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 anomic region (950–700 cm⁻¹) of the FT-IR spectrum, both EPSs exhibited obvious characteristic absorption of 810 cm⁻¹, indicating the existence of mannanose. 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 ¹³C-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 anomic 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 6.592×10⁶ (Fr-I EPS) and 1.920×10⁶ (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 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'-TCCGTAAGGTT-AACCTGCGG-3') and ITS4R (5'-CAGACTTG/GAT(A/G)TAT(A/G/G)CAG-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 extracts, 0.46 g/l KH2PO4, 1 g/l K2HPO4, 0.5 g/l MgSO4.7H2O) 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, aerator rate 2 vvm, and agitation speed 150 rev/min.

Preparation and Fractionation of EPS
The final fermentation broth from the 5-l fermenter were centrifuged at 10,000 xg 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 xg 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 x 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 x 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 recorded 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. Methylolation analyses were performed according to the Hakomori method using sodium methylsulfonate carbamion [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 methylsulfonate carbamion (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 C18 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 NaBD₄ 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.

³¹C-Nuclear Magnetic Resonance (NMR) Spectroscopy
The EPS samples (approx. 20 mg) obtained from column chromatography were dissolved in 99.96% D₂O (0.7 ml) and a 5-mm tube was used. One-dimensional ³¹C 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 D₂O 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 (Na₂-EDTA) and 0.01% sodium azide and filtered through 0.25 µm filter membranes (Millepore 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 degasser (Degasys, DG-1200, uniflow; HPLC Technology, Macclesfield, U.K.), a high performance pump (Model 590 Programmable Solvent Delivery Module; Waters Corp., Milford, MA, U.S.A.), an injection valve (Rhodyne 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 df/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 df/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 anomic 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
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.

<table>
<thead>
<tr>
<th>Sugar (%)</th>
<th>Fr-I EPS</th>
<th>Fr-II EPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribose</td>
<td>n.d.</td>
<td>Trace</td>
</tr>
<tr>
<td>Arabinose</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Xylose</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Mannose</td>
<td>78.90</td>
<td>68.35</td>
</tr>
<tr>
<td>Galactose</td>
<td>21.10</td>
<td>26.23</td>
</tr>
<tr>
<td>Glucose</td>
<td>Trace</td>
<td>5.42</td>
</tr>
</tbody>
</table>

* n.d. means derivative not present.
* Trace means less than 1% present.

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 carboxyl 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 mannos 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-galactitol (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 mannososes 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.

¹C-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 ¹C-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.

<table>
<thead>
<tr>
<th>Methylated sugar</th>
<th>Major mass spectral fragments (m/z)</th>
<th>Fr-I EPS</th>
<th>Fr-II EPS</th>
<th>Linkage modes¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-Me-Man</td>
<td>43,84,71,87,101,117,129,145,161,205</td>
<td>0.8</td>
<td>1.5</td>
<td>Man₁ ←</td>
</tr>
<tr>
<td>2,4,6-tri-O-Me-Man</td>
<td>43,45,87,101,117,129,161,233</td>
<td>11.2</td>
<td>15.1</td>
<td>Man₁ →</td>
</tr>
<tr>
<td>2,3,4-tri-O-Me-Man</td>
<td>43,87,99,101,117,129,161,189,233</td>
<td>5.7</td>
<td>7.0</td>
<td>Man₁ →</td>
</tr>
<tr>
<td>1,3,4-tri-O-Me-Man</td>
<td>43,85,71,87,101,117,129,145,161,205</td>
<td>14.5</td>
<td>38.3</td>
<td>Man₁ →</td>
</tr>
<tr>
<td>2,4-di-O-Me-Man</td>
<td>43,87,101,117,129,189</td>
<td>48.7</td>
<td>38.1</td>
<td>Man₁ →</td>
</tr>
</tbody>
</table>

¹Calculated from peak areas and response factors of hydrogen flame ionization detector on GLC [32].
²Based on derivd O-methylalditol acetates.
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 13C-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 mannos 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 13C-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 mannos (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.
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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 $6.592 \times 10^4$ and $1.920 \times 10^4$ 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

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**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.

<table>
<thead>
<tr>
<th>Parameters$^a$</th>
<th>Fr-I EPS (error %)</th>
<th>Fr-II EPS (error %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_w$ (g mol$^{-1}$)</td>
<td>$6.592 \times 10^4$ (0.6)</td>
<td>$1.920 \times 10^4$ (1.7)</td>
</tr>
<tr>
<td>$M_m$ (g mol$^{-1}$)</td>
<td>$6.592 \times 10^4$ (0.6)</td>
<td>$1.920 \times 10^4$ (1.7)</td>
</tr>
<tr>
<td>$M_i$ (g mol$^{-1}$)</td>
<td>$6.592 \times 10^4$ (1.3)</td>
<td>$1.997 \times 10^4$ (3.0)</td>
</tr>
<tr>
<td>$M_w/M_i$</td>
<td>1.001 (0.8)</td>
<td>1.038 (2.4)</td>
</tr>
<tr>
<td>$R_s$ (nm)</td>
<td>24.2 (1.4)</td>
<td>57.5 (1.5)</td>
</tr>
<tr>
<td>$R_n$ (nm)</td>
<td>24.1 (1.4)</td>
<td>59.0 (1.4)</td>
</tr>
<tr>
<td>$R_g$ (nm)</td>
<td>24.1 (1.4)</td>
<td>60.4 (1.4)</td>
</tr>
</tbody>
</table>

$^a$ $M_w$, $M_m$, and $M_i$ refer number-, weight-, and $z$-average molecular weight, respectively. $M_w/M_i$ represents the polydispersity ratio. $R_s$, $R_n$, and $R_g$ refer to number-, weight-, and $z$-average root-mean-squared radius of gyration, respectively.
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:

$$\log r_g = k + a \log M_w$$

where, $r_g$ is the RMS radius of EPS, $M_w$ 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 to 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 watersoluble galactomannans obtained in this study were relatively smaller than those reported in other studies [32].

There existed a great difference in molecular weights, polydispersity ($M_w/M_n$), and deviation of the three different RMS radii of gyration ($R_g$, $R_\alpha$, and $R_\eta$) 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.

Acknowledgments

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