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Structural and Molecular Characterization of Extracellular Polysaccharides Produced by a New Fungal Strain, *Trichoderma erinaceum* DG-312

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Abstract Two groups of exopolysaccharides (designated as Fr-I EPS and Fr-II EPS) were isolated from the culture filtrate of new fungal strain *Trichoderma erinaceum* DG-312 by Sepharose CL-6B chromatography. The structures of the exopolysaccharides were investigated using gas chromatography (GC), Fourier transform-infrared (FT-IR) spectroscopy, GC-MS analysis, and NMR. GC analysis indicated that Fr-I EPS was composed of mainly mannose (78.9%) and galactose (21.1%), whereas Fr-II EPS contained mannose (68.4%), galactose (26.2%), and glucose (5.4%). In the anomeric region (950–700 cm^{-1}) of the FT-IR spectrum, both EPSs exhibited obvious characteristic absorption of 810 cm^{-1}, indicating the existence of mannose. The spectra of α- and β-configurations were assigned at 880 and 914 cm^{-1}, respectively. The results of GC-MS analyses confirmed that both EPSs were complex heteropolysaccharides with a (1→3)-linked mannan backbone. The C-1 region that appeared in the ^{13}C-NMR spectra of these EPSs indicated a typical anomeric carbon signal. The Fr-I EPS showed two anomeric carbon signals at 102.6 and 99.6 ppm, whereas the Fr-II EPS displayed four anomeric carbon signals at 102.5, 99.6, 98.5, and 94.3 ppm. The molecular characteristics of the EPSs were further investigated using a size exclusion chromatography/multi-angle laser light scattering (SEC/MALLS) system. The SEC/MALLS system revealed that the average molar masses of the EPSs were 6.592×10^4 (Fr-I EPS) and 1.920×10^4 (Fr-II EPS) g/mol, and the molecular conformation of both EPSs in aqueous solution was random coils.

Key words: Exopolysaccharides, GC-MS, NMR, SEC/MALLS, structural analysis, *Trichoderma erinaceum*

In recent years, fungal polysaccharides have attracted a great deal of interest, because some of them have been recognized as a traditional remedy for the treatment of several diseases, and their diverse physiological activities have been elucidated [3, 4, 27]. Together with the knowledge of physicochemical properties, the structure of polysaccharides is also very important in understanding their physiological activities [5, 15]. The fungal polysaccharides have a peculiarity, in that they significantly differ in chemical structure and biological activity, depending on species and their culture conditions [3, 5, 15].

Although most physiologically important fungal polysaccharides have been identified as β-(1→3)- or β-(1→6)-linked glucans [4, 20], other heteropolysaccharides with diverse linkages have frequently been reported. For example, the immune-enhancing polysaccharides of *Morchella esculenta* have been known as (1→2)-linked galactomannan [9].

During the search of mushroom-pathogenic organisms, we isolated a new fungus from the gills of edible mushroom *Sarcodon aspratus* (Berk) S. Ito and identified it as *Trichoderma* sp. In the cultivation of mushroom, *Trichoderma* species have been widely known as major competitive or weed moulds and occasionally also as pathogens [25, 26]. In addition, many *Trichoderma* strains have been identified as having potential applications in biological control and in the production of valuable biomaterials such as antibiotics and industrial enzymes [3, 10, 32].

In our preliminary studies, we found that EPSs produced from this fungus had a strong anti-inflammatory activity against inflamed mice (unpublished data), which encouraged us to clarify the relationship between the molecular characteristics and anti-inflammatory activity of EPSs. The aim of the present study, therefore, was to investigate the structural and molecular features of the two water-soluble EPSs produced by submerged culture of *T. erinaceum* DG-312. To the best of our knowledge, this is the first report to describe a possible industrial application of extracellular polysaccharides obtained from *Trichoderma erinaceum*.

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MATERIALS AND METHODS

Isolation and Identification of the Fungus

While searching mushroom-pathogenic organisms, we isolated a fungus from the gills of edible mushroom *Sarcodon aspratus* (Berk) S. Ito in a mountainous restrict of Kyungbuk province, Korea. The isolated strain was phylogenetically identified by ITS-5.8S rDNA sequencing analysis. The chromosomal DNA of the strain was isolated from the fresh mycelium using a Wizard genomic DNA purification kit (Promega, Madison, WI, U.S.A.) according to the manufacturer's protocol. The resulting genomic DNA was amplified using *Taq* polymerase (Applied Biosystem, Foster City, CA, U.S.A.), and primers ITS1 (5'-TCCGTAGGTG-AACCTGCGG-3') and ITS4R (5'-CAGACTT(G/A)TA(C/T) ATGGTCCAG-3') [36] on a Techne gene thermocycler (GMI Inc. Ramsey, Minnesota, U.S.A.) under the following condition: 95°C-5 min, 45°C-1 min, 72°C-2 min (1 cycle); 95°C-1 min, 45°C-30 sec, 72°C-2 min (29 cycle); 72°C-10 min (1 cycle). The PCR products were purified using the Wizard SV Gel and PCR clean-up system (Promega). The resulting products were cloned into the pGEM-Teasy vector (Promega) and sequenced in both directions using M13 forward and reverse primers with an automated DNA sequencer (ABI PRISM® 3700 Applied Biosystems, Foster City, CA, U.S.A.). The obtained nucleotide sequence of the ribosomal sequence was compared with those of GeneBank using the NCBI Blast program, and sequence homology was comparatively analyzed using the Clustal X program [33]. Consequently, the isolated strain was identified as *Trichoderma erinaceum* and named DG-312 (GeneBank data homology search result >99%).

Fungal Cultures

A culture of *T. erinaceum* DG-312 was maintained on potato dextrose agar (PDA) slants stock culture stored in 25% glycerol solution at –20°C for about 2 months. Slants were incubated at 25°C for 4 days, then stored at 4°C and subcultured every 4 weeks. The fungus was initially grown on PDA medium in a petridish, and then transferred into the seed culture medium (MCM medium: 20 g/l glucose, 2 g/l meat peptone, 2 g/l yeast extract, 0.46 g/l KH_2PO_4, 1 g/l K_2HPO_4, 0.5 g/l $MgSO_4 \cdot 7H_2O$) by punching out 5 mm of the agar plate culture with a self-designed cutter. Shake flask cultures were carried out in 250-ml flasks containing 50 ml of the MCM medium at 25°C for 4 days, using 4% (v/v) inocula [22]. For preparation of EPSs, the fermentation medium was inoculated with 4% (v/v) of the seed culture and then cultivated for 4 days in a 5-l stirred-tank bioreactor (Ko-BioTech Co., Seoul, Korea) with a working volume of 3-l under the following conditions: controlled pH at 5.0, temperature 25°C, aeration rate 2 vvm, and agitation speed 150 rev/min.

Preparation and Fractionation of EPS

The final fermentation broths from the 5-l fermenter were centrifuged at 10,000 ×*g* for 20 min, and the resulting supernatant was mixed with 4 volumes of absolute ethanol, stirred vigorously, and left overnight at 4°C. The precipitated EPSs were centrifuged at 10,000 ×*g* for 20 min, discarding the supernatant. The ethanol precipitates of the EPS components were dissolved in 0.2 mol/l NaCl buffer to a concentration of 10 g/l, and loaded onto a Sepharose CL-6B column (2.4 cm×100 cm, Sigma Chemical Co., St. Louis, MO, U.S.A.). The column was eluted with the same buffer at a flow rate of 0.6 ml/min. The total sugar contents in the EPSs were determined by a phenol-sulfuric acid method using glucose as the standard [8]. Total protein was determined by the Lowry method with bovine serum albumin as the standard [23]. The protein moiety in the EPSs was monitored by measuring absorbance at 280 nm, whereas the carbohydrate moiety was monitored at 480 nm. The active fractions of the EPSs were pooled and lyophilized for further analysis.

Compositional Analysis

The sugar composition was analyzed by gas chromatography (Varian STAR 3600CX, Varian Co. Model: Star 3600CX, Lexington, MA, U.S.A.) equipped with a flame-ionization detector on an SP™-2380 capillary column (15 m×0.25 mm, Supelco Co., Bellefonte, PA, U.S.A.) with He as a carrier gas. For analysis of neutral sugars, the EPSs were hydrolyzed with 2 M trifluoroacetic acid (TFA) (3 h at 121°C). The resulting monosaccharides were quantified by gas liquid chromatography.

FT-IR Spectroscopy

All FT-IR spectra were recorded on a Mattson Instrument Genesis II (Mattson Inc., Fremont, CA, U.S.A.) from 400 to 4,000 cm^{-1}. For transmission infrared spectroscopy, a powder of freeze-dried EPS (1 mg) was milled with KBr (300 mg) into powder and pressed into a pellet. Spectra were corrected for wavenumber-dependent signal-detection efficiency of the setup using a white light spectrum of a temperature-calibrated tungsten band lamp.

Linkage Analysis by GC-MS

Linkage and substitution sites were determined by GC-MS of alditol acetates and partially methylated alditol acetates, respectively. Methylation analyses were performed according to the Hakomori method using sodium methylsulfinyl carbanion [16, 35]. The EPS (15 mg) was dissolved in dimethyl sulfoxide (0.1 ml) by ultrasonication in a nitrogen atmosphere. The solution was treated with methysulfinyl carbanion (0.1 ml) for 4 h at room temperature, and then with methyl iodide (0.1 ml) for 12 h at room temperature. After methylation, the samples were purified on Sep-Pak C_{18} cartridges (Waters Co., Milford, MA, U.S.A.). Sep-

Paks were preconditioned with (i) 5 ml of methanol, (ii) 5 ml of ethyl acetate, (iii) 5 ml of methanol, and (iv) 10 ml of water before the sample was applied. The permethylated EPS was hydrolyzed with 2 M TFA (1.5 ml) for 1 h at 121°C. The mixture of partly O-methylated aldoses was reduced with $NaBD_4$ and then acetylated to give O-methylalditol acetate, which was successively examined by GC-MS. The resulting partially methylated alditol acetate was converted to the corresponding alditol acetates and analyzed by gas chromatography-mass spectrometry (GC-MS). GC-MS (70 eV) was performed on a Shimadzu QP5050 instrument (Shimadzu Co., Nakagyo-ku, Kyoto, Japan) equipped with the same capillary column. Peaks were identified on the basis of relative retention time and fragmentation patterns. The mol % for each sugar was calibrated using the peak areas.

^{13}C-Nuclear Magnetic Resonance (NMR) Spectroscopy

The EPS samples (approx. 20 mg) obtained from column chromatography were dissolved in 99.96% D_2O (0.7 ml) and a 5-mm tube was used. One-dimensional ^{13}C spectra of EPSs were recorded using a Bruker Avance Digital 400 MHz spectrometer (Bruker Co., Billerica, MA, U.S.A.) with a reverse probe and a gradient unit. In order to detect branched C-6 or C-3 carbon mannosyl units, the apparatus was carefully checked to operate in the best condition. The sample was dissolved in D_2O at 25°C and kept during the measurement to prevent complete gelation which would result in a disappearance of the signals. A 90°C shifted squaresine-bell was used in all cases, with zero-filling once. All data were processed using Bruker 1D-WINNMR version 5.0 software (Bruker Co., Billerica, MA, U.S.A.).

SEC/MALLS Analysis

The molecular weights of the EPS were estimated by SEC coupled with a MALLS system (DAWN DSP; Wyatt Technology, Santa Barbara, CA, U.S.A.). The EPS samples were dissolved in a phosphate/chloride buffer (ionic strength =0.1, pH 6.8) containing 0.04% ethylenediaminetetraacetic acid-disodium salt (Na_2-EDTA) and 0.01% sodium azide and filtered through 0.025 μm filter membranes (Millex HV type; Millipore Corp., Bedford, MA, U.S.A.) prior to injection into the SEC/MALLS system [17, 22]. The chromatographic system consisted of a degassor (Degasys, DG-1200, uniflow; HPLC Technology, Maccles-field, U.K.), a high performance pump (Model 590 Programmable Solvent Delivery Module; Waters Corp., Milford, MA, U.S.A.), an injection valve (Rheodyne Inc., Cotati, CA, U.S.A.) fitted with a 100 μl loop, the SEC columns (Shodex Protein KW-803, 804; Showa Denko K.K., Tokyo, Japan) connected in series, and an RI detector (Water 410). Chromatography was performed at room temperature. The flow rate was 0.5 ml/min, and the injection volume and concentration were 100 μl and 3 mg/ml, respectively. During the calculation of molecular weights of each EPS, the value of *dn/dc*, the so-called 'specific refractive index increment', was used according to the guide from the Wyatt Technology and data in the literature [36], in which the estimated *dn/dc* was 0.14 ml/g. Calculation of molecular weight was performed using the Astra 4.72 software (Wyatt Technology).

RESULTS AND DISCUSSION

Preparation and Compositional Analysis of EPSs

Figure 1 shows the typical time profiles of mycelial growth and EPS production during submerged culture of *T. erinaceum* in a 5-l stirred-tank bioreactor. The maximum concentrations of mycelial biomass and EPSs were 9.44 g/l and 2.72 g/l at 72 h, respectively. Two types of purified EPSs (designated as Fr-I and Fr-II EPS) were obtained from the culture filtrates using a gel filtration chromatography on Sepharose CL-6B as previously described (data not shown; see Materials and Methods). The Fr-I EPS was a white powder and soluble in water, whereas Fr-II EPS was a yellow powder and also soluble in water. The results of compositional analysis of the two purified EPSs are shown in Table 1. The constituent sugars of the Fr-I EPS were 78.9% of mannose and 21.1% of galactose. In contrast, Fr-II EPS consisted of three major sugars; 68.35% of mannose, 26.23% of galactose, and 5.42% of glucose. It has been widely known that the carbohydrate composition of fungal polysaccharides differs significantly between species and their culture conditions [28, 30, 48].

FT-IR Spectroscopy

The FT-IR spectra of the EPSs, measured in KBr pellets, are shown in Fig. 2. In the anomeric region (950–700 cm^{-1}), both EPSs exhibited characteristic absorption at 810 cm^{-1}, indicating the existence of mannose [2, 18, 28]. The obvious absorption peaks at 914 and 880 cm^{-1} in Fr-I EPS and Fr-II

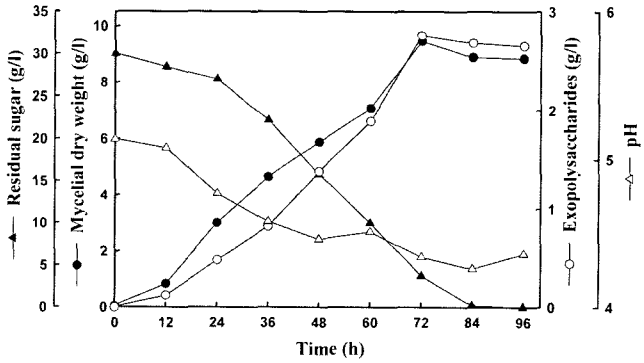

Fig. 1. Time profiles of mycelial biomass and EPS production in *Trichoderma erinaceum* DG-312 using a 5-l stirred-tank bioreactor.

Table 1. Results of the compositional analysis of the two exopolysaccharides (Fr-I EPS and Fr-II EPS) produced by a mycelial culture of *Trichoderma erinaceum* DG-312.

Sugar (%)	Fr-I EPS	Fr-II EPS
Ribose	n.d[a]	Trace[b]
Arabinose	Trace	Trace
Xylose	Trace	Trace
Mannose	78.90	68.35
Galactose	21.10	26.23
Glucose	Trace	5.42

[a]n.d means derivative not present.
[b]Trace means less than 1% present.

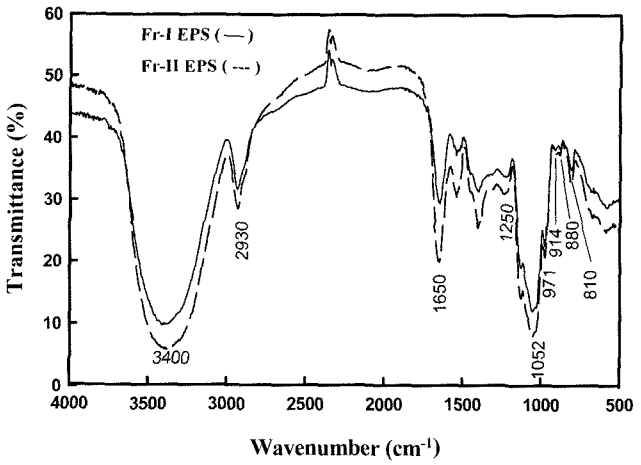

Fig. 2. The FT-IR spectra of the two exopolysaccharides (Fr-I EPS and Fr-II EPS) produced by a submerged culture of *Trichoderma erinaceum* DG-312.

EPS revealed the co-existence of α and β configurations. Both EPSs exhibited characteristic IR absorption at 1,250 and 1,650 cm^{-1}, and their spectral patterns of EPS were nearly identical. In the FT-IR spectra of the two EPSs, the band corresponding to the (C=O) vibration in the carboxyl group at 1,650 cm^{-1} indicates that this carbonyl group is obviously hydrogen-bonded [29, 40]. Furthermore, the band-like structure in the region of 2,930 cm^{-1}, together with the (C-H) vibrations, and a continuous absorption beginning at approximately the region of 3,400 cm^{-1}, are characteristic of a carbohydrate ring [14]. The component bands that are disclosed concern mainly mannans at 971 and 1,052 cm^{-1} [13, 29]. This result showed that FT-IR spectroscopy allows to rapidly and easily obtain an indication of the major components of EPSs.

Structural Characterization of EPS

The Fr-I EPS and Fr-II EPS were individually hydrolyzed, and the partially methylated alditol acetate from the total product was identified by GC-MS with a fragmentation pattern in MS. The results of GC-MS analysis of partially methylated alditol acetate are shown in Table 2.

Hydrolysis of the permethylated Fr-I EPS yielded six compounds: viz. 2,3,4,6-tetra-O-methyl (0.8%), 2,4,6-tri-O-methyl (11.2%), 2,3,4-tri-O-methyl (5.7%), 2,3,6-tri-O-methyl (19.1%), 1,3,4-tri-O-methyl (14.5%), and 2,4-di-O-methyl-D-Man (48.7%). Moreover, the mass spectrum fragment of the galactose unit of Fr-I EPS was characterized as 1,3,4-tri-O-methyl-galacitol (data not shown). On the basis of the methylation analysis, it can be concluded that the basic structure of a galactomannan has a main chain of (1→3)-linked mannopyranosyl with single side chains of galactopyranosyl units attached to the main chain through (2→6) linkages. In this case, about 48.7% of the mannopyranosyl units on average were branched. The Fr-II EPS contained equimolar mannoses of 1,3,4-tri-O-methyl-mannopyranose and 2,4-di-O-methyl-D-mannopyranose. It was elucidated that Fr-II EPS contains a nonreducing, (1→3) linked, (1→6) linked, (2→6) linked mannopyranosyl residue with 3,6-substituted mannopyranose residues, whereas Fr-I EPS possesses more branching points than Fr-II EPS.

^{13}C-NMR measurement was further carried out to prove the presence of (1→6) linkages of the branching in the EPSs. Absorptions of (1→6)-linked C-6 carbons are expected to appear between signals of free C-6 carbon and other carbons. The ^{13}C-NMR spectra of Fr-I EPS and Fr-II EPS are shown in Fig. 3. It contains the regions of the anomeric carbon atoms corresponding to the mannose (102.6 ppm)

Table 2. Identification of partially methylated alditol acetate of the two exopolysaccharides (Fr-I EPS and Fr-II EPS) produced by a mycelial culture of *Trichoderma erinaceum* DG-312.

Methylated sugar	Major mass spectral fragments (*m/z*)	Mol %[a]		Linkage modes[b]
		Fr-I EPS	Fr-II EPS	
2,3,4,6-tetra-O-Me-Man	43,45,71,87,101,117,129,145,161,205	0.8	1.5	Man^1 →
2,4,6-tri-O-Me-Man	43, 45,87,101,117,129,161,233	11.2	15.1	→$^3Man^1$→
2,3,4-tri-O-Me-Man	43,87,99,101,117,129,161,189,233	5.7	7.0	→$^6Man^1$→
2,3,6-tri-O-Me-Man	43,45,87,99,101,113,117,233	19.1	n.d	→$^4Man^1$→
1,3,4-tri-O-Me-Man	43,45,71,87,101,117,129,145,161,205	14.5	38.3	→$^6Man^2$→
2,4-di-O-Me-Man	43,87,101,117,129,189	48.7	38.1	→$^{3,6}Man^1$→

[a]Calculated from peak areas and response factors of hydrogen flame ionization detector on GLC [32].
[b]Based on derived O-methylalditol acetates.

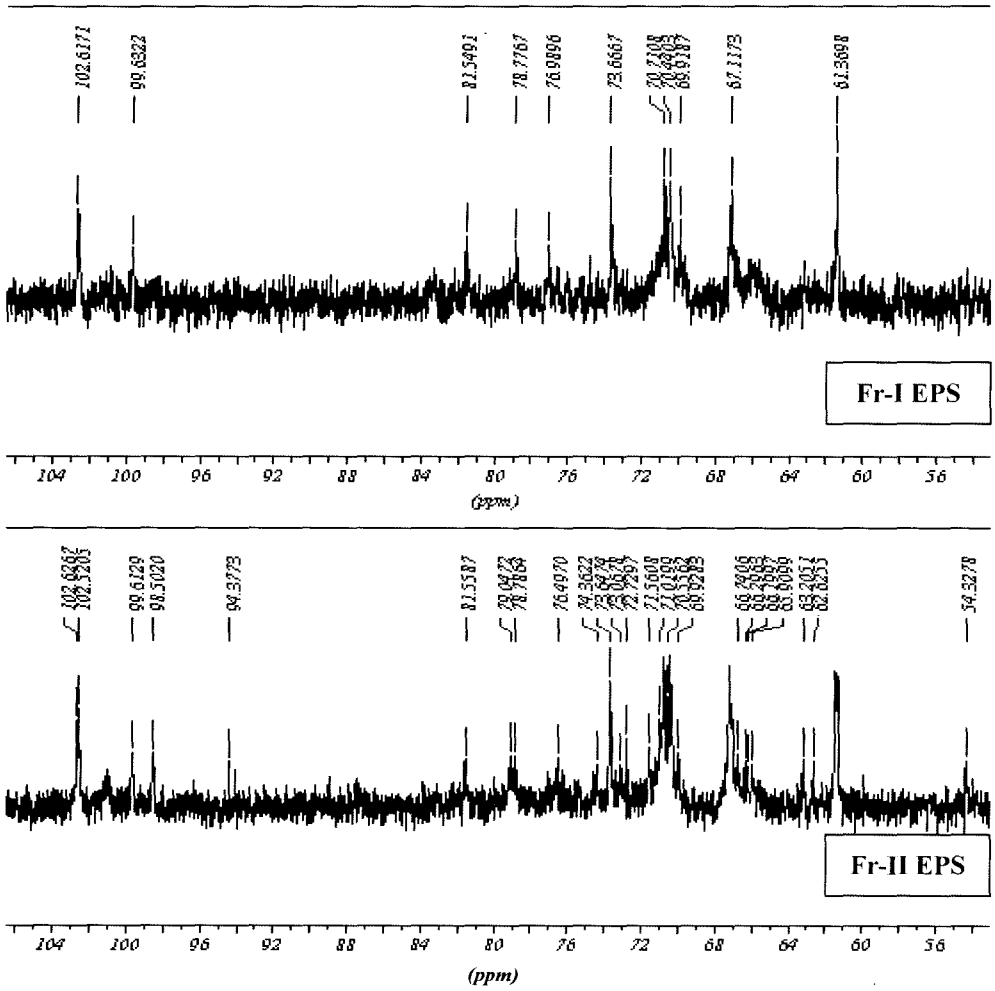

Fig. 3. ^{13}C-NMR spectra of the two exopolysaccharides (Fr-I EPS and Fr-II EPS) produced by a submerged culture of *Trichoderma erinaceum* DG-312.

and galactose (99.6 ppm) units [19, 37]. Carbon signals of Fr-II EPS unquestionably appeared in the anomeric carbon regions of the mannose and galactose units. The unit of mannopyranose had a β-configuration, agreeing with typical high-field C-1 signals higher than an α-configuration of galactopyranose in its ^{13}C-NMR spectrum. As reported earlier [1, 6, 11, 19], the C-3 signals of a main chain of (1→3)-linked mannan indicated higher resonance than the C-3 signals of a main chain of differently linked mannan units. Furthermore, the signals of β-D-mannopyranosyl branched at C-6 in the main chain of (1→3)-linked mannopyranosyl units appeared at 81.5 ppm, which is a higher resonance than the signals of unbranched mannopyranosyl units. A chemical shift of β-(1→3)-linked C-3 and branched C-6 linked mannose showed a downfield shift due to the glycosylation effect, and appeared at 81.54 and 67.11 ppm, respectively. Therefore, it is quite certain that the branching point in our sample was the C-6 carbon of the mannosyl unit in the main chain.

Overall, the data were obtained by compositional analysis, FT-IR spectroscopy, combined methylation-MS analysis, and ^{13}C-NMR spectroscopy. The Fr-I EPS produced from *T. erinaceum* has a main chain of β-(1→3)-D-mannan, to which single galactose units are attached as side chains at C-6. Furthermore, Fr-II EPS has galactose and glucose units in its side chains. Nevertheless, the results provide still a limited amount of information concerning the branching in the galactomannans.

Recently, immune-macrophage-enhancing activity of galactomannan from the edible fungus *M. esculenta* has been reported [9]. The major derivatives of galactomannan by *M. esculenta* included (1→2)-linked mannose (40.4%), (1→4)-linked glucose (19.8%), terminal 1-galactose (11.1%), and (2→3)-linked mannose (10.9%). However, the galactomannan obtained from *T. erinaceum* identified in this study is different to other natural galactomannans in many respects, such as molecular weight, chemical composition, and linkage mode.

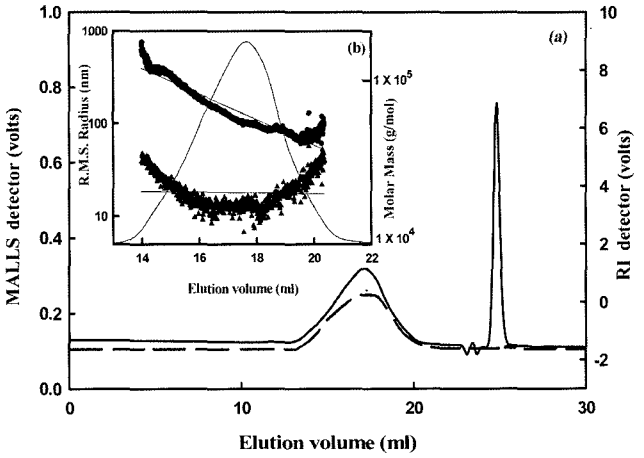

Fig. 4. (a) A typical elution chromatogram of Fr-I EPS showing the response of the refractive index (RI) detector (—) and MALLS detector (---). The high peak appearing at elution volume of around 25 ml is the baseline noise from the buffer solution. **(b)** Molecular weight (●) and RMS (root mean square) (▲) radius *versus* elution volume curve of Fr-I EPS in a SEC/MALLS system.

Molecular Characterization of EPS

The SEC/MALLS approach could be useful in providing greater insight into the characterization of the fungal polysaccharides without carrying out elaborate fractionation procedures prior to analysis [17, 22]. Figures 4(a) and 5(a) show the typical refractive indexes and light scattering detector profiles for each EPS. The molecular mass values of the two eluted fractions were calculated within each peak range. These ranges were defined by the common detection limit for the MALLS and RI chromatograms in the peak regions. The logarithmic plots of molecular weight and root mean square (RMS) radius of gyration of each EPS as a function of elution volume were also presented in Figs. 4(b) and 5(b). For both EPSs, the molecular weights continuously decreased as the elution volume increased, in accordance to the SEC mechanism. The amount of scatter in molecular weight data slightly increased in the later ranges of elution for both EPSs. This phenomenon is presumably due to the combination of small sized molecules, the decrease in Rayleigh scatter with particle size, and the low quantity of material present, making the system too dilute to be measured accurately [17, 22]. Eventually, the weight average molar masses (M_w) of Fr-I EPS and Fr-II EPS were determined to be 6.592×10^4 and 1.920×10^4 g/mol, respectively (Table 3). Figure 6 shows the overall slope for each EPS in the double logarithmic plots of the RMS radius of gyration *vs.* molecular mass. The study of the dependence of the RMS radius of gyration on molecular weight can give additional

Table 3. Relevant molecular parameters of the two groups of exopolysaccharides (Fr-I EPS and Fr-II EPS) produced by a submerged mycelial culture of *Trichoderma erinaceum* DG-312 in MALLS analysis.

Parameters[a]	Fr-I EPS (error %)	Fr-II EPS (error %)
M_n (g mol^{-1})	6.589×10^4 (0.6)	1.849×10^4 (1.7)
M_w (g mol^{-1})	6.592×10^4 (0.6)	1.920×10^4 (1.7)
M_z (g mol^{-1})	6.596×10^4 (1.3)	1.997×10^4 (3.0)
M_w/M_n	1.001 (0.8)	1.038 (2.4)
R_n (nm)	24.2 (1.4)	57.5 (1.5)
R_w (nm)	24.1 (1.4)	59.0 (1.4)
R_z (nm)	24.1 (1.4)	60.4 (1.4)

[a] M_w, M_n, and M_z refer number-, weight-, and z-average molecular weight, respectively. M_w/M_n represents the polydispersity ratio. R_n, R_w, and R_z refer to number-, weight-, and z-average root-mean-squared radius of gyration, respectively.

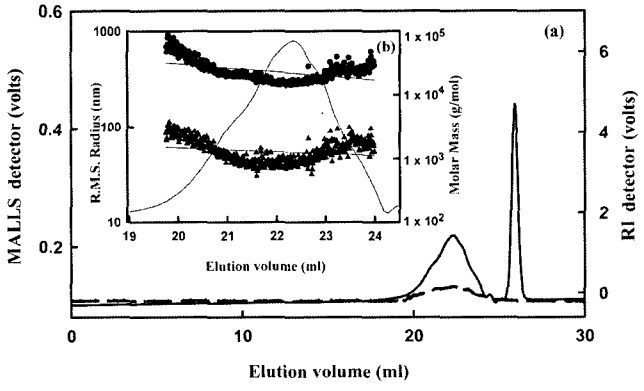

Fig. 5. (a) A typical elution chromatogram of Fr-II EPS showing the response of the refractive index (RI) detector (—) and MALLS detector (---).The high peak appearing at elution volume of around 26 ml is the baseline noise from the buffer solution. **(b)** Molecular weight (●) and RMS (root mean square) (▲) radius *versus* elution volume curve of Fr-II EPS in a SEC/MALLS system.

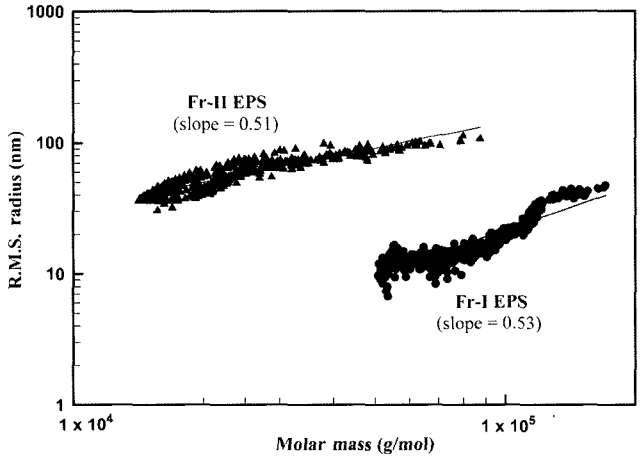

Fig. 6. The double logarithmic plots of RMS (root mean square) radius *versus* molecular mass of the two exopolysacchairdes (Fr-I EPS and Fr-II EPS) produced by submerged culture of *Trichoderma erinaceum* DG-312.

information on the polymer structure [36, 39]. That is, the gross molecular conformation of each EPS in this study can be elucidated from the double logarithmic plot of the RMS radius of gyration *vs.* the molecular mass according to the following equation:

$$\text{Log } r_i = k + a \log M_i$$

where, r_i is the RMS radius of EPS, M_i is the molar mass of EPS, k is the intercept on the y axis (RMS radius of gyration), and a is the slope providing a hint about the conditions of the polymeric chain in aqueous solution. Slope values of 0.33 would indicate compact globular structure, and 0.5 indicates a flexible random coil polymer. For rigid rods, their corresponding value of slope is unity [36]. It should be noted here that most real coils of biopolymers are usually slightly more extended, shifting the slopes from 0.5 to 0.55–0.6 in a good solvent [36, 39]. The slopes of Fr-I EPS (0.53) and Fr-II EPS (0.51) imply that they exist as random coils in an aqueous solution. Molecular weight and gyration radii for the two water-soluble galactomannans obtained in this study were relatively smaller than those reported in other studies [12].

There existed a great difference in molecular weights, polydispersity (M_w/M_n), and deviation of the three different RMS radii of gyration (R_n, R_w, and R_z) between the two EPSs (Table 3). The polydispersity of both EPSs was much closer to unity, suggesting that these EPSs were less polydispersed (almost monodispersed), unlike other groups of biopolymers [25].

The structure and molecular mass of an EPS has been found to play a critical role in its biological activity [7]. In this regard, it is worth pointing out that the characterization data for an EPS from a submerged culture of higher fungi should be separately obtained for each culture condition.

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REFERENCES

1. Ahrazem, O., A. Prieto, J. A. Leal, J. J. Barbero, and M. Bernabe. 2002. Fungal cell-wall galactomannans isolated from *Geotrichum* spp. and their teleomorphs, *Dipodascus* and *Galactomyces*. *Carbohydr. Res.* **337:** 2347–2351.
2. Åkerholm, M. and L. Salmen. 2001. Interactions between wood polymers studied by dynamic FT-IR spectroscopy. *Polymer* **42:** 963–969.
3. Arja, M.-O., P. Marja, L. Raija, and S. Pirkko. 2005. Enhanced production of cellobiohydrolases in *Trichoderma reesei* and evaluation of the new preparations in biofinishing of cotton. *J. Biotechnol.* **116:** 305–317.
4. Bao, X., C. Liu, J. Fang, and X. Li. 2001. Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst. *Carbohydr. Res.* **332:** 67–74.
5. Bohn, J. A. and J. N. BeMiller. 1995. (1→3)-β-D-glucans as biological response modifiers: A review of structure-functional activity relationships. *Carbohydr. Polym.* **28:** 3–14.
6. Chaubey, M. and V. P. Kapoor. 2001. Structure of a galactomannan from the seeds of *Cassia angustifolia* Vahl. *Carbohydr. Res.* **332:** 439–444.
7. Domer, J. E. and R. E. Garner. 1991. Fungal wall components and immunostimulation, pp 157–167. *In* Latge, J. P. and Boucias, D. (eds.). *Fungal Cell Wall and Immune Response.* Springer-Verlag; Berlin.
8. Dubois, M., K. A. Gilles, J. K. Hamiton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugar and related substance. *Anal. Chem.* **28:** 350–356.
9. Duncan, C. J. G., N. Pugh, D. S. Pasco, and S. A. Ross. 2002. Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*. *J. Agric. Food Chem.* **50:** 5683–5685.
10. Eufrocinio, C. M., B. Takeshi, I. K. Shin, F. Ei-ichiro, and K. Akio. 2002. Biosynthetic studies of the tetramic acid antibiotic trichosetin. *Tetrahedron* **58:** 6655–6658.
11. Fedonenko, Y. P., O. N. Konnova, G. V. Zatonsky, A. S. Shashkov, S. A. Konnova, E. L. Zdorovenko, V. V. Ignatov, and Y. A. Knirel. 2004. Structural of the O-polysaccharide of the lipopolysaccharide of *Azospirillum irakense* KBC1. *Carbohydr. Res.* **339:** 1813–1816.
12. Ganter, J. L. M. S. and F. Reicher. 1999. Water-soluble galactomannans from seeds of *Mimosaceae* spp. *Bioresource Technol.* **68:** 55–62.
13. Galichet, A., G. D. Sockalingum, A. Belarbi, and M. Manfait. 2001. FTIR spectroscopic analysis of *Saccharomyces cerevisiae* cell walls: Study of an anomalous strain exhibiting a pink-colored cell phenotype. *FEMS Microbiol. Lett.* **197:** 179–186.
14. Grošev, V. M., R. Božac, and G. J. Puppels. 2001. Vibrational spectroscopic characterization of wild growing mushroom and toadstools. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **57:** 2815–2829.
15. Gutiérrez, A., A. Prieto, and A. T. Martínez. 1996. Structural characterization of extracellular polysaccharides produced by fungi from the genus *Pleurotus*. *Carbohydr. Res.* **281:** 143–154.
16. Hakomori, S. I. 1964. A rapid permethylation of glycolipid and polysaccharide catalyzed by methysulfinyl carbanion in dimethyl sulfoxide. *J. Biochem.* **55:** 205–208.
17. Hwang, H. J., S. W. Kim, C. P. Xu, J. W. Choi, C. W. Kim, and J. W. Yun. 2003. Production and molecular characteristics of four groups of exopolysaccharides from submerged culture of *Phellinus gilvus*. *J. Appl. Microbiol.* **94:** 708–719.

18. Jin, Y., L. Zhang, L. Chen, Y. Chen, P. C. K. Cheung, and L. Chen. 2003. Effect of culture media on the chemical and physical characteristics of polysaccharides isolated from *Poria cocos* mycelia. *Carbohydr. Res.* **338:** 1507–1515.
19. Joshi, H. and V. P. Kapoor. 2003. *Cassia grandis* Linn. f. seed galactomannan: Structural and crystallographical studies. *Carbohydr. Res.* **338:** 1907–1912.
20. Kim, G. Y., H. S. Park, B. H. Nam, S. J. Lee, and J. D. Lee. 2003. Purification and characterization of acidic proteoheteroglycan from the fruiting body of *Phellinus linteus* (Berk. & M.A. Curtis) Teng. *Bioresource Technol.* **89:** 81–87.
21. Kim, S. W., H. J. Hwang, J. P. Park, C. H. Song, and J. W. Yun. 2002. Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. *Lett. Appl. Microbiol.* **34:** 56–61.
22. Kim, S. W., C. P. Xu, H. J. Hwang, J. W. Choi, C. W. Kim, and J. W. Yun. 2003. Production and characterization of exopolysaccharides from an enthomopathogenic fungus *Cordyceps militaris* NG3. *Biotechnol. Prog.* **19:** 428–435.
23. Lowry, O. H., N. J. Rosebrough, L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
24. Lucey, J. A., M. Srimivasan, H. Singh, P. A. Munro. 2000. Characterization of commercial and experimental sodium caseinates by multiangle laser light scattering and size-exclusion chromatography. *J. Agric. Food Chem.* **48:** 1610–1616.
25. Morris, E., O.P.E. Doyle, and K. J. Clancy. 1995. A profile of *Trichoderma* species. I. Mushroom compost production. *Mushroom Sci.* **14:** 611–618.
26. Morris, E., O.P.E. Doyle, and K. J. Clancy. 1995. A profile of *Trichoderma* species. II. Mushroom growing units. *Mushroom Sci.* **14:** 619–626.
27. Park, Y. H., E. K. Moon, Y. K. Shin, M. A. Bae, J. K. Kim, and Y. H. Kim. 2000. Antitumor activity of *Paecilimyces japonica* is mediated by apoptotic cell death. *J. Microbiol. Biotechnol.* **10:** 16–20.
28. Peng, Y., L. Zhang, F. Zeng, and Y. Xu. 2003. Structure and antitumor activity of extracellular polysaccharides from mycelium. *Carbohydr. Polym.* **54:** 297–303.
29. Reis, A. V., O. A. Cavalcanti, A. F. Rubrira, and E. C. Muniz. 2003. Synthesis and characterization of hydrogels formed from a glycidyl methacrylate derivative of galactomannan. *Int. J. Pharmaceutics* **267:** 13–25.
30. Sone, Y., R. Okuda, N. Wada, E. Kishida, and A. Misaki. 1985. Structures and antitumor activities of the polysaccharides isolated fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. *Agric. Biol. Chem.* **49:** 2641–2653.
31. Sweet, D. P., R. H. Shapiro, and P. Albersheim. 1975. Quantitative analysis by various G.L.C. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydr. Res.* **40:** 217–225.
32. Theresa A. B. and J. B. Greg. 2003. A review of the non-target effects of fungi used to biologically control plant diseases. *Agric. Ecosyst. Environ.* **100:** 3–16.
33. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalies and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.
34. Carbone, I. and L. M. Kohn. 1993. Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the Selerotiniaccae. *Mycologia* **85:** 415–427.
35. Waeghe, T., A. Darvill, M. Mcnell, and P. Albershim. 1983. Determanation, by methylation analysis, of the glycosyl-linkage compositions of microgram quantities of complex carbohydrates. *Carbohydr. Res.* **123:** 281–304.
36. Waytt, P. J. 1993. Light scattering and the absolute characterization of macromolecules. *Anal. Chim. Acta* **272:** 1–40.
37. Whiteny, S. E. C., J. E. Brigham, A. H. Darke, J. S. G. Reid, and M. J. Gidley. 1998. Structural aspects of the interaction of mannan-based polysaccharides with bacterial cellulose. *Carbohydr. Res.* **307:** 299–309.
38. Xu, C. P. and J. W. Yun. 2004. Influence of aeration on the production and the quality of the exopolysaccharides from *Paecilomyces tenuipes* C240 in a stirred-tank fermenter. *Enzyme Microb. Technol.* **35:** 33–39.
39. Zhang, P., L. Zhang, and S. Cheng. 2002. Solution properties of an α-(1→3)-D-glucan from *Lentinus edodes* and its sulfated derivatives. *Carbohydr. Res.* **337:** 155–160.
40. Zhang, M., L. Zhang, and P. C. K. Cheung. 2003. Molecular mass and chain conformation derivatives of β-glucan from sclerotia of *Pleurotus tuber-regium*. *Biopolymers* **68:** 150–159.