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Journal of Ethnopharmacology 96 (2005) 19–29
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Review

# The traditional Chinese medicine *Cordyceps sinensis* and its effects on apoptotic homeostasis

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Received 23 May 2004; received in revised form 20 September 2004; accepted 20 September 2004
Available online 5 November 2004

**Abstract**

*Cordyceps sinensis* is a medicinal fungus of Traditional Chinese Medicine. While there are a wide range of reported uses of *Cordyceps sinensis* in the literature, the reports that extracts of this fungus may alter apoptotic homeostasis are most intriguing. However, there are significant challenges regarding research surrounding *Cordyceps sinensis*, such as the difficulty identifying the various species of *Cordyceps* and the many conflicting reports of pharmacological function in the literature. In this review we outline what is known about the ability of *Cordyceps sinensis* to alter apoptotic homeostasis, attempt to reconcile the differences in reported function, identify the challenges surrounding future *Cordyceps sinensis* research, and delineate options for overcoming these critical hurdles.


*Keywords:* *Cordyceps sinensis*; Apoptosis; Traditional medicine; Plant; Fungus

**Contents**



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doi:10.1016/j.jep.2004.09.029

## 1. Introduction

The past 20 years has seen a phenomenal growth in the interest in and use of complementary or alternative medicine (CAM), and in the United States 42% of people utilize some form of CAM (Eisenberg et al., 1998). Of the total CAM market, herbal and specialty dietary supplements command a substantial portion with 29% of men and 36% of women reporting current use (Gunther et al., 2004). In 1994 the herbal supplement market boomed, partly as a result of the passage of the Dietary Supplement Health and Education Act (Center for Food Safety and Applied Nutrition, 2004), driving supplement sales up 70% from 1994 to 1997 (Radimer et al., 2000) and leading to peak sales of US$ 3.3 billion in 1999 (Harnack et al., 2001). This interest in natural compounds is certainly valid as these natural products undoubtedly contain biologically active components: plant-based pharmaceuticals have resulted in approximately one-half of the anticancer drugs developed since 1960 (Kim and Park, 2002) and have led to over 100 other successful pharmaceuticals (Farnsworth, 1994). While there is a wide range of available herbal supplements, one of the most interesting supplements is the not yet well-characterized *Cordyceps sinensis* (Berk.) Sacc.

The *Cordyceps sinensis* fungus first gained worldwide attention when it was revealed that several Chinese runners who broke world records in 1993 had included this fungus as part of their training program (Hollobaugh, 1993, Steinkraus and Whitfield, 1994; Starr et al., 1993). In the ten years since the initial reports, *Cordyceps sinensis* has received tremendous attention from the public. Purported effects of the fungus suggested a wide range of biological functions such as use as an aphrodisiac (Bhattarai, 1993), analgesic (Koyama et al., 1997) immune modulator (Gong et al., 2000), and free radical scavenger (Yamaguchi et al., 2000a). However, these effects have not been well analyzed.

Recently, aseptic mycelial cultivation has resulted in establishing a number of *Cordyceps sinensis* derivative cultures. The two most well studied of the cultures, referred to by the anamorph names *Paecilomyces hepiali* (strain CS-4) and *Cephalosporium sinensis*, may present the opportunity to produce a *Cordyceps sinensis* derivative product in a sustainable fashion. While these strains undoubtedly support ecologically sustainable use of *Cordyceps sinensis*, the actual similarities between wild *Cordyceps sinensis* and the cultures are not clear.

## 2. *Cordyceps sinensis* background and ethnomedical use

*Cordyceps sinensis* is endemic to alpine habitats (3600–5000 m in elevation) on the Tibetan plateau in southwestern China (Fig. 1), where it is a parasite on larvae of moths (Lepidoptera) in the genera *Hepialus* and *Thitarodes* (Kinjo and Zang, 2001). After a host larval infection with either meiotic or mitotic spores, the fungus multiplies in the host by yeast-like budding, eventually killing the host; the fungus then grows in the form of threadlike hyphae. Following overwintering, the fungus ruptures the host body, forming a sexual sporulating structure (a perithecial stroma) that is connected to the dead larva below ground and grows upward to emerge above the soil surface. It is this stroma, either with or without the host larva, which is traditionally used for medicinal purposes (Fig. 2).

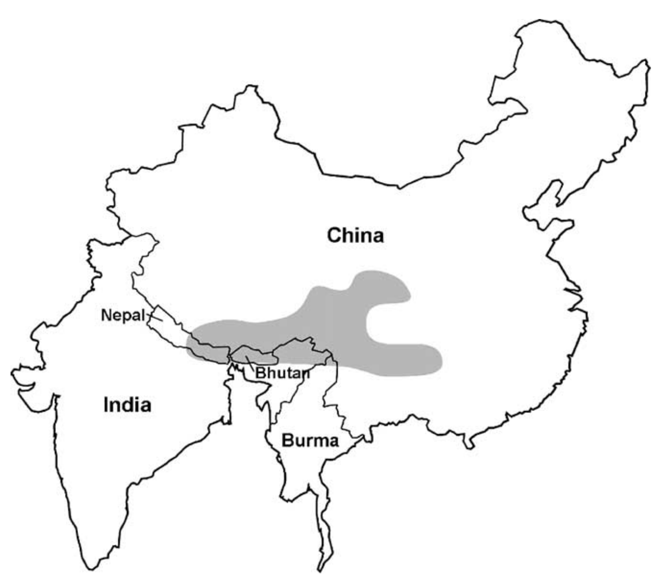


Fig. 1. Home range of *Cordyceps sinensis*.

Fig. 2. Fruiting bodies of *Cordyceps sinensis* and dried caterpillars.

### 2.1. Phylogenetic analysis of the genus Cordyceps

*Cordyceps* is a genus of perithecial ascomycetes (Phylum Ascomycota) classified in the Clavicipitaceae, a family supported by molecular phylogenetic analyses as a monophyletic group derived from the order Hypocreales (Artjariyasripong et al., 2001; Rehner and Samuels, 1995; Spatafora and Blackwell, 1993; Suh et al., 1998). *Cordyceps* species are parasites of insects or fungi, often exhibiting a high degree of host specificity; as a result of this host specificity, the anamorphic forms of some species (e.g., *Beauvaria bassiana*) are widely used as insect biocontrol agents (Huang et al., 2002). According to molecular phylogenetic analyses, *Cordyceps* does not represent a single evolutionary lineage; instead, *Cordyceps* appears to represent several lineages within the Clavicipitaceae (Ito and Hirano, 1997; Artjariyasripong et al., 2001; Sung et al., 2001; Zare et al., 2000). Similarly, the *Cordyceps* species associated with Lepidopteran hosts do not represent a monophyletic group (Nikoh and Fukatsu, 2000; Park et al., 2001; Sung et al., 2001). Furthermore, there appears to be a high degree of genetic variation within the *sinensis* species (Chen and Hseu, 1999). These levels of variation create a significant challenge in verifying samples for analysis of *Cordyceps sinensis*.

### 2.2. Ethnomedical use of Cordyceps sinensis

*Cordyceps sinensis* has a long history of medicinal use in China. This fungus is thought to have been discovered 2000 years ago (Liu et al., 2001) and its use was documented formally in the Qing dynasty *Bencao Congxin* (New Compilation of Materia Medica) in 1757. However, *Cordyceps sinensis* is a unique traditional medicine in that there exists little primary ethnomedical data describing medical use in the literature. Current ethnomedical reports on the uses of *Cordyceps sinensis* are limited to the application as a general tonic in China (Huang et al., 1981; Jiang, 1991; Hanssen and Schadler, 1982) and as an aphrodisiac in Nepal (Bhattarai, 1992a, 1993, 1992b, 1994, 1989). In contrast to the ethnomedical data, the literature surrounding the biological effects of *Cordyceps sinensis* is diverse (reviewed in Zhu et al., 1998a, 1998b). However, some of the most intriguing reports regarding the biological functions of *Cordyceps sinensis* center on its ability to alter apoptotic homeostasis.

## 3. Apoptotic homeostasis and disease states

### 3.1. Review of apoptosis

Apoptosis, or programmed cell death, is an essential event in organism development (Hidalgo and Ffrench-Constant, 2003; Vaux and Korsmeyer, 1999) and homeostasis (Kucharczak et al., 2003, Cory and Adams, 2002); however, it is becoming clear that numerous disorders such as stroke (Zheng et al., 2003), myocardial infarction (Krijnen et al., 2002), and HIV (Buenz and Badley, in press) incorporate apoptosis in their etiology and pathogenesis. There are numerous events that can induce a cell to undergo apoptosis (Nagata, 1997) and since the implication of apoptosis in various disease states as an effector mechanism, the ability to inhibit apoptosis has emerged as an important potential therapy. Interestingly, while inducing apoptosis has already proven an efficient method to treat cancer (Hu and Kavanagh, 2003), the ability to inhibit apoptosis in a clinical setting is just starting to be explored (Liston et al., 2003).

The potential for a cell to undergo apoptosis exists in a balance between endogenous factors characteristic of apoptosis induction, such as Bax (Degli Esposti and Dive, 2003; Scorrano and Korsmeyer, 2003), and factors characteristic of apoptosis inhibition, such as Bcl-2 (Cory et al., 2003; Gross et al., 1999). Once a cell receives sufficient pro-apoptotic stimuli, or lack of anti-apoptotic stimuli, the effector caspases, a family of cysteine aspartate proteases, are activated (Fischer et al., 2003). Cells undergoing apoptosis experience a cascade of events outlined in Fig. 3 that ultimately result in phenotypic changes such as phosphotydylserine expression on the outer cell leaflet (Maderna and Godson, 2003), nuclear condensation, and DNA fragmentation (Nagata et al., 2003) shown in Fig. 4.

### 3.2. Traditional medicines and apoptosis regulation

Many traditional medicines (e.g., *Nelumbo nucifera* (Jung et al., 2003) and *Bacopa monnieri* (Russo et al., 2003)), are reported to scavenge reactive oxygen species and the role of reactive oxygen species in the apoptotic pathway is of particular interest regarding herbal supplements. Reactive oxygen species and their regulatory molecules are important components of the immune system and cell function, such as superoxide radicals generated by activated neutrophils as a pathogen defense mechanism (Babior, 1978), and cell signaling (Ichiki et al., 2003). However, there are also reports of altered oxygen-based free radical levels in disease states. These

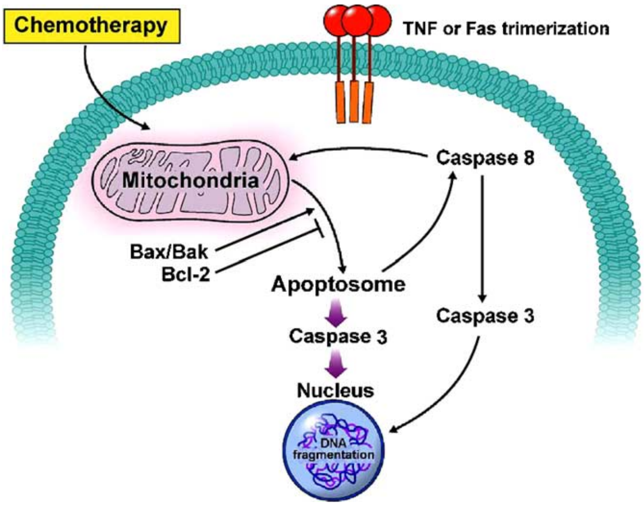


Fig. 3. Fas/CD95 apoptotic pathway. Death receptor trimerization induces a signaling cascade that ultimately results in chromosomal condensation, DNA fragmentation, and cellular apoptosis.

altered levels of reactive oxygen species in disease states such as cancer (Behrend et al., 2003) and stroke (Sugawara and Chan, 2003) have resulted in hypotheses that mitigation of excessive reactive oxygen species could be therapeutically important. While both the exact role of reactive oxygen species in disease states and whether they are a primary insult or a downstream result of the disease states have yet to be determined. It appears that the regulation of reactive oxygen species as therapy will be of significant interest in the future.

Undoubtedly there are herbal supplements that influence apoptotic homeostasis. For example, an isolated compound of the traditional medicine European feverfew (*Chrysanthemum parthenium)* has been shown to decrease susceptibility to apoptotic stimuli through down regulation of the Fas receptor and the Fas ligand agonist through inhibition of NF-κB (Li-Weber et al., 2002). However, the ability of *Cordyceps sinensis* to alter the apoptotic pathway is not so straightforward. Currently, there are reports of *Cordyceps sinensis* extracts both inhibiting (Table 1) and inducing apoptosis (Table 2). Most of these reports reflect a phenomenon level of observation, such as treatment with *Cordyceps sinensis* resulting in decreased caspase-3 activity (Shahed et al., 2001). While these reports do not examine the mechanism of action, they do facilitate a necessary foundation to allow examination of the molecular mechanism.

## 4. *Cordyceps sinensis* inhibits apoptosis

The reports of clinical trials suggest that *Cordyceps sinensis* potentially contains agents that may inhibit apoptosis (reviewed in Zhu et al., 1998a). These clinical results have driven work to assess the ability of *Cordyceps sinensis* to inhibit apoptosis in vitro; however, the results of these studies are conflicting. It has been reported that *Cordyceps sinensis* can scavenge reactive oxygen species (Zhang et al., 1995) by inhibiting malondialdehyde formation by the peroxynitrite generator SIN-1 (Yamaguchi et al., 2000a). These results have been confirmed through in vitro xanthine oxidase, hemolysis, and lipid peroxidation assays (Li et al., 2001). Furthermore, an isolated extract of *Cordyceps sinensis* H1-A has been shown to inhibit apoptosis induced by dimethyl sulfoxide (DMSO), which is known to induce apoptosis through permeabilizing the cell membrane and upregulating nitric oxide synthase (Trubiani et al., 2003). However, extracts of *Cordyceps sinensis* were unsuccessful in inhibiting hydrogen peroxide-induced apoptosis (Buenz et al., 2004), a reactive oxygen species model (Fauconneau et al., 2002).

Alternately, *Cordyceps sinensis* has also been reported to down-regulate apoptotic genes and modulate apoptosis in a

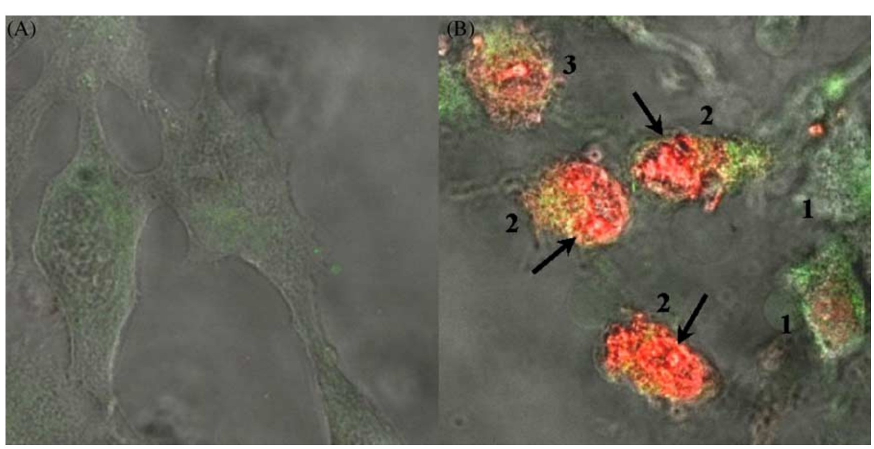


Fig. 4. HeLa cells stained with Annexin V-FITC (green) and propidium iodine (red). (A) Control cells show little phosphatidylserine expression (green) on the outer leaflet, no nuclear propidium iodine intercalation (red), and typical cellular morphology. (B) Cells treated with 30 uM starurosporine for 4 h are induced to undergo apoptosis. (1) Early stage apoptotic cells show increased phosphatidylserine expression on the outer leaflet and slight morphological condensation; however, the nuclear membrane is still intact and thus the cells do not stain propidium iodine positive. (2) In later stages of apoptosis, the integrity of the nuclear membrane is compromised and the DNA stains propidium iodine positive. Nuclear condensation and blebbing are also evident (arrows). 3) In the final stages of apoptosis, the cellular and nuclear membranes are totally compromised and the cell appears necrotic.

Table 1
Reported antiapoptotic effects of *Cordyceps sinensis*

| Reported function | Model | Concentration | Reference |
|---|---|---|---|
| Anticytotoxic activity | Mouse | 150 mg/kg | Yu et al. (1993) |
| Antioxidant activity | Mouse | 50 mg/kg | Yamaguchi et al. (2000b) |
| Antiproliferation activity | Cell culture | 100 μg/mL | Zhao Long and Xiao Xia (2000) |
| Cell proliferation inhibition | Cell culture | 10 μg/mL | Chen et al. (1997) |
| Cell proliferation inhibition | Human adult | 71 μg/mL | Kuo et al. (1996) |
| Cell proliferation inhibition | Human adult | 40 μg/mL | Lin et al. (1999) |
| Cell proliferation inhibition | Mouse | 1.0 % of diet | Lin et al. (1999) |
| Gene expression inhibition | Rat | 0.5 mL per animal | Shahed et al. (2001) |
| Hemolysis inhibitory activity | Cell culture | 1.5 mg/mL | Li et al. (2001) |
| Lipid peroxide formation inhibition | Cell culture | 5.0 mg/mL | Li et al. (2001) |
| Natural killer cell inhibition | Human adult | 12.9 μg/mL | Kuo et al. (1996) |
| Radical scavenging effect | Cell culture | 5.0 mg/mL | Shahed et al. (2001) |
| Radical scavenging effect | Cell culture | 0.08 mg/mL | Li et al. (2001) |
| Tumor necrosis factor inhibition | Human adult | 2.7 μg/mL | Kuo et al. (1996) |

rat kidney ischemia reperfusion model. Shahed et al. (2001) showed a significant decrease in Fas, Fas ligand, and Tumor Necrosis Factor-α (TNF-α) expression along with decreased caspase-3 activity. Similarly, *Cordyceps sinensis* has been reported to inhibit TNF-α expression (Kuo et al., 1996). However, when apoptosis was initiated through CH-11, a Fas agonist antibody (Alderson et al., 1994), aqueous and alcohol extracts of *Cordyceps sinensis* were unable to rescue cells induced through Fas receptor ligation (Buenz et al., 2004).

Furthermore, it has been shown that in certain cell types, inhibition of proliferation or cell cycle arrest results in cells becoming resistant to apoptosis (Chaturvedi et al., 1999). In turn, the reports that *Cordyceps sinensis* inhibits proliferation of leukemic U937 cells (Chen et al., 1997) and glomerular mesangial cells (Zhao Long and Xiao Xia, 2000; Lin et al., 1999) may result by conferring a relatively apoptotic resistant state to cells. While the mechanisms of this inhibition have yet to be characterized, it is reasonable to propose that the alteration may potentially involve p53 (Fridman and Lowe, 2003) or NF-κB (Karin et al., 2004).

## 5. *Cordyceps sinensis* induces apoptosis

The ability to induce apoptosis has been identified and utilized in successful cancer chemotherapeutics (Hu and Kavanagh, 2003); Table 2 outlines the literature suggesting or stating the ability of *Cordyceps sinensis* to induce apoptosis.

Recently, Yang et al. (Yang et al., 2003) described the ability of the previously isolated 410 kDa polysaccharide fraction of *Cordyceps sinensis* termed H1-A (Yang et al., 1999) to induce apoptosis through inhibiting phosphorylation of Bcl-2 and Bcl-xL. These anti-apoptotic Bcl-2 family members are known to sequester cytosolic pro-apoptotic proteins such as Bax. As addressed above, this fraction also inhibited apoptosis induced by dimethyl sulfoxide (DMSO) (Yang et al., 2003). However the report consisting of the inhibition of Bcl-2 and Bcl-xL and subsequent inhibition of apoptosis by DMSO (Yang et al., 2003) seems counterintuitive. Thus, further work is necessary to clarify the physiologic role of the H1-A extract.

Finally, there are reports of direct cytotoxic activity (Nakamura et al., 1999; Kuo et al., 1994; Sato, 1989). However, these studies report inhibition at the phenomena level and do not address specific mechanisms. Yet it is interesting that cordycepin, a compound originally isolated from the *Cordyceps sinensis* relative *Cordyceps militaris* (Cunningham et al., 1950), is known to exert cytotoxic effects through nucleic acid methylation (Kredich, 1980). While isolation of this single active compound may set the stage for work to identify molecular mechanisms of action, the actual presence of cordcycepin in *Cordyceps sinensis* has been difficult to confirm. Cordycepin has been shown to be present in *Cordyceps sinensis* through nuclear magnetic resonance (Chen and Chu, 1996); however, other groups have not been able to detect this compound (Shiao et al., 1994).

Table 2
Reported apoptotic effects of *Cordyceps sinensis*

| Reported function | Model | Dose | Reference |
|---|---|---|---|
| Antitumor activity | Mouse | Not stated | Zang et al. (1985) |
| Antitumor activity | Mouse | 5.0 g/kg | Xu et al. (1992) |
| Cell proliferation stimulation | Cell culture | 10 μg/mL | Chen et al. (1997) |
| Cytotoxic activity | Cell culture | 2.0 μg/mL | Kuo et al. (1994) |
| Cytotoxic activity | Cell culture | 500 μg/mL | Sato (1989) |
| Cytotoxic activity | Cell culture | 10 μg/mL | Nakamura et al. (1999) |
| Metastasis inhibition | Mouse | 100 mg/kg | Nakamura et al. (1999) |

## 6. The challenge of identifying *Cordyceps sinensis*

The most significant challenge working with *Cordyceps sinensis* is the lack of a well-defined mechanism to identify sample material. Although *Cordyceps* species have been included in a number of recent molecular phylogenetic analyses (Artjariyasripong et al., 2001; Ito and Hirano, 1997; Lumbsch et al., 2000; Nikoh and Fukatsu, 2000; Nikoh and Fukatsu, 2001; Obornik et al., 2001; Park et al., 2001; Spatafora and Blackwell, 1993; Suh et al., 2001; Suh et al., 1998; Sung et al., 2001; Zare et al., 2000), most of these studies have either not included *Cordyceps sinensis*, or have included *Cordyceps sinensis* without enough other taxa to allow phylogenetic resolution to be achieved among *Cordyceps sinensis* and other closely related *Cordyceps* species. Most DNA-based studies that include *Cordyceps sinensis* have examined genetic differentiation at the population level rather than at the species level (Chen et al., 2001; Kinjo and Zang, 2001; Liu et al., 2001). Using a neighbor-joining analysis of ribosomal DNA internal transcribed spacer (rDNA-ITS) sequences, Park et al. (Park et al., 2001) determined *Cordyceps sinensis* to be closely related to *Cordyceps ophioglossoides*, a parasite of "false truffle" fungi in the genus *Elaphomyces*. Based on this evidence, as well as the placement of a *Hirsutella* anamorph (though with low bootstrap support) within a larger phylogenetic clade that includes *Cordyceps ophioglossoides* (Sung et al., 2001), a possible phylogenetic placement for *Cordyceps sinensis* is within a basal clade in the Clavicipitaceae that contains both entomopathogenic and fungicolous fungi. However, direct molecular evidence for the proper phylogenetic placement of *Cordyceps sinensis* in relation to a polyphyletic *Cordyceps* is currently lacking, and *Cordyceps* and the family Clavicipitaceae are greatly in need of systematic revision.

Many ascomycetes, including species of *Cordyceps*, have both an asexual (anamorphic) and sexual (teleomorphic) form. *Cordyceps* species have been shown in mycological culture studies to be associated with a number of anamorphic genera including *Paecilomyces*, *Beauvaria*, *Metarhizium*, *Verticillium*, and *Tolypocladium* (Hodge et al., 1996; Huang et al., 2002; Nikoh and Fukatsu, 2000; Nikoh and Fukatsu, 2001; Obornik et al., 2001; Sung et al., 2001; Zare et al., 2000), and phylogenetically related to several species of yeast-like endosymbionts of insects (Suh et al., 2001). Determining the anamorphic state of *Cordyceps sinensis* has posed difficulty for researchers; as a result, 22 names in 13 anamorph genera have previously been associated with *Cordyceps sinensis* (Jiang and Yao, 2002). However, recent molecular evidence (Chen et al., 2001; Chen et al., 2002; Liu et al., 2001) and generation of the anamorphic state from germinated *Cordyceps sinensis* ascospores (Liu et al., 2001) supports *Hirsutella sinensis* Liu et al. as being the correct anamorph of *Cordyceps sinensis*. Many anamorphic fungi, including *Hirsutella sinensis*, can be cultured under laboratory conditions. Because *Cordyceps sinensis* is considered to be declining in the wild due to overharvest (Liu et al., 2003), culture of the anamorphic state may therefore be a means of producing material for research and medicinal preparations in the face of a shortage of teleomorph material.

Several *Cordyceps* species have been described that appear morphologically similar to *Cordyceps sinensis*, including *Cordyceps nepalensis* M. Zang & Kinjo, *Cordyceps multiaxialis* M. Zang & Kinjo, *Cordyceps gansuensis* Zhang et al., and *Cordyceps crassispora* Zang et al. However, these species may simply represent morphological variants of *Cordyceps sinensis* (Kinjo and Zang, 2001; Liu et al., 2001). Whether these morphotypes correspond to differences in pharmacological activity has not been determined. Other *Cordyceps* species (e.g., *Cordyceps militaris*) have been shown to have some of the same medicinal properties as *Cordyceps sinensis* (Wu et al., 2000), but appear to be less highly regarded by consumers and practitioners.

## 7. Strain typing and relations to product quality

The lack of molecular evidence for proper phylogenetic placement of *Cordyceps sinensis* precludes establishing a consensus strain of *Cordyceps sinensis*. Similarly, conducting reliable clinical trials and ascertaining the quality of commercial herbal and other natural products depends upon accurately identifying source materials. A number of factors could contribute to poor quality of *Cordyceps sinensis* products, including intentional substitution of other *Cordyceps* species, substitution of counterfeit material, or unintentional misidentification of field-collected *Cordyceps*. In addition, as demand for *Cordyceps sinensis* products grows and the supply of wild material declines, mycelium of the asexual (anamorphic) stage grown under artificial culture conditions is increasingly used in medicinal products. Among the source materials found to have been sold under the name Chongcao in commercial markets are other *Cordyceps* species (e.g., *Cordyceps militaris*, *Cordyceps liangshanensis*, *Cordyceps gunnii*, *Cordyceps hawkesii*, and *Cordyceps ramosa*), anamorphic fungi including *Hirsutella sinensis*, the anamorph of *Cordyceps sinensis*, as well as *Paecilomyces sinensis* and *Tolypocladium* sp., and rhizomes from the plant species *Stachys geobombycis* and *Stachys sieboldii* (Chen et al., 2002; Cheng et al., 1998). Even within *Cordyceps sinensis*, differences in pharmacological activity have been noted between strains (Kinjo and Zang, 2001).

Because commercial products generally contain dried, powdered material, identifying source material using morphological characters is normally impossible. DNA fingerprinting methods offer a dependable means of identifying source materials from fresh specimens and commercial preparations (Zerega et al., 2002). Such methods are able to distinguish isolates at the species, or even strain, level depending upon the specific method used (e.g., (Terashima et al., 2002)). The effectiveness of DNA fingerprinting for authenticating source material has been demonstrated for a number of pharmacologically useful plants, including skullcap (*Scutellaria* spp.; (Hosokawa et al., 2000)), *Echinacea* (Nieri et al., 2003), black cohosh (*Actaea racemosa*; (Zerega et al., 2002)), *Lycium* (Zhang et al., 2001), ginseng (*Panax ginseng*; (Hon et al., 2003, Ngan et al., 1999)), *Withania* (Negi et al., 2000), St. John's wort (*Hypericum perforatum*; (Mayo and Langridge, 2003)), opium poppy (*Papaver somniferum*; (Saunders et al., 2001)), coca (*Erythroxylum* spp.; (Johnson et al., 2003)), and yarrow (*Achillea millefolium*; (Wallner et al., 1996)). DNA fingerprinting has been successfully used in fungi to distinguish strains of human pathogenic fungi (Buffington et al., 1994; Mcewen et al., 2000), plant pathogens (Barnes et al., 2001; Chen et al., 2000; Morris et al., 2000; Schmidt et al., 2003; Zeller et al., 2003), food spoilage agents (Kure et al., 2003), biocontrol agents (Avis et al., 2001; Hermosa et al., 2001), and edible mushrooms (Terashima et al., 2002).

Previous genetic analyses of multiple *Cordyceps sinensis* populations have examined ribosomal DNA (rDNA) sequence diversity (Chen and Hseu, 1999; Chen et al., 2001; Chen et al., 2002; Kinjo and Zang, 2001; Liu et al., 2001) and patterns of genetic variability exhibited by randomly amplified polymorphic DNA (RAPD) markers (Chen et al., 1999; Cheng et al., 1998). Variability in ribosomal ITS sequences and 18S ribosomal DNA restriction fragment length polymorphism (RFLP) patterns has been shown to be informative for differentiating *Cordyceps sinensis* from other *Cordyceps* species and from market counterfeits (Chen et al., 1999; Chen et al., 2001; Chen et al., 2002; Kinjo and Zang, 2001; Liu et al., 2001), and for generating *Cordyceps sinensis*—specific DNA probes (Chen et al., 2002); however, rDNA sequence variation is too low to allow accurate genotyping at the strain level (Chen et al., 2001; Kinjo and Zang, 2001; Liu et al., 2001). Randomly amplified polymorphic DNA markers are able not only to distinguish *Cordyceps sinensis* from other *Cordyceps* species, but to distinguish individual *Cordyceps sinensis* populations (Chen and Hseu, 1999; Cheng et al., 1998). More recently developed DNA fingerprinting methods such as amplified fragment length polymorphisms (AFLP; Vos et al., 1995) uncover more genetic polymorphism over a larger part of the genome than do ribosomal DNA sequences. Additionally, the results of AFLP fingerprinting have been shown to have a higher degree of repeatability than those of RAPD fingerprinting (Ranamukhaarachchi et al., 2000; Jones et al., 1997; Barker et al., 1999). AFLP fingerprints have thus far not been obtained for *Cordyceps sinensis*, and represent a potentially useful tool for characterizing *Cordyceps sinensis* samples. Further population-level genetic characterization is extremely important for *Cordyceps sinensis* material in order to determine the geographic origins of source material, select standardized strains for clinical experiments, distinguish anamorph cultures from fungal contaminants, and facilitate conservation of genetic diversity in natural populations.

## 8. The future of apoptosis regulation through *Cordyceps sinensis*

There are three prominent factors that may contribute to the discrepancies in reports regarding the ability of *Cordyceps sinensis* to inhibit apoptosis. First, as there is no consensus strain, it is possible that certain populations contain different biologically active compounds. Second, there is the potential that *Cordyceps sinensis* extracts contain a pro-drug and there is a necessary metabolism step in order to generate the biologically active form of the drug. Third, as there are multiple methods of extraction utilized in the literature such as ethanol (Xu et al., 1992), methanol (Kuo et al., 1994), alkaline extract (Kiho et al., 1996), hot water (Manabe et al., 1996), and cold water (Chen et al., 1997), different constituents may be assayed depending on extraction method. However, these challenges are certainly not intractable. The *Cordyceps sinensis* consensus strain could be established through AFLP-based DNA fingerprinting as has been done for other medicinal plants. Similarly, the issue regarding an active metabolite could be addressed through either establishing a liposome system containing enzymes known to be important in drug metabolism such as members of the P450 family (De Graaf et al., 2002) or, alternately, it may be possible to treat the *Cordyceps sinensis* extract with a liver homogenate to generate the active metabolites of the extracts (De Graaf et al., 2002).

Furthermore, the research surrounding *Cordyceps sinensis* is lacking molecular studies. Rather, there are numerous reports of treating model systems with *Cordyceps sinensis* and measuring outcome. However, establishing a consensus strain is an essential foundation to defining molecular mechanisms. Currently the use of voucher specimens and marker compounds does allow identification of species phenotypically and biochemically, respectively. However, a principle marker compound for *Cordyceps sinensis* is cordycepic acid (mannitol-D) and while the presence of cordycepic acid is indicative of *Cordyceps sinensis*, the inclusion of a single marker compound does not necessarily guarantee the presence of other potentially active compounds. Thus, developing an AFLP based DNA fingerprinting program would allow positive identification of not just *Cordyceps sinensis*, but also identification of sub-populations of the species.

*Cordyceps sinensis* has been used as a traditional medicine throughout history and, undoubtedly, as investigation into this fungus continues, more active components with potential therapeutic value will be isolated.

## Acknowledgements

We would like to thank Jacklynn Conway and Jane Meyer, Mayo Clinic, Rochester, Minnesota, USA, for their administrative assistance; Holly Johnson, University of Illinois at Chicago / National Institutes of Heath Center for Botanical Dietary Supplements Research, Chicago, Illinois, USA, for her assistance with the Natural Products Alert Database queries; and Sy Chalpin, Hi-Health, Scottsdale, Arizona, USA, for providing *Cordyceps sinensis* for our work.

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