Comparison of structural features and antioxidant activity of polysaccharides from natural and cultured *Cordyceps sinensis*

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Running Title: Polysaccharide structure and antioxidant activity

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Abstract

Four polysaccharides (named as P1, P2, and P3 from three natural *Cordyceps sinensis* and P4 from cultured *C. sinensis*) were obtained by hot-water extraction and ethanol precipitation and their structural characteristics as well as antioxidant potentials were compared. Results revealed that the backbone of P1, P2, and P3 comprised α-1,4-glucose, with a branching point mainly at position 6 and terminating at glucose. On the other hand, the structure of P4 was highly complex, mainly comprising glucose, galactose, and mannose, with 1,4-glucose and 1,4-galactose as the main chain. For *in vitro* antioxidant assays, all the four polysaccharides showed similar scavenging capacity against DPPH and hydroxyl radicals, whereas P1 had a relative low ferric reducing ability, possibly related to a combination of factors such as the phenolic compounds and amino acids that conjugated in polysaccharides.

Keywords

Natural/cultured; *Cordyceps sinensis*; Polysaccharide; Structure; Antioxidant

Introduction

Cordyceps is a parasitic fungus that grows on the larva of insects. It has been reported that there are more than 350 species belonging to the genus of *Cordyceps* all over the world, out of which about 120 species have been found in China (1, 2). *C. sinensis*, in particular, has been commonly known as a valued Chinese traditional herb for hundreds of years. It was first recorded in "Ben-Cao-Cong-Xin" written by Yiluo Wu in the Qing dynasty, but it was not known to the western society until the 17^{th} century (3). It is traditionally used in China for lung nourishing and kidney improvement as well as relieving cough and reducing phlegm (4-6). However, it grows quite slowly and requires a very specific habitat, resulting in limited productivity and very high cost. Therefore, modern researchers have tried to isolate several mycelial strains from wild *C. sinensis* and produced it using fermentation technology. To date, there are more than 10 genera isolated from natural *C. sinensis*, and some of the fermented products are manufactured on a large scale (7, 8).

Polysaccharide is considered to be one of the major bioactive constituents in both natural and cultured *C. sinensis*. Pharmacological studies have found that polysaccharides isolated from *C. sinensis* exhibit immunomodulatory, antitumor, hypoglycaemic, and anti-fibrosis activities (9). The structure of polysaccharides, such as Molecular weight (Mw), degree of branching, and chain conformation, were found to be closely related to their biological activities (10, 11). However, little literature has been devoted to comparing the chemical composition and structural features between natural *C. sinensis* and its mycelium produced by fermentation. Currently, both

natural and cultured *C. sinensis* products are sold as healthy foods in South East Asia. Whether the polysaccharide fractions have similar structural features and whether the cultured mycelium could be regarded as a substitute of natural counterpart has attracted research attention. In our previous study, differences in terms of amino acid composition, minerals, as well as contents of adenine, adenosine, and mannitol, have been observed not only between natural and cultured *C. sinensis* but also among natural samples (12). It indicated that the originality and growth environment had an impact on the chemical composition of *C. sinensis*.

In order to conduct a comprehensive and detailed evaluation among them, we first evaluated the chemical composition and bioactive constituents' contents to compare the bioactivities among them. Subsequently, we investigated polysaccharides with regard to structural information and pharmacological properties. Therefore, in this study, we isolated the polysaccharides from three natural and one cultured mycelium of *C. sinensis* and determined the Mw, monosaccharide composition, and glycosidic linkages in order to compare their structural characteristics. In addition, *in vitro* antioxidant activity was studied for the purpose of exploiting new food sources of antioxidants.

Materials and Methods

Materials

Natural *C. sinensis* were obtained from the Qinghai Province (Golog, Yushu, and surrounding area of Xining) at different altitudes of above 4,800 m, above 4,200 m, and between 2,800 and 3,300 m, named as S1, S2, and S3, respectively. Cultured *C.*

sinensis (named as S4) was purchased from Guoyao Company (Jiangxi, China). T-series dextrans (T-10, T-40, T-70, T-500, and T-2000) were purchased from Pharmacia Biotech (Uppsala, Sweden). Ascorbic acid, DPPH, TPTZ, and all the monosaccharide standards including L-fucose (Fuc), L-rhamnose (Rha), D-arabinose (Ara), D-galactose (Gal), D-glucose (Glc), D-mannose (Man), D-xylose (Xyl), D-fructose (Fru), D-ribose (Rib), D-galacturonic acid (GalA), and D-glucuronic acid (GlcA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium borodeuteride ($NaBD_4$, 98 atom% D) was purchased from Acros Organics (Morris Plains, NJ, USA). All other reagents were of analytical grade unless specified otherwise.

Preparation of polysaccharides

Grounded natural samples and mycelium were soaked with 80% ethanol overnight to remove pigment and other ethanol-soluble constituents, respectively. The residues were then dried and extracted three times with hot water (95–100°C) for 2h.. All the supernatant was collected and concentrated in a rotary evaporator at 55°C under reduced pressure. The crude polysaccharides were obtained by precipitation with four volumes of anhydrate ethanol and named as C1, C2, C3, and C4 corresponding to S1, S2, S3, and S4, respectively.

The crude polysaccharides were further treated three times with Sevag reagent (chloroform/1-butanol, v/v = 4:1) to remove proteins. Next it was dialyzed against tap water for 48 h and distilled water for 24 h and finally lyophilized to give purified polysaccharide fractions, namely P1, P2, P3, and P4 from C1, C2, C3, and C4,

107 respectively.

108 **Determination of molecular size distribution**

109 The molecular weight distributions of polysaccharides were determined by
110 high-performance gel-permeation chromatography (HPGPC) performed using an
111 Agilent 1260 LC instrument (Agilent Technologies Inc., Santa Clara, CA, USA)
112 equipped with a refractive index detector (RID; Agilent, Santa Clara, CA, USA), a
113 variable wavelength detector (VWD; Agilent, Santa Clara, CA, USA), and an
114 Ultrahydrogel^{TM} Linear column (7.8 mm × 300 mm) (Waters Corp., Milford, MA,
115 USA). The polysaccharides were eluted with 0.1 M sodium chloride (dissolved in
116 0.02% NaN_3 aqueous solution) at a flow rate of 0.6 mL/min. The column temperature
117 was maintained at 35.0 ± 0.1°C. The polysaccharides were filtered through a 0.45-μm
118 membrane prior to injection. The Mw of the samples was calculated according to the
119 calibration curve established using dextran standards (T-10, T-40, T-70, T-500, and
120 T-2000).

121 **Identification of monosaccharide composition**

122 Monosaccharide composition was analyzed by high-performance anion-exchange
123 chromatography coupled with pulsed amperometric detection (HPAEC-PAD) on a
124 Dionex ICS-5000 System (Dionex Corp., Sunnyvale, CA, USA) equipped with a
125 CarboPac PA20 Guard (3 mm × 30 mm) and Dionex CarboPac PA20 column (3 mm ×
126 150 mm). Polysaccharides (10 mg) were hydrolyzed with 2 M sulfuric acid (H_2SO_4)
127 at 100°C for 6 h. The hydrolysate was diluted to suitable concentrations and filter
128 through 0.45-μm filters prior to injection and 10 μL of this solution was quantitatively

analyzed on the basis of monosaccharide standards. Separation was performed with gradient elution comprising a mixture of distilled water, NaOH, and CH_3COONa (13). Chromeleon software was used to control the instrument and process the data.

Analysis of the FTIR spectra

The FTIR spectrum of polysaccharides was analyzed on a Thermo Nicolet 5700 infrared spectrometer (ThermoElectron, Madison, WI, US) in the range of 4,000–400 cm^{-1} using the KBr disc method.

Methylation analysis

The methylation procedure of polysaccharides was performed according to the method by Ciucanu and Kerek (14) with minor modifications. In brief, approximately 3 mg of dried samples were completely dissolved in 0.5 mL anhydrous DMSO and then dried NaOH powder (20 mg) was added to the solution. After stirring for 3 h, 1 mL iodomethane was added dropwise to the mixture in an ice bath. The reaction mixture was further stirred under dark conditions for 2.5 h followed by addition of 0.2 mL water to stop the reaction. The methylated polysaccharides were extracted with chloroform, passed through a sodium sulfate column to remove water, and finally dried under a stream of nitrogen. Complete methylation was confirmed by the disappearance of hydroxyl absorption in the FTIR spectrum. The methylated polysaccharide was then hydrolyzed by 4 M trifluiriacetic acid, reduced with $NaBD_4$, and acetylated with CH_3COOH to produce partially methylated alditol acetates (PMAA), which were analyzed by gas chromatography- mass spectrometry (GC–MS) (Agilent Technology 7890A/5975C, USA).

Amino acid composition analysis

The amino acid composition of polysaccharides was investigated by an automatic amino acid analyzer (L-8900, Hitachi, Japan). Polysaccharides were hydrolyzed with 6 M HCl under nitrogen atmosphere at 110°C for 24 h. The resulting hydrolysates were then evaporated and redissolved in water and filtered through a 0.22-μm membrane prior to analysis.

Determination of total phenolic content

The total phenolic content of polysaccharides was measured according to the Folin–Ciocalteu colorimetric method (15), with slight modifications. In brief, 1 mL polysaccharide solution, 1 mL Folin–Ciocalteu reagent, and 3 mL 10% Na_2CO_3 solution were mixed and further diluted to 10 mL. After incubation at 25 °C under dark conditions for 30 min, the absorbance of the solution was measured at 764 nm. Different concentrations of gallic acid (0.01–0.05 mg/mL) were used as a standard curve and total phenolic content of polysaccharides was presented as gallic acid equivalents (GAE) in milligrams per gram of dry weight polysaccharide.

Antioxidant activities

DPPH radical scavenging activity

The scavenging activity of polysaccharides against DPPH radical was determined as described by Mao *et al*. (16) with slight modification. In brief, 2 mL of different concentrations of polysaccharides (0.25–4 mg/mL) were mixed with equal volumes of DPPH-ethanol solution and incubated for 30 min at room temperature. The mixture was measured at 517 nm with ascorbic acid as a positive control, and the scavenging

173 percentage was calculated by the following equation:

174 Scavenging activity (%) = $(1 - (A_s - A_{s0})/A_0) \times 100$,

175 where A_0, A_s, and A_{s0} are the absorbance of DPPH reagent, polysaccharide, and

176 polysaccharide without DPPH, respectively.

177 ***Hydroxyl radical scavenging activity***

178 The hydroxyl radical scavenging activities of polysaccharides were determined

179 according to the method of Gao *et al*. (17) with minor modification. In brief, 1 mL

180 polysaccharide solutions with different concentrations (0.25–4 mg/mL) were mixed

181 with 1 mL $FeSO_4 \cdot 7H_2O$ (9 mM), 1 mL salicylic acid-ethanol (9 mM), and 1 mL H_2O_2

182 (9 mM). Then the mixture was incubated at 37 °C for 30 min and the absorbance was

183 measured at 510 nm. Distilled water and ascorbic acid were used as a blank and

184 positive control, respectively. The ability to scavenge hydroxyl radicals was calculated

185 according to the following equation:

186 Scavenging activity (%) = $(1 - (A_s - A_{s0})/A_0) \times 100$,

187 where A_0, A_s, and A_{s0} are the absorbance of the blank (water instead of

188 polysaccharide), sample, and control (water instead of H_2O_2) groups, respectively.

189 ***The ferric reducing ability of plasma (FRAP)***

190 The total antioxidant potential of polysaccharides was assayed by the method of

191 Benzie and Strain (18). FRAP reagent was comprised 300 mM acetate buffer (pH 3.6),

192 10 mM TPTZ, and 20 mM $FeCl_3$ solution in a ratio of 10:1:1. A 300 μL freshly

193 prepared FRAP stocking solution was pre-warmed to 37°C for 10 min, and a reagent

194 blank was recorded at 593 nm. Then 30 μL of distilled water and 10 μL of

polysaccharide solutions (0.25–4 mg/mL) was added to the FRAP stocking solution under dark conditions and a second reading was taken after 30 min. Different concentrations of $FeSO_4$ solution ranging from 100 to 1,000 μM were used to establish a standard curve. The antioxidant capacity of each sample was expressed as μmol Fe (II)/g dry weight of polysaccharide.

Statistical analysis

All assays were performed at least in triplicate. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Tukey's HSD test was used to determine the significant difference at a level of $P < 0.05$.

Results and Discussion

Extraction and molecular weight distribution

The yields of crude polysaccharide extracted from three natural *C. sinensis* and cultured *C. sinensis* were 9.43%, 9.78%, 8.94%, and 14.32% by weight, respectively. After removal of protein by Sevag method, the subsequent yields were 27.58%, 31.57%, 38.30%, and 49.36% by weight, respectively.

HPGPC results revealed that the three natural polysaccharides exhibited similar elution profiles (data not shown), indicating that there were no differences in molecular weight distribution among polysaccharides from natural *C. sinensis*. As shown in Fig. 1 (A), the molecular weight distribution profile of polysaccharide from natural material mainly comprised three fractions with Mw of approximately 950 K, 15 K, and 1,000 Da, respectively. The peak at the retention time of 18.5 min (from RI

signal) was considered to be the existence of low molecular residues such as monosaccharides and oligosaccharides. These residues were successfully removed through dialysis treatment. After treating with Sevag reagent, most of the fraction that had the highest UV signal at 280 nm was removed, and the fraction with a high Mw remained dominant. On the contrary, the polysaccharide from cultured *C. sinensis*, P4, presented a broad distribution profile with a shoulder peak as shown in Fig. 1 (B). The average Mw was much lower compared to P1, P2 and P3, approximately 28 KDa. The result showed that there was no significant difference in the molecular weight distribution of P1, P2, and P3, and the major fraction was a high-molecular-weight polymer, but it was completely different from P4.

Monosaccharide composition

After complete acid hydrolysis, the monosaccharide compositions of polysaccharides from natural and cultured *C. sinensis* were analyzed by HPAEC-PAD. As shown in Table 1, P1, P2, and P3 were all mainly composed of Glc with a small amount of Gal and Man, although the percentages of Glc varied slightly, ranging from 81.72% to 89.22% for the crude polysaccharides and from 78.83% to 84.05% for deproteinated polysaccharides, respectively. On the other hand, P4 had a much complicated monosaccharide composition and mainly comprised Gal, Glc, and Man with a percentage of 36.40%, 28.99%, and 24.81%, respectively. Besides, it also contained a small amount of Ara (3.34%) and GalA (7.55%). Therefore, the result revealed a rather distinct difference between polysaccharides from natural and cultured *C. sinensis*.

239 **FTIR spectra**

240 All the polysaccharide exhibited a broad intense characteristic peak at approximately
241 3,380 cm^{-1} for the hydroxyl group, a weak band at near 2,930 cm^{-1} that was attributed
242 to C–H stretching, and a group of absorptions at 1,400–1,200 cm^{-1} for the C–H
243 bending vibration (Fig. 2). The absorption band centered at approximately 1,650 cm^{-1}
244 was caused by associating with water. P4 had weak absorption at 1,745cm^{-1},
245 indicating the presence of carboxyl groups, which was consistent with the results of
246 monosaccharide composition analysis. Furthermore, a characteristic band at 887 cm^{-1}
247 was noticed in P4, indicating the existence of a β-configuration of sugar units,
248 whereas in P1, P2 and, P3 absorption was observed at approximately 845 cm^{-1}
249 corresponding to α-configuration. As expected, the FTIR spectra of P1, P2, and P3
250 were similar, but they had some differences from that of P4.

251 **Methylation analysis**

252 Methylation analysis revealed that all the three natural polysaccharides contained
253 similar types of glycosidic linkages, mostly 1→4 linkage pattern with branching
254 points at position 6 and terminating at glucose (Table 2). A small amount of terminal
255 and 1→3 linked Glc as well as 1→2 linked Man were also identified in the three
256 samples, whereas P1 also contained a small amount of 1→4 linked Gal and 1→6 and
257 1→4,6 linked Man. However, a small amount of terminal and 1→3,6 linked Gal as
258 well as 1→2,3 linked Man was only found in P2 and P3. On the other hand, the
259 results revealed P4 to be highly complex and that it could be a mixture of two or more
260 polysaccharide fractions. 1→4 linked Glc and 1→4 linked Gal may be part of the

main chain of P4. Man units were found as 1→2, 1→6, 1→2,6, and 1→4,6 linkages. Ara was found as terminal and 1→5 linked residues. In addition, terminally linked Gal as well as terminally 1→3 and 1→4, 6 linked Glc was presented in P4. On the basis of these results, it was possible to conclude that the repeating unit of polysaccharides from natural *C. sinensis* contained a backbone mainly comprising 1→4 linked Glc residues with branches attached to O-6 of some Glc. Although 15.44% of this linkage was found in P4, the content of 1→4,6 linked Glc was relative low with only 1.42%. Furthermore, the branching point of P4 may possibly occur in Man residues with 1→2,6 and 1→4,6 linkages. This finding obviously revealed the similarity of glycosidic linkages among polysaccharides from three natural *C. sinensis*, despite the different growth environments that mainly referred to diversity of altitude. Another meaningful finding of this study was the distinctive differences of linkage patterns between natural and cultured materials. Unlike the observed variations of chemical composition reported previously (12), structure of the polysaccharide moiety was not influenced by the growing habitats for natural *C. sinensis* collected from the Qinghai Province. However, the polysaccharide from cultured *C. sinensis* was completely different from the natural ones on the basis of the sugar residues and glycosidic linkages.

Amino acid composition

The amino acid composition of polysaccharides from natural and cultured *C. sinensis* was investigated (Table 3). There were 17 kinds of amino acids contained in P2 and P3, whereas P1 did not contain tyrosine and P4 did not contain methionine. However,

283 the quantities of these two amino acids were not very high in the other
284 polysaccharides, which were 0.18, 0.18, and 0.36 mg/g of tyrosine and 1.19, 0.90, and
285 1.19 mg/g of methionine for P2, P3, and P4, respectively. All four polysaccharides
286 were abundant in glutamic acid, aspartic acid, and proline, and the content was the
287 lowest in P4. Moreover, there were relatively high contents of threonine and serine,
288 indicating the possible existence of O-glycosidic linkages.

289 **Total phenolic content**

290 Phenolic compounds, including phenolic acid, polyphenols, monophenols, and other
291 phenolic derivatives, have a strong ability to scavenge free radical and potentially
292 prevent oxidative stress-related diseases (19, 20). Although the extraction process did
293 not aim to isolate phenolic compounds, the polysaccharides obtained from all four
294 materials appeared to contain a certain amount of phenolic compounds, as shown in
295 Fig. 3 (A). The total phenolic content of P1, P2, and P3 was 3.22±0.05, 5.16±0.02,
296 and 7.63±0.26 mg GAE/g, respectively. P4 presented a total phenolic level of
297 7.05±0.46 mg GAE/g, showing no significant difference with the value of P3, but this
298 level was significantly different from that of P1 and P2. A significant difference
299 among the phenolic contents of the three natural *C. sinensis* was also observed, which
300 can probably be attributed to the different habitats because factors such as moisture,
301 altitude, and temperature influence the composition and content of phenolic
302 compounds.

303 **Antioxidant capacity**

304 Reactive oxygen species (ROS) are commonly produced in the human body and their

excessive generation leads to oxidative stress. Deactivation of radicals occurs via two major mechanisms: single electron transfer and hydrogen atom transfer (21). Therefore, the *in vitro* antioxidant activity of polysaccharides from natural and cultured *C. sinensis* was evaluated through three methods, which are compared with a standard antioxidant (ascorbic acid). As shown in Fig. 3 (B), all four polysaccharides displayed significant scavenging effect against DPPH radical in a dose-dependent manner. However, they were found to exhibit low effect on scavenging hydroxyl radicals compared to ascorbic acid, as shown in Fig. 3 (C). The polysaccharide from cultured mycelium was the most effective of them and scavenged only 40% of hydroxyl radicals. The FRAP values of the four polysaccharides increased in the order of P3 > P4 > P2 > P1 with values of 56.79, 53.74, 44.51, and 30.19, respectively (Fig. 3 (D)), corresponding to the trends of total phenolic contents, indicating that the observed antioxidant potential may be related to the phenolic constituents.

Natural polysaccharides have been reported to always be conjugated with other constituents, such as amino acid, protein, lipids, and phenolic compounds (22). Positive correlation between antioxidant actions and the total phenolic or protein content in the polysaccharide has been observed in several reports (23, 24). The antioxidant activity of protein molecules, in general, may be attributed to the amino acid because several amino acids, such as tyrosine, methionine, histidine, lysine, and tryptophan, are proved to be capable of donating protons to electron-deficient radicals (25-27). In our previous study, we compared the antioxidant activity of water extract among four samples (12). Results showed that cultured *C. sinensis* had relatively

more effective abilities against scavenging DPPH and hydroxyl radicals as well as a higher reducing ferrous power than natural *C. sinensis*, but these effects were not significantly different among the three natural samples. After increasing the polysaccharide purity by ethanol precipitation and Sevag reagent treatment, these antioxidant activities did not improve significantly. On the other hand, although the content of protein and total phenolic decreased dramatically in P1, P2, P3, and P4 compared with these water extracts, the effects did not appear to reduce significantly. These findings indicated that the antioxidant activity of polysaccharides from *C. sinensis* might be associated with a combination of factors because the polysaccharides always contained certain amount of other antioxidative constituents.

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Statement of Human and Animal Rights

This study did not involve any experimentation with human or animal subjects performed by any of the authors.

Disclosure

Wang, Nie, Kan, Chen, Cui, Phillips, Phillips, and Xie declare that they have no conflict of interest.

References

1. Jiang Y, Yao Y. Current understanding of molecular systematics of *Cordyceps.* J. Fungal Res. 2: 58-67 (2004).
2. Sung JM, Lee HK, Yang KJ. Classification of *Cordyceps spp*. by morphological characteristics and protein banding pattern. Korean J. Mycol. 23: 92-104 (1995).
3. Li SP, Yang FQ, Tsim KWK. Quality control of *Cordyceps sinensis*, a valued traditional Chinese medicine. Pharmaceut. Biomed. 41: 1571-1584 (2006).
4. Chen PX, Wang SN, Nie SP, Marcone M. Properties of *Cordyceps sinensis*: A review. J. Funct. Foods. 5: 550-569 (2013).
5. Zhu JS, Halpern GM, Jones K. The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis* Part I. J. Altern. Complem. Med. 4: 289-303 (1998).
6. Zhu JS, Halpern GM, Jones K. The scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis* Part II. J. Altern. Complem. Med. 4: 429-457 (1998).
7. Liang ZQ. Current situation and ponderation of *Cordyceps* Fr. research and exploitation in China. Acta Edulis Fungi. 2: 53-62 (2001).
8. Yan JK, Wang WQ, Wu JY. Recent advances in *Cordyceps sinensis* polysaccharides: Mycelial fermentation, isolation, structure, and bioactivities: A review. J. Func. Foods. 6: 33-47 (2014).
9. Nie SP, Cui SW, Xie MY, Phillips AO, Phillips GO. Bioactive polysaccharides

from *Cordyceps sinensis*: Isolation, structure features and bioactivities. Bioact. Carbohyd. Dietary Fibre. 1: 38-52 (2013).

10. Tao YZ, Zhang LN, Cheung PC. Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. Carbohyd. Res. 341: 2261-2269 (2006).

11. Zhang LN, Li XL, Xu XJ, Zeng FB. Correlation between antitumor activity, molecular weight, and conformation of lentinan. Carbohyd. Res. 340: 1515-1521 (2005).

12. Wang JQ, Kan LJ, Nie SP, Chen HH, Cui SW, Phillips AO, Phillips GO, Li YJ, Xie MY. A comparison of chemical composition, bioactive components and antioxidant activity of natural and cultured *Cordyceps sinensis.* LWT-Food Sci. Techn. 63: 2-7 (2015).

13. Dai J, Liang L, Yin H. Analysis of monosaccharde compositions in polysaccharides from *D. salina* by high-performance anion-exchange chromatography. Food Ferment. Ind. 32: 131-135 (2006).

14. Ciucanu I, Kerek F. A simple and rapid method for the permethylation of carbohydrates. Carbohyd. Res. 131: 209-217 (1984).

15. Singleton V, Orthofer R, Lamuela-Raventos R. Analysis of total phenols and other oxidation substrates and antioxidants by means of folinciocalteu reagent. Method Enzymol. 299: 152-178 (1999).

16. Mao GH, Zou Y, Feng WW, Wang W, Zhao T, Ye CW, Zhu Y, Wu XS, Yang LQ, Wu XY. Extraction, preliminary characterization and antioxidant activity

of Se-enriched Maitake polysaccharide. Carbohyd. Polym. 101: 213-219 (2014).

17. Gao CJ, Wang YH, Wang CY, Wang ZY. Antioxidant and immunological activity in vitro of polysaccharides from *Gomphidius rutilus* mycelium. Carbohyd. Polym. 92: 2187-2192 (2013).

18. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239: 70-76 (1996).

19. Fogarasi AL, Kun S, Tankó G, Stefanovits-Bányai É, Hegyesné-Vecseri B. A comparative assessment of antioxidant properties, total phenolic content of einkorn, wheat, barley and their malts. Food Chem. 167: 1-6 (2015).

20. Weng CJ, Yen GC. Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: phenolic acids, monophenol, polyphenol, and their derivatives. Cancer Treat. Rev. 38: 76-87 (2012).

21. Niu YG, Shang PP, Chen L, Zhang H, Gong L, Zhang XW, Yu WJ, Xu YH, Wang Q, Yu LL. Characterization of a novel alkali-soluble heteropolysaccharide from tetraploid *Gynostemma pentaphyllum* makino and its potential anti-inflammatory and antioxidant properties. J. Agric. Food Chem. 62: 3783-3790 (2014).

22. Chen H, Zhang M, Qu Z, Xie B. Antioxidant activities of different fractions of polysaccharide conjugates from green tea (*Camellia Sinensis*). Food Chem. 106: 559-563 (2008).

23. Wang J, Hu S, Nie S, Yu Q, Xie M. Reviews on mechanisms of in vitro

antioxidant activity of polysaccharides. Oxid. Med. Cell Longe. 2016 (2015).

24. Zhang H, Cui SW, Nie SP, Chen Y, Wang YX, Xie MY. Identification of pivotal components on the antioxidant activity of polysaccharide extract from *Ganoderma atrum*. Bioact. Carbohyd. Dietary Fibre. 7: 9-18 (2016).

25. Elias RJ, Kellerby SS, Decker EA. Antioxidant activity of proteins and peptides. Crit. Rev. Food Sci. 48: 430-441 (2008).

26. Minelli A, Bellezza I, Grottelli S, Galli F. Focus on cyclo (His-Pro): history and perspectives as antioxidant peptide. Amino Acids. 35: 283-289 (2008).

27. Pérez RA, Iglesias MT, Pueyo E, González M, de Lorenzo C. Amino acid composition and antioxidant capacity of Spanish honeys. J. Agric. Food Chem. 55: 360-365 (2007).

Figure Captions

Figure 1. HPGPC profiles of the polysaccharides from (A) natural *Cordyceps sinensis* and (B) cultured mycelium monitored by RID (straight line) and VWD (dotted line) at 280 nm.

Figure 2. FTIR spectrum of the polysaccharides.

Figure 3. (A) Total phenolic contents of polysaccharides calculated as mg GAE/g. The scavenging activities of polysaccharides on (B) DPPH and (C) hydroxyl radicals. (D) Ferric reducing ability of polysaccharides. Data is expressed as the mean ± SD of triplicate determinations and different letters represent significant differences ($P < 0.05$).

Tables

Table 1

Monosaccharide compositions of the polysaccharides [a]

Sample	Ara, %	Gal, %	Glc, %	Man, %	GalA, %
C1	ND[b]	3.44	88.01	8.55	ND
C2	ND	3.04	81.72	15.24	ND
C3	ND	2.62	89.22	8.16	ND
C4	trace	38.48	31.99	23.15	6.38
P1	ND	7.07	80.92	12.00	ND
P2	ND	4.83	84.05	11.12	ND
P3	ND	6.14	78.83	15.03	ND
P4	3.34	36.40	28.99	24.81	7.55

[a] calculated as a molar percentage.

[b] "ND" means not detected.

Table 2

The glycosidic linkages of the polysaccharides determined by GC–MS

Glycosyl residues	Linkage patterns	Peak area percentage (%)			
		P1	P2	P3	P4
Ara	t-Ara*f*	ND[a]	ND	ND	1.91
	5-Ara*f*	ND	ND	ND	3.24
Glc	t-Glc*p*	11.60	15.05	13.70	10.26
	3-Glc*p*	5.07	2.23	2.52	5.85
	4-Glc*p*	51.59	58.28	60.55	15.44
	6-Glc*p*	ND	0.81	0.84	ND
	3,6-Glc*p*	1.62	0.60	ND	ND
	4,6-Glc*p*	9.11	12.59	11.42	1.42
Gal	t-Gal*f*	ND	1.61	1.17	ND
	t-Gal*p*	ND	ND	ND	6.63
	4-Gal*p*	8.48	1.71	0.97	22.54
	3,6-Gal*p*	ND	1.54	1.41	ND
Man	2- Man*p*	3.51	3.02	2.95	5.17
	6- Man*p*	2.61	0.42	ND	7.20
	2,3- Man*p*	ND	2.15	2.63	ND
	2,6- Man*p*	0.82	ND	ND	3.11
	4,6- Man*p*	2.89	ND	ND	9.20

[a] "ND" means not detected.

Table 3

Amino acid composition of the polysaccharides

Amino acid (mg/g)	P1	P2	P3	P4
Aspartic acid	10.45	17.57	8.32	8.45
Threonine[a]	10.48	11.55	9.17	7.15
Serine	9.25	9.51	10.40	4.62
Glutamic acid	20.97	22.58	18.32	10.08
Glycine	11.79	10.89	9.91	5.56
Alanine	9.89	10.82	9.49	4.86
Cystine	1.57	1.45	1.57	0.24
Valine[a]	9.49	8.32	10.07	3.98
Methionine[a]	1.19	0.90	1.19	ND [b]
Isoleucine[a]	4.20	4.20	4.98	1.44
Leucine[a]	5.31	6.43	7.48	1.44
Tyrosine	ND [b]	0.18	0.18	0.36
Phenylalanine[a]	3.30	2.97	3.63	1.16
Lysine[a]	12.72	11.26	13.08	3.95
Histidine	6.51	5.43	9.15	2.02
Arginine	10.71	9.41	14.46	2.61
Proline	23.20	13.93	24.35	6.91
total amino acids (TAA)	151.03	147.39	155.77	64.82
total essential amino acids (EAA)	46.70	45.62	49.62	19.12
EAA/TAA (%)	30.92	30.95	31.86	29.49

[a] means essential amino acid.

[b] "ND" means not detected.

Figures

Figure 1

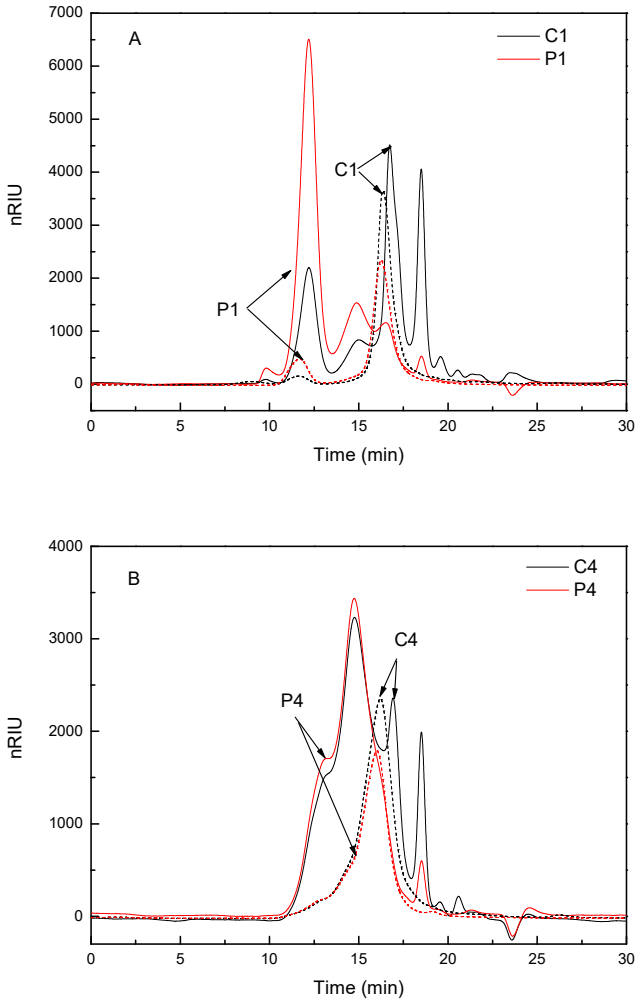


Figure 2

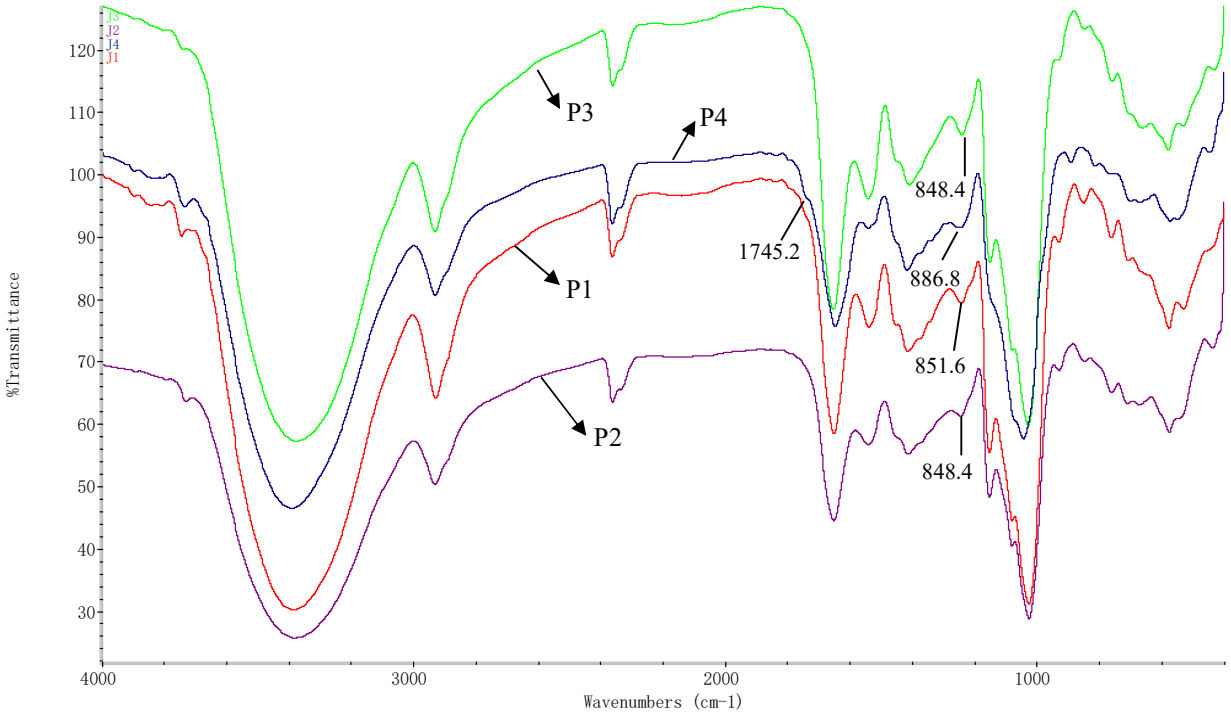
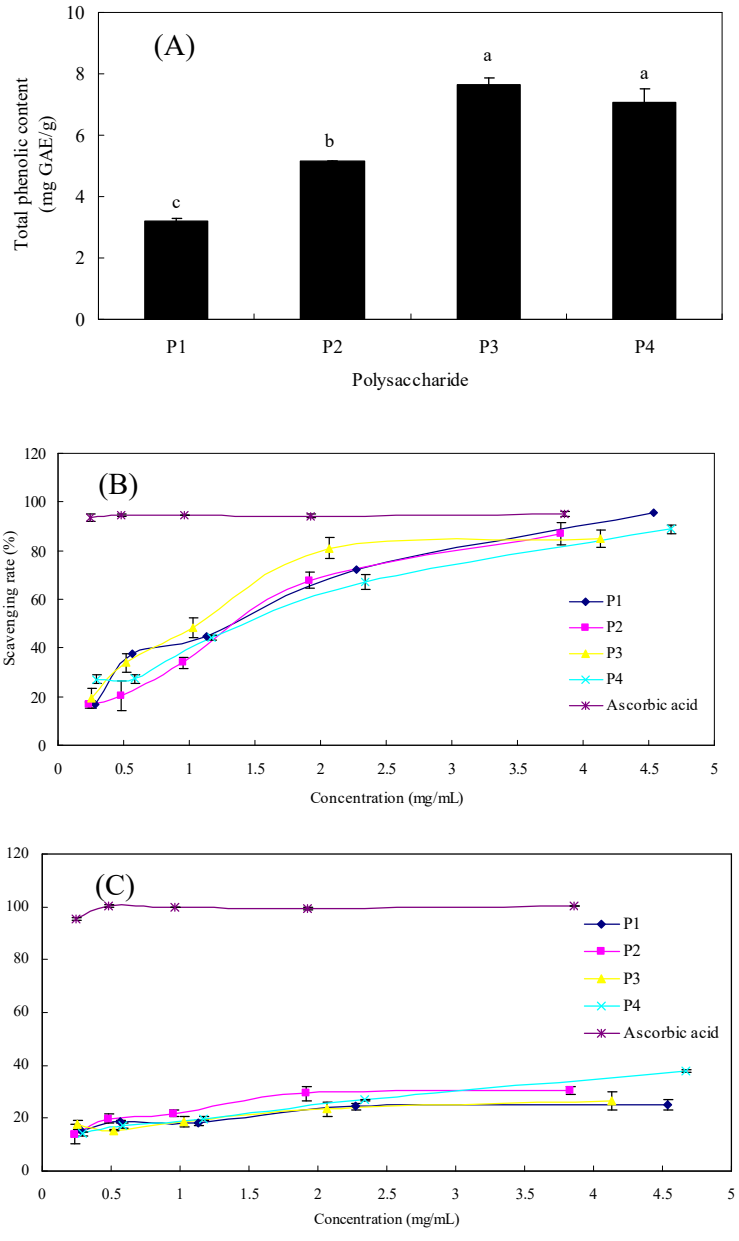
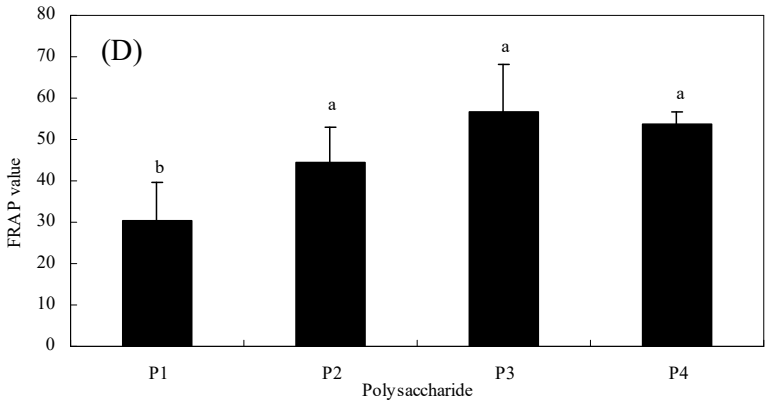

J3
J2
J4
J1
120
110
100
90
80
70
60
50
40
30
%Transmittance
4000
3000
2000
1000
Wavenumbers (cm-1)
P3
P4
P1
P2
1745.2
848.4
886.8
851.6
848.4

Figure 3


(A)
Total phenolic content (mg GAE/g)
10
8
6
4
2
0
a
a
b
c
P1
P2
P3
P4
Polysaccharide
(B)
Scavenging rate (%)
120
100
80
60
40
20
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
P1
P2
P3
P4
Ascorbic acid
Concentration (mg/mL)
(C)
Scavenging effect (%)
120
100
80
60
40
20
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
P1
P2
P3
P4
Ascorbic acid
Concentration (mg/mL)


(D)
FRAP value
80
70
60
50
40
30
20
10
0
b
a
a
a
P1
P2
P3
P4
Polysaccharide