Structures of Exopolysaccharides Involved in Receptor-Mediated Perception of *Mesorhizobium loti* by *Lotus japonicus*

Artur Muszyński[1#], Christian Heiss[1], Christian T. Hjuler[3,5], John T. Sullivan[2], Simon J. Kelly[2,3,4], Mikkel B. Thygesen[3,5], Jens Stougaard[3,4], Parastoo Azadi[1], Russell W. Carlson[1] and Clive W. Ronson[2,3#]

From the [1]Complex Carbohydrate Research Center, University of Georgia, Athens GA, USA, the [2]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand, the [3]Centre for Carbohydrate Recognition and Signaling, Aarhus University, Aarhus, Denmark, the [4] Department of Molecular Biology and Genetics, Aarhus University, Aarhus C, Denmark, and the [5] Department of Chemistry, University of Copenhagen, Copenhagen, Denmark

Running title: Structures of high and low molecular mass EPS of *Mesorhizobium loti* R7A

[#] To whom correspondence should be addressed: Dr. Artur Muszyński, Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Rd, Athens, Georgia 30602, USA; Telephone: +1 (706) 542-9496; FAX: +1 (706) 542-4412; E-mail: muszynski@ccrc.uga.edu; or Prof. Clive Ronson, Department of Microbiology and Immunology, University of Otago, 720 Cumberland St, Dunedin, New Zealand, Telephone: +64 3-479-7701 email: Clive.Ronson@otago.ac.nz

Keywords: *Mesorhizobium loti,* exopolysaccharide, acetylation, determinate symbiosis, octasaccharide, polysaccharide structure, rhizobia, riburonic acid

---

SUPPLEMENTAL DATA

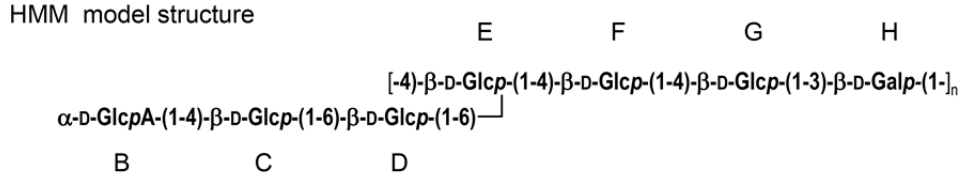
SUPPLEMENTAL TABLES

Supplemental Table 1. Comparison of observed chemical shifts of residue E and H in both HMM and LMM EPS with calculated values obtained from CASPER (1). Since Rib*f*A was not available in the CASPER database, we omitted it from the structure that was used for the chemical shift calculation.

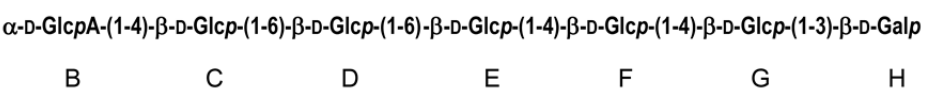
EPS	Residue	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6a,b C6
HMM	E, 4,6-β-Glc	4.50	3.30	3.68	3.72	3.76	4.23/3.90
		103.3	73.6	75.3	78.9	74.2	68.9
	4,6-β-Glc*	4.56	3.39	3.69	3.73	3.81	4.27/3.96
		103.23	73.80	75.10	79.43	74.82	68.79
	H, 3-β-Gal	4.49	3.68	3.76	4.13	3.76	n.d.
		103.3	70.7	82.8	68.9	74.2	
	3-β-Gal*	4.51	3.72	3.82	4.21	3.76	3.81/3.80
		103.49	70.68	83.25	69.15	75.88	61.82
LMM	E, 6-β-Glc	4.50	3.32	3.51	3.44	3.68	4.20/3.83
		103.0	73.4	76.0	70.1	75.3	69.5
	6-β-Glc*	4.53	3.34	3.53	3.49	3.67	4.20/3.88
		103.41	74.08	76.54	70.43	75.96	69.37
	Hβ, 3-β-Gal	4.62	3.64	3.78	4.16	3.70	n.d.
		96.6	71.1	82.8	68.7	75.4	
	3-β-Gal*	4.58	3.60	3.74	4.15	3.68	3.72/3.67
		97.05	71.75	83.45	69.35	75.65	61.85

*chemical shifts derived from CASPER database. The model structures presented below were used for chemical shift predictions.

HMM model structure



LMM model structure



Supplemental Table 2. High resolution MS analysis of LMM EPS octasaccharide. For mass spectra and structure assignments refer to Supplemental Figure 4

EPS octasaccharide		Residues	Composition	OAc groups	Calcd.	Found	Δ (ppm)
HR-MS		A-H	$[RibAGlcAHex_7OAc_3+Na]^+$	3	1629.4549	1629.4558	-0.55
		A-H	$[RibAGlcAHex_6OAc_4+Na]^+$	4	1503.4126	1503.4142	-1.06
		A-H	$[RibAGlcAHex_6OAc_3+K]^+$	3	1477.3760	1477.3767	-0.47
		A-H	$[RibAGlcAHex6OAc_3+Na]^+$	3	1461.4020	1461.4027*	-0.47
		A-H	$[RibAGlcAHex_6OAc_3+NH_4]^+$	3	1456.4466	1456.4474	-0.54
		A-H	$[RibAGlcAHex_6OAc_3+NH_4-H_2O]^+$	3	1438.4361	1438.4366	-0.34
		A-H	$[RibAGlcAHex_6OAc_2+K]^+$	2	1435.3654	1435.3661	-0.49
		A-H	$[RibAGlcAHex_6OAc_2+Na]^+$	2	1419.3915	1419.3917	-0.14
		A-H	$[RibAGlcAHex_6OAc_2+NH_4-H_2O]^+$	2	1396.4255	1396.4256	-0.07
		A-H	$[RibAGlcAHex_6OAc+Na]^+$	1	1377.3809	1377.3816	-0.50
		A-H	$[RibAGlcAHex_6OAc_3+2Na]^{2+}$	3	742.1956	742.1957	-0.13
Fragmentation ion							
No.	Type						
I	Y_7	B-H	$[GlcAHex_6OAc_2+Na]^+$	2	1273.3699	1273.3701	-0.15
II	Y_7	B-H	$[GlcAHex_6OAc+Na]^+$	1	1231.3594	1231.3599	-0.40
III	B_6	A-F	$[RibAGlcAHex_4OAc_3+Na]^+$	3	1137.2964	1137.2964	0.00
IV	Y_7/B_7	B-G	$[GlcAHex_5OAc_2+Na]^+$	2	1111.3171	1111.3172	-0.08
V	Y_7/B_7	B-G	$[GlcAHex_5OAc_2+Na-H_2O]^+$	2	1093.3066	1093.3059	0.64
VI	Y_6/B_7	C-G	$[Hex_5OAc_2+Na]^+$	2	935.2850	935.2848	0.21
VII	Y_3 or Y_4/B_7	3 Hex	$[Hex_3-OH]^+$	0	487.1657	487.1632	5.13
MS/MS		Residues	Composition	OAc groups	Calcd.	Found	Δ (ppm)
*MS/MS of m/z 1461.4026		A-H	$[RibAGlcAHex_6OAc_3+Na]^+$	3	1461.4026	1461.4017	0.04
		A-H	$[RibAGlcAHex_6OAc_3+Na-H_2O]^+$	3	1443.3914	1443.3899	-2.13
Fragmentation ion							
No.	Type						
VIII	B_7	A-G	$[RibAGlcAHex_5OAc_3+Na]^+$	3	1299.3492	1299.3473	-1.61
IX	Y_7	B-H	$[GlcAHex_6OAc_2+Na]^+$	2	1273.3699	1273.3705	-2.15
X	Y_7	B-H	$[GlcAHex_6OAc_2+Na-H_2O]^+$	2	1255.3594	1255.3592	-1.89
XI	Y_7/B_7	B-G	$[GlcAHex_5OAc_2+Na]^+$	2	1111.3171	1111.3169	-0.52
XII	Y_7/B_7	B-G	$[GlcAHex_5OAc_2+Na-H_2O]^+$	2	1093.3066	1093.3056	0.42
XIII	Y_5 or Y_6/B_7	5 Hex	$[Hex_5OAc_2+Na]^+$	2	935.2850	935.2848	-1.35
XIV	Y_7/B_6	B-F	$[GlcAHex_4OAc_2+Na-H_2O]^+$	2	931.2537	931.2536	-0.01

*This parent ion was MS/MS fragmented.

REFERENCES

1. Lundborg, M., and Widmalm, G. (2011) Structural analysis of glycans by NMR chemical shift prediction. *Anal. Chem.* **83**, 1514-1517

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

Supplemental Figure 1. The mass spectra for the PMAA derivatives from HMM EPS of 4-linked Glc*A*p (A), terminally linked Glc*p*A (B), terminally linked Rib*f*A (C), and reducing end 3-linked Gal*p* (D) after carboxyl group reduction.

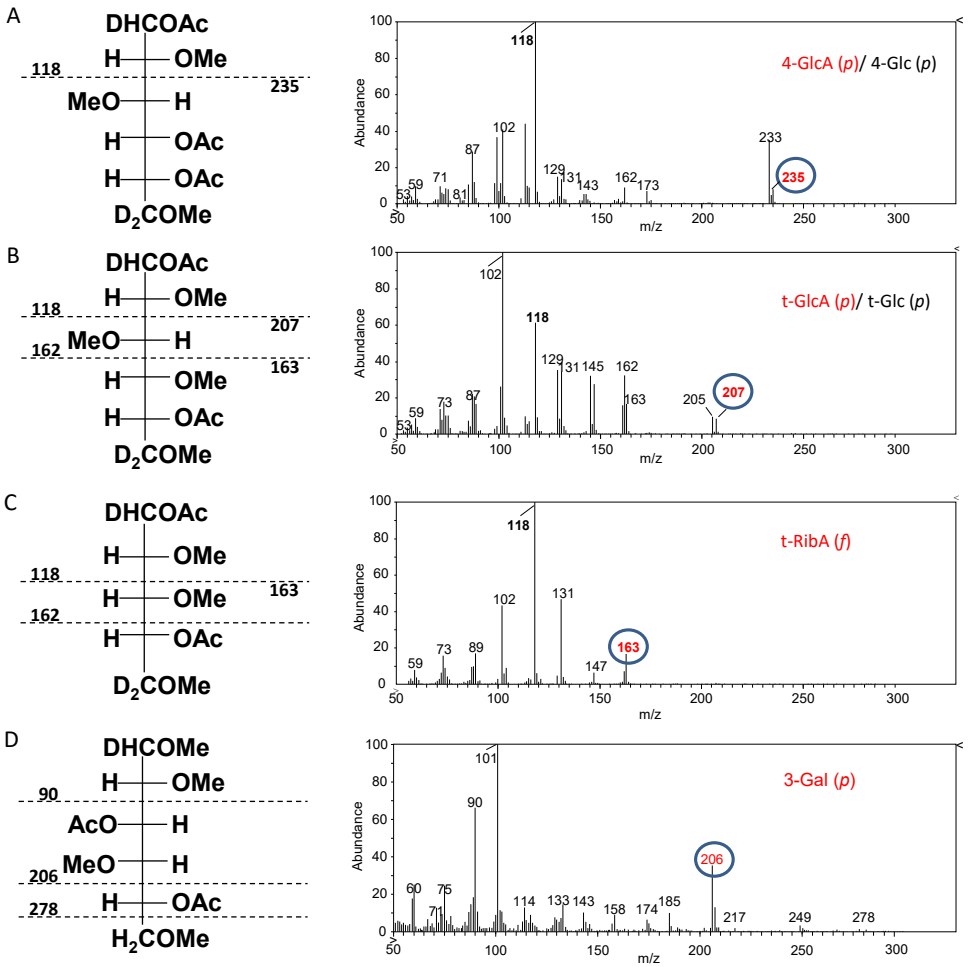
Supplemental Figure 2. The ^{1}H NMR spectrum of intact (*O*-acetylated) HMM EPS from *M. loti* R7A at 70℃. The inset demonstrates viscosity of EPS dissolved in water at RT.

Supplemental Figure 3. The COSY spectrum of de-*O*-acetylated HMM EPS from *M. loti* R7A. The glycosyl residues A-H are as indicated in Table 1. The enlarged region presents the H1/H2 couplings for the β-linked hexosyl residues. Legend: "Hex", gluco or galactosyl residue

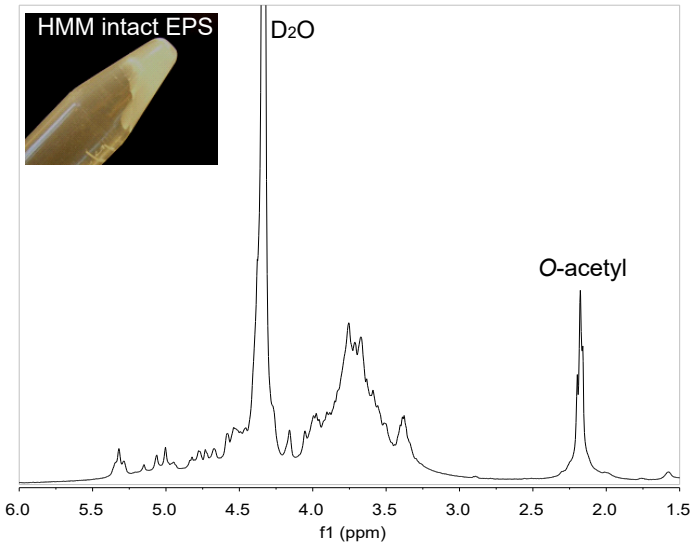
Supplemental Figure 4. SEC elution profile of LMM crude exopolysacchride recovered from *M. loti* R7A *ndvB* G/RDA culture media, and comparison with elution profile of a standard mixture of neutral hexose (G1), hexa-oligosacchrides (G3, G5, G7) and 40-kDa dextran (Vo). The main fraction recovered from culture precipitate was that of *O*-acetylated octasaccharide. A maximum Superdex Peptide 10/300GL column exclusion limit =7000 Da.

Supplemental Figure 5. XR ESI-FT-ICR-MS analysis of LMM EPS octasaccharide, with fragmentation ions indicated with roman numerals I-XIV as listed in Supplemental Table 2.) The panel shows an overview of the MS data obtained for EPS octasaccharide, with an expansion of the mass range m/z 1350-1550 displayed in panel B. C) MS/MS for the ion 1461.4026, showing further fragmentation of the octasaccharide. D) Summary of proposed ICR-MS fragmentation

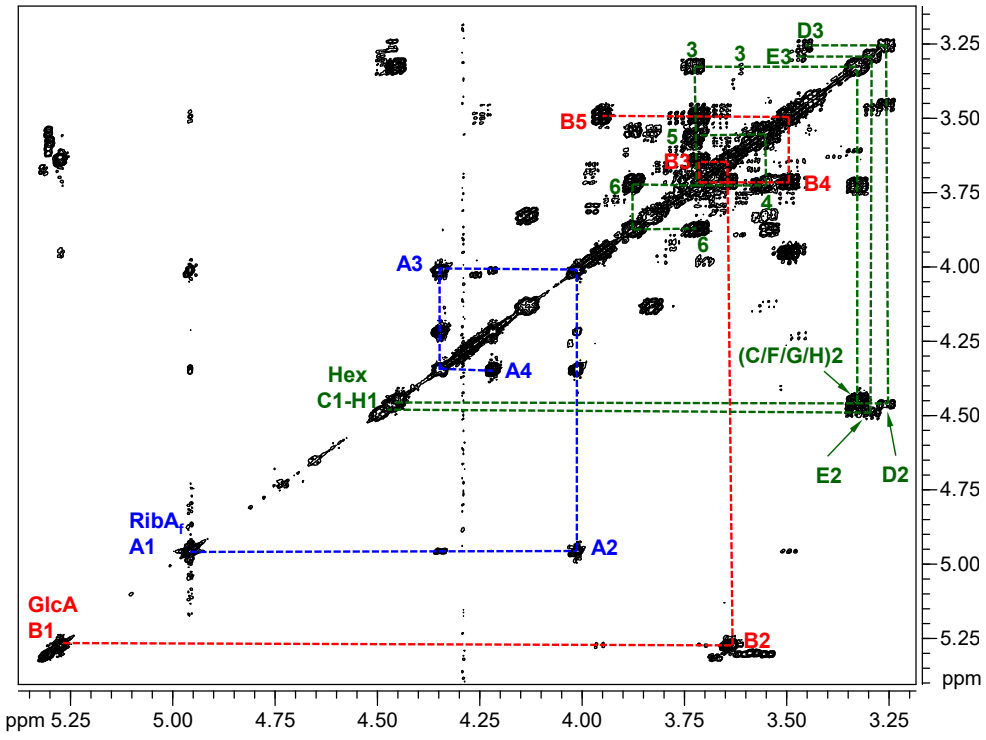
Supplemental Figure 6. Comparison and alignment of *exo* genes involved in biosynthesis of EPS in *M. loti* R7A, *S. meliloti* 1021, *S.fredii* NGR234, *R.tropici* CIAT899.



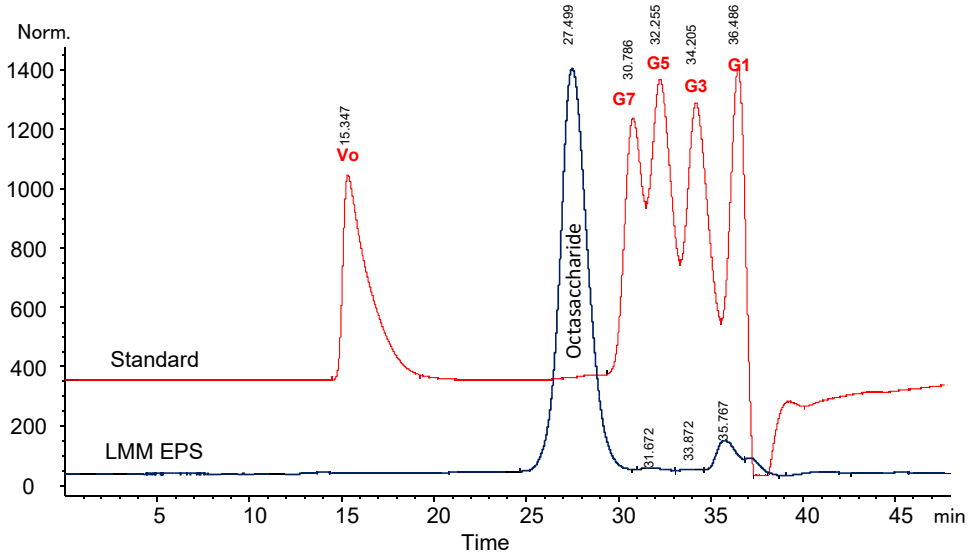
Supplemental Figure 1



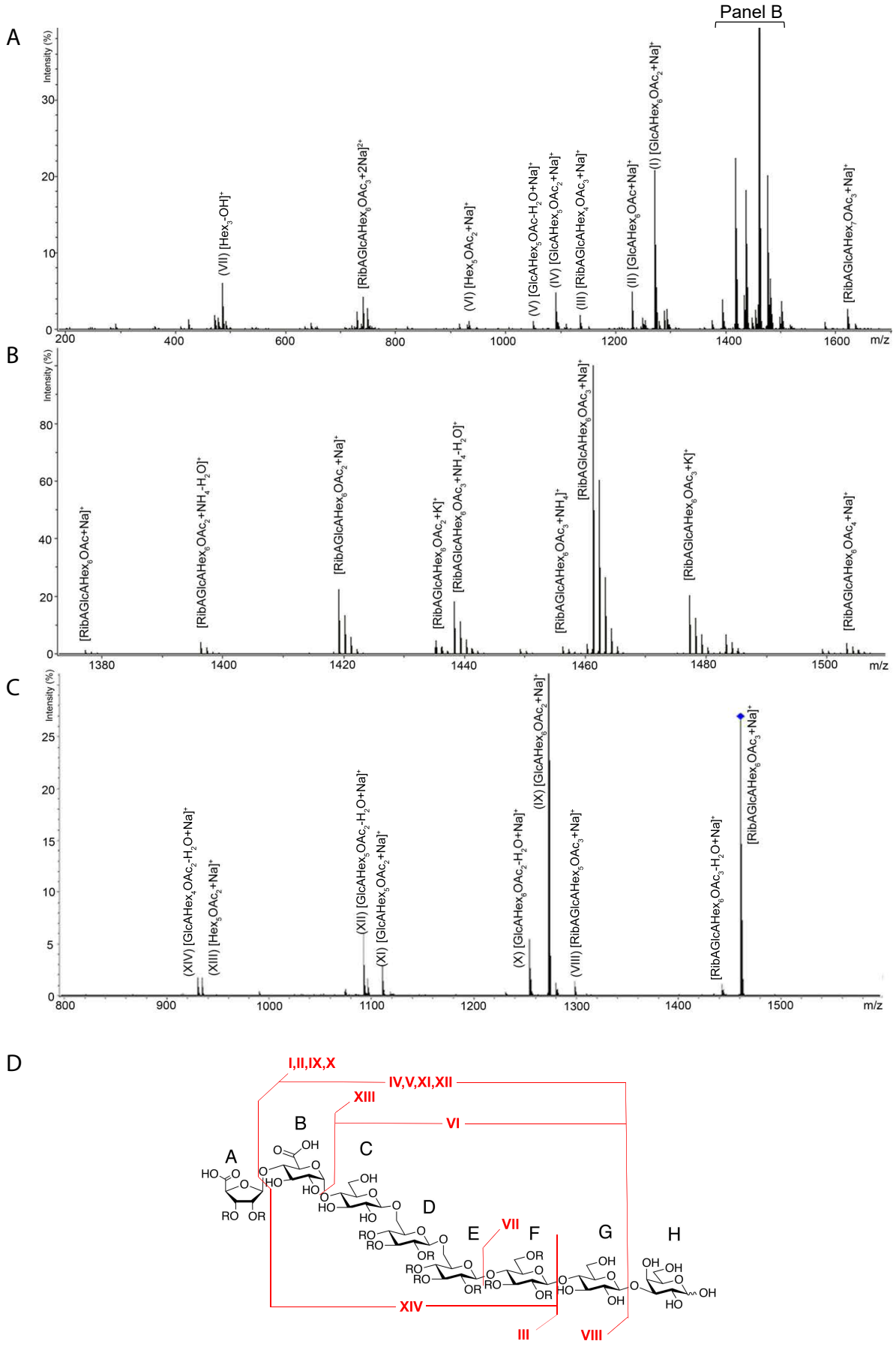
Supplemental Figure 2



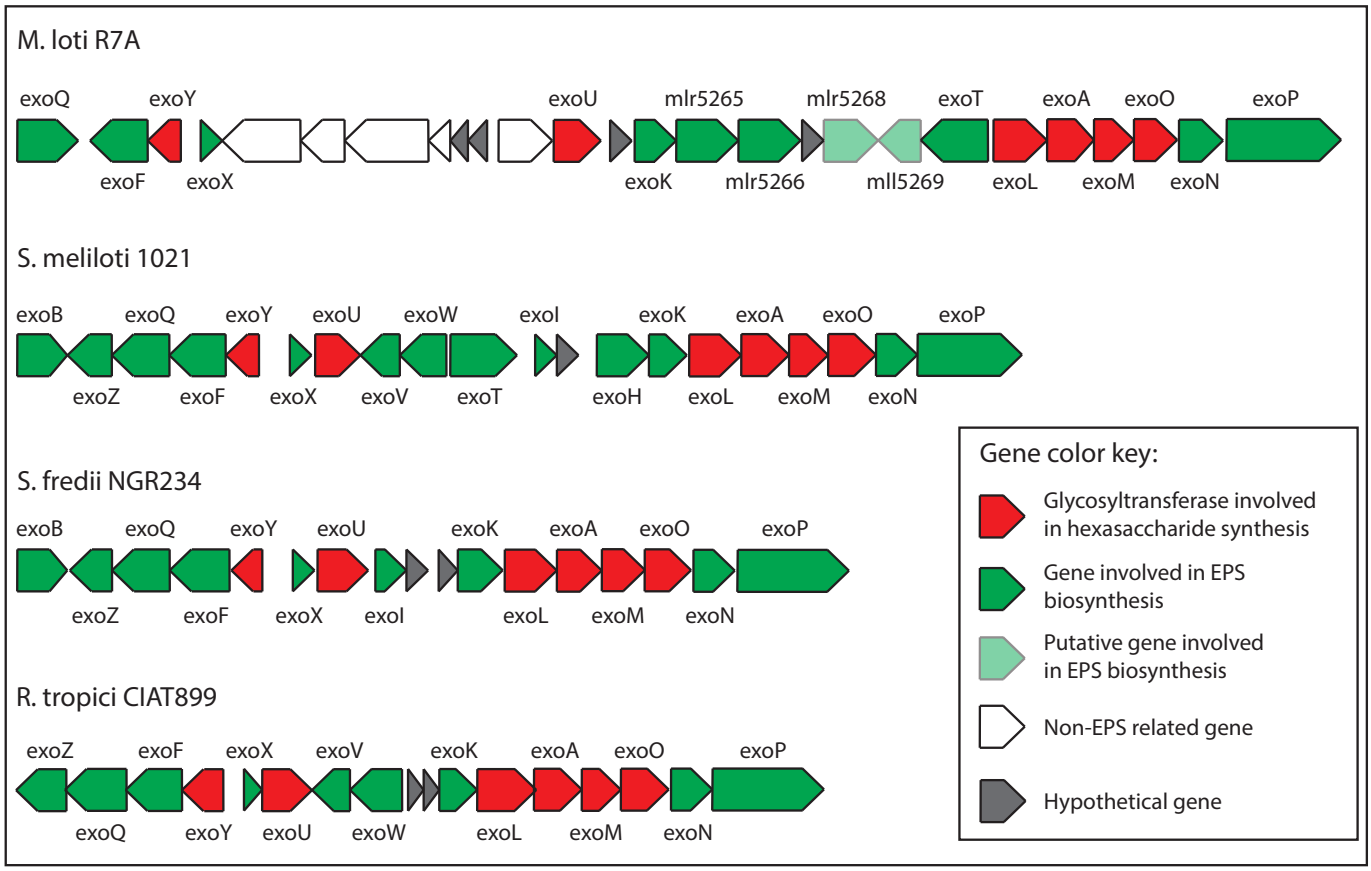
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6