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# A Systematic Review of the Mysterious Caterpillar Fungus *Ophiocordyceps sinensis* in DongChongXiaCao (冬蟲夏草 Dōng Chóng Xià Cǎo) and Related Bioactive Ingredients

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## ABSTRACT

The caterpillar fungus *Ophiocordyceps sinensis* (syn.† *Cordyceps sinensis*), which was originally used in traditional Tibetan and Chinese medicine, is called either "yartsa gunbu" or "DongChongXiaCao (冬蟲夏草 Dōng Chóng Xià Cǎo)" ("winter worm-summer grass"), respectively. The extremely high price of DongChongXiaCao, approximately USD $20,000 to 40,000 per kg, has led to it being regarded as "soft gold" in China. The multi-fungi hypothesis has been proposed for DongChongXiaCao; however, *Hirsutella sinensis* is the anamorph of *O. sinensis*. In Chinese, the meaning of "DongChongXiaCao" is different for *O. sinensis*, *Cordyceps* spp.,‡ and *Cordyceps* sp*ƒ*. Over 30 bioactivities, such as immunomodulatory, antitumor, anti-inflammatory, and antioxidant activities, have been reported for wild DongChongXiaCao and for the mycelia and culture supernatants of *O. sinensis*. These bioactivities derive from over 20 bioactive ingredients, mainly extracellular polysaccharides, intracellular polysaccharides, cordycepin, adenosine, mannitol, and sterols. Other bioactive components have been found as well, including two peptides (cordymin and myriocin), melanin, lovastatin, γ-aminobutyric acid, and cordysinins. Recently, the bioactivities of *O. sinensis* were described, and they include antiarteriosclerosis, antidepression, and antiosteoporosis activities, photoprotection, prevention and treatment of bowel injury, promotion of endurance capacity, and learning-memory improvement. *H. sinensis* has the ability to accelerate leukocyte recovery, stimulate lymphocyte proliferation, antidiabetes, and improve kidney injury. Starting January 1st, 2013, regulation will dictate that one fungus can only have one name, which will end the system of using separate names for anamorphs. The anamorph name "*H. sinensis*" has changed by the *International Code of Nomenclature for algae, fungi, and plants* to *O. sinensis*.

**Key words:** Bioactive Ingredients, *Cordyceps sinensis*, DongChongXiaCao, *Hirsutella sinensis*, *Ophiocordyceps sinensis*

Notification
†The term *"Cordyceps sinensis"* has been renamed to its synonym *"Ophiocordyceps sinensis"* by Sung *et al.* in 2007. In the discussion, *"Cordyceps sinensis"* is still used to represent *"Ophiocordyceps sinensis"* out of respect to the original authors of the articles that we cited.
‡*Cordyceps* spp. indicates any species that belongs to the genus *Cordyceps*.
*ƒ**Cordyceps* sp. indicates the unidentified species that belong to the genus *Cordyceps*

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## INTRODUCTION

The caterpillar fungus *Ophiocordyceps sinensis* (syn. *Cordyceps sinensis*) is one of the entomogenous Ascomycetes and parasitizes the larvae of Lepidoptera to form the well-known traditional Tibetan medicine "yartsa gunbu" or, in traditional Chinese medicine, "DongChongXiaCao (冬蟲夏草 Dōng Chóng Xià Cǎo)"("winter worm-summer grass," [Figure 1]). DongChongXiaCao is a well-described remedy that has been used in traditional Chinese medicine for over 700 years.[1] The wild fungus, which possesses a plant-like fruiting body and originates from dead caterpillar that fill with mycelia [Figure 2], has generally been called *C. sinensis* or *Cordyceps* spp. ("ChongCao" in Chinese) due to its insect-shape appearance. *O. sinensis* (previously named *C. sinensis*) is a slow-growing fungus and needs to be grown at a comparatively low temperature, i.e., below 21ºC. Both temperature and growth rate are crucial factors that identify *O. sinensis* from other similar fungi.[2] In recent decades, curative and health-care products derived from the so-called "Cordyceps" are extremely popular in China in various forms such as capsules, oral liquids, and drinks.[3] Most of these products, derived from submerged mycelial *O. sinensis* cultures [Figure 3], are the popular merchandise items on the market.

It has been shown that *O. sinensis* can be used to treat conditions such as hyposexuality, night sweats, hyperglycemia, hyperlipidemia, asthenia, arrhythmias, and other heart, respiratory, renal and liver diseases.[3] Although "natural *O. sinensis* specimens" have significant pharmaceutical effects, the commercial cultivation of this fungus on larvae of moth to produce fruiting body has not yet been successful.[4] Therefore, the biology of *O. sinensis* remains a secret, and its commercial cultivation is still a dream.[5]

In the past years, several new names have been proposed for *O. sinensis*-like species from alpine regions, such as *O. gansuënsis*, *O. crassispora*, *O. kangdingensis*, *O. multiaxialis, O. nepalensis*, and others, but there is not sufficient to distinguish these species from *O. sinensis*.[6] Fungi other than *O. sinensis* originating

**Figure 1.** Wild DongChongXiaCao (black part: the fruiting body; brown part: the caterpillar corpus)

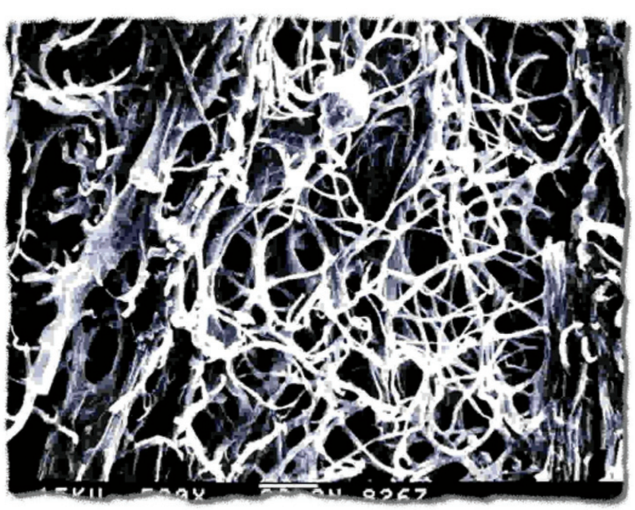

**Figure 2.** Scanning electron micrograph of mycelia filling the inside of a fruiting body of wild DongChongXiaCao

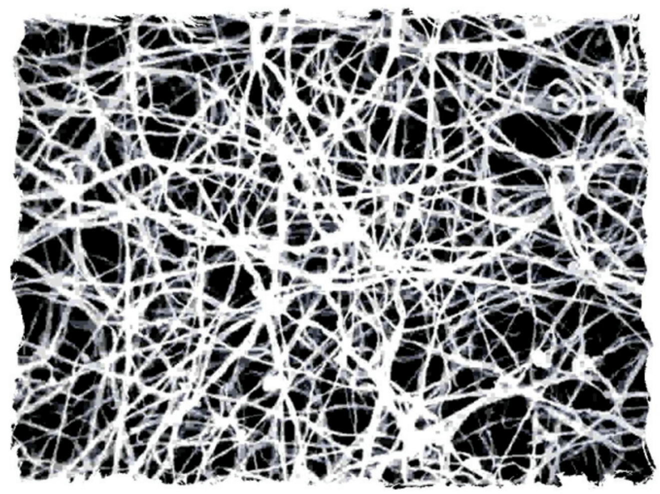

**Figure 3.** Scanning electron micrograph of mycelia from the medium of a submerged culture of *Ophiocordyceps sinensis*


**Figure 4.** Label showing the precious price (CNY ¥698 per gram) of wild DongChongXiaCao sold in August, 2012 in Beijing, China

from natural *O. sinensis* specimens could be important resources for the development of alternative products.[7] For example, it is claimed that the Bailing Capsule, a cultured product isolated from DongChongXiaCao, has a similar chemical composition to natural *O. sinensis*; in addition, the Bailing Capsule possesses anti-inflammatory, antihypoxia, and antitumor properties and has the ability to regulate the endocrine system, enhance immune function, and protect the kidneys, lung, liver, and other organs.[8]

As a traditional oriental medicine, DongChongXiaCao is endemic to alpine habitats on the Tibetan Plateau, located predominantly in Tibet, the Tibetan autonomous prefectures of neighboring provinces, and the high Himalayas.[9] In recent years, DongChongXiaCao has been regarded as the Himalayan Viagra, which has caused the price to reach USD $6.77 per piece of wild medicine.[10] Over the past 10 years, its value has increased dramatically. For example, collectors must pay as much as USD $12,500 per kg for top-quality material.[11] In 2008-2009, the price of *C. sinensis* crude drug was around USD $13,000 per kg, which caused it to be regarded as "soft gold" in China.[12] Furthermore, it is believed that the price of this fungus reached USD $20,000 to 40,000 per kg in the international market.[13] As of August 2012, the price per gram of wild DongChongXiaCao in Beijing is up to CNY ¥698 [Figure 4], or USD $111,560 per kg. This price already surpasses that of real gold. According to the government statistics for 2004, 50,000 kg of this drug were collected, which contributed more than USD $225 million to the Tibet Autonomous Region's GDP,[14] these data suggested that about 40% of the rural cash income in the Tibet Autonomous Region comes from DongChongXiaCao collection.

## REVIEW ARTICLES AND SPECIAL REPORTS OF *CORDYCEPS*

Since 1998, there have been more than 25 reviews or special reports published discussing *Cordyceps*, and 14 of them have focused on *C. sinensis.* For example, these studies have emphasized: terminology, life strategy, and ecology;[5] traditional uses and medicinal potential in Sikkim;[10] the reliability of fungal materials;[2] ecology, trade, and development in Tibet;[15] production and sustainability on the Tibetan Plateau and in the Himalayas;[16] origin of scientific name, morphological characteristics, micromorphological characteristics of the teleomorph, identification, hosts, and synonymy;[6] ethnomycological use, collection, discovery, protection, and the range of diseases treated in Northern Yunnan Province in China;[9] host spectrum, distribution, artificial rearing host, infection technology, and substitute products;[17] clinical efficacy for chronic kidney diseases;[18] markers and analytical methods for quality control;[19] history, use, and implications;[20] pharmacological functions;[21] safety, effects on the nervous system, glucose metabolism, effects on the respiratory, hepatic, cardiovascular, immune systems, immunological disease, inflammatory conditions, cancer, and diseases of the kidney;[22] and *in vitro* and *in vivo* studies, open-label and double-blinded clinical trials on the respiratory, renal, hepatic, cardiovascular, immunological, and nervous systems, and in its effects on cancer, glucose metabolism, inflammatory conditions, and toxicological studies.[23] Two papers have focused on *C. militaris*, placing emphasis on: biological aspects including the host range, mating system, cytology and genetics, insect- and noninsect nutritional requirements, environmental influence on stroma development, and commercial development;[24] and active principles and culture techniques.[25] The other publications focus on *Cordyceps*, examining: pharmacological functions and development of products;[3] production, isolation, purification, structure elucidation, and pharmacological action of polysaccharide;[1] chemical constituents;[26] preparations and chemical structures of polysaccharide;[27] taxonomic concepts, preparations, apoptosis, chemical constituent profiling, hosts, and poisoning;[28] history, medicinal uses, chemical composition, and cultivation;[29] pharmacological actions;[30] and the pharmacological basis of "Yin-nourishing (養陰 Yǎng Yīn)" and "Yang-invigorating (壯陽 Zhuàng Yáng)" actions.[31]

## TERMINOLOGY OF "DONGCHONGXIACAO" WITH REGARD TO *CORDYCEPS*

"DongChongXiaCao" is commonly known as "yarsa gumba" in Tibetan, because "yarsa" means winter and "gumba" means summer. "Gunba" or "gonba" has also been used to replace "gumba," and the fungus is named "keera jhar" (insect herb) in the Indian mountains.[13]

However, the term "DongChongXiaCao" in Chinese has been recognized as having different meanings, as follows:

1. The traditional Chinese medicine originating from *O. sinensis* (syn. *C. sinensis*).
2. The health food originating from *O. sinensis.*
3. The fungus *O. sinensis.*
4. The fungi *Cordyceps* spp.
5. The fungus *Cordyceps* sp.
6. The fungi *Ophiocordyceps* spp.
7. The fungus *Ophiocordyceps* sp.
8. The wild and crude medicine that has a caterpillar shape with fruiting body.
9. The mycelia of *O. sinensis* derived from submerged culture.
10. The mycelia of *H. sinensis* derived from submerged culture.
11. The traditional Chinese medicine originating from the larvae of Hepialidae (Lepidoptera) infected by *O. sinensis*.
12. The traditional Chinese medicine originating from the larvae of *Thitarodes* (syn. *Hepialus*) infected by *O. sinensis*.
13. The traditional Chinese medicine originating from the larvae of *T. armoricanus* (syn. *H. armoricanus*) infected by *O. sinensis*.
14. The traditional Chinese medicine originating from the larvae of Hepialidae (Lepidoptera) infected by *Ophiocordyceps* spp.
15. The traditional Chinese medicine originating from the larvae of Hepialidae (Lepidoptera) infected by *Cordyceps* spp.

The term "ChongCao" (meaning Insect Grass) in Chinese is the abbreviation of "DongChongXiaCao," which related products is popular in the market in Taiwan. For consumers, it is confusing whether "ChongCao" is equivalent to "DongChongXiaCao," the traditional Chinese medicine "DongChongXiaCao," or the fungi *Cordyceps* spp. In addition, *C. militaris*, a type species of *Cordyceps*, has been regarded as a substitute for "DongChongXiaCao" and is named "North DongChongXiaCao," "BeiChongCao," "ChongCao mycelium," or "ChongCao fruiting body." There is no doubt that *O. sinensis* is not equivalent to the traditional Chinese medicine "DongChongXiaCao," because the latter has a caterpillar shape with a fruiting body. In the market, natural products with caterpillar shapes and fruiting bodies that are called "DongChongXiaCao" are extensively distributed; however, the origins of most of these microorganisms and their hosts remain uncertain. In the scientific view, the traditional Chinese medicine wild "DongChongXiaCao" is not strictly equivalent to *O. sinensis* unless the species has been identified. In fact, for most literature, the term "*C. sinensis*" used in the materials section might refer to wild DongChongXiaCao or to its fruiting bodies or cultured mycelia. Dong and Yao[2] reviewed 152 papers from PubMed on *O. sinensis* since 1998 and found that at least 116 papers (over three-quarters) used unreliable, uncertain, or unspecified materials, including so-called cultivated fruit bodies that were apparently not *O. sinensis* strains, based on temperature and growth period.

## RENAMING, ANAMORPH, AND GENETIC DIVERSIFICATION OF *C. SINENSIS*

### Renaming of *C. sinensis*

The colony characteristics of *O. sinensis* cultures are significant different from *Cordyceps* spp. Mostly *Cordyceps* species possess brightly colored and fleshy stromata. The family of Cordycipitaceae has been validated based on the type species of *Cordyceps*, *C. militaris*, and a new family, Ophiocordycipitaceae, based on the Genus *Ophiocordyceps* Petch, the majority of whose species produce dark pigments and tough to pliant stromata, often possesses aperithecial apices including *C. sinensis*.[32] Based on the publication referenced above, *C. sinensis* has been transferred to *Ophiocordyceps* and has been renamed *O. sinensis*.

### The anamorph of *C. sinensis*

Twenty-two names spanning 13 genera associated with the anamorph of *C. sinensis* have been described.[33] However, *H. sinensis* and *C. sinensis* belong to different stages of the life cycle of the same organism: *H. sinensis* is the anamorph of *C. sinensis*, rather than *Paecilomyces sinensis* or other species.[34] The rDNA ITS sequences of *C. sinensis* collected from different geographical regions are almost identical and are significantly different from substitutes.[35] *H. sinensis* has been confirmed as the anamorph of *C. sinensis* by both DNA sequences and microcyclic conidiation, and additionally, two species, *C. multiaxialis* and *C. nepalensis*, were shown to share identical or almost identical ITS sequences with *C. sinensis*.[36]

The relationship between teleomorphs of *Cordyceps* spp. and their presumed anamorphs has been investigated by analyzing 5.8S and ITS rDNA sequences. Both sequence analyzes demonstrated that *H. sinensis* is the anamorph of *C. sinensis*.[37] When analyzing the sequences of ITS1, ITS2 and 5.8S rDNA regions, some species with different names had similar morphologies to *C. sinensis*, which suggested that these species might be synonymous with *C. sinensis*.[38] Based on the results of the 5.8S rDNA and ITS region analyses, it is clear that the ITS sequences within *C. sinensis* are highly homologous, regardless of the geographical origin. The evolutionary distance values between *C. sinensis* and *H. sinensis* were found to be the same as those for *C. sinensis* from different geographic regions, and *C. sinensis* should only have *H. sinensis* as its asexual stage, regardless of sample origin.[39] Even though many studies indicate that *H. sinensis* is the only anamorph of *C. sinensis*, molecular evidence demonstrates the existence of both *H. sinensis* and *Paecilomyces hepiali* DNA in the caterpillars and fruiting bodies of natural *C. sinensis*, strongly supports the multi-fungi hypothesis for natural *C. sinensis*.[40]

### Genetic diversification of C. sinensis

A high diversity in the fungal community structure occurs in natural *O. sinensis*.[5] The significant genetic divergence in *O. sinensis* was found to be greater among southern isolates than among northern isolates in China.[41] The genetic similarity indices range from 0.282 to 0.782, which indicate that there is a high level of diversity among natural *C. sinensis* samples.[42] In addition, a total of 141 markers, 99.3% of which were polymorphic, were identified in 180 individual samples of natural *C. sinensis* from 18 populations, and these 18 populations can be divided into five groups based on genetic distance and by grouping pattern matches with geographic distributions along the latitudinal gradient.[43] It has been observed that differences in medicinal effects among *C. sinensis* populations may be attributed to the existence of genetically differentiated chemotypes in morphotaxon.[44]

## CHEMICAL CONSTITUENTS, PROXIMATE COMPOSITION AND VOLATILE COMPOUNDS

### Chemical constituents

The chemical constituents of natural *Cordyceps* include cordycepic acid, glutamic acid, amino acids, polyamines, cyclic dipeptides, saccharides and sugar derivatives, sterols, nucleotides and nucleosides, 28 saturated and unsaturated fatty acids, fatty acid derivatives and other organic acids, vitamins, and inorganic elements.[13] Palmitic acid, linoleic acid, oleic acid, stearic acid, and ergosterol are the main components of natural and cultured *Cordyceps*, these fatty acids, as well as 14 investigated compounds, can be used to discriminate the hierarchical cluster, as the palmitic acid and oleic acid contents in natural *Cordyceps* are significantly higher than those in the cultured *Cordyceps*.[45]

### Proximate composition and others

Significant differences in proximate composition, such as for protein, fat, carbohydrate, and moisture content, were observed between the corpus and the fruiting body of wild DongChongXiaCao and the fermented mycelia of *C. sinensis*. There is a conspicuously high carbohydrate content of 39.4% for fermented mycelia, whereas carbohydrates comprise 24.20% of the corpus and 24.9% of the fruiting body in wild DongChongXiaCao.[46] Fermented mycelia had lower protein and fat contents (14.8 and 6.63%, respectively) than the corpus (29.1 and 8.62%, respectively) and the fruiting body (30.4 and 9.09%, respectively) of wild DongChongXiaCao.[46] However, Smironv *et al,*[47] showed that the mycelia of *C. sinensis* had high protein (29%) and low lipid contents (7%), and, similar to other studies. They also indicated that the mycelia were rich in endopolysaccharides (EPS, 15%), phospholipids (up to 28% of total lipids), and unsaturated fatty acids (C18:1 - up to 44%; C18:2 - 53% of total fatty acids).

The compositions of natural fruiting bodies of *C. sinensis* (NFCS) and mycelia from submerged cultures (MSMC) and shaking cultures (MSKC) of *H. sinensis* were compared by Li *et al.*[48] They indicated that the crude fat, crude protein, total, and essential amino acid contents could be ranked in the following descending order: MSKC > MSMC > NFCS, and additionally, unsaturated fatty acids in MSMC account for 65.9% of total fatty acids, which is noticeably lower than for NFCS (86.9%) and MSKC (76.5%). The total content of four nucleosides (adenosine, guanosine, uridnine, and inosine) in MSMC (6.20 mg/g) was significantly higher than NFCS (1.80 mg/g) and MSKC (1.60 mg/g).[48] Moreover, the sugar and protein contents of EPS from *Hirsutella* sp. were 92.7 and 5.2%, respectively, and the monosaccharide components of EPS are mannose, galactose, and glucose with a molar ratio of 4.0:8.2:1.0, and its molecular weight is 23 kDa.[49]

### Volatile compounds

From the mycelia of *H. sinensis* cultured with solid-state media (SSM) and submerged fermentation (SF), 51 volatile compounds were identified, and phenols, acids, and alkanes were the major classes of compounds, while butylated hydroxytoluene was the most abundant volatile compound and accounted for 47.38% and 46.12% of the total volatile compounds in mycelia cultured by SSM and SF, respectively.[50]

## BIOACTIVE INGREDIENTS AND BIOACTIVITIES

A review of the literature regarding the bioactive ingredients and bioactivities of *O. sinensis* reveals that eight types of materials have been used, including (1) the crude powder, (2) the crude powder of the fruiting body, (3) the extracts from the crude powder, (4) the extracts from the crude powder of the fruiting body, (5) the crude powder of mycelia, (6) mycelial extracts, (7) the supernatants of submerged fermented cultures and (8) the whole broth of submerged fermented cultures. The diversity of these materials brings up the major concern of whether the knowledge we have acquired regarding *O. sinensis* is applicable to wild “Dong Chong Xia Cao” or the fermented mycelia or mycelial fermentation products (including cultured broth) of *O. sinensis*, *H. sinensis*, or other species.

Over 20 bioactive ingredients from mycelia, culture supernatants, or fruiting bodies have been published, as shown in Table 1. In summary, these ingredients include (1) extracellular polysaccharides, (2) intracellular polysaccharides, (3) cordycepin, (4) adenosine, (5) guanosine, (6) cordymin, (7) lovastatin, (8) γ-aminobutyric acid (GABA), (9) sitosterol, (10) ergosterol, (11) ergosta-4,6,8(14),22-tetraen-3-one(ergone), (12) 5α,8α-epidioxy-22E-ergosta-6,22-dien-3β-ol, (13) 5α, 8α- epidioxy-22E-ergosta-6,9(11),22-trien-3β-ol, (14) 5α,6α-epoxy-5α-ergosta-7,22-dien-3β-ol, (15) 5α,8α-epidioxy- 24(R)-methylcholesta-6,22-dien-3β-D-glucopyranoside, (16) ,6-epoxy-24(R)-methylcholesta-7,22-dien-3β-ol, (17) myriocin, (18) melanin, (19) cordysinin A, (20) cordysinin B, (21) cordysinin C, (22) cordysinin D, (23) cordysinin E, and (24) serine protease.

The bioactivities of *O. sinensis*, which have been identified from materials derived from different preparations or extracts that were tested in *in vitro*, *in vivo*, or *ex vivo* studies, are shown in Table 2. Over 30 different bioactivities have been reported for *O. sinensis*, including (1) immunomodulatory, (2) immunosuppressive, (3) anticomplementary, (4) antitumor, (5) anti-inflammatory, (6) antioxidant, (7) antibacterial, (8) hepatoprotection, (9) kidney benefitting, (10) antidiabetes, (11) hypocholesterolemia, (12) antiarteriosclerosis, (13) antithrombus, (14) hypotension and vasorelaxant, (15) lung benefitting, (16) photoprotection, (17) antidepression, (18) antiosteoporosis, (19) anticerebral ischemia, (20) antifatigue, (21) antiasthma, (22) steroidogenesis, (23) erythropoiesis, (24) antiarrhythmia, (25) antiaging, (26) testosterone production, (27) sedation, and (28) adjunction, as well as the ability to do the following: (29) prevent and treat injury to the bowel, (30) promote endurance capacity, (31) improve learning-memory, (32) prevent allograft rejection, and (33) attenuate lupus.

It has been found that most local folk/traditional healers use *Cordyceps* to treat 21 ailments, including erectile dysfunction, female aphrodisia, malignant tumors, bronchial asthma, bronchitis, diabetes, cough and cold, jaundice, alcoholic hepatitis, and others.[10] The traditional uses of caterpillar fungus among the Bai, Naxi, Lisu, and Tibetan people living in the mountainous Northern Yunnan Province are to improve eyesight, to treat calcium deficiency (specific to children), diabetes and associated nephropathy, and indigestion (specific to children), to speed up labor parturition, and to strengthen the immune system.[9] Moreover, *C. sinensis* exhibits broad biological and pharmacological actions in hepatic, renal, cardiovascular, and immunological systems, and possesses anticancer activity as well.[21]

### Polysaccharides

Evidence indicates that *Cordyceps* polysaccharides may effectively improve immune system function and possess liver protection, antihyperglycemia, hyperlipidemia, antitumor and antioxidant activities.[1]

#### *Extracellular polysaccharides*

There are several different Extracellular polysaccharides (EPS) from *O. sinensis* that are based on their molecular weights (MW). EPS obtained from submerged *O. sinensis* culture supernatant with MW ranging from 5 to 200 kDa has antioxidant and immunomodulatory activities and may attenuate renal failure, described as follows. The EPS designated EPS-1 with an average MW of 38 kDa was hydrolyzed in diluted sulfuric acid solution at pH 1 and 90°C to yield two major MW fractions: 3.0 kDa and 30 kDa possessed high (30-80%) antioxidant and radical-scavenging

**Table 1.** Bioactive ingredients, bioactivities and material sources of *O. sinensis*

| No. | Bioactive Ingredient | Bioactivities | Material Source | References |
|---|---|---|---|---|
| 1 | Extracellular polysaccharides or exopolysaccharide | Immunomodulatory and antitumor | Culture supernatant | [51-53] |
| | | Immunomodulatory | | [54-58] |
| | | Antioxidant | Culture supernatant | [59,60] |
| 2 | Intracellular polysaccharides | Immunostimulatory and antitumor | Mycelium | [61] |
| | | Immunomodulatory and antioxidant | Mycelium | [62] |
| | | Immunomodulatory | Mycelium | [63-65] |
| | | Hypoglycemic | Mycelium | [66,67] |
| | | Hypoglycemic and antioxidant | Mycelium | [68] |
| | | Antioxidant and antitumor | Mycelium | [69] |
| | | Antioxidant | Mycelium | [70,71] |
| | | Antioxidant | Fruiting body | [72] |
| | | Protection of chronic renal failure | Mycelium | [73] |
| | | Cholesterol esterase inhibitory activity | Mycelium | [74] |
| | | Lower plasma triglyceride and cholesterol | Mycelium | [75] |
| 3 | Cordycepin | Steroidogenesis | Culture supernatant | [76,77] |
| | | Antimetastatic activity | Culture supernatant | [78] |
| | | Antitumor | Culture supernatant | [79,80] |
| | | Immunomodulatory | Culture supernatant | [81] |
| 4 | Adenosine | Immunomodulatory | Mycelium | [82] |
| 5 | Guanosine | Immunomodulatory | Mycelium | [82] |
| 6 | Cordymin | Antioxidant; Anti-inflammation | - | [83] |
| 7 | Lovastatin | Hypolipidemic | Mycelium | [19] |
| 8 | γ-aminobutyric acid (GABA) | Neurotransmitter | Mycelium | [19] |
| 9 | Sitosterol | Cytotoxic | Mycelium | [85] |
| 10 | Ergosterol | Cytotoxic | Mycelium | [85] |
| 11 | Ergosta-4,6,8(14),22-tetraen-3-one(ergone) | Cytotoxic | - | [86] |
| 12 | 5α,8α-epidioxy- 22E-ergosta-6,22-dien-3β-ol | Cytotoxic | Mycelium | [85] |
| 13 | 5α,8α-epidioxy-22E-ergosta-6, 9(11),22-trien-3β-ol | Cytotoxic | Mycelium | [85] |
| 14 | 5α,6α-epoxy-5α-ergosta-7,22- dien-3β-ol | Cytotoxic | Mycelium | [85] |
| 15 | 5α,8α-epidioxy-24(R)-methylcholesta-6,22-dien-3β-D- glucopyranoside | Antitumor | Mycelium | [87] |
| 16 | 5,6-epoxy-24(R)-methylcholesta-7,22-dien-3β-ol | Antitumor | Mycelium | [87] |
| 17 | Myriocin | Immune inhibitor | - | [88] |
| 18 | Serine protease | Fibrinolytic | Culture supernatant | [89] |
| 19 | Melanin | Antioxidant | Mycelium | [2] |
| 20 | cordysinin A | Anti-inflammatory | Mycelium | [90] |
| 21 | cordysinin B | Anti-inflammatory | Mycelium | [90] |
| 22 | cordysinin C | Anti-inflammatory | Mycelium | [90] |
| 23 | cordysinin D | Anti-inflammatory | Mycelium | [90] |
| 24 | cordysinin E | Anti-inflammatory | Mycelium | [90] |
| 25 | Cordyceamide A | Cytotoxic | Culture supernatant | [91] |
| 26 | Cordyceamide B | Cytotoxic | Culture supernatant | [91] |

activities.[60] An acidic polysaccharide AEPS-1, fractionated from the EPS produced by *C. sinensis* Cs-HK1 in mycelial culture, was composed of glucopyranose (Glcp) and pyrano-glucuronic acid (GlcUp) in an 8:1 M ratio plus a trace amount of mannose, which has an average MW of 36 kDa and significantly stimulated the release of several major cytokines that may have immunomodulatory properties.[57]

The polysaccharide CPS-2, mostly composed of α-(1→4)-d-glucose and α-(1→3)-d-mannose and branched with α-(1→4, 6)-d-glucose every 12 residues, has an average MW of 43.9 kDa and has been shown to significantly relieve renal failure.[73] A polysaccharide with a MW of 82 kDa that was isolated from the culture medium of *Cordyceps*, namely cordysinocan, and contained glucose, mannose, and galactose in a 2.4:2:1 ratio induced cell proliferation and the secretion of interleukin (IL)-2, IL-6, and IL-8.[54] In another study, EPS consisting of mannose, glucose, and galactose in a ratio of 23:1:2.6 with a MW of about 104 kDa had the ability to stimulate cytokine expression in immunocytes.[56] Moreover, EPS composed of polysaccharide-protein complexes with a β-D-glucan backbone and a wide range of MWs (5 kDa to more than 200 kDa) had moderate antioxidant activities.[59]

### *Intracellular polysaccharides*

Intracellular polysaccharides (IPS), extracted from *O. sinensis*

**Table 2.** Bioactivities, experimental modes, materials and extraction solvents of *O. sinensis*

| No. | Bioactivity | Experiment mode | Material | Extraction solvent | References |
|---|---|---|---|---|---|
| 1 | Immunomodulatory | *In vivo* | Artificial CS*1 | - | [92] |
| | | *In vivo* | CS | - | [93] |
| | | *In vivo* | CS | Water | [94] |
| | | *In vivo* | CS | Water | [95] |
| | | *In vivo* | CS | - | [96] |
| | | *Ex vivo* | EPS*2 | - | [97] |
| | | *Ex vivo* | CS | - | [98] |
| | | *Ex vivo* | EPS | - | [98] |
| | | *In vitro* | CS | - | [99] |
| | | *In vitro* | CS | | [100] |
| | | *In vitro* | Mycelium | - | [101] |
| | | *In vitro* | Mycelium | Water | [102] |
| | | *In vitro* | Mycelium | Methanol | [103] |
| | | *In vitro* | Mycelium | Methanol | [104] |
| | | *In vitro* | Fruiting body | Methanol | [105] |
| 2 | Immunosuppression | *In vitro* | Mycelium | - | [106] |
| | | Clinical | CS | - | [107] |
| 3 | Anticomplementary | *In vitro* | EPS | - | [108] |
| 4 | Antitumor | *In vitro* | Polysaccharide-rich fraction | - | [109] |
| | | Clinical | CS | - | [110,111] |
| | | *In vitro* | Mycelium | Methanol | [112] |
| | | *In vitro* | Mycelium | Methanol | [113] |
| | | *In vivo* | CS | - | [114] |
| | | *In vitro* | CS | Water | [115] |
| | | *In vitro* | Mycelium | Ethyl acetate | [116] |
| | | *In vitro* | Mycelium | Water | [117] |
| 5 | Anti-inflammation | *In vivo* | Cordymin | - | [83] |
| | | *In vitro* | Mycelium | - | [118] |
| | | *In vitro* | Cordysinins A-E from mycelium | - | [90] |
| | | *In vitro* | CS | - | [119] |
| | | *In vitro* | Mycelium | Methanol | [112] |
| | | *In vivo* | Extract | - | [120] |
| 6 | Antioxidation | *In vitro* | EPS | - | [108] |
| | | *In vitro* | EPS | - | [109] |
| | | *In vitro* | Mycelium | Water | [121] |
| | | *In vitro* | Mycelium | Water | [122] |
| | | *In vitro* | Fruiting body | Water | [123] |
| | | *In vivo* | Cordymin | - | [83] |
| | | *In vivo* | Mycelium | - | [124] |
| 7 | Antibacterial | *In vitro* | Mycelium | - | [125] |
| | | *In vivo* | Mycelium | - | [126] |
| 8 | Hepatoprotection | *In vitro* | Fruiting body | - | [167] |
| | | *In vivo* | Compound Codyceps-TCM-700C | - | [128] |
| | | *In vivo* | The fruiting bodies | - | [129] |
| | | *In vivo* | CS | - | [130] |
| | | *In vivo* | CS | - | [131] |
| | | *In vivo* | Mycelium | - | [132] |
| | | *In vivo* | Mycelium | - | [133] |
| 9 | Kidneys benefits | *In vivo* | CS | - | [134] |
| | | *In vivo* | CS | - | [135] |
| | | *In vivo* | CS | - | [136] |
| | | *In vivo* | CS | - | [137] |
| | | *In vivo* | CS | - | [138] |
| | | *In vitro* | CS | - | [139,140] |
| | | *In vitro* | Natural product of CS (H1-A) | - | [141] |

Table 2 (Contd...)

| No. | Bioactivity | Experiment mode | Material | Extraction solvent | References |
|---|---|---|---|---|---|
| | | Clinical | Bailing Capsule, fermented agent of CS | - | [142] |
| 10 | Antidiabetic | *In vivo* | Mycelium | - | [143] |
| | | *In vivo* | CS | - | [144] |
| | | *In vivo* | Vanadium-enriched CS (VECS). | - | [145] |
| | | *In vivo* | Vanadium-enriched CS(VECS) | - | [146] |
| | | *In vivo* | Fruiting body | - | [147,148] |
| | | *In vivo* | Fruiting body | - | [149] |
| | | *In vivo* | Mycelium(cordymax™ Cs-4) | - | [150] |
| | | *In vivo* | Mycelium | Sodium hydroxide | [151] |
| 11 | Hypocholesterolemic | *In vivo* | Mycelium | Water | [152] |
| 12 | Antiarteriosclerosis | *In vivo* | Extract | - | [153] |
| | | In vivo | Fruiting body | Water | [154] |
| 13 | Antithrombus | *In vivo* | Mycelium | Alcohol | [155] |
| 14 | Hypotensive and vasorelaxant | *In vivo* | CS | Phosphate buffer saline | [156] |
| 15 | Lung benefit | *In vivo* | CS | - | [157] |
| | | *In vivo* | CS | - | [158] |
| | | *In vivo* | Cultured CS | - | [159] |
| | | *In vivo* | CS | Alcohol | [146] |
| | | *In vivo* | Mycelium | - | [160] |
| 16 | Photoprotection | *In vitro* | Mycelium | - | [161] |
| | | *In vivo* | Mycelium | Methanol | [162] |
| | | *In vivo* | Mycelium | Water | [163] |
| 17 | Antidepression | *In vivo* | Vanadium-enriched Mycelium | - | [145,146] |
| | | *In vivo* | Mycelium | Water | [164] |
| | | Clinical | Mycelium | - | [165] |
| 18 | Antiosteoporosis | *In vivo* | CS | - | [166] |
| 19 | Anticerebral ischemia | *In vivo* | Extract | - | [167] |
| 20 | Prevention and treatment of injury to the bowel | *In vivo* | Extract | Water | [168] |
| 21 | Promotion to endurance capacity | *In vivo* | Mycelium | - | [169] |
| | | Clinical | Mycelium(cordymax™ Cs-4) | - | [63] |
| 22 | Antifatigue | Clinical | Cultured CS | - | [170] |
| 23 | Antiasthmatic | *In vitro* | Extract | - | [171] |
| 24 | Learning-memory Improvement | *In vivo* | Extracts | - | [172] |
| 25 | Steroidogenesis | *In vitro* | CS | - | [173] |
| | | *In vivo* | CS | - | [174] |
| | | *In vitro* | Mycelium | - | [175] |
| | | *In vitro* | Mycelium | | [176] |
| | | *In vitro* | CS | Water | [177] |
| 26 | Erythropoiesis | *In vitro* | CS | - | [178] |
| 27 | Antiarrhythmic | *In vivo* | CS | Alcohol | [179] |
| 28 | Antiaging | *In vivo* | Extract | - | [180] |
| 29 | Allograft rejection prevention | *In vivo* | CS | - | [181] |
| 30 | Lupus attenuation | *In vivo* | Mycelium | - | [182] |
| | | *In vitro* | A pure compound (H1-A) from CS | - | [183] |
| 31 | Testosterone production | *In vivo* | CS | - | [176,184] |
| 32 | Sedation | *In vivo* | Natural CS | - | [185] |
| 33 | Adjunction | Clinical | CS | - | [186] |

*[1]CS: *Cordyceps sinensis*
*[2]EPS: Extracellular polysaccharide

mycelia and containing 1,3-β-D-, 1,3-α-D-, or 1,4-α-D-glucan with 1,6-branched chains, had MWs ranging from 7.7 to 1180 kDa depending on the extract conditions. Structural analyses showed that insoluble polysaccharides with 1, 3-β-D-glucan contained some 1, 6-branched chains with an average particle diameter of 1.5 μm.[187] The D-glucan consisted of a backbone with (1→4)-D-glucosyl residues and carried a single (1→6)-linked D-glucosyl residue; and these α-D-glucosidic linkages were present in the polysaccharide with short exterior chains.[188] The neutral mannoglucan with a MW of 7.7 k Da consisted of Man and Glc units in the molar ratio of 1:9, a α-D-glucan backbone with (1→4)- and (1→3)-linkages, and side chains of α-D-(1→6)-Manp, which attached to the backbone via O-6 of α-(1→3)-Glcp residues.[189]

The polysaccharide CS-F10, purified from a hot water extract, was composed of galactose, glucose, and mannose in a molar ratio of 43:33:24, and its MW was estimated to be about 15 kDa, which has a comb-type structure with α-D-glucopyranosyl residues on the termini of the side-chains and characteristic sugar residues, such as 1, 5-linked β-D-galactofuranosyl residues.[190] Hot water extracts of mycelia (WIPS) and alkaline extracts of mycelia (AIPS) were characterized as α-d-glucans with a backbone of (1→4)-linked α-D-Glcp and similar MWs (WIPS 1180 kDa; AIPS 1150 kDa); and WIPS had a short branch of (1→6)-linked α-D-Glcp, and AIPS was a linear glucan, which is distinct from the branched structures of most glucans from medicinal fungi.[61]

IPS from *C. sinensis* mycelia with MWs ranging from 8.1 to 460 kDa have antioxidant, anti-inflammatory, immunomodulatory, hypoglycemic, and hypocholesterolemic activities, described as following. Antioxidant polysaccharide CPS1 was found to be a glucomannogalactan with a monosaccharide composed of glucose, mannose, and galactose in a ratio of 2.8:2.9:1, and its total carbohydrate content and average MW were 99.0% and 8.1 kDa, respectively.[72] The anti-inflammatory polysaccharide fraction CME-1, has a MW of 27.6 kDa and containing mannose and galactose in a ratio of 4:6.[191] The hypoglycemic polysaccharides obtained from a hot-water extract and alkaline extracts were found to be composed of galactose, glucose, and mannose in a ratio of 62:28:10 with a MW 45 kDa.[67] In addition, the isolated IPS composed of D-Glc, D-Man, L-Ara, and D-Gal in a molar ratio of 8:90:1:1 with an average MW of 83 kDa has been shown to have immunomodulatory potential.[65] A polysaccharide with MW 210 kDa may protect against free radical-induced neuronal cell toxicity.[192] Another polysaccharide with a MW of 210 kDa that was isolated and named CSP-1 is composed of glucose, mannose, and galactose in the ratio of 1:0.6:0.75 and was shown to have strong antioxidation activity and the abilities to decrease blood glucose and insulin secretion in diabetic animals.[68] The heteropolysaccharide PS-A, composed of D-glucose, D-galactose, and D-mannose in a molar ratio of 2:1:1 with a MW of 460 kDa, was shown to possess strong inhibitory activity against cholesterol esterase and may be a potential agent to control hypercholesterolemia.[74]

## Nucleosides

It has been demonstrated that the nucleoside contents, including uracil, uridine, hypoxanthine, inosine, guanosine, adenosine, adenine, and cordycepin, of natural and cultured *Cordyceps* could be separated into two individual sub-groups, which suggested that the chemical characteristics of the cultured mycelia of different fungal strains isolated from natural *C. sinensis* were similar but were different from natural mycelia.[193] The perithecium of *C. sinensis* was found to have considerably higher amounts of total nucleosides and nucleobases compared to other parts of this fungus.[194] The content of the four active nucleosides, specifically adenosine, guanosine, cytidine, and thymidine, in *C. sinensis* was lower than that of cultured *Cordyceps*.[195] Moreover, there is a positive correlation between nucleoside content in *C. sinensis* and the growth altitude.[46]

### *Adenosine*

Adenosine was abundant in the fruiting body and was considerably more abundant than in the corpus of natural DongChongXiaCao and the mycelia of *C. sinensis*.[196] It has been shown that the amount of adenosine in *Cordyceps* ranges from 0.28 to 14.15 mg/g.[197] The concentration of adenosine 2.45 ± 0.03 mg/g in *C. militaris* fruiting bodies was found to be higher than those of 1.643 ± 0.03 mg/g in natural *C. sinensis*, while the content of the fermented mycelia 1.592 ± 0.03 mg/g of *C. militaris* was similar to those of natural *C. sinensis*.[198]

### *Cordycepin*

Studies have shown that cordycepin is abundant in cultured *C. militaris* (2.28 ± 0.84 mg/g) and sparse in natural *C. sinensis*; however, there is an undetectable amount in cultured *C. sinensis*.[199] The amount of cordycepin in *Cordyceps* was found to range from 0.006 mg/g to 6.36 mg/g.[196] In cultured mycelia and the fruiting body of *Cordyceps*, cordycepin contents were lower and varied from 0.006 to 1.64 mg/g.[196,198] The cordycepin content of the mycelia of *C. sinensis* cultured in potato dextrose agar (PDA) medium and Finger millet medium were 0.075 mg/g and 0.021 mg/g, respectively; however, the cordycepin content in natural *C. sinensis* was higher than in the mycelia cultured with PDA medium.[200] It has been indicated that the concentration of cordycepin in *C. militaris* fruiting bodies is higher than in natural *C. sinensis*, and the concentration of cordycepin in fermented *C. militaris* mycelia is similar to that in natural *C. sinensis*: the mean cordycepin contents in the fruiting bodies of *C. militaris* and *C. sinensis* were found to be 2.65 and 1.64 mg/g, respectively, and the content in *C. militaris* mycelia was 1.59 mg/g.[198] In addition, cultured mycelia and natural specimens of *C. sinensis* contained similar amounts of cordycepin.[42] When extracted with 50% methanol-chloroform, the cordycepin content of cultured mycelia varied from 0.002% to 0.029% (i.e., 0.02-0.29 mg/g) in twenty-one isolates, and the cordycepin content of natural Dong Chong Xia Cao varied from 0.004% to 0.006% (i.e., 0.04-0.06 mg/g).[201]

## Mannitol

Mannitol is the so-called "ChongCao Acid" in Chinese and is also improperly named as "cordycepic acid." When searching for the terms "mannitol" plus "*Cordyceps*" in the Scopus database, a limited number of articles can be found. Li *et al,*[202] indicated that the D-mannitol contents in natural fruiting bodies of *C. sinensis*, mycelia from shaking cultures, and fruiting bodies from artificial cultivation of *H. beakdumountainsi* were 8.9, 11.5, and 10.2%, respectively. For *in vitro*-cultured *C. sinensis*, the amount of D-mannitol yielded was almost the same as that in natural samples.[42] Moreover, when *C. sinensis* was cultured with millet, the D-mannitol content achieved levels as high as that in *C. militaris* fruiting bodies.[203] There was no obvious difference in the amount of nucleosides between cultured *O. sinensis* mycelia and natural products; however, natural products were shown to have a significantly higher D-mannitol content compared with submerged culture mycelia.[204]

Likewise, when searching for the terms "cordycepic acid" plus "*Cordyceps*" in the Scopus database, a limited articles can be found. The chemical constituents of natural *Cordyceps*, including cordycepic acid, have been described.[13] Dong *et al.*[205] showed that the superoxide dismutase activity and the content of cordycepic acid in the fruiting bodies of *C. militaris* were dependent on the sodium selenite concentration in the culture medium. It has been reported that *C. sinensis* has many bioactive components, such as 3′-deoxyadenosine, cordycepic acid, and *Cordyceps* polysaccharides.[3] In addition, yields of cordycepic acid from *C. jiangxiensis*, *C. taii*, and *C. gunnii* were found to be 11.81%, 8.72%, and 4.73%, respectively, of the dry weight of mycelia.[206]

**Amino acids**

The total amount of amino acids in fermented mycelia was determined to be 9.23%, which is lower than that in wild DongChongXiaCao (18.1%) but is similar to that in the fruiting body of wild DongChongXiaCao.[46] The three principal amino acids in the corpus-fruiting body are glutamic acid, aspartic acid, and arginine, and their contents are 2.64–2.66%, 1.70–1.84%, and 1.53–1.60%, respectively.[46] A mixture with 18 synthetic amino acids to mimic the amino acid composition in natural *C. sinensis* showed the same sedative action as natural *C. sinensis*.[185]

**Sterols**

The ergosterol content in the stroma of *C. sinensis* was found to be approximately 0.92 g/L and was about three times higher than that found in the sclerotium.[207] The fruiting body (CsA) and the host caterpillar (CsB) of *C. sinensis* had similar ergosterol compositions, but the level of ergosteryl esters in CsB was much higher than in CsA, these data indicated that CsA and CsB might exist in different growth phases and have different physiological functions for the growth and multiplication of *C. sinensis*.[208] It has been shown that ergosta-4,6,8(14),22-tetraen-3-1 may induce G2/M cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells.[86] 5α,8α-epidioxy-22E-ergosta-6,22-dien-3β-ol, 5α,8α-epidioxy-22E-ergosta-6,9(11),22-trien-3β-ol, and 5α,6α-epoxy-5α-ergosta-7,22-dien-3β-ol isolated from the ethyl acetate fraction with a peroxide ring or an epoxide ring had substantial cytotoxic activity.[209] In addition, 5α,8α-epidioxy-24(R)-methylcholesta-6, 22-dien-3β-D-glucopyranoside, and 5,6-epoxy-24(R)-methylcholesta-7,22-dien-3β-ol from the methanol extract of *C. sinensis* had antitumor activity.[87]

**Aurantiamides**

Two aurantiamides, a new class of potent analgesic and anti-inflammatory agents, named cordyceamides A and B were isolated from the culture liquid of *C. sinensis* along with one known compound, aurantiamide acetate. The structures of these compounds were elucidated as N-benzoyl-l-tyrosinyl-l-phenylalaninol acetate and N-benzoyl-l-tyrosinyl-l-p- hydroxyphenylalaninol acetate by 1D and 2D-NMR techniques and by the comparison with the literature.[91]

**Peptides**

***Cyclodipeptides***

A cyclodipeptide named cordycedipeptide A, which is a natural compound that was isolated from the culture liquid of *C. sinensis*, had cytotoxic activity against L-929, A375, and Hela cells.[210]

***Cordymin***

The peptide cordymin from *C. sinensis*, which has a neuroprotective effect in the ischemic brain due to inhibited inflammation and increased antioxidant activity related to lesion pathogenesis, can be used as a potential preventive agent against cerebral ischemia-reperfusion injury.[83]

***Myriocin***

Myriocin (also known as the antibiotic ISP-1) is a new type of immune inhibitor extracted from *C. sinensis*, it was shown to significantly inhibit the upregulated expression of cyclin D1 induced by high concentrations of glucose, restoring the expression of cyclin D1.[88]

**Melanin**

The antioxidant activity of melanin, derived from a black pigment, was isolated from the fermentation broth of *O. sinensis* and showed much stronger scavenging abilities for 1,1-diphenyl-2-picrylhydrazyl (DPPH•) and ferrous ion chelation compared to the mycelial water extract.[211]

**Lovastatin, γ-aminobutyric acid (GABA), and ergothioneine**

Lovastatin, GABA, and ergothioneine are secondary metabolites of fungal growth. Chen *et al.*[84] indicated that mycelia of *C. sinensis* contained 1365 mg/kg of lovastatin, 220.5 mg/kg GABA, and detectable ergothioneine and had different hypolipidemia, hypotension, and antioxidant activities.

**Cordysinins**

Five cordysinins, A-E, from the mycelia of *C. sinensis* have been identified and have been shown to have anti-inflammatory activities, and 1-(5-Hydroxymethyl-2-furyl)-β-carboline was shown to most significantly inhibit superoxide anion generation and elastase release.[90]

## SHAKING CULTURE AND SUBMERGED FERMENTATION OF *O. SINENSIS*

The maximal production of mycelia, EPS, and exo-biopolymer are important concerns for producers, especially for industry. Several factors that may affect the production of *O. sinensis* in shaking culture and submerged fermentation, including strain, medium, and culture conditions, have been discussed in different studies.

The amounts of mycelial biomass, EPS, and exo-biopolymer range from 11.10 to 62.3 g/l, 0.43 to 3.21 g/l and 22.0 to 28.4 g/l, respectively, described as follows.

It has been observed that Tween 80 can exhibit a remarkable effect on EPS production by increasing EPS yield more than two-fold at 1.5% (w/v); the effects of Tween 80 can probably be attributed to the stimulation of EPS biosynthesis and release from fungal cells.[212] In addition, evidence has shown that palmitic acid may significantly increase the production of biomass and extracellular polysaccharides to 11.10 g/l and 0.43 g/l, respectively.[191] Adding ammonium to the mycelial culture of strain Cs-HK1 may enhance intracellular cordycepin accumulation, which may be attributed to the uptake of ammonia for nucleoside synthesis, and EPS production, which may be attributed to the increased uptake of glucose for EPS biosynthesis.[213]

The optimal medium for the production of mycelia and exo-biopolymers by strain CS 16 is 2% sucrose, 0.9% yeast extract, 0.3% $K_2HPO_4$, and 0.4% $CaCl_2$, and this medium was shown to yield maximal mycelia and exo-biopolymer productions of 54.0 g/l and 28.4 g/l, respectively, in shaking-flask cultures.[214] In bioreactor cultures, rapid differentiation and cell lysis occurred when agitation speed was increased. When the agitation speed was maintained at 350 rpm, 62.3 g/l of mycelia and 22.0 g/l of exo-biopolymers were obtained from strain CS 16 in submerged fermentation.[215] In shaking cultures, the maximum polysaccharide production was 3.05 and 3.21 g/l in a flask and a 5-L jar fermentor, respectively, when fungi were supplied with optimal medium.[216] It is believed that the optimal medium for mycelial growth is 50 g/l sucrose, 10 g/l peptone, and 3 g/l yeast extract, which would produce over 22 g/l of mycelial biomass after 40 days of submerged culture at a temperature below 20°C.[217]

## CULTURE AND BIOACTIVITY OF *HIRSUTELLA SINENSIS* OR *HIRSUTELLA* SP.

There is little literature published on *H. sinensis*, the anamorph of *O. sinensis*. This might be due to geographic limitations, difficulty in isolation and cultivation, and slow growth under low temperatures.

### Culture

It was shown that most strains of *H. sinensis* prefer to grow on Sabouraun's dextrose agar, and some of the strains prefer potato-dextrose agar as the medium for optimal development.[218] The optimized conditions and medium for submerged fermentation of *Hirsutella* sp. have been described as follows: initial pH 5.5, potato extract 20% (w/v), sucrose 2.5%, peptone 0.5%, $K_2HPO_4$ 0.2%, $MgSO_4$ 0.05%, and fermentation for 4 days, the highest production of EPS and mycelial biomass may reach 2.17 and 10.06 g/l, respectively.[49]

### Bioactivity

*H. sinensis* extract (HSW) was shown to have a remarkable action *in vitro* and *in vivo* in a model of kidney injury;[219] the extract downregulated transforming growth factor-β1 in renal tissue, antagonized tubular epithelial-myofibroblast transdifferentiation and renal interstitial fibrosis, and improved renal function in chronic aristolochic acid nephropathy rats.[220] Furthermore, the fermented mycelia of *H. sinensis* significantly prevented spontaneous type 1 diabetes in non-obese diabetic mice.[221] Additionally, *H. sinensis* was shown to significantly increase animal survival after a lethal dose of radiation, accelerate leukocyte recovery, and stimulate immune lymphocyte proliferation.[222]

## ECOLOGY AND HOSTS

*O. sinensis* is confined to the Tibetan Plateau and its surrounding regions, including Tibet, Gansu, Qinghai, Sichuan, and Yunnan provinces in China, in certain areas of the southern flank of the Himalayas, and in the countries of Bhutan, India, and Nepal. These regions are at altitudes of over 3,000 m. The fungus is distributed from the southernmost site in Yulong Naxi Autonomous County in northwestern Yunnan Province to the northernmost site in the Qilian Mountains in Qilian County, Qinghai Province and from the east edge of the Tibetan Plateau in Wudu County, Gansu Province to the westernmost site in Uttarakhand, India.[202] The yearly yield of *C. sinensis* in the Naqu district, which is the principal growth zone in Tibet, is 7000 kg, and the elevation of the distribution area is from 5000 m to 4100 m, and the ecological geographical distribution is mainly affected by vegetation, soil, temperature, and humidity.[223]

As hosts, the larvae of *Thitarodes* spp. play a vital role in supplying nutritional materials for the growth of *O. sinensis*. In the Tibetan Plateau, more than 40 species of the genus *Thitarodes* have been recorded since 1958.[224] According to another publication, 57 taxa are recognized as potential hosts of *O. sinensis*, including 1 *Bipectilus*, 1 *Endoclita*, 1 *Gazoryctra*, 12 *Hepialus*, 2 *Magnificus*, 3 *Pharmacis,* and 37 *Thitarodes*.[225] The recorded altitude ranges of the recognized potential host insects were found to vary from 2800 to 5100 m, and the distribution areas of these species covered 26 provinces in China and more than 12 other countries.[225] Due to long-term adaptive evolution, *O. sinensis* and its host insects have developed a living requirement for special high altitude-associated climates and soil conditions, such as sub-ambient atmosphere and soil temperatures and specific solar radiation, barometric pressure, and hypoxic conditions.[224] Studies show that there is genetic differentiation of *C. sinensis* among different latitudes.[226] The interspecific genetic differentiations are obvious in *Hepialu*, and the genus *Hepialus* might be considered the polyphyletic origin for Cytb sequences, which have abundant variations among the host insects of *C. sinensis* at both the specific and generic levels.[227]

## CONCLUSIONS

*O. sinensis* is a complex fungus with multiple biological functions. Over 20 bioactive ingredients have been found in *O. sinensis*, such as extracellular polysaccharides, intracellular polysaccharides, cordycepin, adenosine, and sterols, derived from its mycelia, culture supernatant, or fruiting body. In addition, over 30 bioactivities have been indicated, including immunomodulatory, antitumor, anti-inflammation, and antioxidant activities, in preparations or solvent extracts in *in vitro*, *in vivo*, or *ex vivo* studies. However, few publications have given regard to the bioactive ingredients, bioactivities, and the medium and culture conditions of *O. sinensis* and *H. sinensis*, which must be incubated for more than 10 days at low temperatures (below 21°C ). The slow growth rate at low temperature is a critical characteristic of *O. sinensis* and *H. sinensis*. It has been demonstrated that these fungi grow poorly at temperatures above 21°C and stop growing at 25°C or above.

The fungus *O. sinensis* is not equivalent to the traditional Chinese medicine DongChongXiaCao; the latter develops a fruiting body following the occupation of a dead caterpillar and grows wild in nature. The host caterpillar provides nutrients for *O. sinensis*, resulting in the formation of a fruiting body in the appropriate climate and environment. The morphology of *O. sinensis* in submerged culture can be microscopically visualized as mycelia, without the fruiting body. The relationship between *O. sinensis* and its host in the traditional Chinese medicine DongChongXiaCao, as well as its pathobiology and ecology, still remains unclear.

The mysterious caterpillar fungus *O. sinensis* has been renamed since 2007; however, many publications still use the name *C. sinensis*. When the names "*Ophiocordyceps sinensis*" and "*Cordyceps sinensis*" are searched for in article titles, abstracts, and keywords in the Scopus database from the year 2008 to 2012, 84 and 255 articles can be found, respectively. Even though it has been confirmed that *H. sinensis* is the anamorph of *C. sinensis*, most articles still use the terms "*Cordyceps sinensis,*" "*Cordyceps,*" and "Cordyceps" to refer to the traditional Chinese medicine DongChongXiaCao. Among all of these publications, not one successfully obtained fruiting bodies from caterpillars artificially infected with *O. sinensis* or *H. sinensis*. The key identification characteristics for *O. sinensis* are the existence of a perithecium, ascus, and ascospore in the fruiting body. If the fruiting body cannot be formed from caterpillars infected by *O. sinensis* or *H. sinensis*, these key characteristics cannot be identified by microscope. Beginning January 1$^{st}$, 2013, regulations will allow one fungus to have only one name, and the system of permitting separate names for anamorphs will end.[228] The anamorph name *H. sinensis* will be changed by the *International Code of Nomenclature for algae, fungi, and plants* (formerly called the *International Code of Botanical Nomenclature*)[229] to *O. sinensis*.

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