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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 *C. sinensis*, exhibiting several activities such as immunomodulation, antitumour, antioxidant and hypoglycaemic. As natural *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 *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

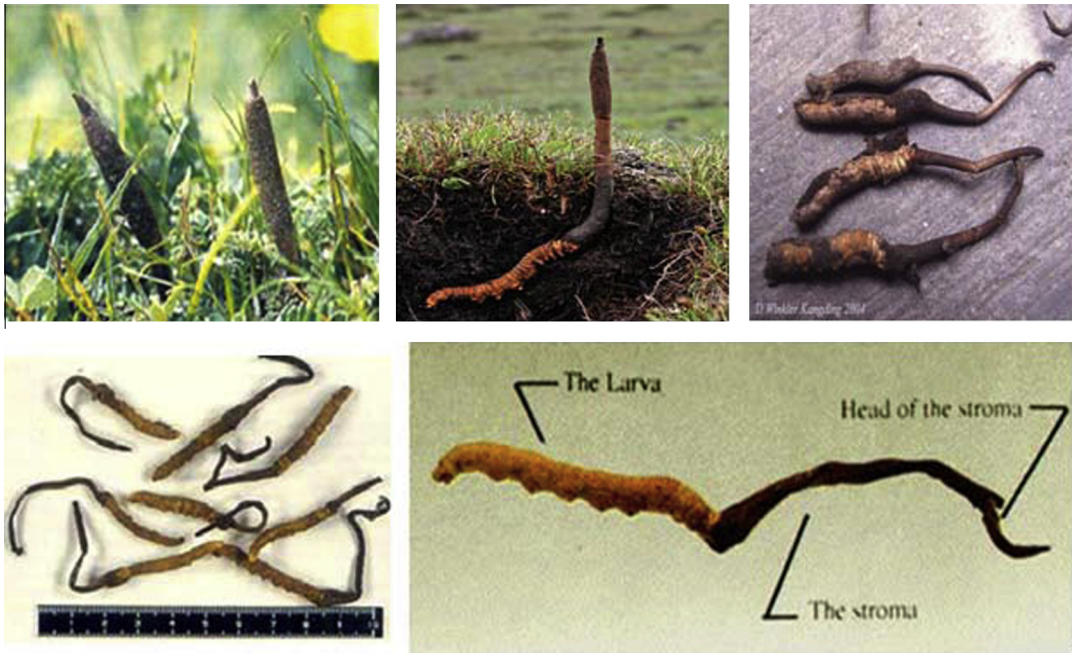

Fig. 1 – *Cordyceps (Ophiocordyceps) sinensis* fruiting body-caterpillar complexes: morphology and natural habitat (Paterson, 2008; Winkler, 2010).

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 (exopolysaccharides, 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, Ukai, & Hirano, 1999; Kiho, Tabata, Ukai, & Hara, 1986; Kiho, Yamane, Hui, Usui, & Ukai, 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

Table 1 – Mycelial biomass and EPS production by mycelial fermentation of Cordyceps sinensis.

Fungi source	Fermentation conditions					Mycelial biomass (g/L)	EPS yield (g/L)	References
	Medium composition	Temperature (°C)	pH	Culture vessel	Period (days)			
C. sinensis CCRC36421	Sucrose 6.17%, corn steep powder 0.5%, $(NH_4)_2HPO_4$ 0.5%, KH_2PO_4 0.15% (w/v)	25	4.4	5-L Jar fermentor: agitation speed, 300 rpm	7		3.2	Hsu, Shiao, Hsieh, and Chang (2002), Hsieh et al. (2005)
C. sinensis	Sucrose 20, corn steep powder 25, $CaCl_2$ 0.78, $MgSO_4{\cdot}7H_2O$ 1.73 (g/L)	20	4.0	5-L Stirred-tank fermenter: aeration rate,2 vvm; agitation speed,150 rpm	16	20.9	4.1	Kim and Yun (2005)
C. sinensis 762	Sucrose 50, peptone 10, yeast extract 3 (g/L)	18		Rotary shaker at 150 rpm	40	22.1		Dong, and Yao (2005)
C. sinensis Cs-HK1	Glucose 40, yeast extract 5, peptone 5, KH_2PO_4 1, $MgSO_4{\cdot}7H_2O$ 0.5 (g/L);NH_4Cl 10 mmol/L	22–25		Shaking incubator at 150 rpm	7	23.2	3.4	Leung et al. 2006; Leung and Wu (2007)
C. sinensis 16	Sucrose 2%, yeast extract 0.9%, K_2HPO_4 0.3%,$CaCl_2$ 0.4% (w/v)	25		Rotary shaker at 150 rpm	5	54.0	28.4	Cha et al., 2007
C. sinensis 1	Sucrose 3%, corn steep powder 5%, bean cake 4%, KH_2PO_4 0.1, $MgSO_4{\cdot}7H2O$ 0.05%, vitamin B1 0.01%	22	6.5	Rotary shaker at 120 rpm	7		5.9	Quan, Wang, Du, and Liu (2007)
C. sinensis 383	Glucose 30, bean cake 20, $MgSO_4$ 2.0, KH_2PO_4 4.0 (g/L)	24	7.0	Rotary shaker at 140 rpm	5		3.9	Lang, Qi, Hou, Zhao, and Jiang (2009)
C. sinensis	Sucrose 20, yeast extract 2.0, KH_2PO_4 1.0, $MgSO_4{\cdot}7H_2O$ 0.6 (g/L)	26	7.0	1000-mL shake flask: aeration rate 1 L/min, agitation speed 130 rpm	4	12.3	24.5	Wu, Chen, and Hao (2009)
C. sinensis CCRC36421	Rice bran 1.5%, molasses 0.5%, CSL 3%, KH_2PO_4 0.1%, $MgSO_4$ 0.05%	25	5.5	5-L Jar fermenter: aeration rate 1.0 vvm, agitation speed, 150 rpm	5–6		48.9	Choi et al (2010)
Hirsutella sinensis	Potato extract 20%, sucrose 2.5%, peptone 0.5%, K_2HPO_4 0.2%, $MgSO_4$ 0.05% (w/v)	24	5.5	Rotary shaker at 180 rpm	4	10.0	2.2	Li, Jiang, and Guan (2010)
C. sinensis CS001	Glucose 30, yeast extract 3, peptone 2, KH_2PO_4 0.6, $MgSO_4{\cdot}7H_2O$ 0.4, vitamin B_1 0.01, palmitic acid 1.0 (g/L)	27	6.5	250-mL shake flask at 160 rpm	7		0.4	Wang, Liu, Zhu, & Kuang, 2011
C. sinensis Cs-HK1	Glucose 40, yeast extract 15, peptone 5, KH_2PO_4 1, $MgSO_4{\cdot}7H_2O$ 0.5 (g/L);NH_4Cl 10 mmol/L; Tween 80 1.5% (w/v)	25	6	Shaking incubator at 150 rpm	7	14.7	7.2	Liu and Wu (2012)
C. sinensis	Glucose 30, peptone 15, KH2PO4 3.0, $MgSO_4{\cdot}7H_2O$ 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,

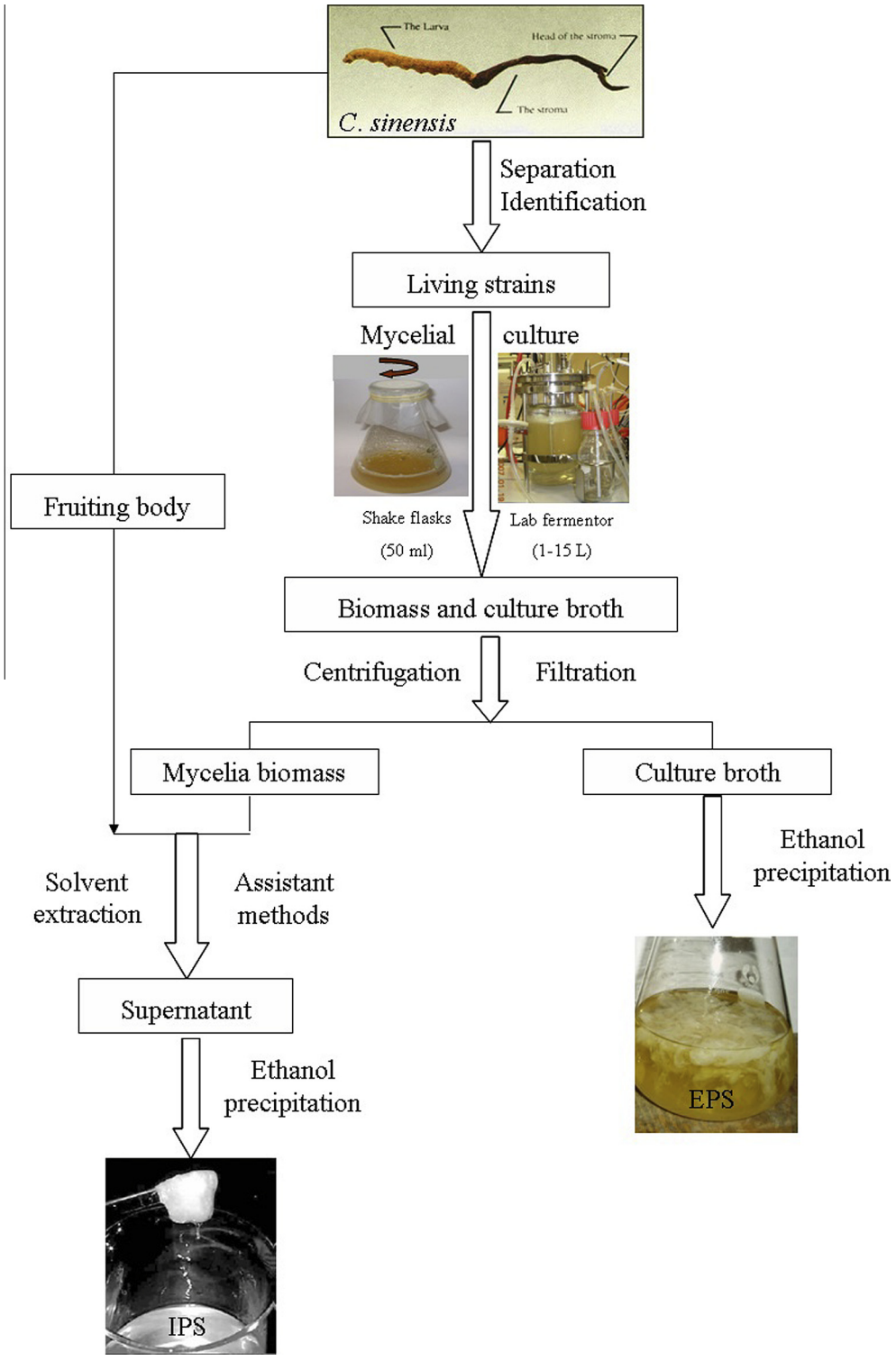

Fig. 2 – Schematic diagram for the extraction of intracellular and extracellular polysaccharides from *Cordyceps sinensis*.

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; , 2006; 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 investigated 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 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 derivation 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 $\sim 10^3$ to $\sim 10^6$ 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*, respectively. Both IPSs contained (1 → 2)-linked D-mannopyranosyl main-chains and (1 → 5)-linked D-galactofuranosyl side-chains. However, significant differences between CS-I and CT-4N were observed, i.e., CS-I contains (1 → 2,3) linkages of D-mannopyranose residues, as well as (1 → 3) and (1 → 6) linkages of galactofuranose, but CT-4 N has (1 → 4,6) linkages of mannopyranose and (1 → 6) linkages of galactopyranose. Thus, galactomannans from *C. sinensis* have a core of D-mannopyranosyl residues and D-galactofuranosyl side chains, and some differences in the linkage mode are observed. In addition, a galactoglucomannan named CS-F10 (Kiho et al., 1999) isolated from *C. sinensis* mycelium had a comb-type structure composed of non-reducing terminal α-D-glucopyranosyl residues, (1 → 5 and/or 6)-linked β-D-galactofuranosyl residues, as well as (1 → 2)-linked and branched α-D-mannopyranosyl residues. Recently, some water-soluble IPSs isolated from

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-I Galactomannan	–	Miyazaki et al. (1977)
Paecilomyces sinensis(Cs-4)	Mycelium	5% Sodium carbonate	Man:Gal = 3:5	23 kDa	CT-4 N Galactomannan	–	Kiho et al., 1986
Paecilomyces sinensis(Cs-4)	Mycelium	Hot water	Gal:Glc:Man = 62:28:10	45 kDa	CS-F30	Hypoglycemic activity	Kiho et al. (1993), Kiho et al. (1996)
Paecilomyces sinensis(Cs-4)	Mycelium	Hot water	Gal:Glu:Man = 43:33:24	15 kDa	CS-F10 Galactoglucomann	Hypoglycemic activity	Kiho et al., 1999
Cephalosporium sinensis Chen sp. nov.	Mycelium	Hot water	Glu:Man:Gal = 1:0.6:0.75	210 kDa	CSP-1	Antioxidant activity; Hypoglycemic activity	Li et al. (2003), Li et al. (2006)
Paecilomyces sinensis(Cs-4)	Mycelium	Hot water	β-Glu	13.6 kDa	Neutral (1 → 3),(1 → 4)-β-D- -glucan	–	Wu et al. (2005)
Paecilomyces sinensis(Cs-4)	Mycelium	Hot water	β-Glu	12.9 kDa	(1 → 3)-β-D-glucan Cordyglucans	Antitumour activity	Wu et al. (2005)
Paecilomyces sinensis(Cs-4)	Mycelium	0.05 M phosphate buffer	α-Glu	184 kDa	SCI-I	–	Wu et al. (2005)
Paecilomyces sinensis(Cs-4)	Mycelium	0.05 M acetate buffer	Glu:Man = 9:1	7.7 kDa	Mannoglucan	Antitumour activity	Wu et al. (2007)
Paecilomyces sinensis(Cs-4)	Mycelium	Hot water	Glc:Man:Gal = 21:2:1	–	CS-Pp 1,3-β-D-glucan with 1,6-branched chain	Immunomodulating	Akaki et al. (2009)
Paecilomyces sinensis(Cs-4)	Mycelium	Hot water	Glc:Man:Gal = 2:1:1	460 kDa	→ 3-α-D-Glcp-1 → 3-β-D-Glcp-1 → 3-β-D-Galp-1 →	Cholesterol esterase inhibitory activity	Kim (2010)
Cephalosporium sinense Chen	Mycelium	Hot water	Glc:Man:Gal = 2.8:2.9:1	8.1 kDa	CPS1 Glucomannogalactan	Antioxidant activity	Wang et al. (2009)
Cephalosporium sinense Chen	Mycelium	Hot water	Man, Glu, Gal, Uronic acid	27 kDa	CAPS	Immunomodulating	Wang et al. (2009)
Cephalosporium sinense Chen	Mycelium	Hot water	Man:Glu: Gal = 4:11:1	43.9 kDa	Galactoglucomanno- -glycan (CPS-2)	Protection of chronic renal failure	Wang et al. (2010)
Tolypocladium sinensis	Mycelium	Hot water	α-Glu	1180 kDa	WIPS α-D-(1 → 4)-glucan	Antitumour and Immuno-stimulating effects	Yan et al. (2011)
Tolypocladium sinensis	Mycelium	1.25 M NaOH / 0.04% $NaBH_4$	α-Glu	1150 kDa	AIPS α-D-(1 → 4)-glucan (86%), (1 → 6) -α-D-glucose (14%)	Antitumour and Immuno-stimulating effects	Yan et al. (2011)
Tolypocladium sinensis	Mycelium	Hot water	Man:Glu:Gal = 3.3:2.3:1	–	APS	Antioxidant activity	Shen et al. (2011)
Paecilomyces sinensis(Cs-4)	Mycelium	Hot water	Glu(95%), Man, Gal	260 kDa	CBHP α-1,4-linked-Glcp	Antifibrotic effect	Nie et al. (2011), Zhang, Li et al. (2012), Zhang, Cheung et al. (2012)
Cordyceps ophioglossoides	Culture filtrate	Ethanol	β-Glu	632 kDa	CO-1	Antitumour activity	Yamada et al., 1984
Paecilomyces sinensis(Cs-4)	Culture broth	Ethanol	Man:Gal:Glc = 10.3:3.6:1	43 kDa	CS-81002	Immunomodulating	Gong et al., 1990
Tolypocladium sinensis	Culture broth	95% Ethanol	Man:Glu: Gal = 23:1:2.6	104 kDa	EPS	Immunomodulatory and antitumour	Yang et al. (2005), Chen et al. (2006), Sheng et al. (2011)
Paecilomyces sinensis	Culture broth	Ethanol	Glu:Man:Gal = 2.4:2:1	82 kDa	Cordysinocan	Immunomodulating	Cheung et al., 2009
Tolypocladium sinensis	Culture broth	95% Ethanol	Glu:Man:Gal = 15.2:3.6:1	40 kDa	EPS-1A	–	Yan et al. (2010)
Tolypocladium sinensis	Culture broth	Ethanol	Glcp:GlcUp = 8:1	36 kDa	AEPS-1	Immunomodulating	Wang, Liu, Zhu, & Kuang, 2011
Tolypocladium sinensis	Culture broth	Ethanol	$ManNH_2$:$GalNH_2$: Gal= 1.0:1.1:0.3	6 kDa	Poly-N-acetylhexosamine	Antioxidant activity	Chen, Siu et al. (2013)

the cultured *C. sinensis* have been identified as glucomannogalactans, 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 mannoglucan 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)-D-glucose and a α-(1 → 3)-D-mannose branched with α-(1 → 4,6)-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 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)-α-D-glucose 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-pyrano-glucuronic 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, Kouwijzer, 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; Wasser, 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 mice. 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

factor-BB-activated human mesangial cells *in vitro*, and prevent the tyrosine phosphorylation of human mesangial proteins. Bcl-2 and Bcl-XL are probably among these proteins as revealed by results of immunoprecipitation and immunoblotting (Yang, Chen, Kuo, & Lin, 1999; Yang, Huang, Hsieh, & Lin, 2003). Similarly, crude polysaccharides obtained from mycelium of *C. sinensis* also affect the induction of apoptosis and expression of p53 gene in SP2/0 cells *in vitro* (Liu et al., 2009). Shen, Shao, Ni, Xu, and Tong (2009) reported the effects of the polysaccharide fraction of *C. sinensis* (PSCS) on triptolide (TPL)-induced apoptosis in HL-60 cells and the molecular events and signaling pathway, enhancing TPL-induced apoptosis and inhibiting the expression of NF-κB and caspases 3, 6, 7, and 9. However, up to now, no studies revealed that EPSs isolated from culture broth of *C. sinensis* could induce apoptosis.

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, Coatrieux, Ingueneau, & 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, Böhm, & Bitsch, 2002).

Li, Li, Dong, and Tsim (2001) studied the antioxidative activity of water extracts of natural *C. sinensis* and mycelial biomass of the *Cordyceps* anamorph fungus *Cephalosporium sinensis* of various sources using xanthine oxidase, haemolysis induction, and lipid peroxidation. Their results show that *Cordyceps* generally possesses strong antioxidative activity in all assays, and cultured *Cordyceps* mycelia have antioxidative activity as strong as that of natural *C. sinensis*. Further testing suggests that several forms of IPSs prepared by ethanol precipitation and DEAE column chromatography have great antioxidative activities. Li et al. (2003) used column chromatography to purify a polysaccharide-enriched fraction isolated from cultured *C. sinensis* that possesses strong oxidative activity. A polysaccharide named CSP-1 is then obtained using activity-guided fractionation. CSP-1 with a MW of 210 kDa is found to have strong antioxidative activity in rat pheochromocytoma PC12 cells, protecting against H_2O_2-induced cell damage, lipid peroxidation, and activation of major antioxidant enzymes, including glutathione peroxidase and superoxide dismutase in cells. Very recently, an APS has been isolated from *Tolypocladium sinensis*, another anamorph strain of *C. sinensis*. This APS is found to markedly protect PC12 cells from H_2O_2-induced cell injury by increasing glutathione peroxidase, catalase, and superoxide dismutase (SOD) activities, as well as by reducing lactate dehydrogenase and malondialdehyde (MDA) levels (Shen, Song, Wu, & Zhang, 2011). In addition, IPSs also affect the antioxidative activity of H22-bearing mice by enhancing the SOD activity of the liver, brain, and serum, as well as the GSH-Px activity of the liver and brain in tumour-bearing mice, and by reducing the MDA level in the liver and brain of tumour-bearing mice (Chen et al., 2006).

A few studies have reported on the antioxidative activities of EPSs isolated from culture broths of *C. sinensis*. Wu et al. recently studied the antioxidant action of an EPS (named Cs-HK1) isolated from the mycelial liquid culture of *C. sinensis* fungus by ethanol precipitation *in vitro*. Results demonstrate that Cs-HK1 shows moderate antioxidative activities, and acidic degradation can improve its antioxidative activities and radical-scavenging capacities (Leung, Zhao, Ho, & Wu, 2009; Yan et al., 2009). Very recently, EPS fractions were isolated from the fermentation broth of Cs-HK1 by gradient precipitation with ethanol at different volume ratios to the liquid medium exhibited moderate antioxidant activities and their activities showed a significant dependence on the protein content (Huang et al., 2013).

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 cultured *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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