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Effect of culture media on the chemical and physical characteristics of polysaccharides isolated from *Poria cocos* mycelia

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Abstract

Mycelia of a wild strain *Poria cocos* were cultured in two media differing in one constituent: bran extract or corn steep liquor, and are designated as wb and wc, respectively. Six polysaccharide fractions were isolated sequentially from the two mycelia by 0.9% NaCl (PCM1), hot water (PCM2), 0.5 M NaOH (PCM3-I and -II) and 88% formic acid (PCM4-I and -II). Their chemical and physical characteristics were determined by infrared spectroscopy (IR), gas chromatography (GC), ^{13}C NMR, light scattering (LS) and viscometry. The results indicated that wb-, wc-PCM1, and PCM2 were heteropolysaccharides mainly composed of α-D-glucose, mannose, and galactose, whereas wb-PCM3-I and wc-PCM3-I were mainly (1→3)-α-D-glucans, and wb- and wc-PCM3-II, PCM4-I and PCM4-II were (1→3)-β-D-glucans. Interestingly, (1→3) α- and (1→3)-β-D-glucans co-existed in the 0.5 M NaOH fraction and were separated individually into the two fractions (PCM3-I and PCM3-II) after neutralizing with acetic acid. The polysaccharides from wc-PCM cultured in media containing corn steep liquor contained relatively more protein. The polysaccharide fractions also existed in conformations including random coil (as in PCM0 and PCM1) and expanded chain (as in PCM3), and differed molecular mass. In addition, two exo-polysaccharides isolated from the two culture media by methanol precipitation (wb- and wc-PCM0) also differed in their monosaccharide composition.

Keywords: *Poria cocos* mycelia; Polysaccharide; Composition; Molecular mass; Conformation; Light scattering

1. Introduction

Poria cocos, a fungus belonging to the Polyporaceae family, is one of the most important herbs in China and other Asian countries. It has been reported that polysaccharides extracted from *Poria cocos* (mainly glucan) have mitogenic, complement activating,[1] antimutagenic,[2] antitumor,[3,4] and immunological activities.[5] The antitumor polysaccharides differ greatly in their chemical composition, molecular mass, and chain conformation.[6,7] and thus, a basic understanding of both their primary and secondary structures is essential for the study of their bioactivities. *Poria cocos* is mainly composed of the polysaccharide pachyman, a (1→3)-β-D-glucan.[8] Kanayama and co-workers[9] have isolated four polysaccharides from *Poria cocos* mycelia, one of which is a highly branched (1→3)-β-D-glucan containing ~20% (1→6)-β-glucan. The other three are heteropolysaccharides consisting of glucose, galactose, xylose, and mannose. Narui and co-workers[10] indicated that the polysaccharide produced by laboratory cultivation is almost identical with that prepared from naturally occurring sclerotium of *Poria cocos*, and is mainly (1→3)-β-D-glucan with few branches. However, the chemical structures and molecular mass of the polysaccharides from *Poria cocos* sclerotium and mycelium are still not fully understood. In particular, few data have been published on their molecular mass, the conformations, and solution properties.

In our laboratory, two β-D-glucans (PC3, PC4) have been isolated from fresh sclerotium of *Poria cocos*[11] and identified as a linear (1→3)-β-D-glucan for PC3 and a (1→3)-β-D-glucan with a few branches and glucuronic

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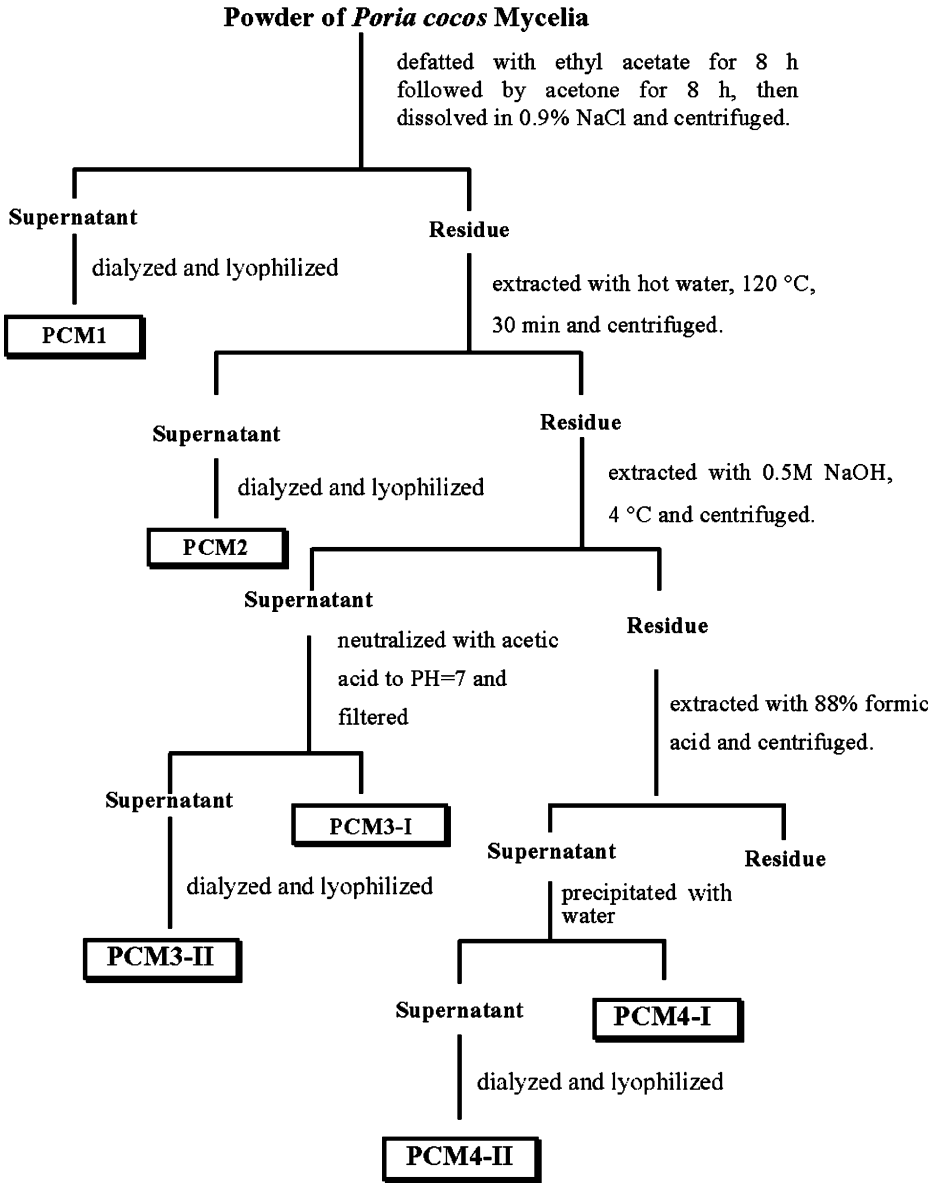

Scheme 1. Extraction of polysaccharides from *Poria cocos* mycelia.

acid for PC4. These polysaccharides are the major fractions of *Poria cocos* polysaccharides. It is worth noting that the glucan PC3 in 0.5 M NaOH aqueous solution could easily aggregate, leading to large apparent molecular mass.[12–14] Dimethyl sulfoxide (Me_2SO) is a good solvent for the β-glucan PC3, which exists predominantly as single chains.[13] The chemical structure, which depends on source, growth conditions and method of isolation has an important bearing on the biological activities of the polysaccharides.[15] However, the dependence of structure and conformation of the *Poria cocos* polysaccharides on source and culture condition has not yet been found. Furthermore, it is still not clear whether α-D-glucan exists in these polysaccharides. Here, we have studied the effect of different culture media used for mycelial cultivation on the chemical composition of the polysaccharides extracted from the mycelia. The chemical structure, molecular mass, conformation and intrinsic viscosity [η] of different polysaccharide fractions isolated were determined by ^{13}C NMR, light scattering, and viscometry.

2. Experimental

2.1. Cultivation of *Poria cocos* mycelia

The cultivation of *Poria cocos* mycelia was performed in Huazhong Agricultural University. The strain coded as No.P0 was obtained from wild *Poria cocos* in Luotian (Hubei, China). The hyphae were inoculated in two

different culture media, the first consisting of D-glucose (25 g), yeast extract (3.2 g), KH_2PO_4 (1 g), $CaCl_2 \cdot 2H_2O$ (0.06 g), $MnCl_2 \cdot 4H_2O$ (5 mg), $MgSO_4 \cdot 7H_2O$ (0.5 g), $ZnCl_2$ (4 mg), $Fe_2(SO_4)_3$ (5 mg), vitamin B_1 (0.1 mg), bran (200 g) extract and water (1 L). The second medium was identical to the first except it had corn steep liquor (10 mL) substituting the bran extract. The hyphae were cultivated under aerobic condition at 24 °C for 14 days. The resulting light-brown mycelia were separated by filtration and washed with water for five times, and then vacuum-dried. In addition, the supernatant from the culture media was also collected to obtain the exo-polysaccharides. The mycelial samples were designated as wb-PCM (wild No.P0, in bran extract) and wc-PCM (wild No.P0, in corn steep liquor).

2.2. Isolation and purification of polysaccharides

Poria cocos mycelia were defatted by Soxhlet extraction with EtOAc for 8 h and then acetone for 8 h. The resulting residue was powdered and then immersed in aq 0.9% NaCl overnight before being centrifuged to give the supernatant, which was then dialyzed and lyophilized to give the first polysaccharide fraction coded as wb- or wc-PCM1. Subsequent isolation of the mycelial polysaccharides by hot water, 0.5 M NaOH and 88% formic acid was carried out as shown in Scheme 1 with the overall extraction process and sample codes outlined in Scheme 1. The collected culture medium was extracted by MeOH to obtain the exo-polysaccharides, coded as wb- or wc-PCM0. Each polysaccharide was purified according to the previous method[16] and finally lyophilized by using lyophilizer (CHRIST Alpha 1-2, Germany).

The concentrated supernatant was decolorized with 30% H_2O_2 and deproteinated by the Sevag method nine times to remove free protein, and then dialyzed (regenerated cellulose tubing; M_w cut-off 8000) against tap water for 5 days and distilled water for 3 days. Each polysaccharide examined by UV spectroscopy (UV-160, Shimadzu, Japan) showed only a main peak at 200 nm for polysaccharide.

2.3. Analysis of chemical composition

Infrared spectra of the polysaccharides were recorded with a Nicolet FT-IR (Spectrum One, Perkin–Elmer Co., USA) spectrometer in the range 4000–400 cm^{-1}, using the KBr-disk method. Protein content in the polysaccharides was measured by a KJELETC 1030 self-analyzer (Switzerland) according to the semi-micro Kjeldahl principle.

Gas chromatography of the alditol acetate derivatives of polysaccharides[17] was performed with an HP 6890 gas chromatograph (Hewlett Packard, USA) using an Alltech DB-225 capillary column (15 m × 0.25 mm) programmed from 180 to 220 °C at 4 °C/min and held at 220 °C for 30 min. The injection sample volume was 2 μL, the carrier gas was helium, and detection was by flame ionization.

High resolution ^{13}C NMR spectra were recorded with an Avance DRX-400 spectrometer (Bruker Co., Germany/Switzerland) at room temperature. The polymer concentration was adjusted to 10% by wt in all experiments. D_2O was used as solvent for PCM0 and PCM1, and (Me_2SO-d_6) for PCM3 and PCM4.

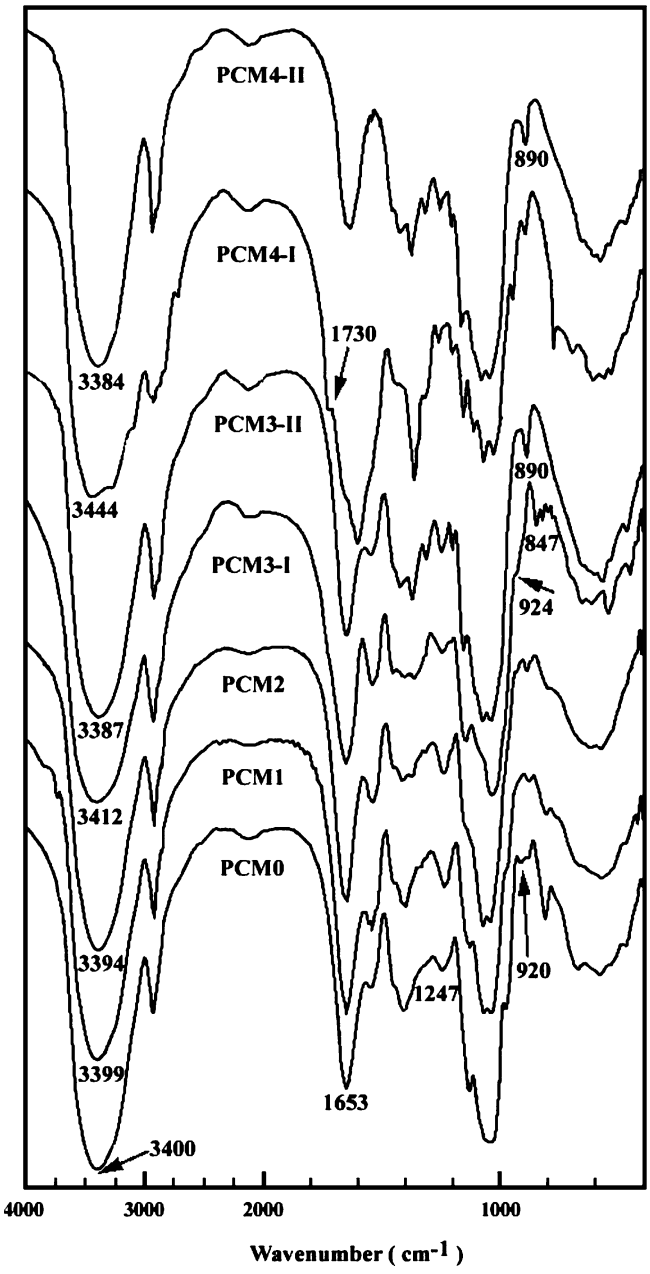

Fig. 1. FT-IR spectra of polysaccharides from wc-PCM.

2.4. Viscometry

Viscosities of the polysaccharide solutions were measured at 30 ± 0.1 °C by using an Ubbelohde capillary viscometer. The 0.5 M NaCl aqueous solutions and Me_2SO (or 0.25 M LiCl–Me_2SO) were used as solvents of the samples, respectively. The kinetic energy correction was always negligible. Huggins and Kraemer plots were used to estimate the intrinsic viscosity [η].

2.5. Laser light scattering (LLS)

The light-scattering intensities of polysaccharides were determined with a multi-angle laser light scattering instrument (MALLS) equipped with a He–Ne laser ($\lambda = 633$ nm; Dawn®DSP, Wyatt Technology Co., USA) in the angles of 42, 49, 63, 71, 81, 90, 99, 109, 118, and 127° at 30 °C. The solutions of desired polysaccharide concentrations were prepared, and optical clarification of the solution was achieved by filtration through an 0.2 μm pore size filter (Whatman, England) into the scattering cell (SV mode). The refractive index increments (dn/dc) were measured with an Optilab refractometer (Dawn®DSP, Wyatt Technology Co., USA) at 633 nm and 30 °C. The dn/dc values of samples in aqueous 0.5 M NaCl and in Me_2SO were determined to be 0.142 and 0.058 mL g^{-1}, respectively. Astra software (Version 4.70.07) was utilized for data acquisition and analysis.

2.6. SEC-LLS measurements

Size-exclusion chromatography combined with laser light scattering (SEC-LLS) measurements were carried out on a Dawn®DSP laser photometer already mentioned, combined with a P100 pump (Thermo Separation Products, San Jose, USA) equipped with TSK-GEL G5000 and G3000 PWXL columns (7.8 mm × 300 mm) in series for aqueous solution, or a G4000 H_6 column (7.5 mm × 300 mm) for Me_2SO at 30 °C. A differential refractive index detector (RI-150) was simultaneously connected. The eluent was aq 0.5 M NaCl or Me_2SO with a flow rate of 1.0 mL/min. All solutions having a polysaccharides concentration of 1.0×10^{-3}–2.0×10^{-3} g/mL were filtered first with a sand filter followed by a 0.45 μm filter (Whattman, England), then kept in sealed glass bottles before being injected onto the SEC column. Astra software (Version 4.70.07) was utilized for the data acquisition and analysis.

3. Results and discussion

3.1. Chemical composition of PCM

The IR spectra for the samples wc-PCM0–wc-PCM4-II are shown in Fig. 1. All samples exhibited the characteristic IR absorption of polysaccharide at 1650 and 1250 cm^{-1}. The IR absorption at 800 cm^{-1} of wc-PCM0 and wc-PCM1 was the characteristic absorption of mannose. The polysaccharides wc-PCM0 and wc-PCM3-I showed IR absorption at 850 and 920 cm^{-1}, characteristic of an α-D-glucan.[18] In contrast, wc-PCM3-II, wc-PCM4-I, and wc-PCM4-II showed clearly IR absorption at 890 cm^{-1}, indicating the existence of a β-D-glucan. Obviously, the samples wc-PCM0, wc-PCM1, wc-PCM2 and wc-PCM3-I contained α-glucan, but wc-PCM3-II, wc-PCM4-I and wc-PCM4-II were

Table 1
Monosaccharide composition, protein content, and yield of the polysaccharides from *Poria cocos* mycelia

Sample	Monosaccharide content in polysaccharide (%)						Protein (%)	Yield (%)
	Fuc	Ara	Xyl	Man	Gal	Glc		
wb-PCM0	–	6.1	3.9	11.4	5.9	71.7	12.8	
wb-PCM1	–	–	–	7.7	19.2	73.1	7.6	1.3
wb-PCM2	–	+	+	0.9	1.3	95.9	8.5	2.0
wb-PCM3-I	–	–	1.0	2.2	+	95.6	–	20.5
wb-PCM3-II	–	2.6	2.0	1.2	2.0	91.4	21.5	3.6
wb-PCM4-I	–	–	+	5.8	–	94.1	–	1.3
wb-PCM4-II	–	–	–	–	23.9	76.1	2.0	1.9
wc-PCM0	4.1	3.0	2.5	61.7	15.0	13.7	19.0	
wc-PCM1	10.5	–	–	24.5	27.5	37.5	30.6	1.8
wc-PCM2	3.4	–	–	12.5	13.4	70.7	29.5	3.1
wc-PCM3-I	–	–	6.4	16.7	–	76.9	20.2	7.7
wc-PCM3-II	–	–	–	+	+	98.9	15.2	3.7
wc-PCM4-I							39.0	4.0
wc-PCM4-II							8.5	2.2

–, not detected; +, trace amount; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

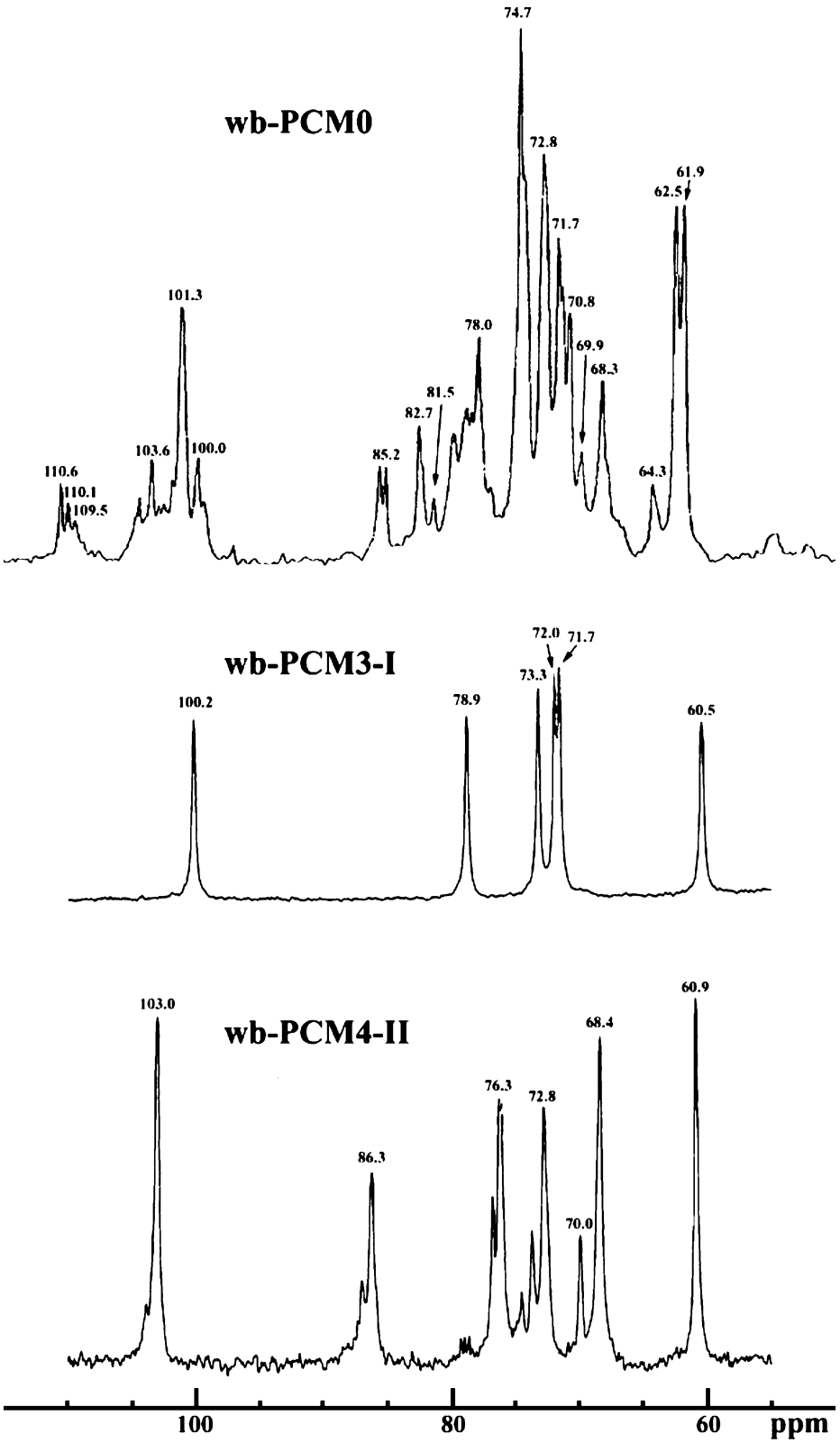

Fig. 2. ^{13}C NMR spectra of the polysaccharides from wb-PCM: wb-PCM0 in D_2O, wb-PCM3-I, and wb-PCM4-II in Me_2SO-d_6.

mainly composed of β-glucan. The IR spectra of the wb-PCM samples showed tendencies similar to those mentioned above. Furthermore, the samples wb-PCM4-I, wb-PCM4-II, and wc-PCM4-II exhibited peaks at 1729–1738 cm^{-1} (COO^-), suggesting the presence of glucuronic acid.[19]

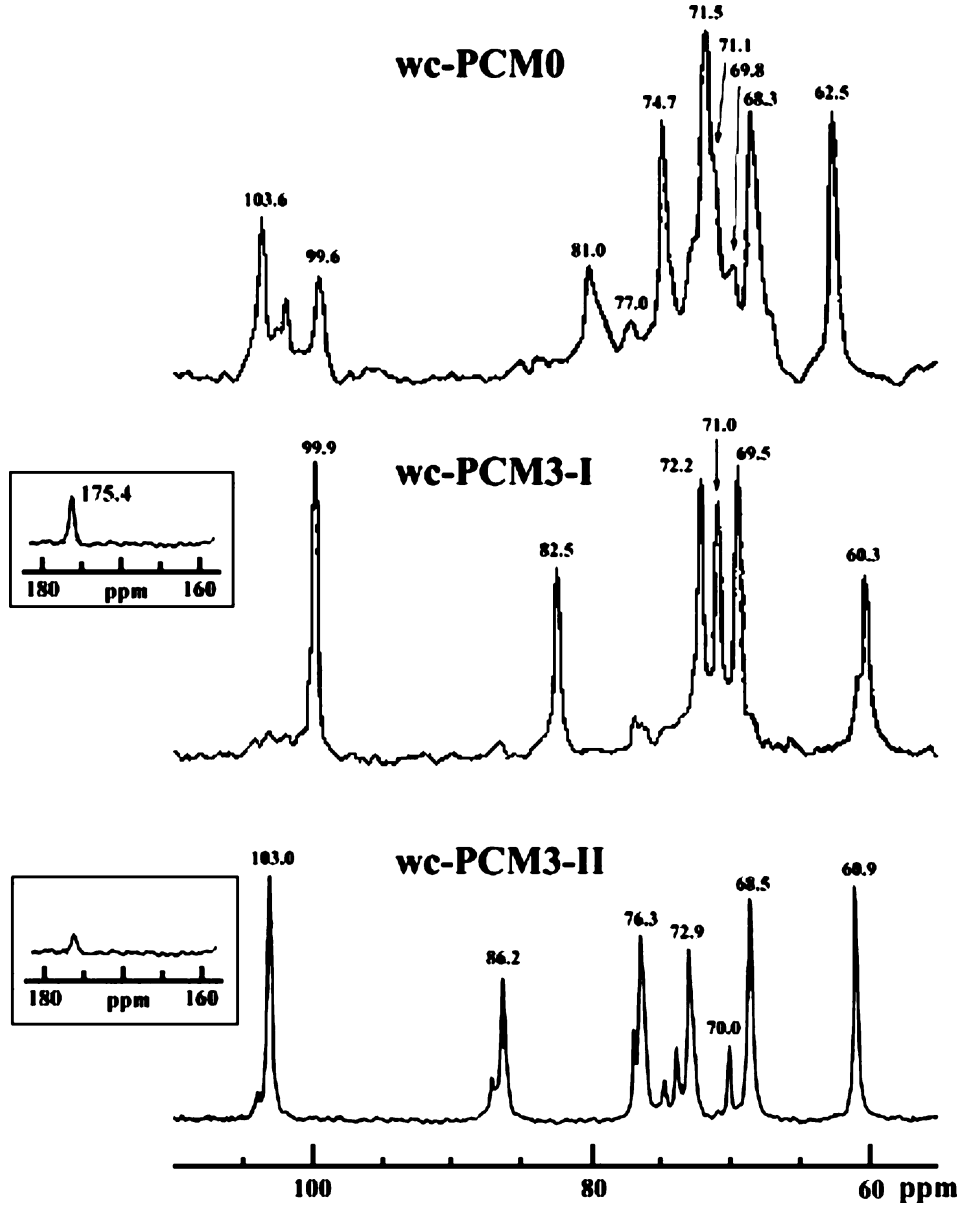

Fig. 3. ^{13}C NMR spectra of the polysaccharides from wc-PCM: wc-PCM0 in D_2O, wc-PCM3-I, and wc-PCM3-II in Me_2SO-d_6.

GC traces of the polysaccharides hydrolyzates, in comparison with standard saccharides showed the monosaccharide component, are summarized in Table 1, together with protein content and yields of the polysaccharides. The results indicated that glucose is the predominant monosaccharide, and its content increases with the progress of isolation. The polysaccharides PCM3-I, PCM3-II, PCM4-I, and PCM4-II consisted mainly of glucose. PCM1 and PCM2 are heteropolysaccharides containing mannose, galactose, glucose and traces of other monosaccharides. In addition, PCM0 contains arabinose, xylose, mannose, galactose, and glucose. Obviously, the protein content in the wc-PCM polysaccharides cultured from the media containing corn steep liquor was higher than that in the wb-PCM from the media containing bran extract. The protein was probably bound to the polysaccharides, because the Sevag procedure was repeated over nine times to remove free protein. Moreover, the overall yield of the wc-PCM polysaccharides was higher than that of wb-PCM. The samples wb-PCM0 and wc-PCM0 all were heteropolysaccharides containing arabinose, xylose, mannose, galactose, and glucose, but the predominant monosaccharide of wc-PCM0 was mannose and that of wb-PCM0 was glucose. In addition, wb-PCM0 contained relatively more galactose.

The ^{13}C NMR spectra of some polysaccharides are shown in Figs. 2 and 3. The strong signals at 101.3, 82.7, 71.7, 70.8, 69.9, 61.9 and 64.3 ppm for wb-PCM0 were assigned as C-1, C-3, C-5, C-2, C-4, C-6 and C-6_s of

Table 2
Comparison of ^{13}C NMR chemical shifts for the polysaccharides in solution state

Sample	Solvent	Chemical shift (ppm)							–CONH– in protein	Source
		C-1	C-2	C-3	C-4	C-5	C-6	$C\text{-}6_s$		
(1→3)-α-D-glucan	Deuterium oxide	100.3	71.4	81.7	70.8	72.7	61.5			Ref. 23
	0.25 M LiCl–Me_2SO-d_6	99.3	70.9	82.2	69.5	71.9	60.4			Ref. 24
wb-PCM3-I	Me_2SO-d_6	100.2	72.0	78.9	71.7	73.3	60.5			This work
wc-PCM3-I	Me_2SO-d_6	99.9	71.0	82.5	69.5	72.2	60.3		175.4	This work
Branched (1→3)-β-D-glucan	Me_2SO-d_6	103.0	72.8	86.2	68.4	76.3	60.9	69.9		Ref. 25
	Me_2SO-d_6	103.0	72.6	86.3	68.5	76.3	61.0	70.7		Ref. 26
wb-PCM4-II	Me_2SO-d_6	103.0	72.8	86.3	68.4	76.3	60.9	70.0		This work
wc-PCM3-II	Me_2SO-d_6	103.0	72.9	86.2	68.5	76.3	60.9	70.0	175.6	This work

branched (1→3)-α-D-glucan.[20,21] The peaks at 100.0 (C-1), 78.0 (C-5), 74.7 (C-3), 72.8 (C-2), 68.3 (C-4) and 62.5 (C-6) ppm are assigned to β-linked D-mannose.[22] The relatively small peaks at 110.6, 110.1, 109.5 (C-1), 85.2 (C-4), and 64.3 (C-6) ppm are assigned to chemical shifts of β-linked D-galactose.[22] The data indicated that the polysaccharides in wb-PCM0 were mainly (1→3)-α-D-glucan, containing other sugar units such as β-linked D-mannose and β-linked D-galactose. As comparing to wb-PCM0, the ^{13}C NMR spectra of wc-PCM0 were different. The strong signals at 103.6, 99.6 (C-1′, C-1), 81.0 (C-2) and 62.5 (C-6) are assigned to a branched α-D-mannopyranan,[22] indicating that wc-PCM0 was mainly comprised of α-D-mannose residues with branching, similar to the results shown in Table 1. These results are in good agreement with IR and GC data, indicating that wb-PCM0, wb-PCM1, wc-PCM0, and wc-PCM1 were heteropolysaccharides, and the glucan content increased progressively along the fractionation scheme. Chemical shift data for the ^{13}C NMR spectra of the samples wb-PCM3-I, wb-PCM4-II, wc-PCM3-I, and wc-PCM3-II, together with data from literature are given in Table 2. The results indicate that the principal component of wb-PCM3-I and wc-PCM3-I was (1→3)-α-D-glucan, and that of wb-PCM4-II and wc-PCM3-II

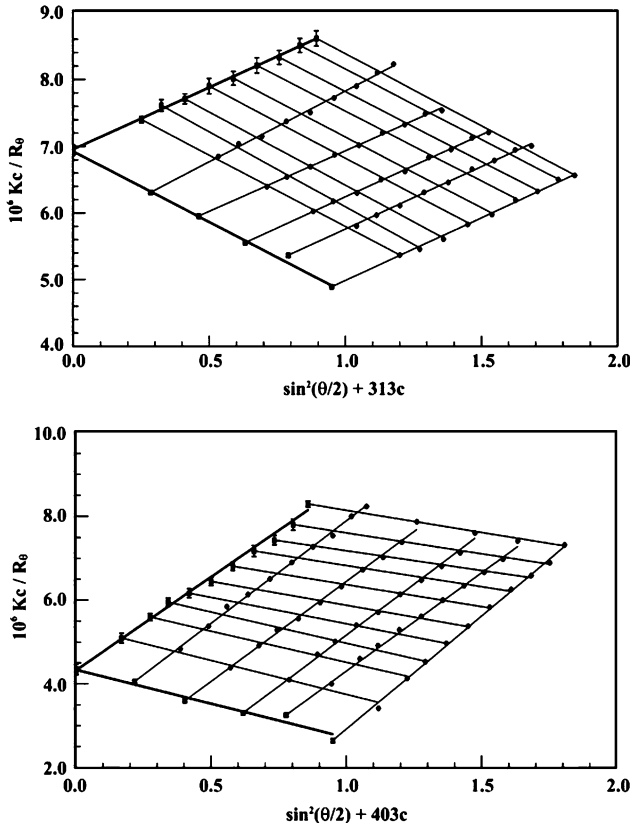

Fig. 4. Zimm plots for wb-PCM0 in 0.5 M NaCl aqueous solution (top) and for wb-PCM3-I in 0.25 M LiCl–Me_2SO (bottom) at 30 °C.

Table 3
Experimental results from LLS and Viscosity for the polysaccharides from *Poria cocos* mycelia at 30 °C

Sample	Solvent	$[\eta]$ ($cm^3\ g^{-1}$)	$M_W \times 10^{-4}$ ($g\ mol^{-1}$)	$\langle s^2\rangle^{1/2}$ (nm)
wb-PCM0	0.5 M NaCl	9.6	14.4	33.6
wb-PCM1	0.5 M NaCl	14.1	33.3 [a]	42.3
wb-PCM2	0.5 M NaCl	27.8		
wb-PCM3-I	0.25 M LiCl–Me_2SO	67.8	23.1	60.1
wb-PCM3-II	Me_2SO	17.0	11.3 [a]	28.4
wb-PCM4-I	Me_2SO		132.3 [a]	96.6
wb-PCM4-II	Me_2SO	88.6	121.1 [a]	119.5
wc-PCM0	0.2 M NaCl	9.4	9.2	20.7
wc-PCM1	0.2 M NaCl	13.7	26.2 [a]	38.0
wc-PCM2	0.2 M NaCl	62.4	89.2 [a]	38.1
wc-PCM3-I	Me_2SO	67.6	54.5	87.3
wc-PCM3-II	Me_2SO	12.5	8.9	39.0

[a] Data obtained from SEC-LLS.

was (1→3)-β-D-glucan.[23–25] The C-6_s signal in the wb-PCM4-II spectrum represents substituted C-6 of branched (1→3)-β-D-glucan,[26–28] indicating a branched glucan. Interestingly, two kinds of α-glucan (PCM3-I) and β-glucan (PCM3-II) co-existed in the 0.5 M NaOH aqueous extract from wc-PCM, thus resembling the polysaccharides isolated from the fruiting bodies of *Lentinus edodes*,[20] in which two kinds of α- and (1→3)-β-D-glucan co-existed in the extract with aqueous 5% NaOH/0.05% $NaBH_4$.

The signal at 175.4 ppm in the ^{13}C NMR spectrum of wc-PCM3-I was assigned to the –CONH– group of protein.[29] There were no obvious chemical shifts of the C atoms in the glucan, indicating that the (1→3)-α-D-glucan was probably bound mainly with protein by non-covalent bonding in the sample wc-PCM3-I. The ^{13}C NMR results supported the conclusion from Table 1, namely that the polysaccharides cultured in corn media contained relatively more protein. The intermolecular interaction between the –OH groups of glucan and –CONH– groups of protein resulted in ^{13}C chemical shift variation of the samples, wb-PCM3-I and wc-PCM3-I. Intra- and inter-molecular hydrogen bonds exist in the α-glucan of wb-PCM3-I, resulting in an ordered structure. The solubility of wb-PCM3-I was also significantly lower than that of wc-PCM3-I, in which the protein perturbed the ordered structure of the α-glucan. Thus, the intra- and inter-molecular splitting interactions in the α-glucan of wb-PCM3-I had an effect on the chemical shift, shifting C-3 upfield (78.9 ppm), and the C-2, C-4, C-5 peaks downfield, as compared with the sample wc-PCM3-I. The chemical shift variations are similar to that of lentinan in Me_2SO and (3:7) Me_2SO–water, in which order–disorder conformation change occurred.[30] Therefore, the variations in culture media significantly affected the chemical components and structures of the polysaccharides from *Poria cocos* mycelia.

3.2. Molecular mass and viscosity

Fig. 4 shows Zimm plots for wb-PCM0 and wb-PCM3-I. Here K is the light scattering constant, R_θ is the reduced Rayleigh ratio at angle θ, and c is polysaccharide concentration. From of LLS, SEC-LLS and viscometry measurements, the weight-average molecular mass M_w, root mean square radius of gyration $\langle s^2\rangle^{1/2}$, and intrinsic viscosity $[\eta]$ values of the polysaccharides in 0.5 M NaCl aqueous solutions, Me_2SO, and 0.25 M LiCl–Me_2SO are summarized in Table 3. Generally, the relatively higher $[\eta]$ and $\langle s^2\rangle^{1/2}$ values found in wb-PCM3-I, wc-PCM3-I, and wb-PCM4-II reflected a relatively expanded chain of the polymer, such as the (1→3)-α-D-glucan from *Lentinus edodes*.[31]

Based on the analysis of M_w and $[\eta]$, the molecules of wb-PCM0, wb-PCM1, wc-PCM0, and wc-PCM1 exist as random coils in aqueous NaCl. The relatively higher $[\eta]$ indicated that the molecules of wb-PCM3-I existed as a more expanded flexible chain in 0.25 M LiCl–Me_2SO than that of the wc-PCM3-I. In general, relatively higher values of $[\eta]$ and $\langle s^2\rangle^{1/2}$ of a polymer with same M_w reflect expanded chains. The relatively lower value of $[\eta]$ for wc-PCM3-I suggested that the protein included in the α-glucan results in a relatively compact coil because of the globular protein conformation.[32] The M_w values for the β-glucan in wb-PCM3-II and wc-PCM3-II are 11.3×10^4 and 8.9×10^4, close to the values of 16.8×10^4 for the (1→3)-β-D-glucan from the *Poria cocos* mycelia.[33] The polysaccharides from *Poria cocos* mycelia cultured in different media thus have dissimilar chemical components, molecular weights, and conformations.

Acknowledgements

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References

1. Yamada, H.; Kiyohara, H.; Takemoto, N.; Zhao, J. F.; Kawamura, H.; Komatsu, Y.; Cyong, J. C.; Aburada, M.; Hosoya, E. *Planta Med.* **1992**, *58*, 166–170.
2. Nunoshiba, T.; Ohtsuka, A.; Nishika, H.; Suemitsu, R. *Sci. Eng. Rev. Doshisha Univ.* **1990**, *30*, 266–272.
3. Chihara, G.; Hamuro, J.; Maeda, Y.; Arai, Y.; Fukuoka, F. *Nature* **1970**, *225*, 943–944.
4. Kanayama, H.; Adachi, N.; Togami, M. *Chem. Pharm. Bull.* **1983**, *31*, 1115–1118.
5. Wang, S. X.; Wen, Y. Y.; Hu, C. X. *Phytother. Res.* **1995**, *9*, 448–451.
6. Borchers, A. T.; Stern, J. S.; Hackman, R. M.; Keen, C. L.; Gershwin, M. E. *Proc. Soc. Exp. Biol. Med.* **1999**, *221*, 281–293.
7. Young, S. H.; Jacobs, R. R. *Carbohydr. Res.* **1998**, *310*, 91–99.
8. New College of Medicine, *Dictionary of Chinese Medicine,* Shanghai Press of Science and Technology, 1977, pp. 1596–1597.
9. Kanayama, H.; Adachi, N.; Fukai, Y. *Yakugaku Zasshi* **1986**, *106* (3), 199–205.
10. Narui, T.; Takahashi, K.; Kobayashi, M.; Shibata, S. *Carbohydr. Res.* **1980**, *87*, 161–163.
11. Zhang, L.; Ding, Q.; Zhang, P.; Feng, H. *Chem. J. Chin. Univ.* **1997**, *6*, 990–993.
12. Zhang, L.; Ding, Q.; Zhang, P.; Zhu, R.; Zhou, Y. *Carbohydr. Res.* **1997**, *303*, 193–197.
13. Ding, Q.; Jiang, S.; Zhang, L.; Wu, C. *Carbohydr. Res.* **1998**, *308*, 339–343.
14. Zhang, L.; Ding, Q.; Meng, D.; Ren, L.; Yang, G.; Liu, Y. *J. Chromatogr. A* **1999**, *839*, 49–55.
15. Srivastava, R.; Kulshreshtha, D. K. *Phytochemistry* **1989**, *28*, 2877–2883.
16. Zhang, L.; Yang, L.; Ding, Q.; Chen, X. *Carbohydr. Res.* **1995**, *270*, 1–10.
17. Englyst, H. N.; Quigley, M. E.; Hudson, G. J. *Analyst* **1994**, *119*, 1497–1509.
18. Sandula, J.; Kogan, G.; Kacurakova, M.; Machova, E. *Carbohydr. Polym.* **1999**, *38*, 247–253.
19. Krcmar, P.; Novotny, C.; Marais, M. F.; Joseleau, J. P. *Intern. J. Biol. Macro.* **1999**, *24*, 61–64.
20. Zhang, P.; Zhang, L.; Cheng, S. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 1197–1202.
21. Zhang, L.; Zhang, M.; Zhou, Q.; Chen, J.; Zeng, F. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2172–2178.
22. Gorin, P. A. J. *Advances in Carbohydrate Chemistry and Biochemistry*; Vol. 38; Academic Press Inc, 1981; pp 13–104.
23. Jansson, P. E.; Kenne, L.; Schweda, E. *J. Chem. Soc. Perkin Trans. I* **1988**, 2729–2736.
24. Chen, J.; Zhou, J.; Zhang, L.; Nakamura, Y.; Norisuye, T. *Polym. J.* **1998**, *30*, 838–842.
25. Kogan, G.; Alfoldi, J.; Masler, L. *Biopolymers* **1988**, *27*, 1055–1063.
26. Misaki, A.; Kakuta, M.; Sasaki, T.; Tanaka, M.; Miyaji, H. *Carbohydr. Res.* **1981**, *92*, 115–129.
27. Deng, C.; Yang, X.; Gu, X.; Wang, Y.; Zhou, J.; Xu, H. *Carbohydr. Res.* **2000**, *328*, 629–633.
28. Bao, X.; Liu, C.; Fang, J.; Li, X. *Carbohydr. Res.* **2001**, *332*, 67–74.
29. Temeriusz, A.; Anulewicz, R.; Wawer, I.; Krygowski, T. M.; Bartoszewicz, B. P.; Rowinska, M. *Carbohydr. Res.* **2001**, *334*, 71–79.
30. Zhang, L.; Li, X.; Zhou, Q. *Polym. J.* **2002**, *34* (6), 1–7.
31. Zhang, P.; Zhang, L.; Cheng, S. *Carbohydr. Res.* **2002**, *337*, 155–160.
32. Wu, Q.; Zhang, L. *J. Appl. Polym. Sci.* **2001**, *82*, 3373–3380.
33. Ding, Q.; Zhang, L.; Wu, C. *J. Polym. Sci. Part B: Polym. Phys.* **1999**, *37*, 3201–3207.