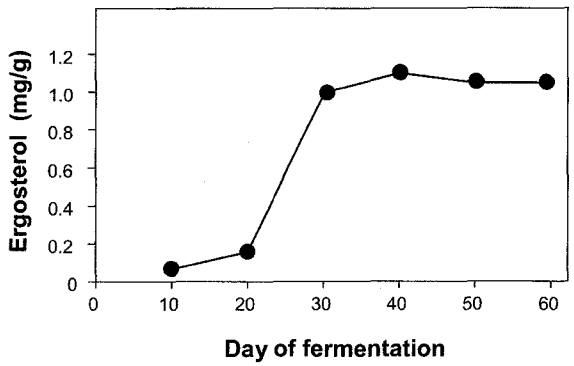

Fig. 4. The amount of ergosterol changes according to the growth of *Cordyceps*. A cultured *Cordyceps* (Li and Tsim, 2004) was tested under different days of fermentation. Ergosterol was revealed by HPLC (Li *et al.*, 2004a) and mean values of five determinations are presented. The SEM is less than 5% of the mean, which is not shown for clarity.

Pharmacological study showed that petroleum extract of *Cordyceps*, rich of ergosterol, possessed anti-arrhythmia effect (Ji *et al.*, 2000). The derivatives of ergosterol have been isolated from the methanol extract of *C. sinensis*, which have been shown to have anti-tumor properties (Bok *et al.*, 1999; Lin *et al.*, 1999). In addition, the ergosterol derivatives also have multiple pharmacological activities, such as cytotoxic activity (Nam *et al.*, 2001) and anti-viral activity (Lindequist *et al.*, 1989); these activities are in line with the quality of *Cordyceps* for both natural or cultured products. Therefore, the level of ergosterol is a useful marker for quality control of *Cordyceps*, at least which represents part of *Cordyceps*' biological functions.

Mannitol is a marker for cultured *Cordyceps*

D-Mannitol is one of the major active compounds in natural *Cordyceps*, and which contributes over 3.4% of the total dry weight. Mannitol has shown to have diuretic, antitussive and anti-free radical activities (Li *et al.*, 1999a). Mannitol is being used to treat many diseases, and therefore which has been used as a marker for *Cordyceps* (Shen and Zhou, 1997). The content of mannitol in *Cordyceps* was usually determined using volumetry, or thin layer chromatography scanning, or colorimetry (Li *et al.*, 1999a). HPLC analysis showed that mannitol was the major component of carbohydrate in natural *Cordyceps*, and the content of mannitol in natural *Cordyceps* was higher than that in the cultured one (Li, *et al.*, 1999b). The content of mannitol determined by HPLC is much lower than that determined by other methods, which could be the HPLC analysis avoids the signals generated from the reduced monosaccharides.

Polysaccharide represents the most biological properties of *Cordyceps*

Cordyceps contains high amount of polysaccharide, which could be ranged from 3 to 8% of the total dry weight (Li *et al.*, 1999b, 2003). *Cordyceps* polysaccharide is considered to process the activities of anti-oxidation (Yamaguchi *et al.*, 2000; Li *et al.*, 2002), immunopotentiation (Liu *et al.*, 1992; Xu *et al.*, 1992; Li and Tsim, 2004), anti-tumor (Zhang *et al.*, 2004) and hypoglycemic (Kiho *et al.*, 1999). Until now, the pharmacological profile of *Cordyceps* correlates very well with the amount of polysaccharide in the herb. Based on the binding to Mono Q® column, four fractions of polysaccharides were isolated from different types of natural and cultured *Cordyceps;* however, the ratio of these four polysaccharide fractions varied in different cultured products of *Cordyceps* (Li *et al.*, 1999 a, b). The molecular weights of polysaccharides isolated from *Cordyceps* were also compared by gel filtration. The polysaccharides in natural *Cordyceps* were predominantly (> 50%) those high molecular weight molecules of over 150 kDa, which were rather distinct as compared to the cultured products.

Based on the activity-guided fractionation, a water soluble protein-containing galactomannan was isolated from the sodium carbonate extract of *Cordyceps*, and its molecular weight was estimated by gel filtration to be ~23 kDa. The isolated compound composed of D-mannose and D-galactose in a molar ratio of 3 : 5, and contained a small proportion of protein. It is a highly branched structure and composes of (1→6)-and (1→2)-linked α-D-mannopyranosyl residues in the main chain (Kiho *et al.*, 1986). Another polysaccharide with hypoglycemic activity, purified from a hot water extract of the cultured mycelium of *C. sinensis*, was a combination of galactose, glucose and mannose in a molar ration of 43 : 33 : 24; its molecular weight was estimated to be about 15 kDa (Kiho *et al.*, 1999).

In searching for active component(s) of having anti-oxidating activity from cultured *Cordyceps*, a polysaccharide of molecular weight ~210 kDa, named CSP-1, was isolated from cultured *Cordyceps* mycelia by ion-exchange and sizing chromatography (Li *et al.*, 2003). The isolated polysaccharide, having strong anti-oxidating activity, contained glucose, mannose and galactose in a ratio of 1 : 0.6 : 0.75. The pre-treatment of isolated polysaccharide on

cultured rat pheochromocytoma PC12 cells showed strong protective effect against hydrogen peroxide (H_2O_2)-induced insult. This report identified a polysaccharide from *Cordyceps* that protected against the free radical-induced neuronal cell toxicity.

Future prospectus

Because of the decreasing supply of natural *Cordyceps*, the isolation of mycelial strain from *Cordyceps* is a trend of many scientists in Orient to achieve a large scale production of *Cordyceps* by fermentation. Indeed, the current health food market is full of fermented products of *Cordycpes;* however, many of them are adulterants. The methodology for authentication of these products has to be well defined, and chemical markers are needed for quality control. Although many so called active constituents have been identified, the exact roles of these chemicals for the functions of *Cordyceps* are not known. At present, multiple markers such as ergosterol, nucleoside, mannitol and polysaccharide are being used for quality control of *Cordyceps*' products. Unfortunately, these markers are far from optimization, and extensive works are needed to define the pharmacological efficiency of these chemical markers.

Another approach in quality control of the herb is using chemical profiling instead of a single compound. By capillary electrophoresis, distinct fingerprints could be revealed in water-soluble constituents derived from different sources of *Cordyceps* (Li *et al.*, 2004b). The result shows that those samples of natural *Cordyceps* are resemblance to each other in the fingerprinting, which are in distinction to the cultured products. This method does not depend on the identities of any chemicals. Thus, the profiles generated from capillary electrophoresis could serve as fingerprints for the quality control of *Cordyceps*. With the needs of the health food market, the fingerprinting of having multi-markers, that represents different *Cordyceps* fractions, should be used for quality control of *Cordyceps*.

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REFERENCES

Bok JW, Lermer L, Chilton J, Klingeman GH, Towers N. (1999) Antitumor sterols from the mycelia of *Cordyceps sinensis*. *Phytochemistry* **51**, 891-898.

Chen CY, Feng GS, Xu YX. (1992) Study on industrial submerged fermentation of *Cordyceps sinensis*. *Chinese Traditional Herbal Drugs* **23**, 409-414,416.

Gong YX, Li SP, Li P, Liu JJ, Wang YT. (2004) Simultaneous determination of six main nucleosides and bases in natural and cultured *Cordyceps* by capillary electrophoresis. *J. Chromatogr. A* **1055**, 215-221.

Ji H, Gong XJ, Lu SG, Cao Q, Li SP, Li P. (2000) Antagonistic effect of extracts from cultural mycelium of *Cordyceps sinensis* on cardiotoxicity induced by ouabain. *J. China Pharm. U.* **31**, 118-120.

Kiho T, Ookubo K, Ukai S, Hara G. (1986) A minor protein-containing galatomannan from a sodium carbonate extract of *Cordyceps sinensis*. *Carbohydr. Res.* **156**, 189-197

Kiho T, Ookubo K, Usui S, Ukai S, Hirano K. (1999) Structural features and hypoglycemic activity of a polysaccharide (CS-F10) from the cultured mycelium of *Cordyceps sinensis*. *Biol. Pharm. Bull.* **22**, 966-970.

Li YH, Li XL. (1991) Determination of esgosterin in *Cordyceps sinensis* and Chong Cao Wuji Wan by HPLC method. *Acta Parmaceutica Sin.* **26**, 768-771.

Li XQ, Bao TT, Wang Y. (1999a) Determination of mannitol in Dongchongxiacao (*Cordyceps sinensis*) by colorimetric method. *Chinese Traditional Herbal Drugs* **30**, 19-21.

Li SP, Li P, Ji H, Zeng Q, Wu ZB. (1999b) Comparison of polysaccharides in natural and cultured *Cordyceps*. *Chinese J. Wild Plant Resour.* **6**, 47-48.

Li SP, Li P, Dong TTX, Tsim KWK. (2001a) Anti-oxidation activity of different types of natural *Cordyceps sinsensis* and cultured *Cordyceps* mycelia. *Phytomedicine* **8**, 207-212.

Li SP, Li P, Dong TTX, Tsim KWK. (2001b) Determination of nucleosides in natural *Cordyceps sinensis* and

cultured *Cordyceps* mycelia by capillary electrophoresis. *Electrophoresis* **22**, 144-150.

Li SP, Li P, Ji H, Dong TTX, Tsim KWK, Zhu Q. (2001c) The nucleosides contents and their variation in natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia. *J. Chinese Pharm. Sci.* **10**, 175-179.

Li SP, Su ZR, Dong TTX, Tsim KWK. (2002) The fruiting body and its host of *Cordyceps sinensis* show close resemblance in main constituents and anti-oxidation activity. *Phytomedicine* **9**, 319-324.

Li SP, Zhao KJ, Ji ZN, Song ZH, Dong TT, Lo CK, Cheung JK, Zhu SQ, Tsim KWK. (2003) A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. *Life Sci.* **73**, 2503-2513.

Li SP, Tsim KWK. (2004) The biological and pharmacological properties of *Cordyceps sinensis*, a traditional Chinese medicine, that has broad clinical applications. In: *Herbal Medicines: Molecular Basis of Biological Activity and Health*, edited by Packer *et al.*, pp. 657, Marcel Dekker Inc., New York.

Li SP, Li P, Lai CM, Gong YX, Kan KKW, Dong TTX, Tsim KWK, Wang YT. (2004a) Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured *Cordyceps* by pressurized liquid extraction and high-performance liquid chromatography. *J. Chromatogr. A* **1036**, 239-243.

Li SP, Song ZH, Dong TTX Lo CK, Zhu SQ and Tsim KWK. (2004b) Distinction of water-soluble constituents from natural and cultured *Cordyceps* mycelia by capillary electrophoresis. *Phytomedicine* **11**, 684-690.

Lin CY, Ku FM, Kuo YC, Chen CF, Chen WP, Chen A, Shiao MS. (1999) Inhibition of activated human mesangial cell proliferation by the natural product of *Cordyceps sinensis* (H1-A): an implication for treatment of IgA mesangial nephropathy. *J. Lab. Clin. Med.* **133**, 55-63.

Lindequist U, Lesnau A, Teuscher E, Pilgrim H. (1989) The antiviral action of ergosterol peroxide. *Pharmazie* **44**, 579-580.

Liu C, Lu S, Ji MR. (1992) Effects of *Cordyceps sinensis* (CS) on *in vitro* natural killer cells. *Zhongguo Zhong Xi Yi Jie He Za Zhi* **12**, 267-269.

Nam KS, Jo YS, Kim YH, Hyun JW, Kim HW. (2001) Cytotoxic activities of acetoxyscirpenediol and ergosterol peroxide from *Paecilomyces tenuipes*. *Life Sci.* **69**, 229-237.

Shen FR, Zhou YS. (1997) Two new species of the genus *Hepialus* from Yunnan, China (Lepidoptera: Hepialiadae). *Acta Zootaxon Sin.* **3**, 37-39.

Siu KM, Mak DH, Chiu PY, Poon MK, Du Y, Ko KM. (2004) Pharmacological basis of 'Yin-nourishing' and 'Yang-invigorating' actions of *Cordyceps*, a Chinese tonifying herb. *Life Sci.* **76**, 385-395.

Wang GD. (1995) *Cordyceps: Ecology, Culture and Application*, Science and Technology Documents Publishing House, Beijing.

Wang BQ, Pang ZG. (1995) Determination of D-mannitiol in Tibet *Cordyceps sinensis* by TLC-scanning. *Chinese Traditional Herbal Drugs* **26**, 189-190.

Wang ZX, Wang XM, Wang TZ. (1995) Current status of pharmacological study on *Cordyceps sinensis* and *Cordyceps* hyphae. *Zhongguo Zhong Xi Yi Jie He Za Zhi* **15**, 255-256.

Xu RH, Peng XE, Chen GZ, Chen GL. (1992) Effects of *Cordyceps sinensis* on natural killer activity and colony formation of B16 melanoma. *Chinese Med. J.* **105**, 97-101.

Yamaguchi Y, Kagota S, Nakamura K, Shinozuka K, Kunitomo M. (2000) Antioxidant activity of the extracts from fruiting bodies of cultured *Cordyceps sinensis*. *Phytother. Res.* **14**, 647-649.

Yin D, Tang X. (1995) Advances in the study on artificial cultivation of *Cordyceps sinensis*. *Zhongguo Zhong Yao Za Zhi* **20**, 707-709.

Yu RM, Yang YC, Yang YP, Wang SF. (1981) Study of chemical components in *Cordyceps sinensis*. *Pharm. Bull.* **16**, 55-57.

Yue DC, Feng XZ, Liu HY, Bao TT. (1995) *Cordyceps sinensis*, In: *Institute of Materia Medica, ed. Advanced Study for Traditional Chinese Herbal Medicine*, Vol 1, pp. 91-113, Beijing Medical University and China Peking Union Medical University Press, Beijing.

Zhang Q, Wu J, Hu Z, Li D. (2004) Induction of HL-60 apoptosis by ethyl acetate extract of *Cordyceps sinensis* fungal mycelium. *Life Sci.* **75**, 2911-2919.

Zheng XY. (2005) *Pharmacopoeia of The People Republic of China*. pp. 86, Chemical Industry Press, Beijing.

Zhu JS, Halpern GM, Jones K. (1998a) The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*: Part I. *J. Altern. Complem. Med.* **4**, 289-303.

Zhu JS, Halpern GM, Jones K. (1998b) The scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis*: Part II. *J. Altern. Complem. Med.* **4**, 429-457.