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MALDI-TOF Analysis of Water Soluble Polysaccharides of an Edible Mushroom, Pleurotus Florida

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ABSTRACT

Matrix-assisted laser desorption /ionization time-of-flight mass spectrometry (MALDI-ToF-MS) has been used to identify structure of the polysaccharide on the basis of its spectral nature. Identification of polysaccharide using MALDI-ToF mass spectra depends on instrumental parameters and sample preparation protocol. Here, the (MALDI-ToF) mass spectrometry analysis of two water-soluble polysaccharides (Fr.I & Fr.II) isolated from the aqueous extract of fruit bodies of edible mushroom, Pleurotus florida have been carried out by using 2,5 dihydroxy benzoic acid (DHB) as matrix.

Keywords: Mushroom polysaccharide, *Pleurotus florida*, MALDI-ToF analysis, 2,5 dihydroxy benzoic acid (DHB) matrix.

1. Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was first described [1] in 1988. MALDI-ToF mass spectrometry has been used routinely for analyzing purified proteins, identifying biomolecules, biomarkers and whole cell-micro organisms [2,3]. Applications also include the characterization of maltose chains in gummy bears [4], fructans in onions [5], methyl galacturonosyl methoxy xylan in stem of Lau [6], high molecular weight oligosaccharides in human milk [7,8], glyconectin carbohydrates in Porifera species [9], heteroglycan in mushroom, *Volvariella bombycina* [10] and glucan in Volvariella diplasia [11]. This technique has an advantage over other techniques because it requires minimum sample volume, less time (< 1 min) for analysis with negligible reagent cost [12,13].

Mushrooms are source of antitumor and immunostimulating polysaccharides [14,15]. Two water-soluble polysaccharides (Fr.I & Fr.II)from the edible mushroom, *Pleurotus florida* have been isolated and characterized by our group and reported [16,17]. I have carried out MALDI-ToF-MS analysis of water-soluble polysaccharides and reported herein.

2. Materials and methods

2.1. Chemicals and reagents

2,5 dihydroxy benzoic acid (DHB) was purchased from Sigma (Saint Louis, Missouri, USA). MilliQ water (18.2 MΩ) was used for sample preparation.

2.2. Isolation and purification of the polysaccharide [16,17]

A mixture of polysaccharides (2.3 g) was isolated from the mushroom, *P. florida* (2 kg) by hot water extraction, followed by precipitation in EtOH and centrifugation. It was then dissolved in minimum volume of water, and exhaustive dialysis was carried out using DEAE cellulose bag (Sigma-Aldrich, retaining M.W.> 12,400). Consequently the high molecular weight polysaccharides remain inside the bag while small molecules diffuse through the pores of the dialysis bag. The solution was then freeze dried and collected (yield, 2 g) for structural analysis. The dried material was not completely soluble in water. So the crude polysaccharide was then allowed to dissolve in 1% NaCl solution to give two fractions, a NaCl-soluble fraction and a NaCl-insoluble fraction. The NaCl-soluble fraction (30 mg) on fractionation through a Sephadex G-75 column yielded two polysaccharide fractions, Fraction-I (7 mg) and Fraction-II (12 mg).

2.3. Preparation of DHB matrix

2 mg 2,5-dihydroxybenzoic acid (DHB) was dissolved in 200μL matrix solvent (acetonitrile-water 2:1,v/v) and 1% sodium trifluro acetate was added to it. Sodium trifluro acetate and acetonitrile percentage was optimized using different concentration of 0.5-3.5 % and 20-80% respectively using the same instrumental settings.

2.4. MALDI-TOF MS analysis [20,21]

The polysaccharide (1.0 mg) was dissolved in 200μL MilliQ (deionized water). Two μL of the sample solution was taken in a vial where DHB matrix (2 μL) was added and centrifuged. One μL of the solution mixture was taken in MALDI sample plate for analysis. MALDI-TOF mass spectrometry was performed on a Voyager-DE PRO (Applied Biosystem) mass spectrometer, equipped with a nitrogen laser operating at 337 nm (laser power 30-35 Joules and accelerating voltage 25kV). Instrument was calibrated with myoglobin (sigma) prior to analysis. The spectra were recorded in the positive reflector mode as the average of 100 laser shots of random positions across a spot using DHB (10 mg/mL) as matrix.

3. Results and discussion

The MALDI-TOF analysis of the polysaccharide (Fr.I)

The analysis was carried out in reflector mode. The molecular weight (~40,000 Da) of the polysaccharide was determined by Gel permeation chromatography. The structure of the water-soluble (Fr.I) was reported [16] as:

→3)-α-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→
6
↑
1
α-D-Glc*p*

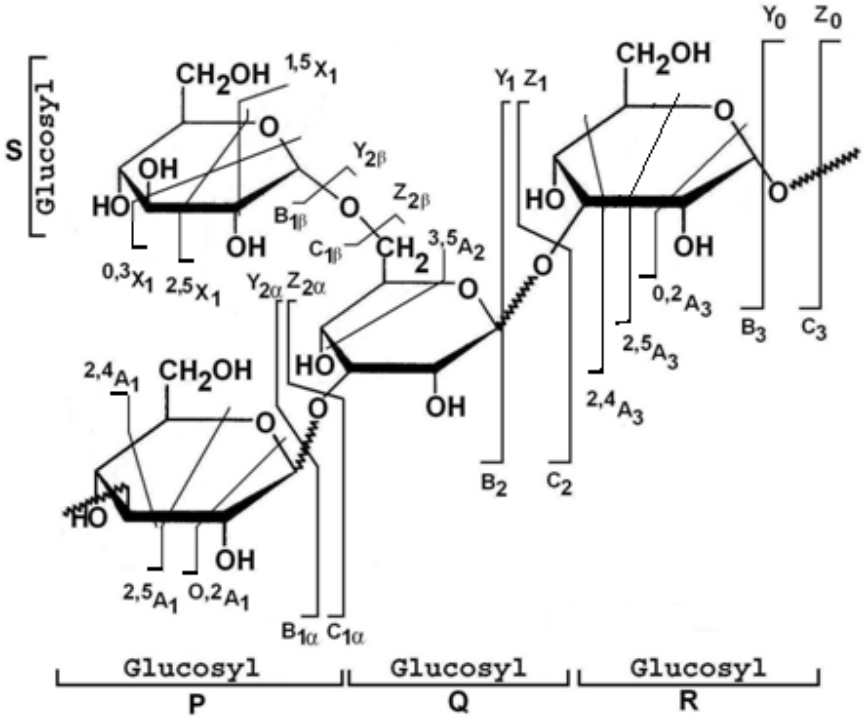

Figure 1(a). Fragmentation patterns (MALDI-TOF MS) of different mass fragments of polysaccharide (Fr.I) isolated from fruit bodies of *P.florida*

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The nomenclature of different fragments [Figure 1 (a)] and their peaks are presented as Harvey et.al [18] showed in case of a high mannose N-linked glycan from ribonuclease B recorded from 2,5 dihydroxy benzoic acid (DHB).

The spectrum [Figure 1 (b)] in reflector mode from *m/z* 500 to 1358 gave distinct mass [Table 1] peaks with reference to myoglobin (MW 30,000) as standard for external calibration. Carbohydrates possess a high affinity towards alkali metal ions and thus in MALDI spectra ($M+Na^+$) is normally observed instead of or in addition to ($M+H^+$) ions of very low abundance. The ion peak of the repeating oligosaccharide at *m/z* 671.1 was observed by breaking of the glycosidic linkage with oxygen at both sides of the repeating unit by double cleavage. The *m/z* 1319.1, 1303.0, 1157.0, 1140.5, 849.2, 833.1, 817.0, 687.0, 655.0 and 509.0 were solely the results of double cleavage phenomenon at glycosidic linkages of more than one oligosaccharide repeating units. The peaks at *m/z* 1260.1, 1244.2, 1200.1, 922.3, 774.4, 758.1, 714.2, 611.8, 596.9, and 551.6 were observed due to the breaking of the glycosidic linkages in one side and of ring of different sugar residues in linear chain of another side through double cleavage. The other fragments at *m/z* 978.2, 581.0, 567.8 and 537.0 appeared due to either breaking of the glycosidic linkages only or along with the ring cleavage of different sugar residues through triple cleavage phenomenon.

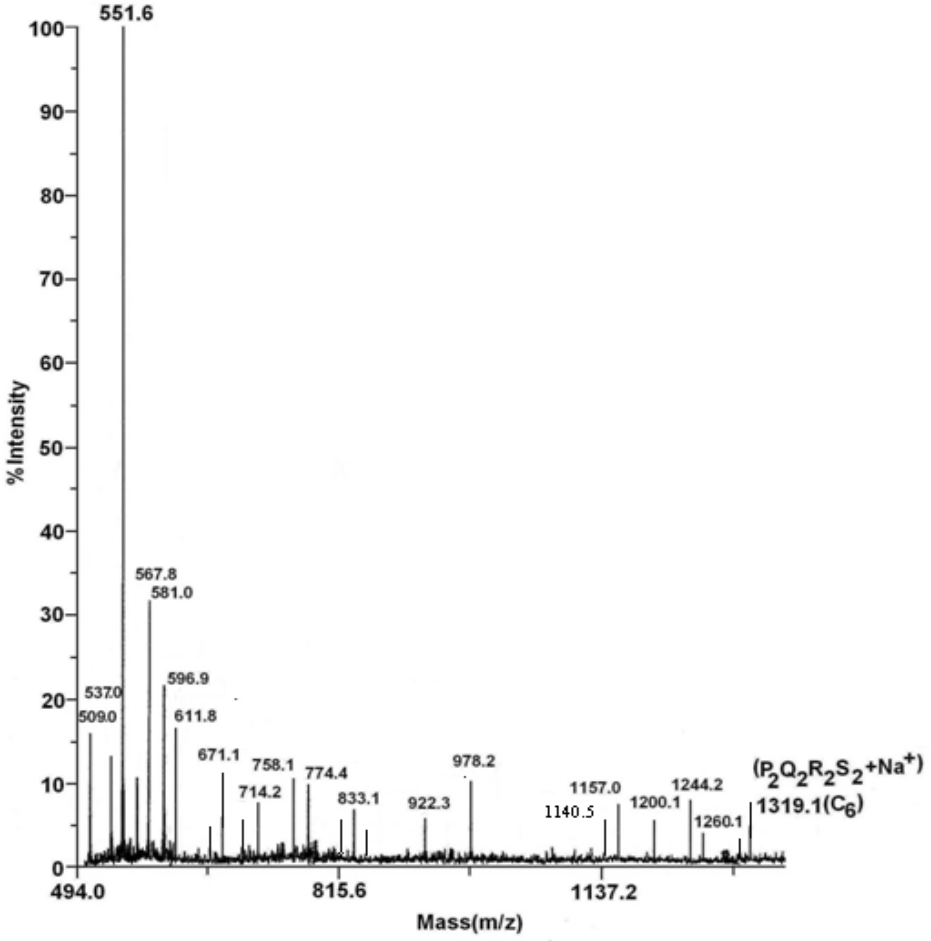

Figure 1(b). MALDI-TOF mass spectrum of polysaccharide (Fr.I) isolated from fruit bodies of *P.florida.* The sample was prepared using DHB (10 mg/mL) matrix dissolved in 2:1 acetonitrile-water containing 1% sodium trifluro acetate.

Fr.I					
Double cleavage					
Glycosidic cleavage	*m/z*		Ring cleavage	*m/z*	
	Expt.	Theo.		Expt.	Theo.
C_6	1319.1	1319.0	$^{0,2}A_6$	1260.1	1260.0
B_6	1303.0	1303.0	$^{2,5}A_6$	1244.2	1244.0
C_5	1157.0	1157.0	$^{2,4}A_6$	1200.1	1200.0
B_5	1140.5	1141.0	$^{3,5}A_5$	922.3	922.0
$C_{4\alpha}$	833.1	833.0	$^{3,5}A_2$	922.3	922.0
$B_{4\alpha}$	817.0	817.0	$^{0,2}A_4$	774.4	774.0
C_3	671.1	671.0	$^{2,5}A_4$	758.1	758.0

B_3	655.0	655.0	$^{2,4}A_4$	714.2	714.0
C_2	509.0	509.0	$^{0,2}A_3$	611.8	612.0
Z_3	671.1	671.0	$^{2,5}A_3$	596.9	596.0
Y_3	687.0	687.0	$^{2,4}A_3$	551.6	551.0
Z_4	833.1	833.0			
Y_4	849.2	849.0			
$Z_{2\alpha}$	833.1	833.0			
$Y_{2\alpha}$	817.0	817.0			
Triple cleavage					
Glycosidic cleavage	*m/z*		Ring cleavage	*m/z*	
	Expt.	Expt.		Expt.	Theo.
C_5/ $Z_{2\beta}$	978.2	978.0	$C_3/^{0,3}X_1$	581.0	581.0
C_5/ $Z_{5\beta}$	978.2	978.0	$C_3/^{2,5}X_1$	567.8	567.0
			$C_3/^{1,5}X_1$	537.0	537.0

Table 1. Different fragmentations of different sugar residues of the repeating unit of the polysaccharide (Fr.I) in MALDI-TOF-MS analysis.

The higher fragments above 1358 showed poor response of *m/z* values in reflector mode. It may be the reason that the higher ranges of mass values were not observed due to collapse of the molecule facing such kind of cleavage incidence.

The MALDI-TOF analysis of the polysaccharide (Fr.II):

The molecular weight of the polysaccharide was observed as ~ 48,000 Da, $[\alpha]_D^{30}$ +80.3 (c 0.08,water) and its structure was reported [17] as:

α-D-Glc*p*
1
↓
4
→6)-α-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→
2
↑
1
β-D-Man*p*

Different fragmentation of Fr.II was described according to Harvey et.al [18] [Figure 2 (a)]. The molecule showed distinct mass peaks from *m/z* 500 to 1358 [Table 2 and Figure 2 (b)] in reflector mode.

The ion peaks of the repeating oligosaccharide at *m/z* 655.0 and 671.0 were observed due to breaking of the glycosidic linkages without or with oxygen at both sides of the repeating unit by double cleavage. The *m/z* 1319.0, 1303.0, 1156.8, 1140.5, 849.0, 833.1, 687.0, 655.0 and 508.7 were solely the results of double cleavage phenomenon at

glycosidic linkages of more than one oligosaccharide repeating units.

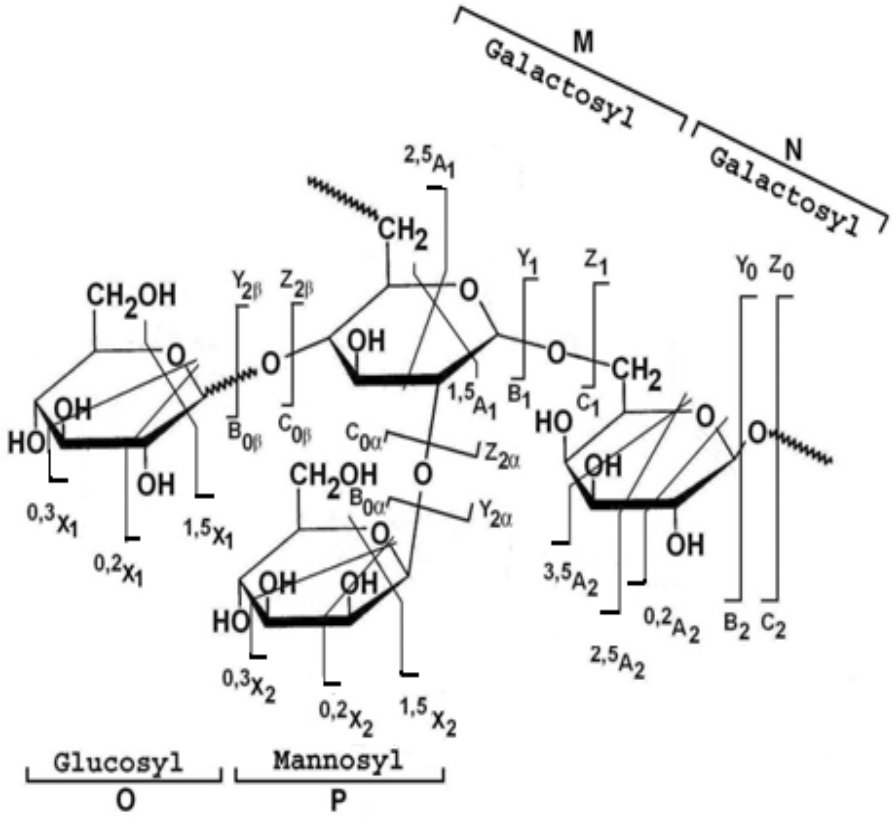

Figure 2(a). Fragmentation patterns (MALDI-TOF MS) of different mass fragments of polysaccharide (Fr.II) isolated from fruit bodies of *P.florida.*

The peaks at *m/z* 1259.6, 1244.0, 1214.0, 1111.5, 920.0, 611.6, 596.0 and 566.8 were observed due to the breaking of the glycosidic linkages in one side and of ring of different sugar residues in linear chain of another side through double cleavage. The other fragments at *m/z* 1229.0, 1199.0, 1185.0, 977.8, 581.0, 551.0 and 537.0 appeared due to either breaking of the glycosidic linkages only or along with the ring cleavage of different sugar residues through triple cleavage phenomenon. α and β are representing two major branches in the polysaccharides.

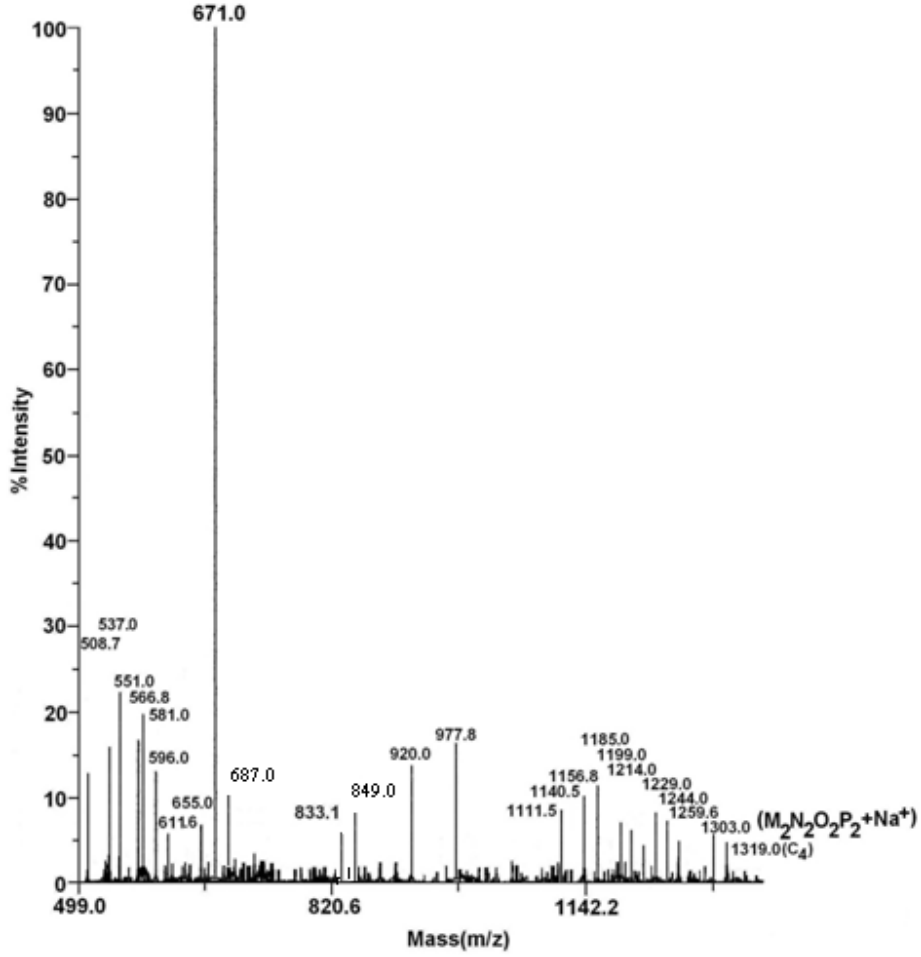

Figure 2(b). MALDI-TOF mass spectrum of polysaccharide (Fr.II) isolated from fruit bodies of *P.florida.* The sample was prepared using DHB (10 mg/mL) matrix dissolved in 2:1 acetonitrile-water containing 1% sodium trifluro acetate.

Fr.II					
Double cleavage					
Glycosidic cleavage	*m/z*		Ring cleavage	*m/z*	
	Expt.	Theo.		Expt.	Theo.
C_4	1319.0	1319.0	$^{0,2}A_4$	1259.6	1260.0
B_4	1303.0	1303.0	$^{2,5}A_4$	1244.0	1244.0
C_3	1156.8	1157.0	$^{3,5}A_4$	1214.0	1214.0
B_3	1140.5	1141.0	$^{1,5}A_3$	1111.5	1112.0
C_2	671.0	671.0	$^{2,5}A_3$	920.0	920.0
B_2	655.0	655.0	$^{0,2}A_2$	611.6	612.0

C_1	508.7	509.0	$^{2,5}A_2$	596.0	596.0
Y_3	849.0	849.0	$^{3,5}A_2$	566.8	566.0
Z_3	833.1	833.0			
Y_2	687.0	687.0			
Z_2	671.0	671.0			
Triple cleavage					
Glycosidic cleavage	*m/z*		Ring cleavage	*m/z*	
	Expt.	Expt.		Expt.	Theo.
C_3/ $Z_{4\beta}$	977.8	978.0	$C_4/^{0,3}X_3$	1229.0	1229.0
C_3/ $Z_{4\alpha}$	977.8	978.0	$C_4/^{0,3}X_4$	1229.0	1229.0
C_3/ $Z_{2\beta}$	977.8	978.0	$C_4/^{0,2}X_3$	1199.0	1199.0
C_3/ $Z_{2\alpha}$	977.8	978.0	$C_4/^{0,2}X_4$	1199.0	1199.0
			$C_4/^{1,5}X_3$	1185.0	1185.0
			$C_4/^{1,5}X_4$	1185.0	1185.0
			$C_2/^{0,3}X_1$	581.0	581.0
			$C_2/^{0,3}X_2$	581.0	581.0
			$C_2/^{0,2}X_1$	551.0	551.0
			$C_2/^{0,2}X_2$	551.0	551.0
			$C_2/^{1,5}X_1$	537.0	537.0
			$C_2/^{1,5}X_2$	537.0	537.0

Table 2. Different fragmentations of different sugar residues of the repeating unit of the polysaccharide (Fr.II) in MALDI-TOF-MS analysis.

4. Conclusions

Thus double or triple cleavages of the polymeric chain showed different mass fragments from where the cross-linking and branching information [19] of these molecules were established.

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