SUPPLEMENTARY MATERIAL

Isolation and structural elucidation of a novel homogenous polysaccharide from *Tricholoma matsutake*

Hua Cheng^a, Yan Jia^a, Ling Wang^a, Xiaoying Liu^b, Guangrong Liu^b, Li Li^a, Congfen He^{a*}

^aBeijing Technology and Business University, School of Science, Beijing Key Laboratory of Plants Resource Research and Development, Beijing 100048, China;

^bInfinitus International (China) Company, Ltd., Guangzhou, Guangdong 510665, China

*Corresponding author

E-Mail: congfenhe@126.com.cn;

Tel.: +86-10-68984893; Fax: +86-10-68985292

Disclosure statement:

The authors declare no conflicts of interest.

Abstract

A crude polysaccharide possessing antitumor, radiation-resistant, and anti-aging attributes was extracted from *Tricholoma matsutake* by water extraction and alcohol precipitation. From this crude polysaccharide, a homogeneous polysaccharide, TMP-5II, was successfully purified by Sephacryl S-300 column chromatography. The average molecular weight (M_w) of TMP-5II was 15.76 kDa. Monosaccharide analysis indicated that the homogeneous polysaccharide contained four different residues: D-glucose, D-galactose, D-mannose, and D-fucose. Attenuated total reflectance infrared spectroscopy revealed characteristics typical of carbohydrate polymers and a peak typical of a β -type glycosidic bond. TMP-5II was selected for structural characterization by nuclear magnetic resonance (NMR) analysis. According to ^1H NMR, ^{13}C NMR, and two-dimensional-NMR analysis, TMP-5II contains two kinds of linkages, β and α , at a ratio of 4:1. Preliminary results was that the polysaccharide had (1-4)-beta-pyran glucose as the main chain, and a branched chain in the O-6 location with fucose (1-2) mannose (1-3)-alpha-pyran galactose.

Table S1 Information on the GC–MS spectrogram of seven acetylated standard monosaccharides

Peak No.	Monosaccharide	Retention time (min)	Peak area ($\times 10^7$)
1	L-rhamnose	28.201	1.180
2	D-fucose	28.550	0.370
3	D-arabinose	28.756	0.362
4	D-xylose	29.503	0.697
5	D-mannose	37.593	1.208
6	D-glucose	38.026	0.565
7	D-galactose	38.387	0.800

Table S2. Monosaccharide composition and molar ratio of TMP-5II

Peak No.	Retention time (min)	Monosaccharide composition	Peak area ($\times 10^7$)	Molar ratio
1	30.678	D-fucose	0.155	1.00
2	42.523	D-mannose	0.637	2.08
3	42.882	D-glucose	1.241	10.52
4	43.572	D-galactose	1.284	7.52

Figure S1 Elution curve of TMP-5 from Sephacryl S-300 column chromatography

Every marker “♦” on the curve represent the absorbance value of polysaccharide content of each fraction collected in column chromatography. Tubes 18 to 22 were collected for TMP-5□, as well as Tubes 29 to 36 for TMP-5II.

Figure S1

Figure S2 The purity evaluation of TMP-5II by GPC-MALLS Curve in red color is 18 angle laser light scattering instrument signal, representation of molecular weight. Curve in blue color is differential detector signal, representation of polysaccharide content. The two curves will be more anastomotic with the higher purity.

Figure S2

Figure S3 The GC-MS spectrogram of seven acetylated standard monosaccharides According to retention time, peak “□” is L-rhamnose, as well as peak “□” to peak “□” are D-fucose, D-arabinose, D-xylose, D-mannose, D-glucose, D-galactose respectively.

Figure S3

Figure S4 The GC–MS spectrogram of hydrolyzed and acetylated TMP-5II
According to the retention time of seven standard monosaccharides, peaks “①” to “④” are D-fucose, D-mannose, D-glucose and D-galactose respectively.

Figure S4

Figure S5 The infrared spectrum of TMP-5II Characteristic absorption of functional groups in accordance with the abscissa, the ordinate vibration intensity of specific chemical bonds for the curve for result analysis. TMP-5II is a kind of polysaccharide for the specific absorption of polysaccharide –CH stretching vibration absorption at 2911cm^{-1} .

Figure S5

Figure S7 The ^{13}C NMR spectrogram of TMP-5II Four C-1 signals at $\delta 98.368$, $\delta 101.665$, $\delta 101.772$ and $\delta 103.073$ dedicate that TMP-5II is composed four kinds of monosaccharide. There should be three signals between $\delta 62$ to 60 , but only two signals $\delta 60.847$ and $\delta 61.227$ for C -6 connection. $\delta 15.783$ is fucose methyl carbon signal. $\delta 75$ to 79 are C signals.

Figure S7

Figure S8 Heteronuclear single-quantum coherence (HSQC) spectrogram of TMP-5II Signals of H in accordance with the abscissa, the ordinate signals of C for the intersection parts for result analysis. Through affiliation of C and H signals, the direct connection condition of C and H.

Figure S8

Figure S9 Heteronuclear multiple bond coherence (HMBC) spectrogram of TMP-5II Signals of H in accordance with the abscissa, the ordinate signals of C for the intersection parts for result analysis. Through affiliation of C and H signals, the indirect connection condition of C and H. Then the structure can be deduced.

Figure S9