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Recent advances in Cordyceps sinensis polysaccharides: Mycelial fermentation, isolation, structure, and bioactivities: A review

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ABSTRACT

Cordyceps (Ophiocordyceps sinensis) sinensis, the Chinese caterpillar fungus, is a unique and precious medicinal fungus in traditional Chinese medicine which has been used as a prestigious tonic and therapeutic herb in China for centuries. Polysaccharides are bioactive constituents of \textit{C. sinensis}, exhibiting several activities such as immunomodulation, antitumor, antioxidant and hypoglycaemic. As natural \textit{C. sinensis} fruiting body-caterpillar complexes are very rare and expensive, the polysaccharides documented over the last 15–20 years from this fungal species were mostly extracted from cultivated fungal mycelia (intracellular polysaccharides) or from mycelial fermentation broth (exopolysaccharides). Extraction and purification of the polysaccharides is a tedious process involving numerous steps of liquid and solid phase separations. Nevertheless, a large number of polysaccharide structures have been purified and elucidated. However, relationships between the structures and activities of these polysaccharides are not well established. This review provides a comprehensive summary of the most recent developments in various aspects (i.e., production, extraction, structure, and bioactivity) of the intracellular and exopolysaccharides from mycelial fermentation of \textit{C. sinensis} fungi. The contents and data will serve as useful references for further investigation, production and application of these polysaccharides in functional foods and therapeutic agents.

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1. Introduction

Cordyceps (Ophiocordyceps) sinensis (Berk.) Sacc., the Chinese caterpillar fungus or DongChongXiaCao (winter worm-summer grass) in Chinese or Tochukaso in Japanese, is a valuable medicinal fungus in traditional Chinese medicine (TCM). *C. sinensis* is a parasitic fungus to the moth larvae (Lepidoptera) of *Hepialus armoricanus* (and Thitarodes). In late summer or early autumn, the larvae are infected by the fungal spores and gradually consumed by the fungal mycelia, and turned into “stiff worms” in winter. In spring and early summer of the following year, a stroma or fruiting body forms on the larva head, grows and emerges out of the ground like a grass (Fig. 1) (Chen, Wang, Nie, & Marcone, 2013; Holliday, & Cleaver, 2008; Li, & Tsim, 2004; Lo, Hsieh, Lin, & Hsu, 2013; Winkler, 2010; Zhang, Li, Wang, Li, & Liu, 2012; Zhu, Halpern, & Jones, 1998). The natural *C. sinensis* fruiting body-caterpillar complexes are mainly distributed on the high plateaus of 3500–5000 m above sea level in Tibet, Qinghai, Sichuan, and Yunnan Provinces in China (Li et al., 2011).

*C. sinensis* has been used in China for more than 700 years, mainly as a tonic to invigorate the lungs and to nourish the kidneys (Dong & Yao, 2008). Modern pharmacological studies have shown its therapeutic effects on a wide range of diseases and conditions, such as respiratory, renal, liver, nervous system, and cardiovascular diseases, as well as tumours, aging, hyposexuality, and hyperlipidaemia (Ding et al., 2011; Ji et al., 2009; Liu, Li, Zhao, Tang, & Guo, 2010; Lo et al., 2013; Marchbank, Ojobo, Playford, & Playford, 2011; Song, Ming, Peng, & Xia, 2010; Yue, Ye, Zhou, Sun, & Lin, 2013; Zhang, Wang, Zhang, & Ye, 2011; Zhu et al., 1998). *C. sinensis* has been listed as an herbal drug in the official Chinese Pharmacopoeia by the Committee of Pharmacopoeia and Chinese Ministry of Health since 1964. During the outbreak of the Severe Acute
Respiratory Syndrome (SARS) in China in 2003, there was a notable increase in the use of *C. sinensis*. Over the last 10 years, the demand as well as the price for *C. sinensis* has increased dramatically in China, Japan, Korea and India (Au et al., 2012; Jeffrey, 2012; Winkler, 2009). As natural *C. sinensis* are very limited and cannot meet the increasing demand, fermentation technology has been widely exploited for large-scale production of *C. sinensis* fungal mycelia and other useful constituents. The fermentation-cultivated mycelia of some fungal strains isolated from the natural *C. sinensis* have been shown to produce the similar pharmacological efficacy to the wild fungal materials and been widely applied to various health food products (Zhu et al., 1998).

The multiple pharmacological effects of *C. sinensis* can be attributed to its chemical ingredients, including polysaccharides, proteins, nucleotides, mannitol, ergosterol, aminophenol, fatty acids, and trace elements. Actually, several reviews on these compounds and their properties and bioactivities of *C. sinensis* have been published in the last few years (Chen, Wang et al., 2013; Paterson, 2008; Shashidhar, Giridhar, Sankar, & Manohar, 2013; Zhao, Xie, Wang, & Li, 2013; Zhong et al., 2009). In particular, polysaccharides represent one of the most abundant components in the fungus and a major group of bioactive constituents which have been extracted and isolated from the fruiting bodies, cultured mycelium, and fermentation broth, which are structurally diverse biomacromolecules with various physiochemical properties. Polysaccharides have been the target for the development and quality control of *C. sinensis* health products. More recently, some reviews on extraction, isolation, structure and bioactivities of polysaccharides from *C. sinensis* have appeared (Lo et al., 2013; Nie, Cui, Xie, Phillips, & Phillips, 2013; Xiao, 2008; Zhong et al., 2009). However, these reviews mainly focus on separation techniques, structural features and bioactivities of intracellular polysaccharides (IPSs) from the fruiting body of natural *C. sinensis* and mycelia of cultured *C. sinensis*, but the mycelial fermentation, physicochemical properties and pharmacological activities of extracellular polysaccharides or exopolysaccharides (EPSs) isolated from culture broth of *C. sinensis* are less involved. In addition, the relationships are still not well established between the molecular structure and the bioactivity of *C. sinensis* derived polysaccharides. Herein, this review summarizes and compares the recent studies on the production, extraction, and purification of IPSs and EPSs from *C. sinensis* as well as the characterization of their structural features, chain conformations, and bioactivities.

### 2. Production of biomass and polysaccharides by mycelial fermentation

Wild or natural *C. sinensis* is becoming increasingly scarce because of reckless harvesting, geographical limitation, and unfavourable weather conditions for its proliferation (Yao, 2004). Cultivation of fungal mycelia is a more reliable alternative for mass production of the fungal materials. Several species of fungi have been successfully isolated from the natural *C. sinensis* such as *Paecilomyces sinensis*, *Cephalosporium sinensis*, *Tolypocladium sinensis*, and *Hirsutella sinensis* (Yin & Tang, 1995). Some of these species have been cultivated in large quantities of mycelial biomass by fermentation technology. The fungal mycelia have been reported to exert similar pharmacological effects to those of wild *C. sinensis* species (Dong & Yao, 2008; Zhu et al., 1998).

To improve the efficiency and productivity of mycelial fermentation processes, many investigators have studied the effects of various process factors on the maximal production of mycelial biomass and EPSs and to optimize the fermentation conditions, such as medium composition, temperature, pH, and culture vessel (Hsieh, Tsai, Hsu, Chang, & Lo, 2005; Kim & Yun, 2005; Liu & Wu, 2012). Table 1 provides a summary of the strains, culture conditions, mycelial biomass and EPS yields of *C. sinensis* which have been reported in the literature. The biomass and EPS yields varied in a wide range from 10 to 54 g/L, and <1.0 to >40 g/L with the fungal species and culture conditions, respectively. In particular, our group has previously demonstrated the optimization of submerged culture conditions for *C. sinensis* (Cs-HK1), such as temperature, initial pH, carbon and nitrogen levels, minerals, and surfactants (Tween 80) (Leung, & Wu, 2007; Leung, Zhang, & Wu, 2006; Liu & Wu, 2012). However, the production of bioactive EPS by liquid fermentation of edible or medicinal fungi (e.g. *C. sinensis*) is still a new area of research without much industrial application. Thus, there is a need to enhance the EPS productivity through effective strategies of process intensification in the future.

### 3. Extraction, isolation and purification of polysaccharides

*C. sinensis* polysaccharides can be classified into two types according to their locations in the fungal cells, intracellular polysaccharides (IPSs) and extracellular polysaccharides (EPSs). IPSs are extracted from the fruiting body (or worm) and mycelium of *C. sinensis* with pure water, aqueous acidic/alkaline solutions, aqueous buffers under heating (Guan, Zhao, Feng, Hu, & Li, 2011; Kiho, Ookubo, Usui, & Hara, 1986; Kiho, Yamane, Hui, Usui, & Ukae, 1996; Wang, Wang et al., 2009; Wu, Hu, Pan, Zhou, & Zhou, 2007; Wu, Ishurd, Sun, & Pan, 2005; Wu, Sun, & Pan, 2005; Wu, Sun, & Pan, 2006; Wu, Sun, Qin, Pan, & Sun, 2006; Yan, Wang, Li, & Wu, 2011). Extraction in hot or boiling water is the most common and convenient method for extracting water-soluble mushroom polysaccharides. However, the major drawbacks of hot water extraction are the high extraction temperature, long extraction time and low extraction efficiency. Various methods have been used to improve the extraction efficiency such as treatment with enzymes, microwave and high power ultrasound (Wang, Wang et al., 2011; Xie, Shan, & Zhang, 2009). The application of high-power or high-intensity ultrasound or ultrasound-assisted extraction (UAE) has been widely studied for extracting polysaccharides from different plant materials. The enhancement of extraction efficiency by UAE is mainly attributed to the mechanical effects of ultrasound, particularly the shear forces arising from acoustic cavitation (Velickovic, Milenovic, Ristic, & Veljkovic, 2006). On the other hand, for extraction of EPSs, the fermentation broth of *C. sinensis* was sequentially...
| Fungi source | Fermentation conditions | Mycelial biomass (g/L) | EPS yield (g/L) | References |
|--------------|-------------------------|-----------------------|----------------|------------|
| **C. sinensis CCRC36421** | Sucrose 6.17%, corn steep powder 0.5%, (NH₄)₂HPO₄ 0.5%, KH₂PO₄ 0.15% (w/v) | 4.4 | 7 | 3.2 | Hsu, Shiao, Hsieh, and Chang (2002), Hsieh et al. (2005) |
| **C. sinensis** | Sucrose 20, corn steep powder 25, CaCl₂ 0.78, MgSO₄·7H₂O 1.73 (g/L) | 4.0 | 16 | 20.9 | 4.1 | Kim and Yun (2005) |
| **C. sinensis 762** | Sucrose 50, peptone 10, yeast extract 3 (g/L) | 22-25 | 40 | 22.1 | Dong, and Yao (2005) |
| **C. sinensis CS-HK1** | Glucose 40, yeast extract 5, peptone 5, KH₂PO₄ 1, MgSO₄·7H₂O 0.5 (g/L);NH₄Cl 10 mmol/L | 22-25 | 7 | 23.2 | 3.4 | Leung et al. 2006; Leung and Wu (2007) |
| **C. sinensis 16** | Sucrose 2%, yeast extract 0.9%, K₂HPO₄ 0.3%,CaCl₂ 0.4% (w/v) | 6.5 | 5 | 54.0 | 28.4 | Cha et al., 2007 |
| **C. sinensis 1** | Sucrose 3%, corn steep powder 5%, bean cake 4%, KH₂PO₄ 0.1, MgSO₄·7H₂O 0.05%, vitamin B1 0.01% | 7 | 7 | 5.9 | Quan, Wang, Du, and Liu (2007) |
| **C. sinensis 383** | Glucose 30, bean cake 20, MgSO₄ 2.0, KH₂PO₄ 4.0 (g/L) | 7.0 | 5 | 3.9 | Lang, Qi, Hou, Zhao, and Jiang (2009) |
| **C. sinensis** | Sucrose 20, yeast extract 2.0, KH₂PO₄ 1.0, MgSO₄·7H₂O 0.6 (g/L) | 7.0 | 4 | 12.3 | 24.5 | Wu, Chen, and Hao (2009) |
| **C. sinensis CCRC36421** | Rice bran 1.5%, molasses 0.5%, CSL 3%, KH₂PO₄ 0.1%, MgSO₄ 0.05% | 5.5 | 5-6 | 48.9 | Choi et al (2010) |
| **Hirsutella sinensis** | Potato extract 20%, sucrose 2.5%, peptone 0.5%, K₂HPO₄ 0.2%, MgSO₄ 0.05% (w/v) | 5.5 | 4 | 10.0 | 2.2 | Li, Jiang, and Guan (2010) |
| **C. sinensis CS001** | Glucose 30, yeast extract 3, peptone 2, KH₂PO₄ 0.6, MgSO₄·7H₂O 0.4, vitamin B₃ 0.01, palmitic acid 1.0 (g/L) | 6.5 | 7 | 0.4 | Wang, Liu, Zhu, & Kuang, 2011 |
| **C. sinensis Cs-HK1** | Glucose 40, yeast extract 15, peptone 5, KH₂PO₄ 1, MgSO₄·7H₂O 0.5 (g/L);NH₄Cl 10 mmol/L; Tween 80 1.5% (w/v) | 6 | 7 | 14.7 | 7.2 | Liu and Wu (2012) |
| **C. sinensis** | Glucose 30, peptone 15, KH₂PO₄ 3.0, MgSO₄·7H₂O 1.5, potato 200 (g/L) | 250-ML shake flask | 25.0 | 2.8 | Yin, Qiao, Qin, Tang, and Jia (2013) |
centrifuged and concentrated, and the resultant material was precipitated by using ethanol, and then centrifuged to harvest the crude EPSs. Fig. 2 summarizes the isolation procedures of IPSs and EPSs from *C. sinensis*.

After extracting crude polysaccharides from *C. sinensis*, the obtained polysaccharide precipitate was partially purified by deproteination and decoloration, and then further purified through column chromatography, such as ion-exchange chromatography, gel filtration chromatograph and affinity chromatography. Elution was conducted with an appropriate running buffer, followed by collection, concentration, dialysis, and lyophilization (Li, Su, Dong, & Tsim, 2002; Li et al., 2003; Wang, Peng et al., 2011; Wang, Wang et al., 2009; Wu et al., 2005; Wu et al., 2006; Wu et al., 2007; Yan, Li, Wang, & Wu, 2007).
2010; Yan et al., 2011). In addition, based on the different solubility of polysaccharides in ethanol, isopropanol, and other solvents, polysaccharides were simply and effectively fractionated. Huang, Siu, Wang, Cheung, and Wu (2013) recently isolated EPS fractions from a fermentation medium of C. sinensis by gradient ethanol precipitation. Their results suggest that the method is simple and workable for the initial fractionation of polysaccharides, proteins, and their complexes with different molecular sizes and for further identification of bioactive components.

## 4. Physicochemical characterization

The physicochemical and structural features of a polysaccharide mainly include monosaccharide composition, molecular weight (MW), configuration of glycosidic linkages, type of glycosidic linkage, position of glycosidic linkage, sequence of monosaccharide, number and location of appended non-carbohydrate groups, and molecular chain conformation (Cui, 2005; Nie, & Xie, 2011; Zhang, Cui, Cheung, & Wang, 2007). Polysaccharides with different monosaccharide constituents and chemical structures have been isolated from wild or cultured C. sinensis. Many research groups have elucidated the chemical structures of purified IPSs and EPSs using infrared spectroscopy, liquid-state nuclear magnetic resonance (NMR) (one and two dimensions), solid-state NMR, gas chromatography (GC), GC-mass spectroscopy (GC–MS), high-performance liquid chromatography (HPLC), acid hydrolysis, methylation analysis, periodate-oxidation, and Smith degradation (Akaki et al., 2009; Kiho, Hui, Yamane, & Ukai, 1993; Kiho et al., 1996; Kiho et al., 1999; Nie et al., 2011; Wang, Yin, Chen, & Wang, 2009, Wang, Wang et al., 2009; Wang, Peng et al., 2011; Wu et al., 2005;; Wu et al., 2007; Yan et al., 2010; Yan et al., 2011; Wu et al., 2005). A wide range of bioactive polysaccharides of different structural characteristics from C. sinensis have been isolated based on differences in source materials, isolation protocols, and fractionation protocols. The sources, molecular properties, chemical structures, and bioactivities are summarized in Table 2.

### 4.1 Monosaccharide composition

Monosaccharide composition analysis usually involves cleavage of glycosidic linkages by acid hydrolysis, derivatization, and detection and quantification by GC. In addition, high-performance anion-exchange chromatography with pulsed amperometric detection and detection and quantification by GC. In addition, high-performance anion-exchange chromatography with pulsed amperometric detection has been gradually developed to supplement traditional methods as it doesn’t require derivatization of monosaccharide with high resolution (Panagiotopoulos, Sempéré, Lafont, & Kerhervé, 2001). Recently, a 1-phenyl-3-methy-5-pyrazolone pre-column derivatization method has been used to determine monosaccharide composition (Chen, Siu, Wang, Liu, & Wu, 2013; Wang, Yin et al., 2010; Wang, Wang et al., 2009).

Although many different IPSs and EPSs have been obtained, the monosaccharide composition is usually glucose (Glu), mannose (Man), and galactose (Gal) in various mole ratios (Cha et al., 2007; Gong et al., 1990; Kiho et al., 1999; Li et al., 2003; Miyazaki, Oikawa, & Yamada, 1977; Nie et al., 2011; Wang, Wang et al., 2009; Wu et al., 2006; Yan et al., 2010). However, the IPSs are also found to only contain D-Glu to be composed of different polyglucans (Akaki et al., 2009; Wu et al., 2005; Wu et al., 2005; Wu et al., 2006; Yan et al., 2011), but so far, only one study has been reported that EPS isolated from culture broth of C. sinensis was a β-glucan (Yamada et al., 1984). In addition, IPSs and EPSs may also contain uronic acid, proteins and inorganic elements (Kiho et al., 1986; Wang, Wang et al., 2011; Wang, Peng et al., 2011). These polysaccharide conjugates isolated from natural or cultured C. sinensis also represent a major class of bioactive compounds and may exert more important pharmacological effects than neutral polysaccharides.

### 4.2 Average molecular weight

Various techniques such as viscometry, osmometry, sedimentation, and HPLC have been used to determine the average polymer molecular weight (MW) and polydispersity index. Among them, high-performance gel permeation chromatography (HPGPC) is a common method for determining the MW of polysaccharides and has also been used by many researchers for MW of IPSs and EPSs. Size-exclusion chromatography with multi-angle laser light scatter detection is also an efficient method for the evaluation of the absolute MW of polysaccharides and provides greater resolution than traditional gel permeation chromatography (Boukari et al., 2009; Hilliou et al., 2009). Different MWs ranging from ~10^3 to ~10^6 Da have been found in various source materials of C. sinensis and experimental conditions (Nie et al., 2013; Yan et al., 2011; Zhao et al., 2013; Zhong et al., 2009; Zhou, Gong, Su, Lin, & Tang, 2009).

### 4.3 Chemical structures

IPSs from natural and cultured C. sinensis usually consist of glucose, mannose, and galactose with 1→4(6)-glucopyranosyl (Glcp), 1→6-mannopyranosyl (Manp), and 1→4(6)-galactopyranosyl (Galp) (Guan et al., 2011; Nie et al., 2013; Zhong et al., 2009; Zhou et al., 2009). The earliest reports on IPSs by Miyazaki et al. (1977) and Kiho et al. (1986) included a galactomannan designated CS-I from a hot-water extract and a water-soluble, protein-containing galactomannan (CT-4N) isolated from a 5% sodium carbonate extract of C. sinensis by gradient ethanol precipitation. Their results suggest that the method is simple and workable for the initial fractionation of polysaccharides, proteins, and their complexes with different molecular sizes and for further identification of bioactive components.
Table 2 – Polysaccharides originated from *Cordyceps sinensis* fungi: source, chemical structures and bioactivities.

| Living strains | Polysaccharides source | Extraction medium | Components | Molecular weight | Linkages and types | Bioactivities | References |
|----------------|------------------------|-------------------|------------|------------------|-------------------|--------------|------------|
| *Paecilomyces sinensis*(Cs-4) | Mycelium | Hot water | Man:Gal = 1:1 | – | CS-1 Galactomannan | – | Miyazaki et al. (1977) |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | 5% Sodium carbonate | Man:Gal = 3:5 | 23 kDa | CT-4 N Galactomannan | – | Kihó et al., 1986 |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | Hot water | Gal:Glc:Man = 62:28:10 | 45 kDa | CS-F30 | Hypoglycemic activity | Kihó et al. (1993), Kihó et al. (1996) |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | Hot water | Glu:Man:Gal = 10:60:75 | 210 kDa | CS-F10 Galactoglucomannan | CSP-1 | Kihó et al., 1999 |
| *Cephalosporium sinensis* Chen sp. nov. | Mycelium | Hot water | Gal:Glc:Man = 43:33:24 | 15 kDa | Neutral (1 → 3), (1 → 4)-β-glucan | Antioxidant activity, Hypoglycemic activity | Li et al. (2003), Li et al. (2006) |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | Hot water | β-Glu | 13.6 kDa | Cordyglucans | – | Wang et al. (2005) |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | 0.05 M phosphate buffer | α-Glu | 184 kDa | SCI-I | – | Wu et al. (2005) |
| *Cephalosporium sinensis* Chen | Mycelium | Hot water | Glc:Man:Gal = 21:2:1 | – | 1,3-α-D-Glcp-1,6-branched chain Glc-1,3-β-D-Glcp-1 | Cholesterol esterase inhibitory activity | Akaki et al. (2009) |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | 0.05 M acetic acid buffer | Man, Glu, Gal, Uronic acid | 27 kDa | CPS1 Glucomannogalactan | Antioxidant activity, Protection of chronic renal failure | Wang et al. (2009), Wang et al. (2010) |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | Hot water | α-Glu | 1180 kDa | WIPS α-(1→4)-glucan | Antitumour and Immuno-stimulating effects | Yan et al. (2011) |
| *Tolypocladium sinensis* | Mycelium | 1.25 M NaOH / 0.04% NaBH₄ | α-Glu | 1150 kDa | AIPS α-(1→4)-glucan (86%), (1→6)-α-D-glucose (14%) | Antitumour and Immuno-stimulating effects | Yan et al. (2011) |
| *Paecilomyces sinensis*(Cs-4) | Mycelium | Hot water | Man:Gal:Glc = 10.3:3.6:1 | 43 kDa | CS-81002 | Immunomodulating | Nie et al. (2011), Zhang, Li et al. (2012), Zhang, Cheung et al. (2012) |
| *Tolypocladium sinensis* | Mycelium | Hot water | Glu(95%), Man, Gal | 260 kDa | CBHP α-1,4-linked-Glcp | Antioxidant activity | Shen et al. (2011) |
| *Cordyceps ophioglossoides* | Culture filtrate | Ethanol | β-Glu | 632 kDa | CO-1 | Antifibrotic effect | Yamada et al., 1984, | |
| *Paecilomyces sinensis*(Cs-4) | Culture broth | Ethanol | Man:Gal:Glc = 21.2:1.2:1 | 104 kDa | EPS-1A | Antimicrobial activity | Wang, Liu, Zhu, & Kuang, 2011 | |
| *Paecilomyces sinensis*(Cs-4) | Culture broth | 95% Ethanol | Glu:Man:Gal = 2:1:1 | 46 kDa | AEPS-1 | Antioxidant activity | Chen, Siu et al. (2013) |
the cultured C. sinensis have been identified as glucomannan-galactans, whose backbone were mainly composed of (1→2) and (1→4)-linkage of mannose, (1→3)-linkage of galactose, (1→_), and (1→3,6)-linkage of glucose (Nie et al., 2011; Wang, Wang et al., 2009). Furthermore, a novel neutral mannoglycan isolated from C. sinensis mycelium has a backbone of predominantly (1→4)-linked α-D-GlcP (61.3%) together with a proportion of (1→3)-linked α-D-GlcP residues (28.0%), with single α-D-Manp units (10.7%) as the side chains attached to C-6 of (1→3)-linked D-Glcp residue (Wu et al., 2007). Similarly, Wang, Yin et al. (2010) reported the chemical structure of a water-soluble polysaccharide (CPS-2) isolated from cultured C. sinensis, which was composed mostly of a α-(1→4)-β-D-glucose and a α-(1→3)-D-mannose branched with α-(1→4,6)-β-D-glucose every twelve residues on average. Based on the above reports, it can be concluded that heteropolysaccharides are the most common bioactive polysaccharides in the fruiting bodies and mycelia of C. sinensis.

Some researchers have also reported that IPSs are polyglucans with different structural characteristics. For instance, Wu et al. (2005) found that cordyglucans obtained from C. sinensis mycelia is mainly composed of a (1→3)-β-D-glucan linked backbone with short (1→6)-β-D-glucan linked branches. Wu et al. (2006) also reported that the structure of a polysaccharide (SCP-I) isolated from C. sinensis mycelium, i.e., SCP-I, is a β-D-glucan containing an α-(1→4)-linked backbone and a branched short α-(1→6)-linkage. Furthermore, Akaki et al. (2009) reported that an insoluble polysaccharide (CS-Pp) purified from the cultured mycelium of C. sinensis was a 1,3-β-D-glucan with some 1,6-branched chains. Recently, Yan et al. (2011) isolated the two polysaccharides, WIPS and AIPS, from hot water and dilute alkaline extracts, respectively, of the mycelial biomass of a C. sinensis fungus Cs-HK1, which were characterized as α-D-glucans with a backbone of (1→4) linked α-D-glucopyranosyl (Glcp) (>60%). WIPS is found to have a short branch of (1→6)-linked α-D-Glcp (~14%), whereas AIPS is a highly linear glucan.

In addition to the IPSs extracted from the mycelium or fruiting bodies of C. sinensis, EPSs isolated from the culture broth of C. sinensis have been reported. Gong et al. (1990) reported an EPS called CS-81002 isolated from the fermentation medium of C. sinensis and characterized as a branched heteropolysaccharide. Recently, Yan et al. (2010) reported the isolation and structure of EPS-1A from a fermentation broth of C. sinensis Cs-HK1. EPS-1A was found to be a slightly branched polysaccharide with its backbone being composed of (1→6)-α-D-glucose residues (~77%) and (1→6)-α-D-mannose residues (~23%). Branching occurred at the O-3 position of (1→6)-α-D-mannose residues of the backbone with (1→6)-α-D-mannose residues and (1→6)-β-D-galactose residues and terminated with β-D-galactose residues. Meanwhile, Wang, Peng et al. (2011) reported that on the acidic polysaccharide AEPS-1, which had a linear backbone of (1→3)-linked α-D-Glcp residues with two branches, namely, α-D-Glcp and α-D-pyranogluconic acid (GlcUp), attached to the main chain by (1→6) glycosidic bonds at every seventh α-D-Glcp unit. In addition, a novel poly-N-acetylhexosamine (polyhexNAC) of about 6 kDa was isolated from the low-MW fraction of EPS produced from the liquid fermentation of C. sinensis Cs-HK1. The molecular structure was elucidated as a [-4-β-D-ManNAc-(1→3)-β-D-GalNAc-(1→) disaccharide repeating unit in the main chain with a Gal branch randomly occurring at the 3-position of ManNAc (Chen, Siu et al., 2013).

4.4. Conformational features

The activities of polysaccharides depend on their MWs, chemical structures, and chain conformations. In general, polysaccharides in aqueous solutions exhibit different forms of chain conformations, such as random coil (Senti et al., 1955), various helical forms, single helix, double helix, triple helix (Kashiwagi, Norisuye, & Fujita, 1981; Sato, Norisuye, & Fujita, 1984; Zhang, & Yang, 1995), and aggregate (Ding, Jiang, Zhang, & Wu, 1998). A few reports are available on the solution properties and chain conformations of C. sinensis polysaccharides. For example, Cai, Li, and Lu (1999) firstly developed a method to study the morphology of a IPS by atomic force microscopy (AFM). Their results show that this IPS has a multi-branched structure and various linkages between adjacent monosaccharides, which make up small rings and helical structures. Very recently, the morphological characteristics and chain conformation of EPS isolated from a mycelial culture of C. sinensis Cs-HK1 have been analyzed by AFM together with the Congo red test, optical rotation, and dynamic light scattering. Results suggest that this EPS forms large interwoven networks in aqueous solution and is primarily connected with triple-helical conformation and occasionally single-helical conformation in solution. However, IPSs obtained from Cs-HK1 mycelium exhibit random coils in aqueous alkaline solutions (Wang, Cheung, Leung, & Wu, 2010; Wang, Peng et al., 2011; Yan et al., 2011). In addition, the random coils or aggregated networks of EPS-1 formed in aqueous solution were transformed into the single helices after sulfation (Yan, Wang, Ma, & Wu, 2013).

The relationships among solution properties, chain conformations of polysaccharides, and their biological activities are difficult to elucidate. The detailed chain conformations of C. sinensis polysaccharides in aqueous solutions require further investigations using other technological means, such as static and dynamic light scattering, viscosity analysis based on the theory of dilute polymer solutions, circular dichroism analysis, transmission electron microscope, scanning electron microscopy, AFM, AFM-based single-molecule force spectroscopy, fluorescence correlation spectroscopy, and NMR spectroscopy (Yang, & Zhang, 2009). Diluted solution theory, molecular modeling, and computer-assisted energy minimization methods have also been used to analyze the chain conformation of polysaccharides (Brant, 1981; Pol-Fachin, Fernandes, & Verli, 2009; Pérez, Kowijzer, Mazeau, & Engelsen, 1996, Strlegel, Plattner, & Willett, 1999).

5. Bioactivities

Based on TCM theories, the major effects of C. sinensis are “to enrich the lung yin and yang”. Its use includes treatment of chronic lower back pain, sensitivity to cold, overabundance of mucus and tears, chronic cough and wheezing, and blood
in phlegm due to consumption as a result of kidney yang (shenyangqu). C. sinensis also has antibacterial activity, reduces asthma, lowers blood pressure, and strengthens heartbeat according to Western medicine (Zhu et al., 1998). Polysaccharides represent a major class of bioactive constituents of C. sinensis, contributing to its health effects and pharmacological activities according to a large number of animal and clinical studies. The multiple bioactivities and health benefits of IPSs and EPSs are summarized and compared in detail below.

5.1 Immunomodulatory activity

Immunomodulation is the most notable biological function of natural polysaccharides, which is associated with their putative role as biological response modifiers (Moradali, Mostafavi, Ghods, & Hedjaroude, 2007). The immuno-stimulating and immunosuppressive properties of IPSs and EPSs have been assessed on natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses (Koh, Yu, Suh, Choi, & Ahn, 2002; Paterson, 2008; Zhong et al., 2009; Zhou et al., 2009). Phagocyte release is an early step in the response of macrophages to pathogen invasion of the human body. Macrophages can also defend against pathogen invasion by secreting proinflammatory cytokines [e.g., tumour necrosis factor (TNF-α) and interleukin (IL)-1] and releasing cytotoxic and inflammatory molecules [e.g., nitric oxide (NO) and reactive oxygen species] (Medzhitov, & Janeway, 2000).

The majority of studies on IPSs and EPSs immunomodulation have been evaluated by activating macrophages. EPSs prepared from cultured C. sinensis induce the production of TNF-α, IL-6, and IL-10 dose-dependently and elevate phagocytes in monocytes and PMN, but IPSs only moderately induce TNF-α release, CD11b expression, and phagocytes at the same concentration (Cheung et al., 2009; Kuo, Chang, Cheng, & Wu, 2007). This finding indicates that the immunomodulatory components of cultured C. sinensis mainly reside in the culture filtrate and are similar to previously reported ones (Gong et al., 1990). Recent reports have shown that polysaccharides isolated from various natural or cultured C. sinensis have the same immunomodulatory activity of stimulation of the release of several major cytokines in the mouse macrophage cell line RAW264.7 and in mouse splenocyte cells by activating the IκB-NF-κB pathway (Akaki et al., 2009; Chen, Zhang, Shen, & Wang, 2010; Wang, Peng et al., 2011). More recently, Chen, Yuan, Wang, Song, and Zhang (2012) reported that an acid polysaccharide fraction (APSF) from C. sinensis fungus could increase the expressions of TNF-α, IL-12, and iNOS, and reduce the expression of IL-10 of Ana-1 cells, convert M2 macrophages to M1 phenotype by activating NF-κB pathway.

5.2 Antitumour activity

Since the first report on the antitumour activity of mushroom polysaccharides in the 1960s (Chihara, 1969), researchers have isolated structurally diverse polysaccharides with strong antitumour activity from plants, animals, and fungi. Mushroom polysaccharides exert inhibitory effects toward many kinds of tumours, such as Sarcoma 180 solid tumour, Ehrlich solid tumour, Sarcoma 37, Yoshida sarcoma, and Lewis lung carcinoma (Wasser, & Weis, 1999). The currently accepted mechanism by which mushroom polysaccharides exert antitumour effects can be summarized as follows: (1) prevention of oncogenesis by oral administration of polysaccharides isolated from medicinal mushrooms, (2) enhancement of immunity against bearing tumours, (3) direct antitumour activity to induce the apoptosis of tumour cells, and (4) prevention of the spread or migration of tumour cells in the body (Moradali et al., 2007; Wassar, 2002; Zhang et al., 2007).

Many studies have demonstrated that both of IPSs and EPSs have strong antitumour activity through the above proposed mechanisms. As a simple method, the prevention of the onset of tumour by oral administration is used to evaluate the antitumour activity of polysaccharides in vivo. IPSs obtained from the mycelia of C. sinensis are effective against sarcoma 180, with almost 90% inhibition in ICR/JCL mice (Wu et al., 2005; Zhang et al., 2007). In addition, EPSs obtained from a cultured broth of C. sinensis significantly lowers c-Myc, c-Fos, and vascular endothelial growth factor expression in B16 melanoma-bearing mouse. Thus, EPSs can inhibit tumour growth in the lungs and liver of mice and can be a potential adjuvant in cancer therapy (Yang et al., 2005).

EPSs exert their antitumour effect mainly through the enhancement and activation of the immune response of the host organism. An EPS isolated from one of the anamorph strains of C. sinensis belonging to Tolypocladium spp. is found to significantly enhance the neutral red uptake capacity of peritoneal macrophages and spleen lymphocyte proliferation in B16-bearing mouse. The metastasis of B16 melanoma cells in lungs and liver is also significantly inhibited. Moreover, this EPS can markedly prevent H22 tumour growth and elevate immunocyte activity in H22 tumour-bearing mice, indicating that EPSs inhibit tumour cells mainly by activating the host’s immune system (Zhang, Li, Qiu, Chen, & Zheng, 2008; Zhang, Yang, Chen, Hou, & Han, 2005). To confirm this finding, Sheng, Chen, Li, and Zhang (2011) investigated the effects of EPSs on immunocytes in vitro, and results indicate that EPS treatment significantly promotes the mRNA and protein levels of TNF-α and IFN-γ. This phenomenon supports the assumption that antitumour activity is related to the promoted cytokine expression of immunocytes. EPSs can also stimulate the maturation and activation of murine and human dendritic cells by inhibiting STAT3 activation (Huang, Song, Yang, Yin, & Zhang, 2011; Song, Lin, Yuan, & Zhang, 2011). Furthermore, EPS may induce dendritic cell sarcoma (DCS) cells to exhibit mature characteristics, and the mechanism involved is probably related to the inhibition of the JAK2/STAT3 signal pathway and promotion of the NF-κB signal pathway (Song et al., 2013). On the other hand, IPSs can also activate many immune cells to modulate the release of cell signal messengers such as cytokines, and increased cytokine production in immune cells has been studied in mice and humans (Chen, Shiao, Lee, & Wang, 1997; Yoon, Yu, Shin, & Suh, 2008).

Finally, the ability to induce apoptosis has been identified and utilized in successful cancer chemotherapeutics (Chen et al., 1997; Yoon et al., 2008), and studies have suggested that C. sinensis can induce apoptosis (Buenz, Bauer, Osmundson, & Motley, 2005). A 410 kDa polysaccharide fraction (IPS) isolated from C. sinensis is found to inhibit cell proliferation, promote the apoptosis of IL-1- and platelet-derived growth...
Oxidation phenomena have been implicated in many illnesses, such as diabetes mellitus, arteriosclerosis, nephritis, Alzheimer's disease, and cancer (Negre-Salvayre, Costeirieux, Ingueuneu, & Salvayre, 2008). Therefore, natural antioxidants isolated from plants, fungi, and marine algae represent most useful nutraceuticals and functional foods for health protection and disease prevention (Gutteridge, & Halliwell, 1994). Antioxidant activity has become one of the focuses of studies on mechanisms of the nutraceutical and therapeutical effects of TCM using various assay methods and activity indices (Dong & Yao, 2008; Schlesier, Harwat, Bo¨hm, & Bitsch, 2002).

5.3. Antioxidant activity

Oxidation phenomena have been implicated in many illnesses, such as diabetes mellitus, arteriosclerosis, nephritis, Alzheimer's disease, and cancer (Negre-Salvayre, Costeirieux, Ingueuneu, & Salvayre, 2008). Therefore, natural antioxidants isolated from plants, fungi, and marine algae represent most useful nutraceuticals and functional foods for health protection and disease prevention (Gutteridge, & Halliwell, 1994). Antioxidant activity has become one of the focuses of studies on mechanisms of the nutraceutical and therapeutical effects of TCM using various assay methods and activity indices (Dong & Yao, 2008; Schlesier, Harwat, Bo¨hm, & Bitsch, 2002).

5.4. Hypoglycemic effect

Many research groups have evaluated the hypoglycaemic effects of natural products, including fungal polysaccharides using the Streptozotocin (STZ)-induced and alloxan-induced animal models (Hwang, & Yun, 2010; Hwang et al., 2005; Yamac et al., 2008). IPSs extracted with hot water and alkalis have significant hypoglycemic effects on normal and alloxan-diabetic mice and STZ-diabetic rats in vivo by reducing the plasma glucose level in both STZ-induced diabetic rats and alloxan-induced diabetic mice, thereby increasing the serum insulin levels in diabetic animals (Li et al., 2006; Wang, & Shiao, 2000; Zhang et al., 2006). IPSs also significantly lowered the levels of plasma triglyceride and cholesterol in mice and increase the activities of hepatic glucokinase, hexokinase, and glucose-6-phosphate dehydrogenase (Kiho et al., 1993; Kiho et al., 1996; Kiho et al., 1999).

5.5. Other bioactivities

As aforementioned, IPSs and EPSs obtained from wild or cultivated C. sinensis demonstrate immunoregulation, antitumour, antioxidation, and hypoglycemic effects, as well as other important bioactivities, including anti-fibrosis, anti-fatigue, kidney protection, increasing plasma testosterone levels, and radiation protection (Nie et al., 2013; Wang, Yin et al., 2009, 2010; Wong, Wu, & Benzie, 2011; Yan, Zhang, & Wang, 2012; Yao et al., 2014; Zhang, Cheung, Al-Assaf, Phillips, & Phillips, 2012; Zhong et al., 2009).

6. Conclusions and future perspectives

C. sinensis is a well-known and precious medicinal fungus in China for its ability to treat a broad spectrum of human diseases, especially those related to the functions of the lung and kidney, the immune system, and for its ability to enhance the quality of life and physical performance. Polysaccharides have been identified as the major active components of C. sinensis with a wide range of bioactivities including immunomodulation, antitumour, antioxidation, and hypoglycemic effects. Fermentation production, isolation, structural characterization, and the bioactivities of polysaccharides from
different wild or natural C. sinensis have been extensively investigated in recent years, mainly in China, Japan, and Korea. However, the relationship between structural features, solution behavior, space conformation, and their bioactivity is unclear due to the structural diversity and complexity of polysaccharide molecules. The fruiting bodies or mycelial biomass as the sources of IPSs, especially EPSs isolated from the culture broth, have attained from different C. sinensis species and in various conditions. Because of the variable properties of raw material and the composition of polysaccharides, it is difficult to maintain consistency, reproducibility and reliability of the results. There is a need to establish standard protocols for collection and preparation of the source material and for the extraction, isolation and purification of polysaccharides. These will be useful for determination of the chemical structures and chain conformations and the biological activities and for applications in food, medicine and cosmetic products.

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REFERENCES

Akai, J. J., Matsu, Y., Kojima, H., Nakajima, S., Kamei, K., & Tamesada, M. (2009). Structural analysis of monocyte activation constituents in cultured mycelia of Cordyceps sinensis. Fitoterapia, 80, 182–187.

Au, D., Wang, L., Yang, D., Mok, D. K., Chan, A. S., & Xu, H. (2012). Application of microscopy in authentication of valuable Chinese medicine I-Cordyceps sinensis, its counterfeiters, and related products. Micro Research Technology, 75, 54–64.

Boukari, I., Putaux, J. L., Catala, B., Barakat, A., Saake, B., Rémond, C., O’Donohue, M., & Chabbert, B. (2009). In vitro model assemblies to study the impact of lignin-carbohydrate interactions on the enzymatic conversion of xylan. Biomacromolecules, 10, 2489–2498.

Brant, D. A. (1981). Solution properties of polysaccharides: Based on a symposium. Division of Carbohydrate Chemistry: American Chemical Society.

Buenz, E. J., Bauer, B. A., Osmundson, T. W., & Motley, T. J. (2005). The traditional Chinese medicine Cordyceps sinensis and its effects on apoptosis homeostasis. Journal of Ethnopharmacology, 96, 19–29.

Cai, L. T., Li, P., & Lu, Z. H. (1999). Observation of the structure morphology of Cordyceps polysaccharide by atomic force microscope. Journal of Chinese Electron Microscopy Society, 18, 103–105 (in Chinese).

Cha, S. H., Lim, J. S., Yoon, C. S., Koh, J. H., Chang, H. I., & Kim, S. W. (2007). Production of mycelia and exo-biopolymer from molasses by Cordyceps sinensis 16 in submerged culture. Bioresource Technology, 98, 165–168.

Chen, Y. J., Shiao, M. S., Lee, S. S., & Wang, S. Y. (1997). Effect of Cordyceps sinensis on the proliferation and differentiation of human leukemic U937 cells. Life Sciences, 60, 2349–2359.

Chen, S., Siu, K. C., Wang, W. Q., Liu, X. X., & Wu, J. Y. (2013). Structure and antioxidant activity of a novel poly-N-acetylhexosamine produced by a medicinal fungus. Carbohydrate Polymers, 94, 332–338.

Chen, P. X., Wang, S., Nie, S., & Marcone, M. (2013). Properties of Cordyceps sinensis: A review. Journal of Functional Foods, 5, 550–569.

Chen, W., Yuan, F., Wang, K., Song, D., & Zhang, W. (2012). Modulatory effects of the acid polysaccharide fraction from one of anamorph of Cordyceps sinensis on Ana-1 cells. Journal of Ethnopharmacology, 142, 739–745.

Chen, J. F., Zhang, W. Y., Lu, T. T., Li, J., Zheng, Y., & Kong, L. D. (2006). Morphological and genetic characterization of a cultivated Cordyceps sinensis fungus and its polysaccharide component possessing antioxidant property in H22 tumour-bearing mice. Life Sciences, 78, 2742–2748.

Chen, W., Zhang, W., Shen, W., & Wang, K. (2010). Effects of the acid polysaccharide fraction isolated from a cultivated Cordyceps sinensis on macrophages in vitro. Cellular Immunology, 262, 69–74.

Cheung, J. K. H., Li, J., Cheung, A. W. H., Zhu, Y., Zheng, K. Y. Z., Bi, C. W. C., Duan, R., Choi, R. C. Y., Lai, D. T. W., Dong, T. T. X., Lai, B. W. C., & Tsim, K. W. K. (2009). Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. Journal of Ethnopharmacology, 124, 61–68.

Chihara, G. (1969). The antitumour polysaccharide lentinian: An overview 222. In T. Aoki et al. (Eds.), Manipulation of host defense mechanism (pp. 687–694).

Choi, J. W., Ra, K. S., Kim, S. Y., Yoon, T. J., Yu, K. W., Shin, K. S., Lee, S. P., & Suh, H. J. (2010). Enhancement of anti-complementary and radical scavenging activities in the submerged culture of Cordyceps sinensis by addition of citrus peel. Bioresource Technology, 101, 6028–6034.

Cui, S. W. (2005). Structural analysis of polysaccharides. In Steve W. Cui (Ed.), Food carbohydrates: Chemistry, physical properties and applications (1 edition). Boca Raton, FL: CRC Press.

Ding, Q., Jiang, S., Zhang, L., & Wu, C. (1998). Laser light-scattering studies of pachyman. Carbohydrate Research, 308, 339–343.

Ding, C., Tian, P. X., Xue, W., Ding, X., Yan, H., Pan, X., Feng, X., Xiang, H., Hou, J., & Tian, Y. (2011). The efficacy of Cordyceps sinensis in long term treatment of renal transplant patients. Frontiers in Bioscience (Elite Edition), 3, 301–307.

Dong, C. H., & Yao, Y. J. (2005). Nutritional requirements of mycelial growth of Cordyceps sinensis in submerged culture. Journal of Applied Microbiology, 99, 483–492.

Dong, C. H., & Yao, Y. J. (2008). In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of Cordyceps sinensis. IWT-Food Science and Technology, 41, 669–677.

Gong, M., Zhi, Q., Wang, T., Wang, X. L., Ma, J. X., & Zhang, W. J. (1990). Molecular structure and immunoactivity of the polysaccharide from Cordyceps sinensis (Berk) Sacc. Chinese Biochemical Journal, 6, 486–492 (in Chinese).

Guan, J., Zhao, J., Feng, K., Hu, D. J., & Li, S. P. (2011). Comparison and characterization of polysaccharides from natural and cultured Cordyceps using saccharide mapping. Analytical and Bioanalytical Chemistry, 399, 3465–3474.

Gutteridge, J. M. C., & Halliwell, B. (1994). Antioxidants in nutrition, health, and disease. Oxford, New York, NY: Oxford University Press.

Hilliou, L., Freitas, F., Oliveira, R., Reis, M. A. M., Lespineux, M., Grandfils, C., & Alves, V. D. (2009). Solution properties of an exopolysaccharide from a medicinal fungus and its polysaccharide component possessing antioxidant property in H22 tumour-bearing mice. Life Sciences, 78, 2742–2748.

Holaday, J., & Cleaver, M. (2008). Medicinal value of the caterpillar fungi species of the genus Cordyceps (Fr.) Link (Ascomycetes). A review. International Journal of Medicinal Mushrooms, 10, 219–234.
Koh, J. H., Yu, K. W., Suh, H. J., Chang, D. M., & Lo, C. T. (2005). Medium optimization for polysaccharide production of Cordyceps sinensis. Applied Biochemistry and Biotechnology, 120, 145–157.

Hsu, T. H., Shiao, L. H., Hsieh, C., & Chang, D. M. (2002). A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChongXiaCao, its counterfeit and mimic, and fermented mycelium of Cordyceps sinensis. Food Chemistry, 78, 463–469.

Huang, Q. L., Siu, K. C., Wang, W. Q., Cheung, Y. C., & Wu, J. Y. (2013). Fractionation, characterization and antioxidant activity of exopolysaccharides from fermentation broth of a Cordyceps sinensis fungus. Process Biochemistry, 48, 380–386.

Huang, J., Song, D., Yang, A., Yin, H., & Zhang, W. (2011). Differentiation and maturation of human dendritic cells modulated by an exopolysaccharide from an anamorph of Cordyceps sinensis. Biomedical & Preventive Nutrition, 1, 126–131.

Hwang, H. J., Kim, S. W., Lim, J. M., Joo, J. H., Kim, H. O., Kim, H. M., & Yun, J. W. (2005). Hypoglycemic effect of crude exopolysaccharides produced by a medicinal mushroom Phellinus baumi in streptozotocin-induced diabetic rats. Life Sciences, 76, 3069–3080.

Hwang, H. S., & Yun, J. W. (2010). Hypoglycemic effect of polysaccharides produced by submerged mycelial culture of Lasiodiplodia theobromae on streptozotocin-induced diabetic rats. Biotechnology and Bioprocess Engineering, 15, 173–181.

Jeffrey, G. (2012). The 'Viagra' transforming local economies in India. BBC News. Retrieved, 09/07, 2012.

Ji, D. B., Ye, J. L., Li, C. L., Wang, Y. H., Zhao, J., & Cai, S. Q. (2009). Antiaging effect of Cordyceps sinensis extract. Phytotherapy Research, 23, 116–122.

Kashiwagi, Y., Norisuye, T., & Fujita, H. (1981). Triple helix of Schizopyllum commune polysaccharide in dilute solution, 4. Light scattering and viscosity in dilute aqueous sodium hydroxide. Macromolecules, 14, 1220–1225.

Kiho, T., Hui, J., Yamane, A., & Ukai, S. (1993). Polysaccharide in fungi. XXXII. Hypoglycemic activity and chemical properties of a polysaccharide from the cultural mycelium of Cordyceps sinensis. Biological and Pharmaceutical Bulletin, 16, 1291–1293.

Kiho, T., Oobu, 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. Biological and Pharmaceutical Bulletin, 22, 966–970.

Kiho, T., Tabata, H., Ukai, S., & Hara, C. (1986). Polysaccharide in fungi. 18. A minor, protein-containing galactomannan from a sodium-carbonate extract of Cordyceps sinensis. Carbohydrate Research, 156, 189–197.

Kiho, T., Yamane, A., Hui, J., Usui, S., & Ukai, S. (1996). Polysaccharides in fungi, XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of Cordyceps sinensis and its effect on glucose metabolism in mouse liver. Biological and Pharmaceutical Bulletin, 19, 294–296.

Kim, S. D. (2010). Isolation, structure and cholesterol esterase inhibitory activity of a polysaccharide, PS-A, from C. sinensis. Journal of the Korean Society from Applied Biological Chemistry, 53, 784–789.

Kim, H. O., & Yun, J. W. (2005). A comparative study on the production of exopolysaccharides between two entomopathogenic fungi Cordyceps militaris and Cordyceps sinensis in submerged mycelial cultures. Journal of Applied Microbiology, 99, 728–738.

Koh, J. H., Yu, K. W., Suh, H. J., Choi, Y. M., & Ahn, T. S. (2002). Activation of macrophages and the intestinal immune system by an orally administered decoction from cultured mycelia of Cordyceps sinensis. Bioscience, Biotechnology, and Biochemistry, 66, 407–411.

Kuo, M. C., Chang, C. Y., Cheng, T. L., & Wu, M. J. (2007). Immunomodulatory effect of exo-polysaccharides from submerged cultured Cordyceps sinensis: Enhancement of cytokine synthesis, CD11b expression, and phagocytosis. Applied Microbiology and Biotechnology, 75, 769–775.

Lang, J., Qi, X., Hou, Y., Zhao, S., & Jiang, G. (2009). Production of exopolysaccharides by Cordyceps sinensis in liquid culture. Journal of Dalian Polytechnic University, 28, 107–110 (in Chinese).

Leung, P. H., & Wu, J. Y. (2007). Effects of ammonium feeding on production of bioactive metabolites (cordycepin and exopolysaccharides) in mycelial culture of a Cordyceps sinensis fungus. Journal of Applied Microbiology, 103, 1942–1949.

Leung, P. H., Zhang, Q. X., & Wu, J. Y. (2006). Mycelium cultivation, chemical composition and antitumour activity of a Tolypocladium sp. fungus isolated from wild Cordyceps sinensis. Journal of Applied Microbiology, 101, 275–283.

Leung, P. H., Zhao, S. N., Ho, K. P., & Wu, J. Y. (2009). Chemical properties and antioxidant activity of exopolysaccharides from mycelial culture of Cordyceps sinensis fungus Cs-HK1. Food Chemistry, 114, 1251–1256.

Li, S. P., & Tsim, K. W. K. (2004). The biological and pharmacological properties of Cordyceps sinensis, a traditional Chinese medicine that has broad clinical applications. In L. Packer, C. N. Ong, & B. Halliwell (Eds.), Herbal and traditional medicine. Molecular aspects of health (pp. 657–683). New York: Marcel Dekker.

Li, R., Jiang, X. L., & Guan, H. S. (2010). Optimization of mycelium biomass and exopolysaccharides production by Hirustella sp in submerged fermentation and evaluation of exopolysaccharides antibacterial activity. African Journal of Biotechnology, 9, 196–203.

Li, S. P., Li, P., Dong, T. T. X., & Tsim, K. W. K. (2001). Anti-oxidation activity of different types of natural Cordyceps sinensis and cultured Cordyceps mycelia. Phytomedicine, 8, 207–212.

Li, S. P., Su, Z. R., Dong, T. T. X., & Tsim, K. W. K. (2002). The fruiting body and its caterpillar host of Cordyceps sinensis show close resemblance in main constituents and anti-oxidation activity. Phytomedicine, 9, 319–324.

Li, Y., Wang, X. L., Jiao, L., Jiang, Y., Li, H., Jiang, S. P., & Guo, J. (2009). A survey of the geographic distribution of Ophiocordyceps sinensis. The Micro Journal of Biology, 49, 913–919.

Li, S. P., Zhang, G. H., Zeng, Q., Huang, Z. G., Wang, Y. T., Dong, T. T. X., & Tsim, K. W. K. (2006). Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured Cordyceps mycelia. Phytomedicine, 13, 428–433.

Li, S. P., Zhao, K. J., Ji, Z. N., Song, Z. H., Dong, T. T. X., Lo, C. K., Cheung, J. K. H., Zhu, S. Q., & Tsim, K. W. K. (2003). A polysaccharide isolated from Cordyceps sinensis, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. Life Sciences, 73, 2503–2513.

Liu, Z., Li, Zhao, D., Yang, H., & Guo, J. (2010). Protective effect of extract of Cordyceps sinensis in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Behavioral and Brain Functions, 6, 61–68.

Liu, Y., Liu, N., Han, B., Tian, X., Su, J., & Zhao, D. (2009). Effect of crude polysaccharides from mycelium of Cordyceps sinensis on expression of p53 gene and induction of apoptosis in SP2/0 cells. Acta Veterinaria et Zootechnica Sinica, 40, 117–121 (in Chinese).

Liu, Y. S., & Wu, J. Y. (2012). Effects of Tween 80 and pH on mycelial pellets and exopolysaccharide production in liquid culture of a medicinal fungus. Journal of Industrial Microbiology and Biotechnology, 39, 623–628.

Lo, H. C., Hsieh, C., Lin, F. Y., & Hsu, T. H. (2013). A Systematic Review of the Mysterious Caterpillar Fungus Ophiocordyceps sinensis in DongChongXiaCao and Related Bioactive
Ingredients. Journal of Traditional and Complementary Medicine, 3, 16–32.

Marchbank, T., Ojobo, E., Playford, G., & Playford, R. (2011). Reparative properties of the traditional Chinese medicine Cordyceps sinensis (Chinese caterpillar mushroom) using HT29 cell culture and rat gastric damage models of injury. British Journal of Nutrition, 105, 1303–1310.

Medzhitov, R., & Janeway, C. (2000). Innate immune recognition: mechanisms and pathways. Immunological Reviews, 173, 89–97.

Miyazaki, T., Oikawa, N., & Yamada, H. (1977). Studies on fungal polysaccharides. 20. Galactomannan of Cordyceps sinensis. Chemical & Pharmaceutical Bulletin, 25, 3324–3328.

Moradali, M., Mostafavi, H., Ghods, S., & Hedjarioude, G. (2007). Immunomodulating and anticancer agents in the realm of macrocystes fungi (macrofungi). International Immunopharmacology, 7, 701–724.

Negre-Salvayre, A., Cottrieux, C., Ingueneau, C., & Salvayre, R. (2008). Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. British Journal of Pharmacology, 153, 6–20.

Nie, S. P., Cui, S. W., Phillips, A. O., Xie, M. Y., Phillips, G. O., Al-Assaf, S., & Zhang, X. L. (2011). Elucidation of the structure of a bioactive hydrophobic polysaccharide from Cordyceps sinensis by methylation analysis and NMR spectroscopy. Carbohydrate Polymers, 84, 894–899.

Nie, S. P., Cui, S. W., Xie, M., Phillips, A. O., & Phillips, G. O. (2013). Bioactive polysaccharides from Cordyceps sinensis: isolation, structure features and bioactivities. Bioactive Carbohydrates and Dietary Fiber, 1, 38–52.

Nie, S. P., & Xie, M. Y. (2011). A review on the isolation and structure of tea polysaccharides and their bioactivities. Food Hydrocolloids, 25, 144–149.

Panagiotopoulos, C., Sempérè, R., Lafont, R., & Kerherve, P. (2001). Sub-ambient temperature effects on the separation of monosaccharide by high-performance anion-exchange chromatography with pulse amperometric detection: application to marine chemistry. Journal of Chromatography A, 920, 13–22.

Paterson, R. R. M. (2008). Cordyceps – a traditional Chinese medicine and another fungal therapeutic biofactory? Phytochemistry, 69, 1469–1495.

Pérez, S., Kouwizier, M., Mazeau, K., & Engelsen, S. B. (1996). Modeling polysaccharides: present status and challenges. Journal of Molecular Graphics, 14, 307–321.

Pol-Fachin, L., Fernandes, C. L., & Verili, H. (2009). GROMOS96 43a1 performance on the characterization of glycoprotein conformational ensembles through molecular dynamics simulations. Carbohydrate Research, 344, 491–500.

Quan, W., Wang, J., Du, S., & Liu, G. (2007). Studies on the production of exopolysaccharides by liquid culture of Cordyceps sinensis. Fajiao Keji Tongxun, 36, 2–4 (in Chinese).

Sato, T., Norisuye, T., & Fujita, H. (1984). Double-stranded helix of xanthan: conformational and hydrodynamic properties in 0.1 M aqueous sodium chloride. Macromolecules, 17, 2696–2700.

Schlesier, K., Harwat, M., Böhm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. Free Radical Research, 36, 177–187.

Senti, F. r., Hellman, N. N., Ludwig, N. H., Babcock, G. E., Tobin, R., Glass, C. A., & Lamberts, B. L. (1955). Viscosity, sedimentation, and light-scattering properties of fraction of an acio- hydroyzed dextran. Journal of Polymer Science, 17, 527–546.

Shashidhar, M. G., Giridhar, P., Sankar, K. U., & Manohar, B. (2013). Bioactive principles from Cordyceps sinensis: A potent food supplement – A review. Journal of Functional Foods, 5, 1013–1030.

Shen, Y., Shao, X., Ni, Y., Xu, H., & Tong, X. (2009). Cordyceps sinensis polysaccharide enhances apoptosis of HL-60 cells induced by triptolide. Journal of Zhejiang University (Medical Sciences), 38, 158–162 (in Chinese).

Shen, W., Song, D., Wu, J., & Zhang, W. (2011). Protective effects of a polysaccharide isolated from a cultured Cordyceps mycelia on hydrogen peroxide-induced oxidative damage in PC12 cells. Phytotherapy Research, 25, 675–680.

Sheng, L., Chen, J., Li, J., & Zhang, W. (2011). An exopolysaccharide from cultivated Cordyceps sinensis and its effects on cytokine expression of immunocytes. Applied Biochemistry and Biotechnology, 163, 669–678.

Song, D., He, Z., Wang, C., Yuan, F., Dong, P., & Zhang, W. (2013). Regulation of the antitumor polysaccharide from an anamorph of Cordyceps sinensis on dendritic cell sarcoma (DCS) cell line. European Journal of Nutrition, 52, 687–694.

Song, D., Lin, J., Yuan, F., & Zhang, W. (2011). Ex vivo stimulation of murine dendritic cells by an exopolysaccharide from one of the anamorph of Cordyceps sinensis. Cell Biochemistry and Function, 29, 555–561.

Song, L. Q., Ming, Y. S., Peng, M. X., & Xia, J. L. (2010). The protective effects of Cordyceps sinensis extract on extracellular matrix accumulation of glomerular sclerosis in rats. African Journal of Pharmacy and Pharmacology, 4, 471–478.

Striegel, A. M., Plattner, R. D., & Willett, J. L. (1999). Dilute solution behavior of dendrimers and polysaccharides: SEC, ESI-MS, and computer modeling. Analytical Chemistry, 71, 978–986.

Velickovic, D. T., Mileenovic, D. M., Ristic, M. S., & Veljkovic, V. B. (2006). Kinetics of ultrasonic extraction of extractable substances from garden (Salvia officinalis L.) and glutinous (Salvia glutinosa L.) sage. Ultrasonics Sonochemistry, 13, 150–156.

Wang, Z. M., Cheung, Y. C., Leung, P. H., & Wu, J. Y. (2010). Ultrasonic treatment for improved solution properties of a high-molecular weight exopolysaccharide produced by a medicinal fungus. Bioresearch Technology, 101, 5517–5522.

Wang, X. L., Liu, G. Q., Zhu, C. Y., & Kuang, S. M. (2011). Enhanced production of mycelial biomass and extracellular polysaccharides in caterpillar-shaped medicinal mushroom Cordyceps sinensis CS001 by the addition of palmitic acid. Journal of Medicinal Plants Research, 5, 2873–2878.

Wang, Z. M., Peng, X., Lee, K. L. D., Tang, J. C., Cheung, P. C. K., & Wu, J. Y. (2011). Structural characterization and immunomodulatory property of an acidic polysaccharide from mycelial culture of Cordyceps sinensis fungus Ca-HK1. Food Chemistry, 125, 637–643.

Wang, S. Y., & Shiao, M. S. (2000). Pharmacological functions of Chinese medicinal fungus Cordyceps sinensis and related species. Journal of Food and Drug Analysis, 8, 248–257.

Wang, Y., Wang, M., Ling, Y., Fan, W. Q., Wang, Y. F., & Yin, H. P. (2009). Structural determination and antioxidant activity of a polysaccharide from the fruiting bodies of cultured Cordyceps sinensis. The American Journal of Chinese Medicine, 37, 977–989.

Wang, L., Wang, G., Zhang, J., Zhang, G., Jia, L., Liu, X., Deng, P., & Fan, K. (2011). Extraction optimization and antioxidant activity of intracellular selenium polysaccharide by Cordyceps sinensis SU-02. Carbohydrate Polymers, 86, 1745–1750.

Wang, Y., Yin, H., Chen, T., & Wang, M. (2009). Preliminary structural identification and protection on renal cell injury of acidic polysaccharide from Cordyceps sinensis. Journal of China Pharmaceutical University, 40, 559–564 (in Chinese).

Wang, Y., Yin, H., Lv, X., Wang, Y., Gao, H., & Wang, M. (2010). Protection of chronic renal failure by a polysaccharide from Cordyceps sinensis. Fitoterapia, 81, 397–402.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumour and immunomodulating polysaccharides. Applied Microbiology and Biotechnology, 10, 13–32.

Wasser, S. P., & Weis, A. L. (1999). Medicinal properties of substances occurring in higher basidiomycetes mushroom: current perspective. International Journal of Medicinal Mushrooms, 1, 31–51.
Winkler, D. (2009). Caterpillar fungus (Ophiocordyceps sinensis) production and sustainability on the Tibetan Plateau and in the Himalayas. Asian Medicines, 5, 291–316.

Winkler, D. (2010). Cordyceps sinensis – a previous parasitic fungus infecting Tibet. Field Mycology, 11, 60–67.

Wong, W. C., Wu, J. Y., & Benzie, I. F. F. (2011). Photoprotective potential of Cordyceps polysaccharides against ultraviolet B radiation-induced DNA damage to human skin cells. British Journal of Dermatology, 164, 980–986.

Wu, C., Chen, Y., & Hao, Y. (2009). Production of mycelia and polysaccharides by liquid fermentation of Cordyceps sinensis. Food Science, 30, 171–174 (in Chinese).

Wu, Y. L., Hu, N., Pan, Y. J., Zhou, L. J., & Zhou, X. X. (2007). Isolation and characterization of a mannoglucon from edible Cordyceps sinensis mycelium. Carbohydrate Research, 342, 870–875.

Wu, Y. L., Ishurd, O., Sun, C. R., & Pan, Y. J. (2005). Structure analysis and antitumour activity of (1→3)-beta-D-glucans (Cordyglucans) from the mycelia of Cordyceps sinensis. Planta Medica, 71, 381–384.

Wu, Y. L., Sun, C. R., & Pan, Y. J. (2005). Structural analysis of a neutral (1→3)–(1→4)-beta-d-glucan from the mycelia of Cordyceps sinensis. Journal of Natural Products, 68, 812–814.

Wu, Y. L., Sun, C. R., & Pan, Y. J. (2006). Studies on isolation and structural features of a polysaccharide from the mycelium of a Chinese edible fungus (Cordyceps sinensis). Carbohydrate Polymers, 63, 251–256.

Wu, Y. L., Sun, H. X., Qin, F., Pan, Y. J., & Sun, C. R. (2006). Effect of various extracts and a polysaccharide from the edible mycelia of Cordyceps sinensis on cellular and humoral immune response against ovalbumin in mice. Phytotherapy Research, 20, 646–652.

Xiao, J. H. (2008). Current status and ponderation on preparations and chemical structures of polysaccharide in fungi of Cordyceps (Fr.) Link. Chinese Traditional and Herbal Drugs, 39, 454–460.

Xie, J., Shan, B., & Zhang, W. (2009). Microwave-assisted extraction of polysaccharides from fermented Cordyceps sinensis mycelium optimized by response surface analysis. Chinese Journal of Biotechnology, 7, 34–38 (in Chinese).

Yamada, H., Kawaguchi, N., Ohmori, T., Takeshita, Y., Taneya, S., & Miyazaki, T. (1984). Structural and antitumour activity of an alkali-soluble polysaccharide from Cordyceps ophioglossoides. Carbohydrate Research, 125, 107–115.

Yan, J. K., Li, L., Wang, Z. M., Leung, P. H., Wang, W. Q., & Wu, J. Y. (2009). Acidic degradation and enhanced antioxidant activities of exopolysaccharides from Cordyceps sinensis mycelial culture. Food Chemistry, 117, 641–646.

Yan, J. K., Li, L., Wang, Z. M., & Wu, J. Y. (2010). Structural elucidation of an exopolysaccharide form mycelial fermentation of a Tolypocladium sp. fungus isolated from wild Cordyceps sinensis. Carbohydrate Polymers, 79, 125–130.

Yan, J. K., Wang, W. Q., Li, L., & Wu, J. Y. (2011). Physicochemical properties and antitumour activities of two c-glucans isolated from hot water and alkaline extracts of Cordyceps (Cs-HK1) fungal mycelia. Carbohydrate Polymers, 85, 753–758.

Yan, J. K., Wang, W. Q., Ma, H. L., & Wu, J. Y. (2013). Sulfation and enhanced antioxidant capacity of an exopolysaccharide produced by the medicinal fungus Cordyceps sinensis. Molecules, 18, 167–177.

Yan, F., Zhang, Y., & Wang, B. (2012). Effects of polysaccharides from Cordyceps sinensis mycelium on physical fatigue in mice. Bangladesh Journal of Pharmacology, 7, 217–221.

Yang, L. Y., Chen, A., Kuo, Y. C., & Lin, C. Y. (1999). Efficacy of a pure compound H1-A extracted from Cordyceps sinensis on autoimmune disease of MRL LPR/LPR mice. The Journal of Laboratory and Clinical Medicine, 134, 492–500.

Yang, L. Y., Huang, W. J., Hsieh, H. G., & Lin, C. Y. (2003). H1-A extracted from Cordyceps sinensis suppresses the proliferation of human mesangial cells and promotes apoptosis, probably by inhibiting the tyrosine phosphorylation of Bcl-2 and Bcl-XL. The Journal of Laboratory and Clinical Medicine, 141, 74–83.

Yang, L., & Zhang, L. M. (2009). Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. Carbohydrate Polymers, 76, 349–361.

Yang, J. Y., Zhang, W. Y., Shi, P. H., Chan, J. P., Han, X. D., & Wang, Y. (2005). Effects of exopolysaccharide fraction (EPSF) from a cultivated Cordyceps sinensis fungus on c-Myc, c-Fos, and VEGF expression in B16 melanoma-bearing mice. Pathology, Research and Practice, 201, 745–750.

Yao, Y. J. (2004). Conservation and rational use of the natural resources of Cordyceps sinensis. Science News, 15, 28–29 (in Chinese).

Yao, X., Meran, S., Fang, Y., Martin, J., Midgley, A., Pan, M. M., Liu, B. C., Cui, S. W., Phillips, G. O., & Phillips, A. O. (2014). Cordyceps sinensis: in vitro anti-fibrotic bioactivity of natural and cultured preparations. Food Hydrocolloids, 35, 444–452.

Yin, H., Qiao, C., Qin, S., Tang, W., & Ha, S. (2013). Optimization of fermentation medium for Cordyceps sinensis. Food Research & Development, 34, 5–8 (in Chinese).

Yin, D. H., & Tang, X. M. (1995). Progresses of cultivation research of Cordyceps sinensis. China Journal of Chinese Materia Medica, 20, 707–709.

Yoon, T. J., Yu, K. W., Shin, K. S., & Suh, H. J. (2008). Innate immune stimulation of exo-polymers prepared from Cordyceps sinensis by submerged culture. Applied Microbiology and Biotechnology, 80, 1087–1093.

Yue, K., Ye, M., Zhou, Z., Sun, W., & Lin, X. (2013). The genus Cordyceps: a chemical and pharmacological review. Journal of Pharmacy and Pharmacology, 65, 474–493.

Zhang, X. L., Cheung, L. B., Al-Assaf, S., Phillips, G. O., & Phillips, A. O. (2012b). Cordyceps sinensis decreases TGF-β1 dependent epithelial to mesenchymal transdifferentiation and attenuates renal fibrosis. Food Hydrocolloids, 28, 200–212.

Zhang, M., Cui, S., Cheung, P., & Wang, Q. (2007). Antitumour polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumour activity. Trends in Food Science & Technology, 18, 4–19.

Zhang, G. Q., Huang, Y. D., Bian, Y., Wong, J. H., Ng, T. B., & Wang, H. X. (2006). Hypoglycemic activity of the fungi Cordyceps militaris, Cordyceps sinensis, Tricholoma mongolicum, and Omphalina lapipecens in streptozotocin-induced diabetic rats. Applied Microbiology and Biotechnology, 72, 1152–1156.

Zhang, W., Li, J., Qiu, S., Chen, J., & Zheng, Y. (2008). Effects of the exopolysaccharide fraction (EPSF) from a cultivated C. sinensis on immunocytes of H22 tumour bearing mice. Fitoterapia, 79, 168–173.

Zhang, Y., Li, E., Wang, C., Li, Y., & Liu, X. (2012a). Ophiocordyceps sinensis, the flagship fungus of China: terminology, life strategy and ecology. Mycology, 3, 2–10.

Zhang, Z., Wang, X., Zhang, Y., & Ye, G. (2011). Effect of Cordyceps sinensis on renal function of patients with chronic allograft nephropathy. Urology International, 86, 298–301.

Zhang, L., & Yang, L. (1995). Solution properties of pachymann from Poria cocos mycelia in dimethyl sulfoxide. Biopolymers, 36, 695–700.

Zhang, W. Y., Yang, J. Y., Chen, J. P., Hou, Y. Y., & Han, X. D. (2005). Immunomodulatory and antitumour effects of an exopolysaccharide fraction from cultivated Cordyceps sinensis.
(Chinese caterpillar fungus) on tumour-bearing mice. Biotechnology and Applied Biochemistry, 42, 9–15.
Zhao, J., Xie, J., Wang, L. Y., & Li, S. P. (2013). Advanced development in chemical analysis of Cordyceps. Journal of Pharmaceutical and Biomedical Analysis. http://dx.doi.org/10.1016/j.jpba.2013.04.025.
Zhong, S., Pan, H. J., Fan, L. F., Lv, G., Wu, Y., Parmeswaran, B., Pandey, A., & Soccol, C. R. (2009). Advances in research of polysaccharides in Cordyceps species. Food Technology and Biotechnology, 47, 304–312.
Zhou, X. W., Gong, Z. H., Su, Y., Lin, J., & Tang, K. (2009). Cordyceps fungi: natural products, pharmacological functions and developmental products. Journal of Pharmacy and Pharmacology, 61, 279–291.
Zhu, J. S., Halpern, G. M., & Jones, K. (1998). The scientific rediscovery of an ancient Chinese herbal medicine: Cordyceps sinensis. Journal of Alternative and Complementary Medicine, 4, 289–303 (Part I); 4 429–457 (Part II).