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Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*

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Abstract

The water-soluble crude polysaccharides were obtained from the fruiting bodies of cultured *Cordyceps militaris* by hot water extraction followed by ethanol precipitation. The polysaccharides were successively purified by chromatography on DEAE–cellulose-52 and Sephacryl S-100 HR columns, giving main three polysaccharide fractions termed P50-1, P70-1, and P70-2. Structural features of P70-1 were investigated by a combination of chemical and instrumental analysis, such as partial acid hydrolysis, methylation analysis, periodate oxidation – Smith degradation, GC–MS, ^{13}C NMR, HPAEC-PAD, and FT-IR. The results indicated that P70-1 has a backbone of (1 → 6)-linked β-D-mannopyranosyl residues, which occasionally branches at O-3. The branches were mainly composed of (1 → 4)-linked α-D-glucopyranosyl and (1 → 6)-linked β-D-galactopyranosyl residues, and terminated with β-D-galactopyranosyl residues and α-D-glucopyranosyl residues. In the in vitro antioxidant assay, P70-1 was found to possess hydroxyl radical-scavenging activity with an IC_{50} value of 0.548 mg/ml.

Keywords: Cultured *Cordyceps militaris*; Fruiting bodies; Polysaccharide structure; HPAEC-PAD analysis; Antioxidant

1. Introduction

Interest in the structure of fungal extracellular polymers has increased since several physiological roles were shown, in fungi morphogenesis, in their association with hosts and by their mobilization as food reserve (Krcmar, Novotny, Marais, & Joseleau, 1999). Many natural polysaccharides and polysaccharide–protein complexes were isolated from fungi and were used as a source of therapeutic agents (Carbonero et al., 2006; Mechacanon, Madla, Kirtikara, & Prasitsil, 2005; Yu et al., 2004) during the past years.

Cordyceps militaris is known as the Chinese rare caterpillar fungus, and has similar pharmacological activities to the well known chinese traditional medicine *Cordyceps sinensis* (Gai, Jin, Wang, Li, & Li, 2004; Zheng & Cai, 2004). It is commonly used in the orient to replenish the kidney and soothe the lung for the treatment of hyposexualities, hyperglycemia, hyperlipidemia, renal dysfunction, and liver disease (Won & Park, 2005; Yu et al., 2004). Recently, several studies have demonstrated that the extracts of *C. militaris* have multiple pharmacological actions such as anti-inflammatory (Won & Park, 2005), improvement of insulin resistance and insulin secretion (Choi, Park, Choi, Jun, & Park, 2004). The antioxidant activity of *C. militaris* is even stronger than *C. sinensis* and *Cordyceps kyushuensis* (Chen, Luo, Li, Sun, & Zhang, 2004). Because natural *C. militaris* is rare and expensive, many scientists have examined its life cycle with the aim of developing techniques for the isolating fermentable strains. Several strains have been isolated from natural

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C. militaris and manufactured in high quality by fungus-cultivation technology (Chai, Bai, & Xie, 2003; Ling, Peng, Zhang, & Zhang, 2003). The products from cultured *C. militaris* have shown similar pharmacological efficacy compared with natural *C. militaris* (Wang, 2002). Cultivated fruiting bodies of *C. militaris* were commonly sold as drug materials and health food products in China and South East Asia (Li, Yang, & Tsim Karl, 2006; Zheng & Cai, 2004).

In the last few years, the structures of several polysaccharides isolated from *Cordyceps* spp. were reported (Mechacanon et al., 2005; Wu, Sun, & Pan, 2006; Xiao et al., 2006). Three polysaccharides (CPS-1, CPS-2, and CPS-3) were isolated from *C. militaris* by our research group recently (Yu et al., 2004, Yu, Wang, Zhang, Zhou, & Zhao, 2004). The sugar composition analysis showed that CPS-1 was composed mainly of Rha, Xyl, Man, Glc, and Gla in a molar ration of 1:6.43:25.6:16.0:13.8, CPS-2 consisted of Rha, Glc, and Gla in a molar ration of 1:4.46:2.43 and CPS-3 was a homogeneous polysaccharide, which was composed of D-glucose with a major linkage form of a α-D-glucose. Although, the structural investigations of three polysaccharides shed light on the monosaccharide compositions of polysaccharides from *C. militaris*, isolation, purification, and activities' determination of more fine polysaccharides, especially detailed studies of the structures, were lacking.

Therefore, the polysaccharides were further fractionated from *C. militaris* and several distinctive polysaccharides were obtained. Among them, P70-1 exerted inhibitory activity towards hydroxyl radicals with an IC_{50} value of 0.548 mg/ml. So, the aim of this research was to investigate the complete structure of P70-1.

2. Experimental

2.1. Material

Cultured *C. militaris* was obtained from Shenyang Zhongtian Bioengineering Corporation, Shenyang, China. The material (No. 97-08-0005) was identified by Professor R.M. Yu, College of Pharmacy, Jinan University, China.

Ascorbic acid (Vitamin C, Vc), hydrogen peroxide (H_2O_2), ferrous sulfate ($FeSO_4$), and brilliant green were purchased from Shanghai Chemical Reagent Company, Shanghai, China. All other reagents were obtained from the Sigma Chemical Co. All reagents were of analytical grade.

2.2. General methods

The total sugar content of P70-1 was determined by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Optical rotations were measured with a Jasco P-1020 polarimeter. IR spectra were recorded with a Tensor 27 Bruker instrument with KBr pellets. ^{13}C NMR spectra were recorded with a Bruker 500 instrument, and the sample was dissolved in D_2O. High-performance anion exchange chromatography (HPAEC) was analyzed on a Dionex ICS-2500 system, coupled with pulsed amperometric detection (PAD), equipped with a Carbo PAC ™PA10 (2.0 × 250 mm) column. GC was analyzed on an Agilent 190911 J-413 HP-5 equipped with FID, inositol as an internal standard; GC–MS was conducted with a Hewlett Packard 5895 instrument, using a fused-silica capillary column (30 × 25 mm) coated with a 0.2-mm film of DB-5. The ionisation potential was 70 eV and the temperature of the ion source was 220 °C.

2.3. Extraction and fractionation of polysaccharides

The dried powder of cultured *C. militaris* (300 g) was defatted with ethanol for 10 h and extracted three times with hot water (50 °C), each time for 10 h. The extract was concentrated to a volume of 100 ml under reduced pressure. Two fractions of crude polysaccharides, termed CP50 and CP70, were obtained by graded ethanol precipitation, at final concentrations of 50% and 70% of ethanol, respectively.

2.4. Isolation and purification of the polysaccharides

CP50 and CP70 were deproteinized with Sevag reagent, 1-butanol/chloroform (v/v = 1:4) (Staub, 1965), decolorized with 30% H_2O_2, and then dialyzed against tap water and distilled water for 48 h, respectively. The resulting polysaccharide solution was concentrated and lyophilized. Ion-change chromatography and gel filtration column chromatography were used for the isolation of these preparations. Each sample (200 mg) was dissolved in 0.025 M Tris–HCl (pH 7.4), centrifuged, and then the supernatant was injected to a column of DEAE–cellulose-52 equilibrated with 0.025 M Tris–HCl (pH 7.4).

After loading with sample, the column was gradient eluted with NaCl aqueous solution (0–1 M), and this process was monitored by the phenol–sulfuric acid method (Dubois et al., 1956). The fractions were further purified by gel filtration chromatography on a column of Sephacryl S-100 HR.

Consequently, three polysaccharides, namely P70-1, P70-2, and P50-1, were obtained. Among these, P70-1, which had $[\alpha]_D^{20} + 230°$(*c* 0.5, water), was used in the subsequent structural and bioactive studies.

2.5. Determination of molecular weights of P70-1, P70-2, and P50-1

The molecular weights of P70-1, P70-2, and P50-1 were determined by a gel chromatographic technique (Wang, Liang, & Zhang, 2001). Standard dextrans T-500, T-70, T-40, and T-10 were passed through a Sephacryl S-300 HR column, and then the elution volumes were plotted against the logarithms of their respective molecular weights. A solution of the polysaccharide (5 mg) in distilled

water (0.5 ml) was applied to the column equilibrated and eluted with distilled water at a fixed flow rate (10 ml/h). The elution volume of the polysaccharide was then plotted in the same graph, and the molecular weights of P70-1, P70-2, and P50-1 were determined.

2.6. Monosaccharide composition analysis of P70-1, P70-2, and P50-1

The polysaccharides (10 mg) were hydrolyzed with 2 M trifluoroacetic acid (TFA) at 100 °C in a sealed-tube for 8 h. Excess acid was removed by co-distillation with MeOH after the hydrolysis was completed. One part of the hydrolysate was analyzed by GC, and the other was measured by HPAEC-PAD.

2.6.1. GC analysis

The hydrolysate and inositol were reduced by $NaBH_4$, followed by acidification with acetic acid. And then co-distilled with MeOH in order to remove excess boric acid and dried over P_2O_5. Thereafter, the sugars were treated with pyridine (2 ml) and Ac_2O (1.5 ml) to convert into their alditol acetate and analyzed by GC, at a temperature program of 50–230 °C with a rate of 2 °C/min.

2.6.2. HPAEC-PAD analysis

The hydrolysate (1 mg) was dissolved in pure water (1 mg/ml). Twenty-five microliters of this solution was used for the ionic-chromatography analysis by HAPEC-PAD of Dionex ICS-2500 System, eluted with a mixture of water and 200 mM NaOH in the volume ratio of 92:8.

2.7. Partial acid hydrolysis of P70-1, P70-2, and P50-1

The polysaccharide sample (100 mg) was hydrolyzed with 0.05 M TFA for 6 h at 100 °C, centrifuged, dialyzed with distilled water for 48 h, and then diluted the solution in the sack with ethanol. After hydrolysis, the precipitate (A) and supernatant (B) in the sack and the fraction out of sack (C) were dried, and then GC analysis was carried out.

2.8. Periodate oxidation – Smith degradation analysis of P70-1

The polysaccharide of 20 mg was allowed to swell overnight in distilled water (10 ml), dispersed using a blender, and on addition of 15 mM $NaIO_4$ (25 ml), an immediate loss of viscosity occurred. The solution was kept in the dark at 4 °C, 30 μl aliquots were withdrawn at 6 h intervals, diluted to 5 ml with distilled water and read in a spectrophometer at 223 nm (Linker, Evans, & Impallomeni, 2001). Complete oxidation, identified with a stable absorbance, was reached in 96 h. Consumption of HIO_4 was measured by a spectrophotometric method (Aspinall & Ferrier, 1957), and formic acid production was determined by titration with 0.053 M NaOH. Glycol (2 ml) was added, and then the experiment of periodate oxidation was over. The rest of the periodate product was exhaustively dialyzed against tap water (48 h) and distilled water (24 h), respectively. The nondialysate was concentrated and reduced with sodium borohydride (40 mg) for 24 h at room temperature, and then the pH of the solution was adjusted to 5.0 by adding 0.1 M acetic acid, dialyzed against distilled water (24 h) and the nondialysate was dried in vacuum. The above product was hydrolyzed with 2 M TFA (3 ml) at 100 °C for 6 h. GC analyzed the components of this polyalcohol as the alditol acetate under the same conditions as those used for the monosaccharide composition analysis (Zhao, Kan, Li, & Chen, 2005).

2.9. Methylation analysis of P70-1

P70-1 (10 mg) was performed by the method of Hakomori (1964). The methylated polysaccharide was treated with 90% aqueous formic acid (3 ml) for 10 h at 100 °C in a sealed tube. After removal of the formic acid, the residues were heated with 2 M trifluoroacetic acid (2 ml) under the same conditions and the hydrolysate was concentrated to dryness. The methylated sugars were reduced with $NaBH_4$, acetylated with acetic anhydride, and analyzed as the alditol acetates by GC–MS. The identification of the methylated sugars linkages were identified on the basis of relative retention time and fragmentation pattern (Needs & Selvendran, 1993; Perret et al., 1992). The molar ratios for each sugar were calibrated using the peak areas and response factor of the flame-ionisation detector in GC.

2.10. In vitro antioxidant assay by hydroxyl radical system

In vitro antioxidant activity was evaluated using the hydroxyl radical system generated by the Fenton reaction. Hydroxyl radical-scavenging activity was measured according to the method described by the reference (He, Luo, Cao, & Cui, 2004). Briefly, the reaction mixture contained 1 ml of brilliant green (0.435 mM), 2.0 ml of $FeSO_4$ (0.5 mM), 1.5 ml of H_2O_2 (3.0%) and samples of varying concentrations. After incubation at room temperature for 20 min, the absorbance of the mixture was measured at 624 nm. Hydroxyl radicals gave a brilliant green colour, so the absorbance change of the reaction mixture indicated the scavenging ability for hydroxyl radicals. The hydroxyl radical-scavenging activity was expressed as:

$$\text{Scavenging rate } (\%) = [(A_s - A_0)]/[(A - A_0)] \times 100\%$$

where A_s is the absorbance in the presence of the sample, A_0 is the absorbance of the control in the absence of the sample, and A is the absorbance without the sample and Fenton reaction system.

2.11. Statistical analysis

Tests were carried out in triplicate for three separate experiments. Values are presented as means ± SD. The

IC_{50} values is the amount of sample needed to inhibit free radicals concentration by 50%, which can be calculated from the concentration–response curves (SigmaPlot 2001 software).

3. Results and discussion

3.1. Isolation, purification, and composition of polysaccharides

CP50 and CP70, two crude polysaccharides, were obtained from cultured *C. militaris* by hot water extraction followed by ethanol precipitation, with yields of 3% and 4%, respectively. After fractionation on DEAE–cellulose 52 and Sephacryl S-100 HR column, P70-1 (250 mg), P70-2 (30 mg) and P50-1 (40 mg) were obtained from the NaCl elution. The homogeneity of the three polysaccharides was elucidated by the following tests. They were all eluted as a single peak from gel-filtration chromatography on Sephacryl S-300 HR column and had the same optical rotation in different low content aqueous ethanol by Jasco P-1020 automatic optical polarimeter at room temperature. Average molecular weight, total sugar content, specific rotations, and monosaccharide compositions of these polysacchrides were determined and shown in Table 1.

Profile of P70-1 in Sephacryl S-300 HR appeared a single elution peak (Fig. 1), as detected by the phenol-sulfuric acid assay. The alditol acetate of P70-1's hydrolysate was measured by GC shown in Fig. 2. HPAEC-PAD chromatogram profiles of standard monosaccharide mixture solution and hydrolysate of P70-1 were shown in Fig. 3. The three monosaccharides, D-Man, D-Gal, and D-Glc, were identified in the hydrolysate of P70-1, and their ratios were 3.12:1.45:1.00 by HPAEC-PAD and 3.22:1.35:1.00 by GC–MS.

3.2. Structural characterization of P70-1

P70-1 was supposed to be in the D-configuration according to the optical rotation, ^{13}C NMR data and GC analysis. The UV absorption spectra of P70-1 showed no absorption at 280 and 260 nm, implying that protein and nucleic acid were absent in this polysaccharide.

Table 1
Components of monosaccharide and properties of the three polysaccharides from cultured *Cordyceps militaris*

Samples	P70-1		P70-2	P50-1
$[\alpha]_D^{20}$	+230.0		+150.0	+150.0
Molecular weight (kDa)	42		26	50
Carbohydrate (wt%)	99.9%		95.0%	95.5%
Monosaccharide component (mol)				
Man	3.12[a]	3.22[b]	1.75[a]	2.80[a]
Gal	1.45[a]	1.35[b]	1.31[a]	2.62[a]
Glc	1.00[a]	1.00[b]	1.00[a]	1.00[a]

[a] Analyzed by HPAEC-PAD.
[b] Analyzed by GC.

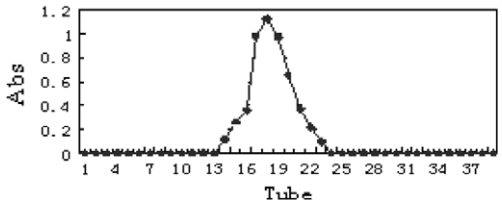

Fig. 1. Profile of P70-1 in Sephacryl S-300 HR column chromatography.

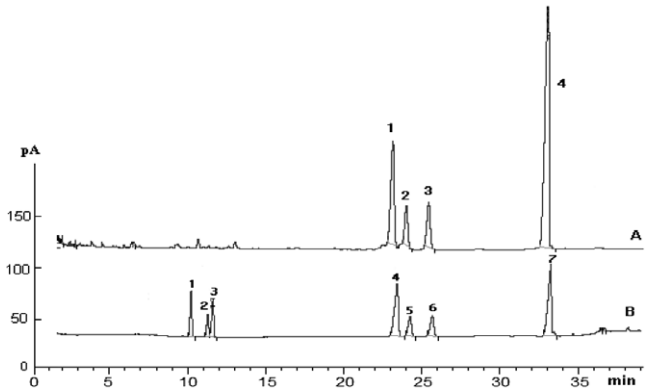

Fig. 2. GC profile of the alditol acetates of standard monosaccharide (B) and the alditol acetates of P70-1 hydrolysate. (A) Peak identity; 1, D-Man (rt: 23.352); 2, D-Glc (rt: 24.196); 3, D-Gal (rt: 25.651); 4, alditol acetate of inositol as an internal standard. (B) Peak identity; 1. L-Rha (rt: 10.232); 2. L-Fuc (rt: 11.285); 3. D-Xyl (rt: 11.628); 4. D-Man (rt: 23.419); 5. D-Glc (rt: 24.241); 6. D-Gal (rt: 25.684); 7. Alditol acetate of inositol as an internal standard.

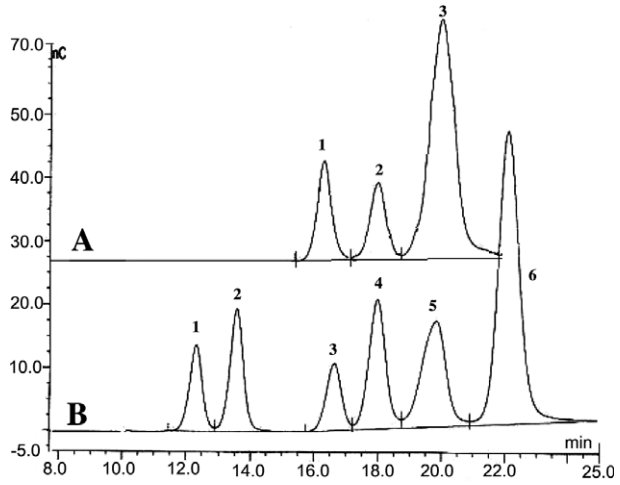

Fig. 3. HPAEC-PAD chromatogram profile of standard monosaccharide mixture solution and hydrolysate of P70-1. (A) Peak identity; 1, D-Gal; 2, D-Glc; 3, D-Man. (B) Peak identity; 1, L-Rha; 2, L-Ara; 3, D-Gal; 4. Glc; 5, D-Man; 6, D-Fru.

IR spectra of P70-1 showed absorption bands at 3381, 2932, 1657 (br), 973, 893, and 849 cm^{-1}. The absorption bands at 849, and 892 cm^{-1} indicated that P70-1 contained both α- and β-type glycosidic linkages in its structure (Barker, Bourne, Stacey, & Whiffen, 1954).

Three fractions were obtained after partial acid hydrolysis of P70-1. The alditol acetates of each fraction hydrolysate were subjected to GC analysis, and the results shown in Table 2. The material retained in the sack after dialyzing of the partial acid hydrolyzate was named as fraction A, and it has the highest molecular size of the three fractions, its structure was proposed closest with P70-1. The component of fraction A, in which the ratio of mannose:glucose:galactose was 7.879:1:1.725, indicated that mannose may be the backbone of the structure of P70-1, and glucose and galactose may be close to this backbone. The analysis results for fraction B and fraction C indicated that the branched structure of P70-1 was composed of glucose and galactose because these sugar residues could be obtained easily after hydrolysis.

The profile of methylated P70-1 on GC–MS appeared in Fig. 4. Methylation analysis of fractions P70-1 showed the presence of six components (Table 3), namely 2,3,4,6-Me_4-Glc, 2,3,4,6-Me_4-Gal, 2,3,4-Me_3-Man, 2,3,6-Me_3-Glc, 2, 3, 4-Me_3-Gal, and 2, 4-Me_2-Man in molar ratio of 1.24:1.28:7.64:1.40:2.29: 2.47 (about 1:1:6:1:2:2). This result showed a good correlation between terminal and branched residues. In addition, these molar ratios also agreed with the overall monosaccharide composition of P70-1 described above.

Table 2
GC analysis results of fractions from partial acid hydrolysis of P70-1

Fractions	Molar ratios		
	D-Mannose	D-Glucose	D-Galactose
A	7.879	1	1.725
B	0.053	1	2.322
C	0	1	1.578

A, precipitation in the sack.
B, supernatant in the sack.
C, fraction out of the sack.

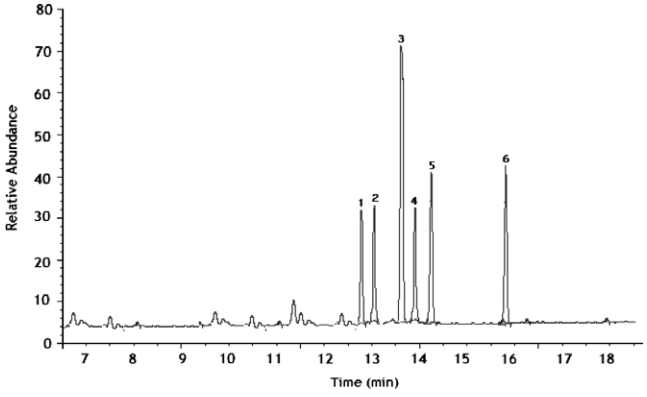

Fig. 4. GC profile of methylated P70-1 on GC-MS. Peak identity: (1) 2,3,4,6-Me_4-Glc, (2) 2,3,4,6-Me_4-Gal, (3) 2,3,4,-Me_3-Man, (4) 2,3,6-Me_3-Glc, (5) 2,3,4-Me_3-Gal, (6) 2,4-Me_4-Man.

The periodate-oxidated product of P70-1 was hydrolyzed and tested by GC. Mannose, glycerol, and erithritol were found on the GC. The presence of mannose indicating a part of mannose is in 1,3-, 1,2,3-, 1,2,4-, 1,3,4-, 1,3,6-, or 1,2,3,4-linkage, which cannot be oxidized by HIO_4. As galactose and glucose were not found in the hydrolyzed product, it could be inferred that galactose and glucose are in linkages that can be oxidized, namely 1-, 1,6-, 1,2-, 1,2,6-, 1,4-, or 1,4,6-linkage. In view of the results from the methylation analysis, it can be concluded that mannose was derived from 1,3,6-linked glucose residues, glycerol from 1,3,6-linked mannose, 1-linked galactose and 1-linked glucose, and erithritol from 1,4-linked glucose.

On periodate oxidation, HIO_4 consumption and formic acid production of the polysaccharide were 1.520 mol/mol sugar residue and 0.721 mol/mol sugar residue, respectively, which was in agreement with the theoretically calculated values (1.615 mol/mol for HIO_4 and 0.769 mol/mol for formic acid) on the basis of the structural features described above.

Based on the data available in the literature (Zhang, 1999), the resonances in the region of 98–106 ppm in the ^{13}C NMR spectrum of P70-1 were attributed to the anomeric carbon atoms of mannopyranose (Man*p*), galactopyranose (Gal*p*) and glucopyranose (Glc*p*), respectively. The peak at 105.0 ppm corresponded to C-1 of β-D-galactopyranose (Gal*p*) residues (Agrawal, 1992). The anomeric

Table 4
Assignment of ^{13} CNMR chemical shifts of P70-1

Sugar residues	Chemical shifts, δ(ppm)					
	C1	C2	C3	C4	C5	C6
α-D-GC*p*(1→	98.2	72.8	79.9	71.3	72.0	61.6
→4)-α-D-GC*p*(1→	99.3	72.0	73.9	79.8	69.8	60.1
→6)-α-D-Man*p*(1→	101.0	72.8	72.8	68.7	71.5	68.5
→6)-β-D-Gal*p*1→	102.2	73.9	75.1	69.8	75.1	69.1
β-D-Gal*p*(1→	105.0	73.9	75.1	71.3	75.1	64.8
→6)-α-D-Man*p*(1→ (3)↑	102.1	72.0	79.8	69.1	71.4	65.8

Table 3
GC-MS results of methylation analysis of P70-1

Methylated sugar	Retention time (min)	Molar ratios	Mass fragments (*m*/*z*) (Relative abundance, %)	Linkage type
2,3,4,6-Me_4-Glc	12.80	1.24	45, 71, 87, 101, 129, 161, 205 (53, 70, 30, 100, 60, 55, 50, 10)	T-
2,3,4,6-Me_4-Gal	13.01	1.28	45, 87, 101, 117, 145, 161, 205 (63, 35, 100, 95, 70, 50, 20)	T-
2,3,4,-Me_3-Man	13.78	7.64	45, 71, 87, 101, 129, 161, 189 (37, 15, 70, 40, 100, 60, 35)	1,6-
2,3,6-Me_3-Glc	13.96	1.40	45, 87, 101, 113, 117, 161, 233 (30, 23, 35, 43, 100, 7, 20)	1,4-
2,3,4-Me_3-Gal	14.29	2.29	45, 87, 101, 189, 233 (7, 23, 98, 40, 100, 20)	1,6-
2,4-Me_2-Man	15.74	2.47	71, 87, 99, 101, 189 (10, 40, 13, 63, 30)	1,3,6-

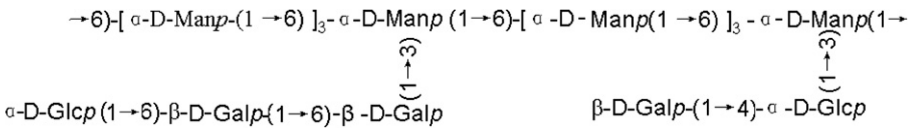

Fig. 5. Predicted structure of P70-1.

carbon atoms signal of the terminal α-D-glucopyranose residues emerged at 98.2 ppm due to link with the O-3 position of β-D-galactopyranose. The assignment of the carbon atoms signals appeared in Table 4.

Both results of partial acid hydrolysis and methylation linkage analysis of P70-1 indicated that (1 → 6)-linked-mannose was the largest amounts residue of the polysaccharide structure, the branched residue was (1 → 3,6)-linked mannose revealing that (1 → 6)-linked-mannose should be possible to form the backbone structure. The relative amounts of (1 → 3,6)-linked-mannose indicated that approximate branch ratios could theoretically be 25%, namely on average one branching point for each four residues of backbone. Residues of branches structure were (1 → 4)-linked-glucose, and (1 → 6)-linked-galactose, which is in agreement with those data of partial acid hydrolysis and Smith degradation.

On the basis of the results obtained above, it was possible to conclude that a repeating unit of P70-1 contains a backbone composed of (1 → 6)-linked-mannose with branches attached to O-3 of glucose and galactose, respectively. The branches probably contain (1 → 4)-linked-glucose, (1 → 6)-linked-galactose and their comparative quantities can be confirmed by molar ratios. Also, methylation analysis of P70-1 revealed the terminal galactose and terminal glucose in molar ratios of 1:1, and the terminal glucose should attached with the (1 → 6)-linked galactose through ^{13}C NMR analysis. From the aforementioned results, it can be concluded that P70-1 is composed of a repeating unit having the possible structure shown in Fig. 5.

3.3. Hydroxyl radical-scavenging activity of P70-1

The hydroxyl radical-scavenging activities of P70-1 and Vc used as a positive control were determined (He et al., 2004), and the results were plotted in Fig. 6. As illustrated in the figure, P70-1 exhibited the scavenging activity towards hydroxyl radicals in a concentration-dependent manner, with an IC_{50} value of 0.548 mg/ml. Under the same conditions, Vc, a free radical scavenger, showed a slightly stronger effect on the hydroxyl radicals, with an IC_{50} value of 0.358 mg/ml.

It is well-known that reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anion and hydrogen peroxide, are related to the pathogenesis of various diseases (Abe & Berk, 1998; Busciglio & Yankner, 1995). Hydroxyl radical is the most reactive among the oxygen radicals and induces severe damage to the adjacent biomolecules (Chance, Sies, & Boveris, 1979). The oxidative injury

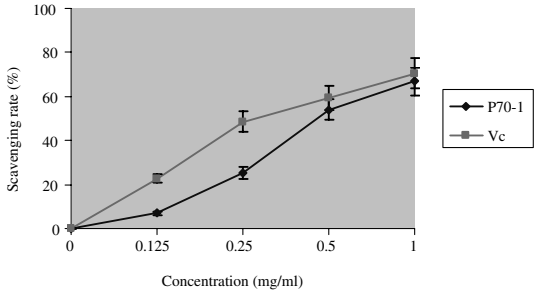

Fig. 6. Hydroxyl radical-scavenging activity of P70-1 and Vc. Values are means ± SD of three determinations.

caused by hydrogen peroxide and superoxide molecules is also associated with the indirect production of hydroxyl radicals via Fenton reaction and/or iron-catalyzed Haber–Weiss reaction (Erel, 2004). Thus, to evaluate the in vitro antioxidant activity of the purified polysaccharide, the hydroxyl radical-scavenging ability was measured by using the Fenton reaction system in the present study.

Polysaccharides from plants, animals and fungi are thought to be a promising group of antioxidative compounds (Yang, Gao, Han, & Tan, 2005). As mentioned above, polysaccharides are one of the main bioactive components in *Cordyceps* spp. Our findings indicated that P70-1 was capable of scavenging hydroxyl radicals, which provided an experimental evidence for supporting the folkloric uses of *C. militaris* as a substitute for *C. sinensis*. To better understand the bioactivity of P70-1, our further study on the mechanism of its scavenging activity against hydroxyl radicals is in the process.

4. Conclusion

The results of this paper showed that the water-soluble polysaccharide P70-1 of cultured *C. militaris* is a heteropolysaccharide and is occasionally branched. The fundamental information obtained from this work is beneficial to the interpretation in the relationship of the polysaccharide structure and its biological functions. The primary results of the in vitro antioxidant assay for the first time demonstrated that P70-1 possesses the hydroxyl radical-scavenging activity, which provides the experimental evidence and scientific explanation for the folkloric uses of *C. militaris* as a substitute for *C. sinensis*.

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