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Structure of polysaccharides from the fruiting body of *Hericium erinaceus Pers*

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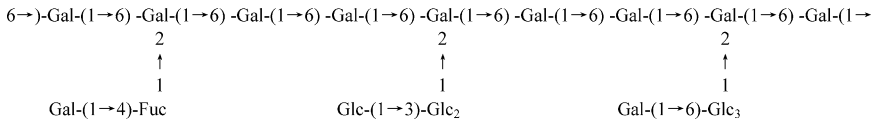
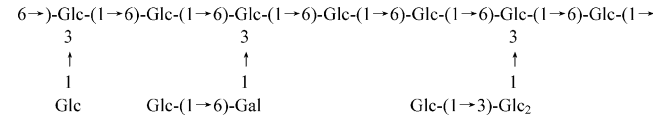
Abstract

The water-soluble crude polysaccharide HP, obtained from the fruiting body of *Hericium erinaceus Pers* by boiling-water extraction and ethanol precipitation, was fractionated by DEAE-Sepharose CL-6B column chromatography, giving two polysaccharide fractions termed HPA and HPB. The polysaccharide of HPA consists of Glc, Gal and Fuc in the ratios 1:2.110:0.423, and HPB contains the monosaccharides Gal and Glc in molar ratios of 1:11.529. On the basis of methylation and GC-MS analysis, periodate oxidation-Smith degradation, and partial acid hydrolysis, the repeating units of HPA and HPB were established:

HPA



HPB



Keywords: *Hericium erinaceus Pers*; Structural analysis; Polysaccharide

1. Introduction

Hericium erinaceus is a well known edible and medicinal mushroom in oriental countries. This mushroom is called 'yamabushitake' and 'houtou' in Japan and China, respectively (Byung, Jun, & Chi, 2003). Recently, *Hericium erinaceus* has attracted great attention owing to its antimicrobial effect (Kim, Pyun, Ko, & Park, 2000), anti-tumor activities (Mizuno, Sation, Nishitoba, & Kawagishi, 1995; Mizuno, Wasa, Ito, Suzuki, & Ukai, 1992), immunomodulatory effect (Liu, Fang, & Xiao, 2002), antioxidant properties (Mau, Lin, & Song, 2002), cytotoxic effect (Kuwahara, Morihiro, Nemoto, & Hiramatsu, 1992), and promotion of synthesis of neurogrowth factor (Kawagishi et al., 1994; Lee et al., 2000). The cultures of *H. erinaceus* or their extracts processed in tablets have been put into production on a large scale, mainly for curing gastric ulcer and chronic gastricism (Lu, Li, & Cang, 2002). *Hericium erinaceus Pers*, belonging to the same genus, is not studied now. In this study, we report on the structural features and properties of two polysaccharide fractions isolated from water extract of the fruiting body of *Hericium erinaceus Pers*. In addition, their activities of curing and preventing against chronic gastricism are identified.

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2. Experimental

2.1. Materials

The fruiting body of *Hericium erinaceus Pers* was supplied by Biological Institute of JiLin Province (China). The columns of Sepharose CL-6B and Sephadex G-100 were Pharmacia products of Sweden. All other reagents were of the highest available quality in China.

2.2. General methods

Optical rotation was measured with a WZZ-T_1 polarimeter (Shanghai Physical Optics Instrument Co.). Paper chromatography (PC) and gas chromatography (GC) were used for identification and quantification. PC was performed on Xinhua No. 1 paper (7 cm × 40 cm) using the solvent system: 4:1:5 *n*-butyl alcohol–acetic acid–water and visualized by spraying with phthalic acid reagent (0.9 ml of aniline and 1.6 g of phthalic acid were dissolved in 100 ml of water-saturated *n*-butanol) and heating at 100 °C for 15 min. GC was performed on a Vavian 3400 instrument (Hewlett-Packard Component, USA) equipped with SE-30 column (50 mm × 0.20 mm × 0.25 μm). The column temperature was kept at 120 °C for 2 min and then increased to 250 °C for 3 min at a rate of 8 °C/min. Gas chromatography-mass spectrometry (GC-MS) was done on a HP5890(II) instrument(Hewlett-Packard Component, USA) with an HPS quartz capillary column (25 m × 0.22 mm × 0.2 nm), and at temperatures programmed from 120 to 140 °C at 1 °C/min. Infrared (IR) spectroscopy of the samples was recorded on SPECORD in a range of 400–4000 cm^{-1}. Gel filtration chromatography was carried out on columns of Sepharose CL-6B and Sephadex G-100 (Pharmacia) using 0.15 M NaCl. The column was calibrated with T-series dextrans of known molecular weights (Mrs). All gel filtration chromatography was monitored with phenol–H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.3. Isolation and purification of polysaccharides

The fruiting body of *Hericium erinaceus Pers* was extracted with 1 l of water at 100 °C for 2 h, and repeated twice. After each extraction, the soluble polymers were separated from residues by filtration, and extracts were combined, concentrated and dialyzed against running water for 48 h. The above extract was submitted to graded precipitation with three volumes of ethanol. The precipitate was collected by centrifugation, washed successively with ethanol and acetone, then dried at 45 °C at reduced pressure, giving HP (8%) as a crude polysaccharide.

HP was added to 200 mg proteinase, dialyzed in distilled water at 37 °C for 48 h (changing water once every 4 h), and then the aqueous sugar solution was treated with Sevag reagent (1-butanol:chloroform = 1:4); after full oscillation and centrifugation, the supernatant solution was retreated in this manner until there was no free protein (Staub, 1965). Then HP was treated with 30% H_2O_2 to decolorize, and dialyzed against tap water and distilled water for 48 h, respectively. The resulting polysaccharide solution was concentrated, frozen and freeze-dried.

Size-exclusion chromatography was used for the fractionation of this preparation. The sample (100 mg) was dissolved in distilled water, centrifuged, and then the supernatant was injected to a column of DEAE-Sepharose CL-6B equilibrated with 0.9% sodium chloride. After loading with sample the column was eluted with NaCl aqueous solution (0.15 M) and different concentrations of NaCl aqueous solution (0.15 and 3.9 M) stepwise at 8 ml/12 min. HPA (17.8 mg) and HPB (26.5 mg) were obtained from NaCl eluate, and then purified by gel filtration chromatography on a column of Sephadex G-100 (2.6 cm × 90 cm).

2.4. Monosaccharide composition and properties

Total carbohydrate, protein, and sugar component of these polysaccharides were determined by the phenol–sulfuric acid, Kjeldahl, and GC, respectively. The molecular weight was calculated by the calibration curve obtained by using various standard dextrans (Wang, Liang, & Zhang, 2001).

2.5. Partial hydrolysis with acid

Polysaccharide sample was hydrolyzed with 0.05 M trifluoroacetic acid, kept at 95 °C for 16 h, centrifuged, dialyzed the supernatant with distilled water for 48 h, and then diluted the solution in the sack with ethanol. After hydrolyzation, the precipitate and supernatant in the sack and the fraction out of sack were dried, and then GC analysis was carried out.

2.6. Periodate oxidation-Smith degradation

For analytical purpose, 25 mg of the polysaccharide (HPA or HPB) were dissolved in 12.5 ml of distilled water and 12.5 ml of 30 mmol/l $NaIO_4$ were added. The solution was kept in the dark at RT, 0.1 ml aliquots were withdrawn at 3–6 h intervals, diluted to 25 ml with distilled water and read in a spectrophometer at 223 nm (Alfred, Leigh, & Giuseppe, 2001). Glycol (2 ml) was added, and then the experiment of periodate oxidation was over. The solution of periodate product (2 ml) was sampled to calculate the yield of formic acid by 0.00523 M sodium hydroxide, and the rest was extensively dialyzed against tap water and distilled water for 24 h, respectively. The content inside was concentrated and reduced with sodium borohydride (80 mg), and the mixture was left for 24 h at room temperature, neutralized to pH 6.0 with 50% acetic acid, dialyzed as described above, and concentrated to a volume (10 ml). One-third of solution described above was freeze-dried and analyzed with GC.

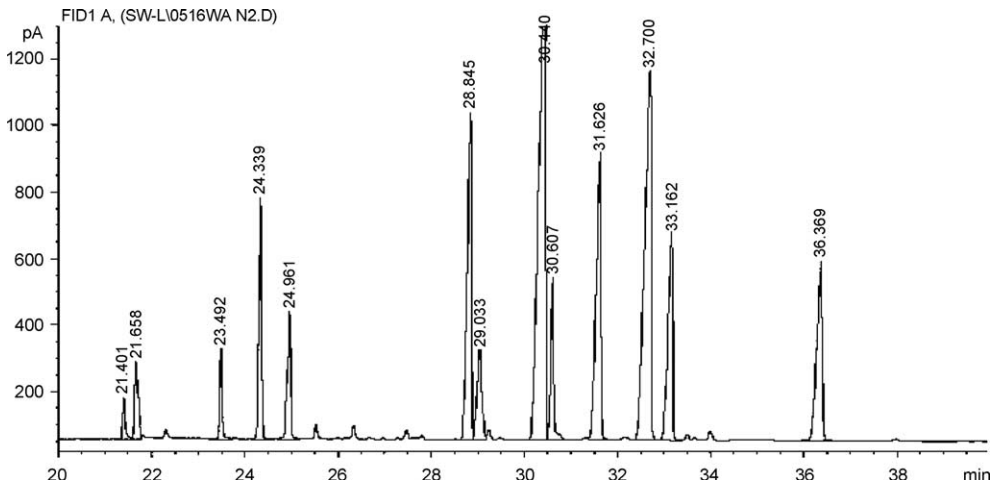


Fig. 1. Gas chromatogram of HP I.

Others were added to the same volume of 1 M sulfuric acid, kept for 40 h at 25 °C, neutralized to pH 6.0 with barium carbonate, and filtered. The filtrate was dialyzed as foresaid, and the content out of sack was desiccated for GC analysis; the content inside was diluted with ethanol, and after centrifugation, the supernatant and precipitate were also dried out for the GC analysis.

2.7. Methylation analysis

Polysaccharide (20 mg) was dissolved in dimethylsulfoxide (6 ml), removed air with nitrogen, stirred for 15 h at 25 °C, and then sodium hydroxide–dimethylsulfoxide combined solution was added (6 ml); after blending well, the mixture was methylated with 3.6 ml methanol iodide, reacting for 7 min exactly, the product was dialyzed against running water and distilled water for 24 h, respectively, then desiccated, and the product was retreated twice as described above.

The solution was concentrated to 10 ml, treated thrice with equivolume chloroform, fully shocked to extract the methylated polysaccharide, after watered, dried over sodium sulfate for 24 h, filtered and evaporated to 1 ml, then examined by IR spectrum. No absorption peak of hydroxyl identified the complete methylation.

The desiccated methylated polysaccharide was subjected to 1 ml formic acid, suffused with nitrogen, kept at 100 °C for 4 h, got rid of excess formic acid by methanol, dried in a vacuum desiccator, then hydrolyzed in 2 M trifluoroacetic acid for 6 h at 100 °C, treated with ethanol then distilled water, reduced with sodium borohydride for 24 h, followed by acetylation with acetic anhydride–pyridine(1:1) at 100 °C for 2 h.

The resulting alditol acetates were subjected to GC and GC-MS analyses. Linkages were identified on the basis of relative retention time and fragmentation pattern (Needs & Selvendran, 1993). The molar ratios for each sugar were calibrated using the peak areas and response factor of the flame-ionisation detector in GC.

3. Results and discussion

3.1. Isolation, purification and composition of polysaccharides

The crude polysaccharide HP was isolated from the hot-water extract of *Hericium erinaceus Pers* by a yield of 8%. After fractionation on DEAE-Sepharose CL-6B column, HPA (17.8%) and HPB (29%) were obtained from the NaCl eluate. The two fractions were purified by gel chromatography on Sphadex G-100 column, respectively, and showing only one symmetrical peak indicating that no other polysaccharide was present in the sample. We came to a conclusion that HPA and HPB were homogeneous by the following tests. They were both eluted as a single peak from gel-filtration chromatography on Sphadex G-100 column which was equilibrated in 0.9% sodium chloride and gave a single spot on cellulose acetate pellicle electrophoresis (borax–sodium hydroxide buffer, pH 10) at 40 V for 50 min with detection using Toluidine Blue; besides they had the same optical rotation in different low content aqueous ethanol by WZZ-T_1 automatic optical polarimeter at room temperature, respectively; moreover average molecular weight of

Table 1
Components of monosaccharides and properties of fractions from *Hericium erinaceus Pers*

Samples	HPA	HPB
Average molecular weights	5.0×10^4	3.0×10^4
$[\alpha]_D$ (deg)	+64	+93.7
Protein (wt%)	nd	13.5
Carbohydrate (wt%)	100	85
Sugar component (mol)		
Glc	1	11.529
Gal	2.110	1
Fuc	0.423	

nd, not detected; Glc, glucose; Gal, galactose; Fuc, fucose.

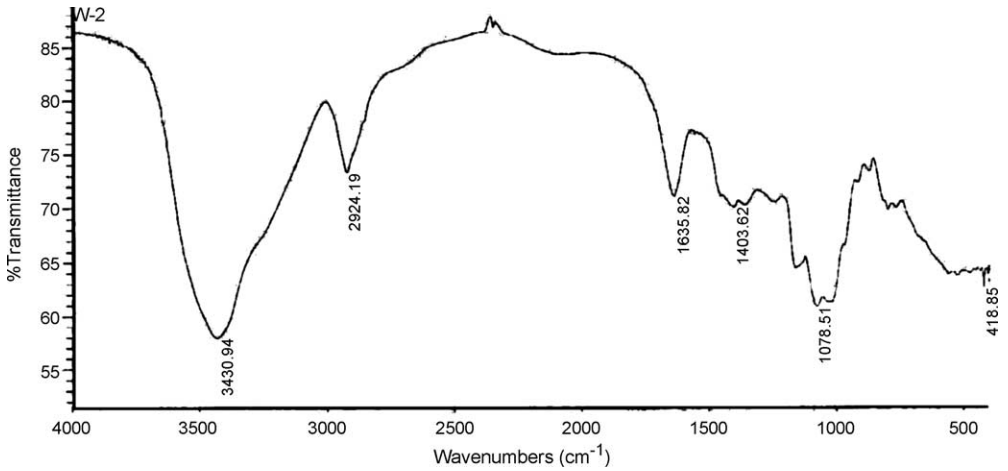


Fig. 2. IR spectrum of HP I.

HPA and HPB were around 5.0×10^4 and 3.0×10^4 on Sphadex G-100 column. Due to these purification identifications, HPA and HPB were uniform on both molecular weight and polarity, and then further structural studies were followed (Zhang, 1987).

Average molecular weight, specific rotations, and sugar compositions (Fig. 1) of HPA and HPB were determined and given in Table 1. Though the protein content of HPB was relatively high, it could be deemed to be protein-bound polysaccharide because the Sevag method has been repeated many times to remove proteins.

Fig. 2 shows the IR spectra of HPA. HPA and HPB exhibited the absorption at 880 cm^{-1}, typical for β configuration.

3.2. Structural characterization of HPA

Fractions HPA_1, HPA_2, HPA_3, and HPA_4, obtained after partial acid hydrolysis of HPA, were subjected to GC analysis, yielding results shown in Table 2. Both HPA_1 and HPA_2 contained the monosaccharides glucose and galactose, indicating that they are the components of backbond structure of HPA. This assay detected glucose in the HPA_3 and large amounts of fucose only in the fraction of HPA_4, indicating fucose and a part of glucose could be in the position of branched structure of HPA.

The polysaccharide of HPA showed abundance HIO_4 uptake, while it was oxidized. The consumption of HIO_4 (0.255 mmol) was about two times more than the amount of formic acid (0.112 mmol) that was produced after 20 h of periodate treatment, indicating the existing of large amounts of monosaccharides which are 1 → linked or (1 → 6)-linked. In addition, it should be concluded that sugar residues oxidized and linkages of 1 → or 1 → 6 account for 93 and 73%, respectively.

The periodate-oxidized products were hydrolyzed and examined by gas chromatography (Table 3). The presence of glucose indicating a part of Glc are (1 → 3)-linked, (1 → 2,3)-linked, (1 → 2,4)-linked, (1 → 3,4)-linked, (1 → 3,6)-linked or (1 → 2,3,4)-linked, namely linkages that cannot be oxidized. Galactose and fucose were absent, then it should be inferred that Gal and Fuc are all linkages that can be oxidized, namely 1 → linked, (1 → 6)-linked, (1 → 2)-linked, (1 → 2,6)-linked, (1 → 4)-linked or (1 → 4,6)-linked.

Results of Smith-degradation analysis of fractions were summarized in Table 3. There was no precipitation in the sack, indicating that the backbond of HPA should be all oxidized by HIO_4. The linkages of backbond are 1 → , 1 → 2, 1 → 6 or 1 → 2,6, which are linkages that can be oxidized to produce glycerin, according to the presence of glycerin out of sack. Gas chromatography of fractions which was obtained from periodate oxidized-Smith degradation of HPA_1 showed the absence of glucose, suggesting Glc that cannot be oxidized are in branched structure.

Methylation analysis of fractions HPA (Fig. 3) showed the presence of seven components, namely 2,3,4,6-Me_4-Gal, 2,3,4,6-Me_4-Glc, 2,3-Me_2-Fuc, 2,4,6-Me_3-Glc, 2,3,4-Me_3-Glc, 2,3,4-Me_3-Gal and 3,4-Me_2-Gal in molar ratios of 0.429:0.245:0.091:0.358:1.585:1.88:1 (Table 4). This showed a good correlation between terminal and branched

Table 2
GC analysis results of fractions from partial acid hydrolysis

Fractions	Molar ratios		
	Fuc	Gal	Glc
HPA_1[a]		4.159	1
HPA_2[b]		0.274	1
HPA_3[c]		0.243	1
HPA_4[d]	1.147	2.173	1
HPB_1[a]			+
HPB_2[b]			+
HPB_3[c]			+
HPB_4[d]		1	5.166

+, be detected.
[a] Precipitation.
[b] Precipitation in the sack.
[c] Supernatant in the sack.
[d] Fraction out of sack.

Table 3
GC results of Smith-degradation of polysaccharides HPA, HPA_1, HPB, and HPB_1

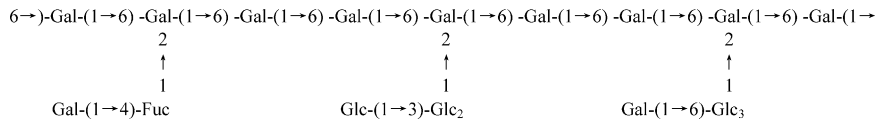
Fractions	Molar ratios													
	HPA				HPA_1[a]				HPB			HPB_1[a]		
	Fuc	Gal	Glc	Gly[b]	Fuc	Gal	Glc	Gly	Gal	Glc	Gly	Gal	Glc	Gly
Full acid hydrolysis			1	7.75				+		1	1.84		1	1.51
Smith-degradation														
Out of sack			1	2.41				+		1	1.38		1	1.06
Supernatant in the sack														
Precipitation in the sack														

[a] Precipitation of partial acid hydrolysis.
[b] Glycerin.

residues. In addition, these molar ratios agree with the overall monosaccharide composition of HPA described above. This assay detected trace amounts of fucose (1.5%), due to its low content in the sample. By methylation linkage analysis, Fuc and Gal are all linkages that can be oxidized by HIO_4, and sugar residues oxidized accounted for 93.6% of the total residues analyzed through the comparison of molar ratios, in accordance with the results of periodate oxidation-Smith degradation.

Both results of partial acid hydrolysis and methylation linkage analysis of HPA indicated that 2,3,4-Me_3-Gal and 2,3,4-Me_3-Glc were major components of the backbond structure. But the branched residue was (1 → 2,6)-linked-galactose, revealing that (1 → 6)-linked-galactose should be possible to form the backbond structure. The relative amounts of (1 → 2,6)-linked-galactose indicating that approximate branch ratios could theoretically be 33%, namely on average one branching point for each three residues of backbond. Residues of branches structure were (1 → 3)-linked-glucose, (1 → 6)-linked-glucose and (1 → 4)-linked-fucose, 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 HPA contains a backbond composed of (1 → 6)-linked-galactose with branches attached to O-2 of some galactose. The branches probably contain (1 → 6)-linked-glucose, (1 → 3)-linked-glucose and (1 → 4)-linked-fucose, and their comparative quantities can be confirmed by molar ratios. Also, methylation analysis of HPA revealed the terminal Gal and terminal Glc in molar ratios of 1:2. The following repeating unit of HPA was established:



3.3. Structural characterization of HPB

Composition analysis showed that HPB contains glucose and galactose, with the molar ratios as shown in Table 1. On partial acid hydrolysis of HPB (Table 2), only glucose was detected in the fractions of HPB_1 and HPB_2, revealing that

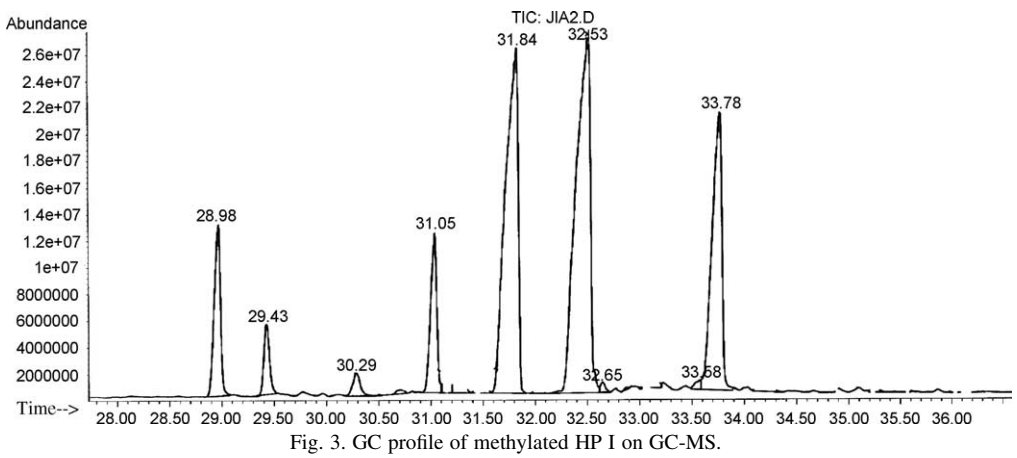
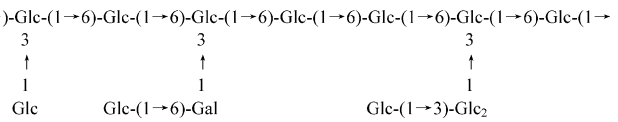


Fig. 3. GC profile of methylated HP I on GC-MS.

Table 4
GC-MS results of methylation analyses of polysaccharides HPA and HPB

Methyl positions	Linkages	Molar ratios	
		HPA	HPB
2,3,4,6-Me_4-Gal	T-	0.492	
2,3,4,6-Me_4-Glc	T-	0.245	1.839
2,3-Me_2-Fuc	1,4-	0.091	
2,4,6-Me_3-Glc	1,3-	0.358	1
2,3,4-Me_3-Glc	1,6-	1.585	4.680
2,3,4-Me_3-Gal	1,6-	1.88	0.789
3,4-Me_2-Gal	1,2,6-	1	
2,4-Me_2-Glc	1,3,6-		3.880

the backbond of HPB is composed of glucose. The fraction of HPB_4 contained large amount of galactose as compared with the molar ratios of sugar composition, indicating that galactose should be present at branch chains. Results of periodate oxidation-Smith degradation (Table 3) showed that a part of glucose and all galactose were oxidized, the backbond of HPB was all oxidized, and glycerin was produced, revealing that the backbond of HPB composed of linkages that can be oxidized to produce glycerin. Methylation analysis revealed that HPB consisted mainly of terminal glucose, 1,3-linked glucose, 1,6-linked glucose, 1,6-linked galactose, and 1,3,6-linked glucose in the molar ratios as shown in Table 4. The proportion of terminal glucose in methylation analysis of HPB was much lower than that in sugar analysis, probably resulting from elimination under the condition of repetitive methylation (Yang & Montgomery, 2001). On the basis of the aforementioned results, it can be concluded that HPB is composed of a repeating unit with three branches having the following structure:



4. Conclusions

According to the above results, it was concluded that the water extract of *Hericium erinaceus Pers* contained predominantly two types of water extractable polysaccharides (HPA and HPB). They are all of β configuration, and their repeating units of structures were proposed as described above. According to their estimated molecular weight, it is possible to assume that HPA and HPB contain 17 and 14 repeating units, respectively.

Many mushroom polysaccharides with activities have been reported (Peng, Zhang, Zeng, & Xu, 2003), most of which are composed of β-(1 → 3)(1 → 6)-linked-glucan or β-(1 → 6)-linked-glucan. Preliminary activity tests showed that HPA and HPB were effective in curing and preventing against chronic gastricism, and the activities of HPA are more than that of HPB. Further studies are necessary to relate such activities with the pharmacological effect of *Hericium erinaceus Pers*.

Acknowledgements

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References

Alfred, L., Leigh, R. E., & Giuseppe, I. (2001). The structure of polysaccharide from infectious strains of *Burkholderia cepacia*. *Carbohydrate Research*, *335*, 45–54.

Byung, K. Y., Jun, B. P., & Chi, H. S. (2003). Hypolipidemic effect of an exo-biopolymer produced from a submerged mycelial culture of *Hericium erinaceum*. *Bioscience Biotechnology and Biochemistry*, *67*(6), 1292–1298.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Kawagishi, H., Shimada, A., Shirai, R., Okamoto, K., Ojima, F., Sakamoto, H., Ishiguro, Y., & Furukawa, S. (1994). Erimacines A, B, and C, strong stimulators of nerve growth factor (NGF) synthesis, from the mycelia of *Hericium erinaceum*. *Tetrahedron Letters*, *35*, 1569–1572.

Kim, D. M., Pyun, C. W., Ko, H. G., & Park, W. M. (2000). Isolation of antimicrobial substances from *Hericium erinaceum*. *Mycobiology*, *28*, 33–38.

Kuwahara, S., Morihiro, E., Nemoto, A., & Hiramatsu, A. (1992). Synthesis and absolute configuration of a cytotoxic fatty acid isolated from the mushroom *Hericium erinaceum*. *Bioscience Biotechnology and Biochemistry*, *56*, 1417–1419.

Lee, E. W., Shizuki, K., Hosokawa, S., Suzuki, M., Suganuma, H., Inakuma, T., Li, J., Ohnishi-Kameyama, M., Nagata, T., Furukawa, S., & Kawagishi, H. (2000). Two novel diterpenoids, erinacines H and I from the mycelia of *Hericium erinaceum*. *Bioscience Biotechnology and Biochemistry*, *64*, 2402–2405.

Liu, C. P., Fang, J. N., & Xiao, X. Q. (2002). Structural characterization and biological activities of SC4, an acidic polysaccharide from *Salvia chinensis*. *Acta Pharmacologica Sinica*, *23*(2), 162–166.

Lu, L., Li, J. L., & Cang, Y. H. (2002). PCR-based sensitive detection of medicinal fungi *Hericium* species from ribosomal internal transcribed spacer (ITS) sequences. *Biological and Pharmaceutical Bulletin*, *25*(8), 975–980.

Mau, J. L., Lin, H. C., & Song, S. F. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International*, *35*, 519–526.

Mizuno, T., Sation, H., Nishitoba, T., & Kawagishi, H. (1995). Antitumor active substances from mushroom. *Food Review International*, *11*(1), 23–61.

Mizuno, T., Wasa, T., Ito, H., Suzuki, C., & Ukai, N. (1992). Antitumor active polysaccharides isolated from the fruiting body of *Hericium erinaceum*, an edible and medicinal mushroom called yamabushitake or houtou. *Bioscience Biotechnology and Biochemistry*, *66*, 937–942.

Needs, P. W., & Selvendran, R. R. (1993). Avoiding oxidative degradation during sodium hydroxydimethyloidine-mediated carbohydrate methylation in dimethyl sulfoxide. *Carbohydrate Research*, *245*, 1–10.

Peng, Y. F., Zhang, L., Zeng, F. B., & Xu, Y. X. (2003). Structure and antitumor activity extracellular polysaccharides from mycelium. *Carbohydrate Polymer*, *54*, 297–303.

Staub, A. M. (1965). Removeal of protein—Sevag method. *Methods in Carbohydrate Chemistry*, *5*, 5–6.

Wang, G. Y., Liang, Z. Y., & Zhang, L. P. (2001). Studies on the structure of JS_1—The water soluble polysaccharide isolated by alkaline from *Hippophae rhamnoides* L. *Chemical Journal of Chinese University*, *22*(10), 1688–1690.

Yang, B. Y., & Montgomery, R (2001). β-Elimination of glucosyl uronic residues during methylation of an acidic polysaccharide from *Erwinia chrysanthemi* CU, 643, 332, 317–323.

Zhang, W. J. (1987). *Technology of biochemical research on compound polysaccharide*. Hangzhou: Zhejiang University Press, pp. 108–111.