Electronic Supplementary Information

Carbohydrate and Lignin are Simultaneously Solubilized from Unpretreated Switchgrass by Microbial Action at High Temperature

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Tables S1 - S6

Figures S1 - S10

Table S1. Molecular characterization of lignin after analytical pyrolysis of switchgrass samples.

		Switchgrass samples							
		SG	**wSG**	**SG1c**	**SG2c**	**SG3c**	**SG1**	**SG2**	**SG3**
Aromatic compound	**Precursor[a]**	**Peak intensity of S, G and H precursor (arbitrary units)**							
Vinyl phenol	H	4.5	4.5	4.7	4.6	5.1	5.0	4.9	4.8
Guaiacol	G	2.7	2.5	2.8	2.9	2.8	3.0	2.8	3.0
Coniferyl alcohol	G	2.9	2.9	3.5	3.3	3.5	3.5	3.3	3.4
Methyl guaiacol	G	1.7	1.7	1.9	1.8	1.9	1.9	1.8	1.9
Vinyl guaiacol	G	3.7	4.1	4.0	4.1	4.1	4.2	4.2	4.1
4-Ethyl guaiacol	G	1.5	1.5	1.6	1.6	1.6	1.6	1.7	1.7
Syringol	S	1.6	1.3	1.6	1.6	1.6	1.7	1.6	1.7
Allyl+propenyl guaiacol	G	2.0	1.9	2.5	2.5	2.7	2.6	2.4	2.5
Ethylsyringol	S	1.9	2.0	2.4	2.2	2.6	2.4	2.3	2.3
4-Methyl-2,6-dimethoxyphenol	S	1.3	1.4	1.7	1.4	1.7	1.6	1.5	1.6
Coniferyl aldehyde	G	1.5	1.4	1.6	1.7	1.7	1.7	1.7	1.7
Coniferyl alcohol	S, G	2.3	2.2	3.0	2.7	3.1	2.9	2.7	3.0
Syringaldehyde	S	1.2	1.0	1.3	1.2	1.2	1.2	1.2	1.3
4-Propenylsyringol	S	1.9	2.0	3.0	2.7	3.2	2.8	2.6	2.8
Sinapyl aldehyde	S	1.0	1.1	1.1	1.1	1.1	1.1	1.0	1.1
Sinapyl alcohol	S	1.0	0.9	1.3	1.2	1.3	1.1	1.2	1.2
	Total lignin, %	19.2	18.8	22.6	22.8	20.8	21.7	23.4	22.6
	S/G ratio	0.7	0.7	0.8	0.7	0.7	0.7	0.8	0.7

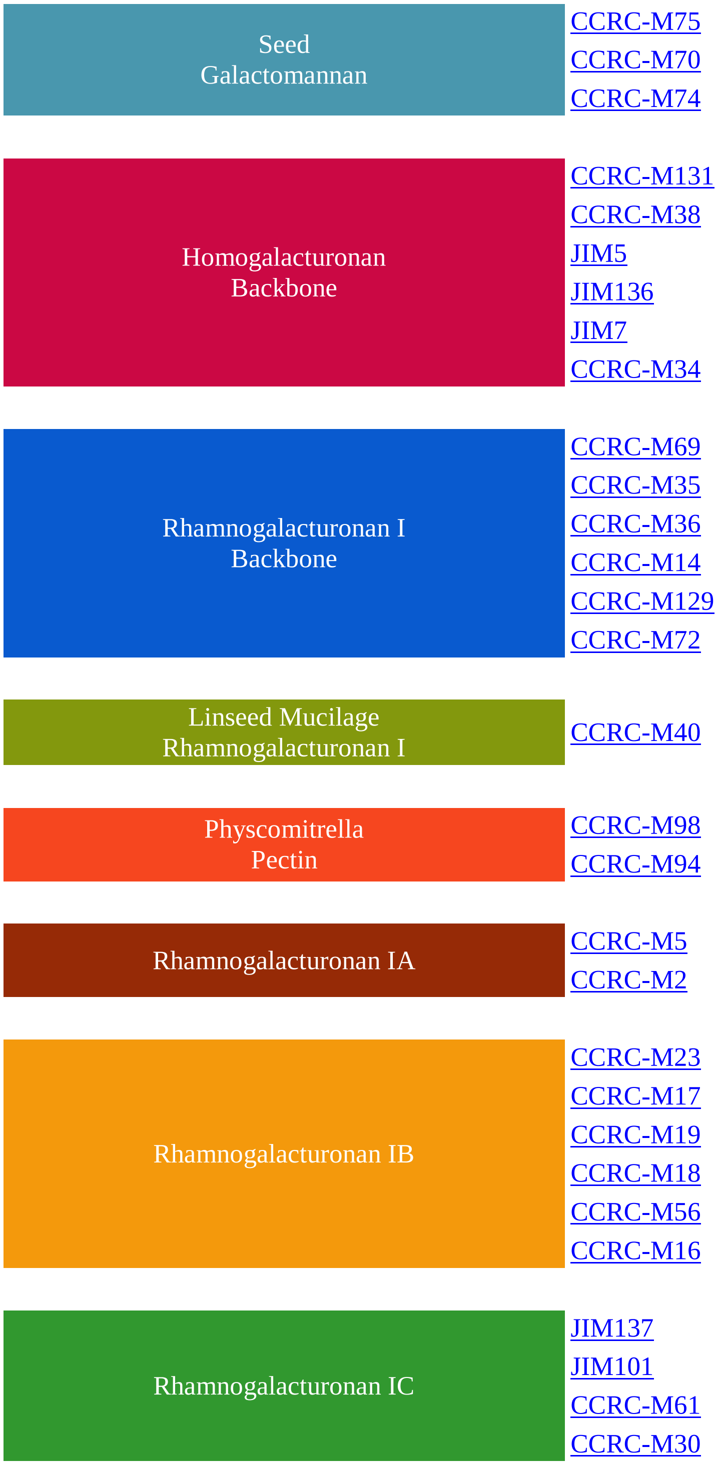
Table S2. Characteristics of solid state NMR spectra shown in Figure S8. The abbreviations are: *S, syringyl (aromatic unit with two methoxy groups); G, guaiacyl (aromatic unit with only one methoxy); H, *p*-hydroxyphenyl (aromatic unit with no methoxy); ne, in non-esterified arylglycerol β-aryl ethers; e, in esterified arylglycerol β-aryl ethers.

Resonance number	Chemical shift (ppm)	Assignments*
1	>172	Carbohydrates & Lignins: -*C*OO-R, CH3-*C*OO-
2	153	Lignins: S3(e), S5(e), H4
2	147	Lignins: G1, G4, S3(ne), S5(ne)
3	135	Lignins: S1(e), S4(e)
3	134.3	Lignins: S1(ne), S4(ne)
3	130	Lignins: H6, H2, H1
	115-106	Lignins: G5, G6, G2, S2, S6, H3, H5
	105-102	Carbohydrates: C1
4	88.6	Carbohydrates: crystalline C4
5	83.6	Lignins: Cβ. Carbohydrates: amorphous C4
	74.8	Lignins: Cα. Carbohydrates: C2,3,5
	72.1	Carbohydrates: C2,3,5
	64.7	Carbohydrates: C6
	61.6	Lignins: Cγ
	55.7	Lignins: O*C*H3
	30.1	Residual proteins or waxes: aliphatic methylene carbons
6	21	Carbohydrates: *C*H3-COO

Table S3. Expanded list of all plant cell wall glycan-directed monoclonal antibodies (mAbs) used in this study for Glycome Profiling (Figure 2). The groupings of antibodies are based on a hierarchical clustering analysis of all mAbs screened against a panel of plant polysaccharide preparations (*9*) that groups the mAbs according to the polysaccharides that they predominantly recognize. The majority of listings link to the Wall*Mab*DB plant cell wall monoclonal antibody database (http://www.wallmabdb.net) that provides detailed descriptions of each mAb, including immunogen used, antibody isotype, epitope structure recognized (where known), supplier information, and any related literature reference(s).

Glycan Group Recognized	**mAb Name**
Non-Fucosylated Xyloglucan	CCRC-M54
	CCRC-M48
	CCRC-M49
	CCRC-M96
	CCRC-M50
	CCRC-M51
	CCRC-M53
	CCRC-M100
	CCRC-M103
	CCRC-M58
	CCRC-M86
	CCRC-M55
	CCRC-M52
	CCRC-M99
	CCRC-M95
	CCRC-M101
	CCRC-M104
	CCRC-M89
	CCRC-M93
	CCRC-M87
	CCRC-M88
	CCRC-M57
	CCRC-M90

Fucosylated Xyloglucan	CCRC-M102 CCRC-M39 CCRC-M106 CCRC-M84 CCRC-M1
Xylan 1/Xyloglucan	CCRC-M111 CCRC-M108 CCRC-M109
Xylan 2	CCRC-M119 CCRC-M115 CCRC-M110 CCRC-M105 CCRC-M117 CCRC-M113 CCRC-M120 CCRC-M118 CCRC-M116 CCRC-M114 CCRC-M154 CCRC-M150
Xylan 3	CCRC-M160 CCRC-M137 CCRC-M152 CCRC-M149 CCRC-M144 CCRC-M146 CCRC-M145 CCRC-M155
Xylan 4	CCRC-M153 CCRC-M151 CCRC-M148 CCRC-M140 CCRC-M139 CCRC-M138



Seed Galactomannan	CCRC-M75 CCRC-M70 CCRC-M74
Homogalacturonan Backbone	CCRC-M131 CCRC-M38 JIM5 JIM136 JIM7 CCRC-M34
Rhamnogalacturonan I Backbone	CCRC-M69 CCRC-M35 CCRC-M36 CCRC-M14 CCRC-M129 CCRC-M72
Linseed Mucilage Rhamnogalacturonan I	CCRC-M40
Physcomitrella Pectin	CCRC-M98 CCRC-M94
Rhamnogalacturonan IA	CCRC-M5 CCRC-M2
Rhamnogalacturonan IB	CCRC-M23 CCRC-M17 CCRC-M19 CCRC-M18 CCRC-M56 CCRC-M16
Rhamnogalacturonan IC	JIM137 JIM101 CCRC-M61 CCRC-M30

Rhamnogalacturonan I Arabinogalactan	CCRC-M60
	CCRC-M41
	CCRC-M80
	CCRC-M79
	CCRC-M44
	CCRC-M33
	CCRC-M32
	CCRC-M13
	CCRC-M42
	CCRC-M24
	CCRC-M12
	CCRC-M7
	CCRC-M77
	CCRC-M25
	CCRC-M9
	CCRC-M128
	CCRC-M126
	CCRC-M125
	CCRC-M123
	CCRC-M122
	CCRC-M121
	CCRC-M112
	CCRC-M21
	JIM131
	CCRC-M22
	JIM132
	JIM1
	CCRC-M15
	CCRC-M8
	MH4.3E5
	JIM16
Arabinogalactan 1	JIM93
	JIM94
	JIM11
	MAC204
	JIM20

Arabinogalactan 2	JIM14 MAC207 JIM19 JIM12 CCRC-M133 CCRC-M107
Arabinogalactan 3	JIM4 CCRC-M31 JIM17 CCRC-M26 JIM15 JIM8 CCRC-M85 CCRC-M81 MAC266 PN 16.4B4
Arabinogalactan 4	JIM133 JIM13 CCRC-M92 CCRC-M91 CCRC-M78
Unidentified	MAC265 CCRC-M97

Table S4. The $\log_2$ ratios for MAB binding to switchgrass samples. Only $\log_2$ ratios ≥ 1.0 or ≤ -1 are shown (corresponding to $\geq$ 2-fold changes). Treatments with no MABs that meet the cut are colored light blue-gray. The maximum value in any treatment is listed in the "Max. Value" column (dark gray). Raw values were floored at 0.1 prior to calculating the ratios.

		Log2 Ratio (wSG/SG)						
mAb group	**mAb name**	**Am OX**	**Na2C03**	**1M KOH**	**4M KOH**	**Chlorite**	**Post-chlorite 4M KOH**	**Max. Value**
AG-2	JIM12		-1.08					-1.08
AG-2	JIM14	2.52						2.52
AG-2	JIM19	1.05						1.05
AG-3	CCRC-M26							
AG-3	CCRC-M85							
AG-3	JIM15							
AG-3	JIM17							
AG-3	JIM8							
AG-3	MAC266	-1.33						-1.33
AG-4	CCRC-M78							
AG-4	CCRC-M92							
AG-4	JIM13							
AG-4	JIM133							
FUC XG	CCRC-M106							
non-FUC XG	CCRC-M87	2.20	2.03					2.20
Pectic backbone	CCRC-M131							
Pectic backbone	CCRC-M14		2.78					2.78
Pectic backbone	CCRC-M35		1.60					1.60
Pectic backbone	CCRC-M36		1.20					1.20
Pectic backbone	CCRC-M38							
Pectic backbone	JIM5							
Pectic backbone	JIM7							
RG-1/AG	CCRC-M112	1.68	1.77				1.10	1.77
RG-1/AG	CCRC-M12	1.51	1.23					1.51
RG-1/AG	CCRC-M121							
RG-1/AG	CCRC-M122							
RG-1/AG	CCRC-M123							
RG-1/AG	CCRC-M125	1.27						1.27
RG-1/AG	CCRC-M126							
RG-1/AG	CCRC-M13	1.04						1.04
RG-1/AG	CCRC-M134							
RG-1/AG	CCRC-M15	1.22						1.22
RG-1/AG	CCRC-M21	1.24						1.24
RG-1/AG	CCRC-M22							
RG-1/AG	CCRC-M32	1.07						1.07
RG-1/AG	CCRC-M33	1.22						1.22
RG-1/AG	CCRC-M41							
RG-1/AG	CCRC-M42	1.11						1.11
RG-1/AG	CCRC-M44	1.57						1.57
RG-1/AG	CCRC-M60							
RG-1/AG	CCRC-M7	1.44	1.12					1.44
RG-1/AG	CCRC-M77	1.92	1.60	1.07				1.92
RG-1/AG	CCRC-M79							
RG-1/AG	CCRC-M80							
RG-1/AG	JIM1				-1.16			-1.16
RG-1/AG	JIM131							
RG-1/AG	JIM132	1.95						1.95
RG-1/AG	JIM16							
RG-I	JIM3		-1.04					-1.04
RG-Ib	CCRC-M16							
RG-Ib	CCRC-M17		1.11					1.11
RG-Ib	CCRC-M23		1.81					1.81
RG-Ic	CCRC-M61		1.11					1.11
RG-Ic	JIM101							
xylan-2	CCRC-M116				1.08			1.08
xylan-2	CCRC-M117							
xylan-2	CCRC-M150							
xylan-2	CCRC-M154							
Xylan-3	CCRC-M144							
Xylan-3	CCRC-M149							
Xylan-3	CCRC-M155							
	CCRC-M97							

Table S4 (continued)

		Log2 Ratio (SG1/SG1c)						
mAb group	**mAb name**	**Am OX**	**Na2C03**	**1M KOH**	**4M KOH**	**Chlorite**	**Post-chlorite 4M KOH**	**Max. Value**
AG-2	JIM12							
AG-2	JIM14	2.26						2.26
AG-2	JIM19							
AG-3	CCRC-M26							
AG-3	CCRC-M85							
AG-3	JIM15							
AG-3	JIM17							
AG-3	JIM8							
AG-3	MAC266							
AG-4	CCRC-M78	1.31	1.98					1.98
AG-4	CCRC-M92							
AG-4	JIM13	1.93						1.93
AG-4	JIM133	1.29						1.29
FUC XG	CCRC-M106							
non-FUC XG	CCRC-M87							
Pectic backbone	CCRC-M131							
Pectic backbone	CCRC-M14							
Pectic backbone	CCRC-M35							
Pectic backbone	CCRC-M36							
Pectic backbone	CCRC-M38							
Pectic backbone	JIM5					1.73		1.73
Pectic backbone	JIM7							
RG-1/AG	CCRC-M112	1.32						1.32
RG-1/AG	CCRC-M12	2.11						2.11
RG-1/AG	CCRC-M121							
RG-1/AG	CCRC-M122	1.03						1.03
RG-1/AG	CCRC-M123	1.01						1.01
RG-1/AG	CCRC-M125	1.01						1.01
RG-1/AG	CCRC-M126	1.35						1.35
RG-1/AG	CCRC-M13	1.54						1.54
RG-1/AG	CCRC-M134	1.09						1.09
RG-1/AG	CCRC-M15							
RG-1/AG	CCRC-M21	1.98						1.98
RG-1/AG	CCRC-M22							
RG-1/AG	CCRC-M32	2.25						2.25
RG-1/AG	CCRC-M33	1.67						1.67
RG-1/AG	CCRC-M41							
RG-1/AG	CCRC-M42	1.98						1.98
RG-1/AG	CCRC-M44	2.04						2.04
RG-1/AG	CCRC-M60							
RG-1/AG	CCRC-M7	2.14						2.14
RG-1/AG	CCRC-M77	2.43				1.16		2.43
RG-1/AG	CCRC-M79	1.30						1.30
RG-1/AG	CCRC-M80	1.12						1.12
RG-1/AG	JIM1	1.39	1.08			1.31		1.39
RG-1/AG	JIM131	3.01	1.39			1.45		3.01
RG-1/AG	JIM132	2.49				1.47		2.49
RG-1/AG	JIM16	1.57				1.18		1.57
RG-I	JIM3							
RG-Ib	CCRC-M16							
RG-Ib	CCRC-M17							
RG-Ib	CCRC-M23							
RG-Ic	CCRC-M61	1.44						1.44
RG-Ic	JIM101	1.99						1.99
xylan-2	CCRC-M116							
xylan-2	CCRC-M117						1.14	1.14
xylan-2	CCRC-M150							
xylan-2	CCRC-M154	1.70						1.70
Xylan-3	CCRC-M144							
Xylan-3	CCRC-M149	-1.33						-1.33
Xylan-3	CCRC-M155							
	CCRC-M97					1.12		1.12

Table S4 (continued)

		Log2 Ratio (SG2/SG2c)						
mAb group	**mAb name**	**Am OX**	**Na2C03**	**1M KOH**	**4M KOH**	**Chlorite**	**Post-chlorite 4M KOH**	**Max. Value**
AG-2	JIM12							
AG-2	JIM14	2.10				1.54		2.10
AG-2	JIM19							
AG-3	CCRC-M26							
AG-3	CCRC-M85		1.08					1.08
AG-3	JIM15					1.13		1.13
AG-3	JIM17		-1.27					-1.27
AG-3	JIM8					1.02		1.02
AG-3	MAC266							
AG-4	CCRC-M78	1.60	1.51					1.60
AG-4	CCRC-M92							
AG-4	JIM13	2.44						2.44
AG-4	JIM133					1.00		1.00
FUC XG	CCRC-M106							
non-FUC XG	CCRC-M87							
Pectic backbone	CCRC-M131	1.58	1.67					1.67
Pectic backbone	CCRC-M14							
Pectic backbone	CCRC-M35							
Pectic backbone	CCRC-M36							
Pectic backbone	CCRC-M38	1.51	1.70					1.70
Pectic backbone	JIM5	2.51				1.77		2.51
Pectic backbone	JIM7			1.66				1.66
RG-1/AG	CCRC-M112	1.86				1.93		1.93
RG-1/AG	CCRC-M12	2.49	1.03					2.49
RG-1/AG	CCRC-M121	1.23						1.23
RG-1/AG	CCRC-M122	1.40	1.13					1.40
RG-1/AG	CCRC-M123	1.52						1.52
RG-1/AG	CCRC-M125	1.46						1.46
RG-1/AG	CCRC-M126	2.46						2.46
RG-1/AG	CCRC-M13	1.95						1.95
RG-1/AG	CCRC-M134	1.79						1.79
RG-1/AG	CCRC-M15							
RG-1/AG	CCRC-M21	2.65						2.65
RG-1/AG	CCRC-M22	1.71						1.71
RG-1/AG	CCRC-M32	2.16						2.16
RG-1/AG	CCRC-M33	2.16						2.16
RG-1/AG	CCRC-M41							
RG-1/AG	CCRC-M42	2.17						2.17
RG-1/AG	CCRC-M44	2.51						2.51
RG-1/AG	CCRC-M60	1.10						1.10
RG-1/AG	CCRC-M7	2.28						2.28
RG-1/AG	CCRC-M77	2.44				1.13		2.44
RG-1/AG	CCRC-M79	1.71						1.71
RG-1/AG	CCRC-M80	1.79						1.79
RG-1/AG	JIM1	1.50	1.29			1.27		1.50
RG-1/AG	JIM131	2.62						2.62
RG-1/AG	JIM132	1.79				1.09		1.79
RG-1/AG	JIM16					1.14		1.14
RG-I	JIM3							
RG-Ib	CCRC-M16	2.10						2.10
RG-Ib	CCRC-M17	1.09						1.09
RG-Ib	CCRC-M23	2.13						2.13
RG-Ic	CCRC-M61		-1.03			1.24		1.24
RG-Ic	JIM101					1.52		1.52
xylan-2	CCRC-M116							
xylan-2	CCRC-M117							
xylan-2	CCRC-M150	1.07						1.07
xylan-2	CCRC-M154	1.46						1.46
Xylan-3	CCRC-M144		1.03					1.03
Xylan-3	CCRC-M149							
Xylan-3	CCRC-M155		1.16					1.16
	CCRC-M97							

Table S4 (continued)

		Log2 Ratio (SG3/SG3c)						
mAb group	**mAb name**	**Am OX**	**Na2C03**	**1M KOH**	**4M KOH**	**Chlorite**	**Post-chlorite 4M KOH**	**Max. Value**
AG-2	JIM12							
AG-2	JIM14	1.36				1.49		1.49
AG-2	JIM19	1.17						1.17
AG-3	CCRC-M26		-1.46					-1.46
AG-3	CCRC-M85		1.24					1.24
AG-3	JIM15					1.11		1.11
AG-3	JIM17					1.27		1.27
AG-3	JIM8					1.03		1.03
AG-3	MAC266							
AG-4	CCRC-M78	1.38	1.01					1.38
AG-4	CCRC-M92		1.19					1.19
AG-4	JIM13	1.55						1.55
AG-4	JIM133					1.39		1.39
FUC XG	CCRC-M106						-1.10	-1.10
non-FUC XG	CCRC-M87							
Pectic backbone	CCRC-M131		1.45					1.45
Pectic backbone	CCRC-M14							
Pectic backbone	CCRC-M35							
Pectic backbone	CCRC-M36							
Pectic backbone	CCRC-M38		1.79					1.79
Pectic backbone	JIM5					1.52		1.52
Pectic backbone	JIM7							
RG-1/AG	CCRC-M112	1.61	1.25			1.56		1.61
RG-1/AG	CCRC-M12	2.06	1.99					2.06
RG-1/AG	CCRC-M121	1.56						1.56
RG-1/AG	CCRC-M122	1.35						1.35
RG-1/AG	CCRC-M123	1.20						1.20
RG-1/AG	CCRC-M125	1.43						1.43
RG-1/AG	CCRC-M126	2.21				1.06		2.21
RG-1/AG	CCRC-M13	1.99						1.99
RG-1/AG	CCRC-M134	1.91						1.91
RG-1/AG	CCRC-M15							
RG-1/AG	CCRC-M21	2.22						2.22
RG-1/AG	CCRC-M22							
RG-1/AG	CCRC-M32	1.37						1.37
RG-1/AG	CCRC-M33	1.73						1.73
RG-1/AG	CCRC-M41	1.04						1.04
RG-1/AG	CCRC-M42	1.61						1.61
RG-1/AG	CCRC-M44	1.46						1.46
RG-1/AG	CCRC-M60	1.00						1.00
RG-1/AG	CCRC-M7	1.89						1.89
RG-1/AG	CCRC-M77	2.00				1.37		2.00
RG-1/AG	CCRC-M79	1.48						1.48
RG-1/AG	CCRC-M80	1.05						1.05
RG-1/AG	JIM1	1.38				1.42		1.42
RG-1/AG	JIM131	1.85				1.66		1.85
RG-1/AG	JIM132					1.14		1.14
RG-1/AG	JIM16					1.58		1.58
RG-I	JIM3							
RG-Ib	CCRC-M16							
RG-Ib	CCRC-M17							
RG-Ib	CCRC-M23					1.16		1.16
RG-Ic	CCRC-M61					1.40		1.40
RG-Ic	JIM101					1.53	1.07	1.53
xylan-2	CCRC-M116							
xylan-2	CCRC-M117							
xylan-2	CCRC-M150							
xylan-2	CCRC-M154							
Xylan-3	CCRC-M144							
Xylan-3	CCRC-M149							
Xylan-3	CCRC-M155							
	CCRC-M97							

Table S5. Glycan accessibility due to abiotic and biotic effects. Maximum $\log_2$ ratios for each sample/control pair, regardless of treatment, was used to filter the list for epitopes that showed ≥ 2-fold or ≤ -2-fold changes (see Table S4). Only epitopes that changed in concentration ≥ 2-fold in all three microbial samples or ≥ 2-fold for wSG/SG were considered significant. The results show increased concentrations (≥ 2-fold) of specific glycan epitopes released after extraction of the residual switchgrass biomass after washing with water (78°C for 18 hr, wSG) compared to the unwashed material (SG), and after three successive incubations (78°C for 5 days, SG1, SG2 and SG3) with *C. bescii* compared to the controls treated similarly but without the microorganism (SG1c, SG2c and SG3c, respectively). The results are categorized as indicating increased glycan extractability due to biotic effects (SG1-SG3, microbial only) and to both abiotic and biotic processes (thermal and microbial). The color-coding for the monoclonal antibodies (MAB) correspond to the groupings described in Fig. 2 (see Supplementary Table 4 for a detailed listing). The green color of the fold-changes darkens with increasing values. The abbreviations are: RG, rhamnogalacturonan; AG, arabainogalactan; HG, homogalacturonan.

Table S5 (continued)

MAB group	MAB name	Fold change			
		wSG/ SG	SG1/ SG1c	SG2/ SG2c	SG3/ SG3c
MICROBIAL ONLY					
AG-4	CCRC-M78		3.9	3.0	2.6
	JIM13		3.8	5.4	2.9
	JIM133		2.5	2.0	2.6
HG Backbone	JIM5		3.3	5.7	2.9
RG-I/AG	CCRC-M122		2.0	2.6	2.5
	CCRC-M123		2.0	2.9	2.3
	CCRC-M126		2.6	5.5	4.6
	CCRC-M134		2.1	3.5	3.8
	CCRC-M79		2.5	3.3	2.8
	CCRC-M80		2.2	3.5	2.1
	JIM1		2.6	2.8	2.7
	JIM131		8.0	6.2	3.6
	JIM16		3.0	2.2	3.0
RG-Ic	JIM101		4.0	2.9	2.9
THERMAL AND MICROBIAL					
AG-2	JIM14	5.7	4.8	4.3	2.8
RG-I/AG	CCRC-M112	3.4	2.5	3.8	3.1
	CCRC-M12	2.9	4.3	5.6	4.2
	CCRC-M125	2.4	2.0	2.7	2.7
	CCRC-M13	2.1	2.9	3.9	4.0
	CCRC-M21	2.4	3.9	6.3	4.7
	CCRC-M32	2.1	4.8	4.5	2.6
	CCRC-M33	2.3	3.2	4.5	3.3
	CCRC-M42	2.2	3.9	4.5	3.1
	CCRC-M44	3.0	4.1	5.7	2.8
	CCRC-M7	2.7	4.4	4.9	3.7
	CCRC-M77	3.8	5.4	5.4	4.0
	JIM132	3.9	5.6	3.5	2.2
RG-Ic	CCRC-M61	2.2	2.7	2.4	2.6

Table S6. Up-regulated genes when *C. bescii* is grown on washed switchgrass (wSG) compared to growth with glucose as the primary carbon and energy source. SP indicates whether the encoded protein is predicted to contain a signal peptide and therefore potentially extracellular.

Gene	Annotated Function	Fold change[a]	SP[b]
Carbohydrate metabolism			
Cbes_1854	Pectate lyase	51	Y
Cbes_1855	Pectate disaccharide-lyase	28	Y
Cbes_1865	Glycoside hydrolase family 9	23	Y
Cbes_0234	Glycoside hydrolase family 5	17	Y
Cbes_0186	Putative esterase	15	N
Cbes_0183	Cellulose 1,4-beta-cellobiosidase	14	N
Cbes_0558	1,4-alpha-glucan branching enzyme	11	N
Cbes_0603	Xylose isomerase	11	N
Cbes_1853	Rhamnogalacturonan lyase	10	Y
Cbes_0147	1-Phosphofructokinase	8	N
Cbes_1864	Glycosyl transferase family 39	7	Y
Cbes_2579	alpha Amylase catalytic region	7	N
Cbes_1866	Mannan endo-1,4-beta-mannosidase, Cellulase	7	Y
Cbes_0187	Glycoside hydrolase family 39	6	N
Cbes_0567	Xylulokinase	6	N
Cbes_0184	Xylan 1,4-beta-xylosidase	5	N
Cbes_0618	Endo-1,4-beta-xylanase	4	Y
Cbes_1857	Glycoside hydrolase family 48	4	Y
Carbohydrate transport			
Cbes_0596	[c]BPD transport systems inner membrane component	67	Y
Cbes_0595	BPD transport systems inner membrane component	37	Y
Cbes_0175	BPD transport systems inner membrane component	24	N
Cbes_2331	BPD transport systems inner membrane component	24	N
Cbes_0614	extracellular solute-binding protein family 1	20	Y
Cbes_2054	BDP transport systems inner membrane component	18	N
Cbes_0181	[c]Extracellular SBP family 1	13	Y
Cbes_2266	ABC transporter related	10	N
Cbes_0615	BDP transport systems inner membrane component	8	Y
Cbes_2265	Monosaccharide-transporting ATPase	8	Y
Cbes_2376	[c]Extracellular SBP family 1	7	Y
Cbes_0179	BDP transport systems inner membrane component	7	N
Cbes_2577	BDP transport systems inner membrane component	7	Y/N
Cbes_0597	[c]Extracellular SBP family 1	6	Y
Cbes_0399	[c]Extracellular SBP family 1	5	Y
Cbes_1688	ABC transporter related	4	N

Table S6 (continued)

Gene	Annotated Function	Fold change[a]	SP[b]
Hypothetical proteins			
Cbes_1881	Hypothetical protein	31	Y
Cbes_1880	Hypothetical protein	31	Y/N
Cbes_2572	Hypothetical protein	30	Y
Cbes_1747	Hypothetical protein	17	N
Cbes_2698	Hypothetical protein	16	N
Cbes_1902	Hypothetical protein	13	N
Cbes_0998	DUF322	9	N
Cbes_0690	DUF107	8	N
Cbes_0153	Hypothetical protein	7	N
Cbes_2075	Hypothetical protein	6	N
Cbes_0231	Hypothetical protein	6	N
Cbes_0908	Hypothetical protein	6	Y
Cbes_1396	Hypothetical protein	5	N
Cbes_1858	Hypothetical protein	5	N
Cbes_1000	Hypothetical protein	5	N
Cbes_1622	Hypothetical protein	5	N
Cbes_0229	Hypothetical protein	4	N
Cbes_2145	Hypothetical protein	4	N
Cbes_1651	Hypothetical protein	4	N
Cbes_1872	Hypothetical protein	4	Y
Cbes_2142	Hypothetical protein	4	N
Regulators			
Cbes_2264	Periplasmic binding protein/LacI transcriptional regulator	14	Y
Cbes_2105	Sigma 54 modulation protein/ribosomal protein S30EA	8	N
Cbes_2230	CheA signal transduction histidine kinase	5	N
Cbes_1771	Response regulator receiver protein	4	N
Cbes_0577	putative CheA signal transduction histidine kinase	5	
Oxidative stress			
Cbes_1983	Ferritin Dps family protein	13	N
Cbes_0163	Rubrerythrin	8	N
Cbes_0477	Rubrerythrin	6	N
Cbes_0164	ferric uptake regulator, Fur family	5	N
Cbes_0820	4Fe-4S ferredoxin iron-sulfur binding domain protein	4	Y
Cbes_2746	hydrolyase, Fe-S type, tartrate/fumarate subfamily	4	N

Table S6 (continued)

Gene	Annotated Function	Fold change[a]	SP[b]
Miscellaneous			
Cbes_2246	AIG2 family protein	9	N
Cbes_1679	phosphoribosyltransferase	8	N
Cbes_1695	glutamine amidotransferase of anthranilate synthase	8	N
Cbes_1874	Fimbrial assembly family protein	8	N
Cbes_1277	RNA-metabolising metallo-beta-lactamase	7	N
Cbes_1832	metal dependent phosphohydrolase	7	N
Cbes_2035	coenzyme F390 synthetase-like protein	7	N
Cbes_1164	Stage V sporulation protein S	6	N
Cbes_0360	Radical SAM domain protein	6	N
Cbes_1654	flagellar protein FlaG protein	6	N
Cbes_0577	putative CheA signal transduction histidine kinase	5	N
Cbes_2108	ribonuclease R	5	N
Cbes_1590	Tetratricopeptide TPR_2 repeat protein	5	N
Cbes_2701	CRISPR-associated RAMP protein, Csm4 family	5	N
Cbes_1002	exodeoxyribonuclease VII, large subunit	5	N
Cbes_1975	GCN5-related N-acetyltransferase	5	N
Cbes_1883	twitching motility protein	5	N
Cbes_1425	ATP synthase F1, gamma subunit	5	N
Cbes_0689	single-stranded-DNA-specific exonuclease RecJ	4	N
Cbes_1120	ribosomal protein L27	4	N
Cbes_2533	carbonic anhydrase family protein	4	Y
Cbes_0291	peptidase S8 and S53 subtilisin kexin sedolisin	4	Y
Cbes_0553	lipolytic protein G-D-S-L family	4	N
Cbes_1960	homoserine kinase	4	N
Cbes_0233	Nucleotidyl transferase	4	N

Figure S1. Growth of *C. bescii* on cellobiose (0.5%, w/v) with (Cell + YE) and without (Cell) yeast extract (0.05%, w/v) and on yeast extract (0.05%, w/v) only (YE).

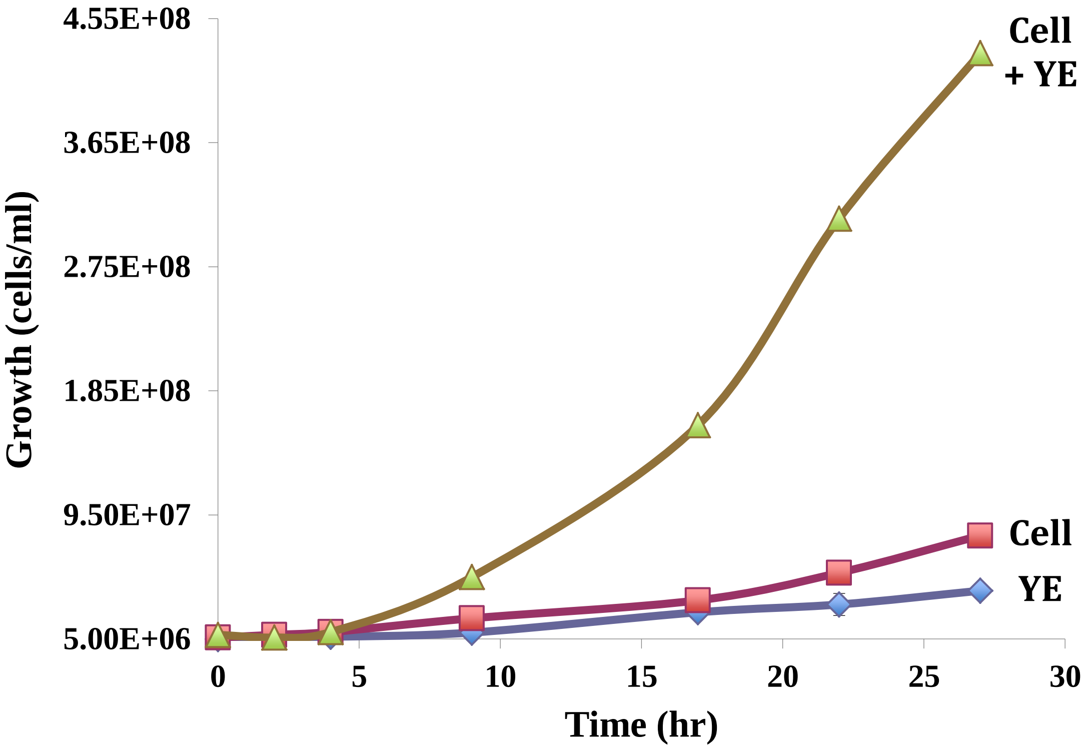


Figure S2. Design of the experiment to investigate the mechanism by which *C. bescii* degrades switchgrass and how high temperature contributes to this process. Switchgrass (SG; sieved 20/80-mesh fraction, obtained from Dr. Brian Davison, Oak Ridge National Laboratory, Oak Ridge, TN) was washed with water at 78°C for 18 hrs at 150 rpm (wSG). The washed switchgrass biomass was rinsed, dried and used as the primary growth substrate for the first *C. bescii* culture. The residual (insoluble) one time spent SG material (SG1) was used to grow a second *C. bescii* culture, yielding insoluble material SG2. This in turn was used to grow a third *C. bescii* culture (SG3). In the corresponding controls (SG1c, SG2c and SG3c), the switchgrass biomass was treated similarly, but without *C. bescii*. All cultures and controls were incubated at 78°C with shaking (150 rpm) for 5 days. The initial concentration of switchgrass biomass in all cultures and controls was 5 g/L.

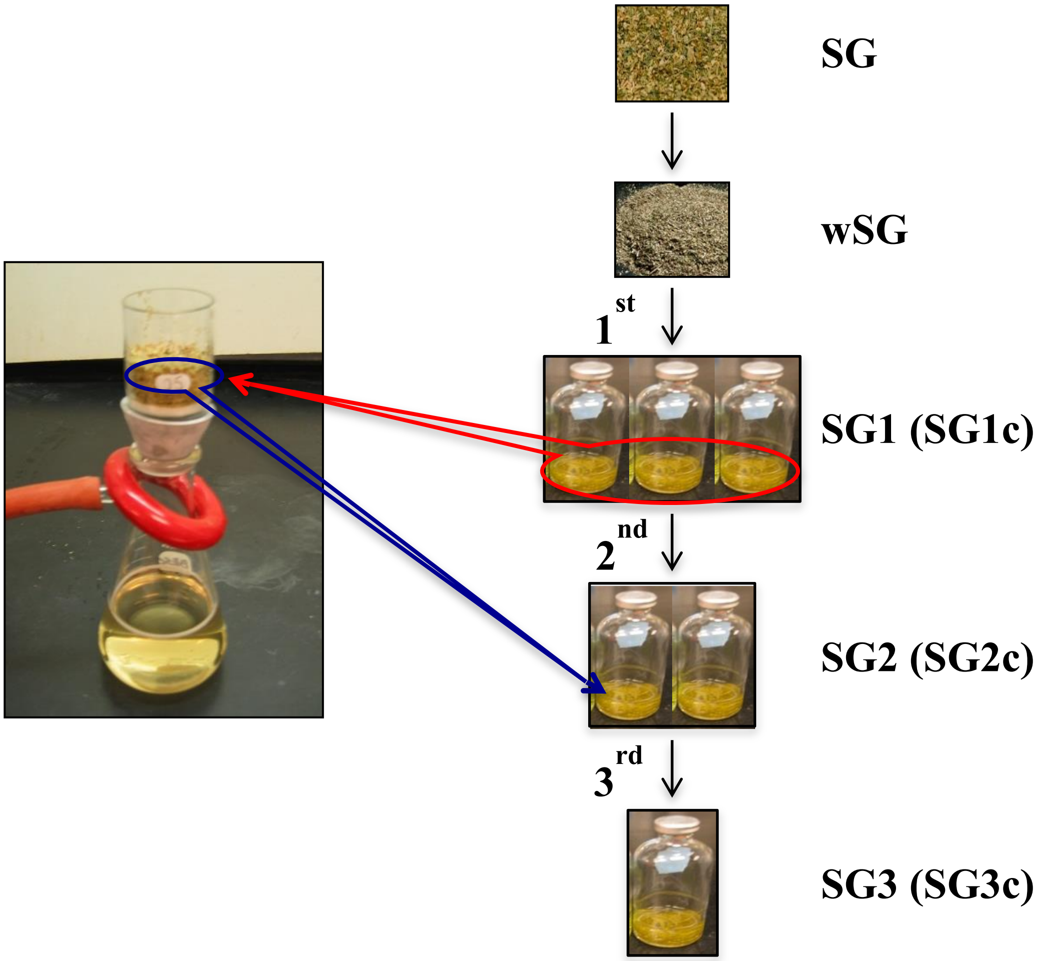


Figure S3. Stimulated Raman Scattering images showing transverse section of a vascular bundle and the statistic distribution of lignin. The percentage is calculated based on pixel intensity of whole image. The samples were untreated switchgrass (SG), after hot-water washing (78°C for 18 hr; wSG) and after treatment with *C. bescii* (SG1). Nine images taken from different sections were used for SG, and seven images for wSG and SG1. Representative images are shown. The scale bar is 30 µm.

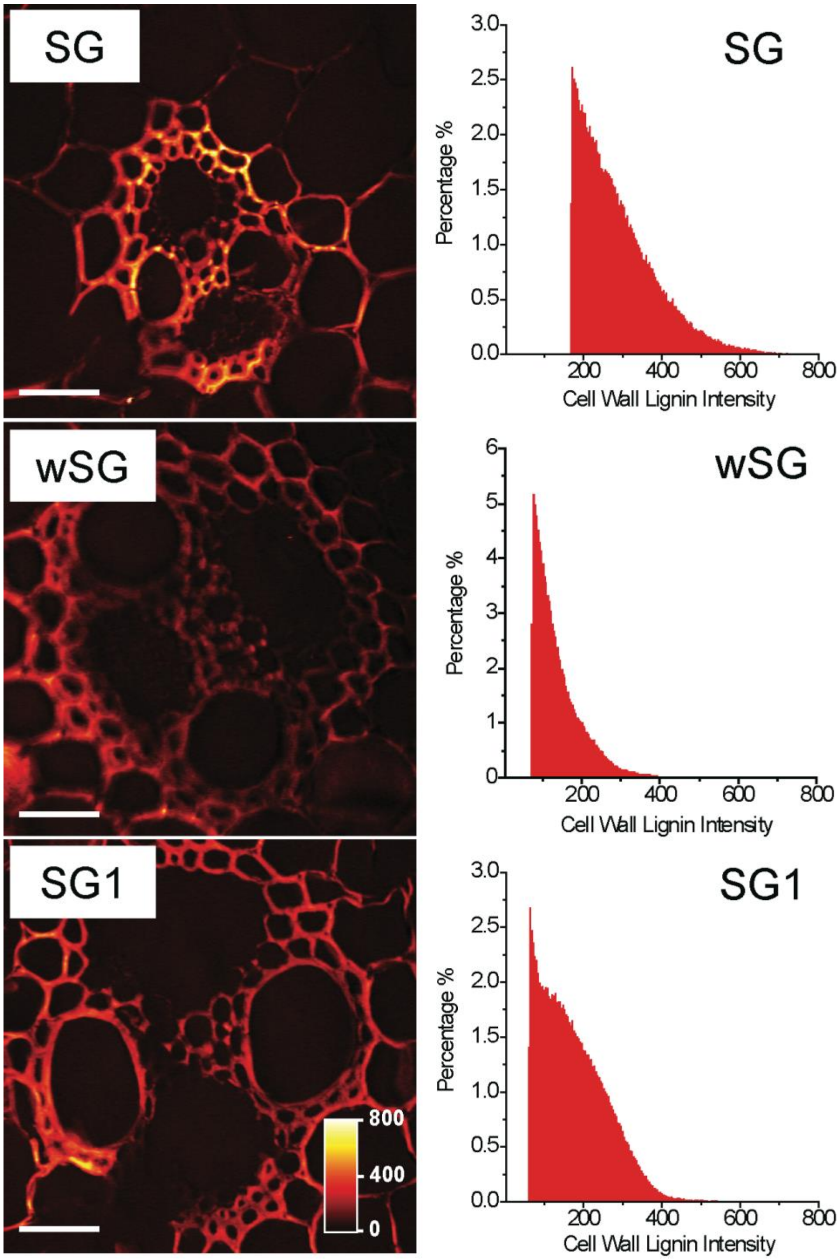


Figure S4. Acetyl bromide-soluble lignin assay of high molecular-weight lignin in cell- and particulate-free supernatant after incubation at 78°C for 5 days. The high molecular-weight fractions of supernatants from media alone (Media), wSG with media (wSG), *C. bescii* grown on wSG (*C.b.*, wSG), or *C. bescii* grown on Avicel (*C.b.*, Avicel) were collected with 50 kDa cut-off centrifugal filters. While some lignin is liberated from wSG during the incubation, more than twice the amount is released from wSG by *C. bescii* in the same conditions. Two biological replicates are shown (n=3, ±SD) represented by the red and blue bars.

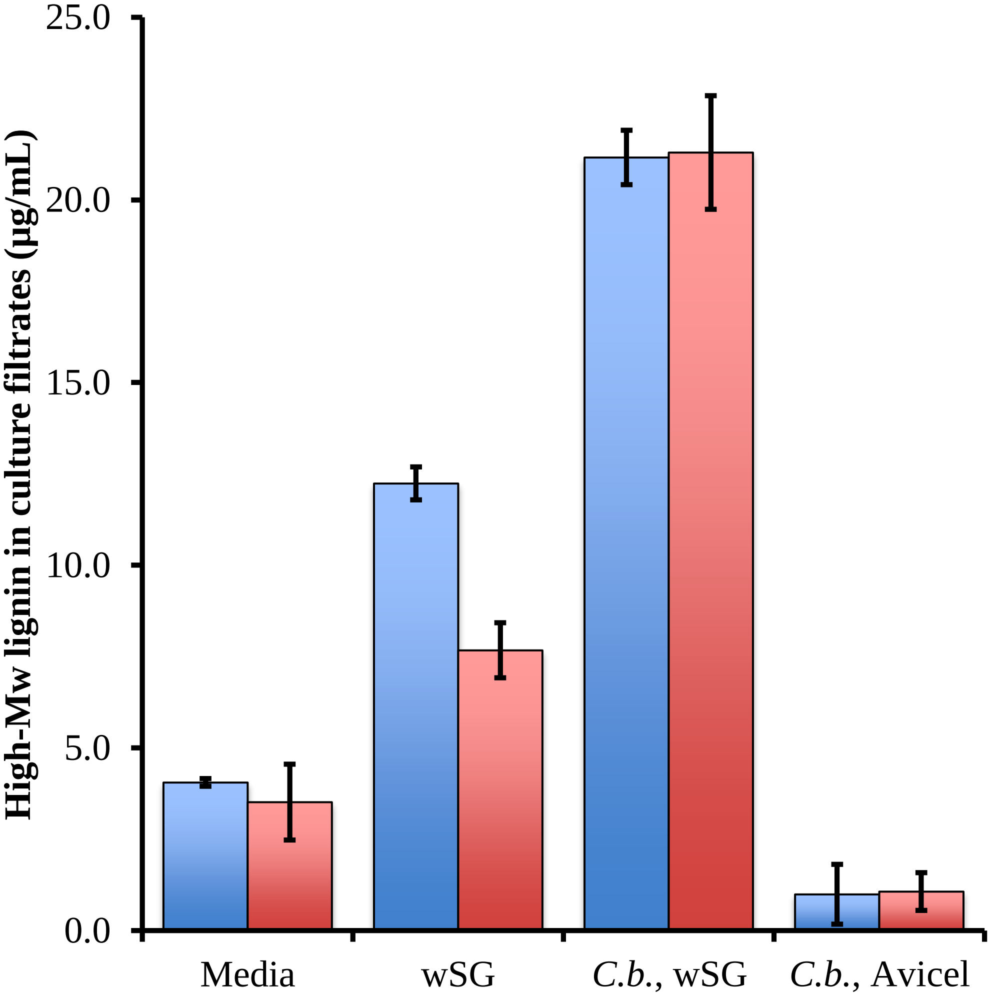


Figure S5. Stimulated Raman Scattering images showing transverse section of a vascular bundle and the statistic distribution of cellulose. The percentage is calculated based on pixel intensity of whole image. The samples were untreated switchgrass (SG), after hot-water washing (78°C for 18 hr; wSG) and after treatment with *C. bescii* (SG1). Nine images taken from different sections were used for SG, and seven images for wSG and SG1. Representative images are shown. The scale bar is 30 µm.

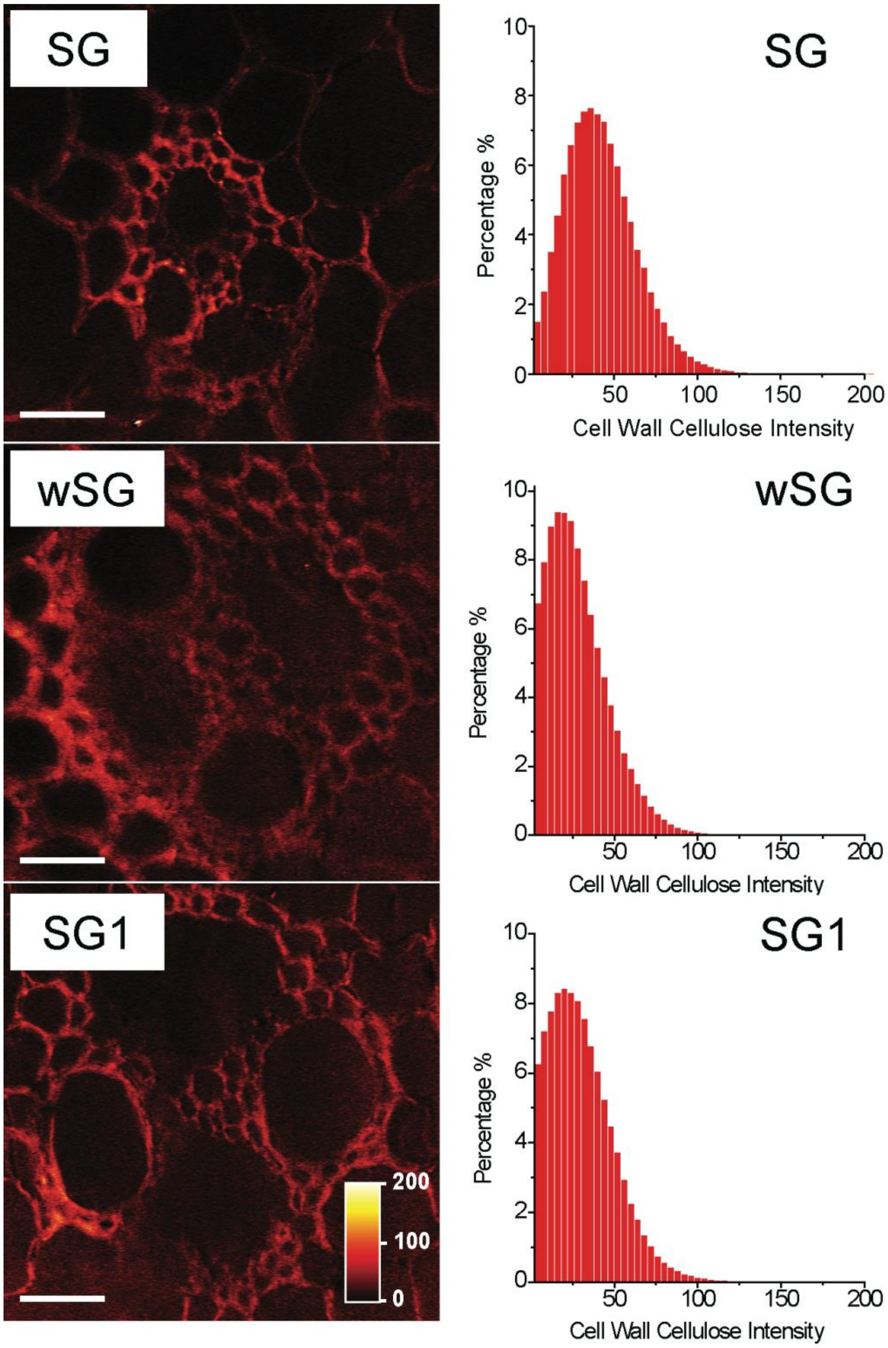


Figure S6. Gel permeation chromatograms of isolated holocellulose from switchgrass samples. Controls and *C. bescii* samples are given in solid and dotted lines, respectively.

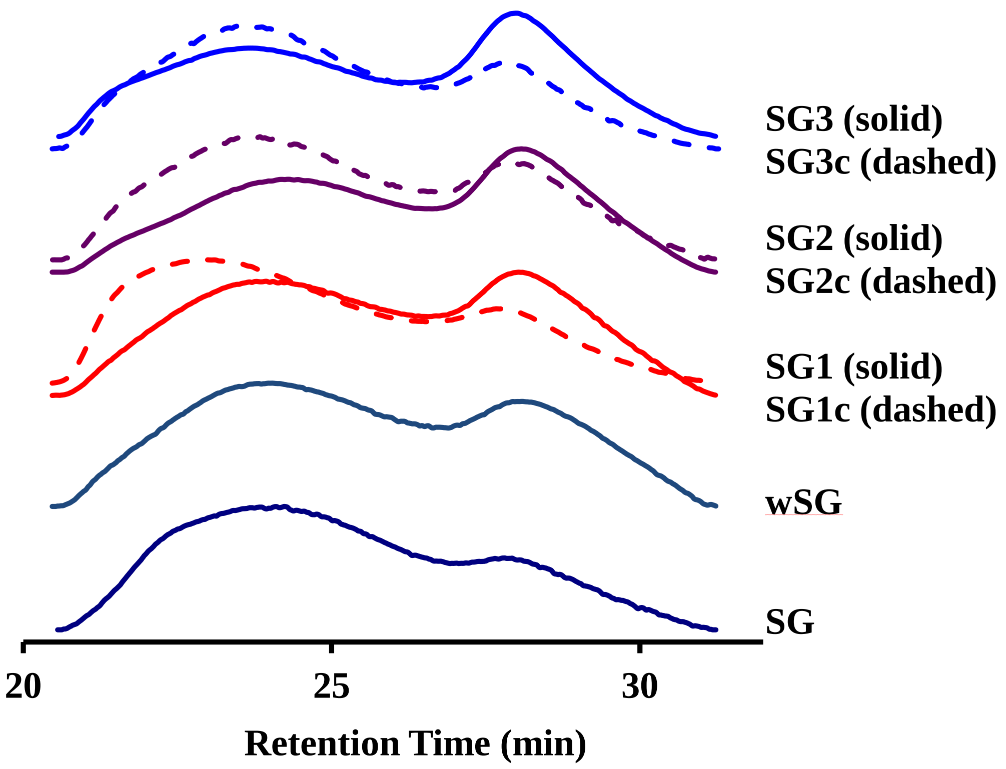


Figure S7. Degree of polymerization of cellulose isolated from the switchgrass samples. DPn is degree of polymerization; DPw is weight-average degree of polymerization.

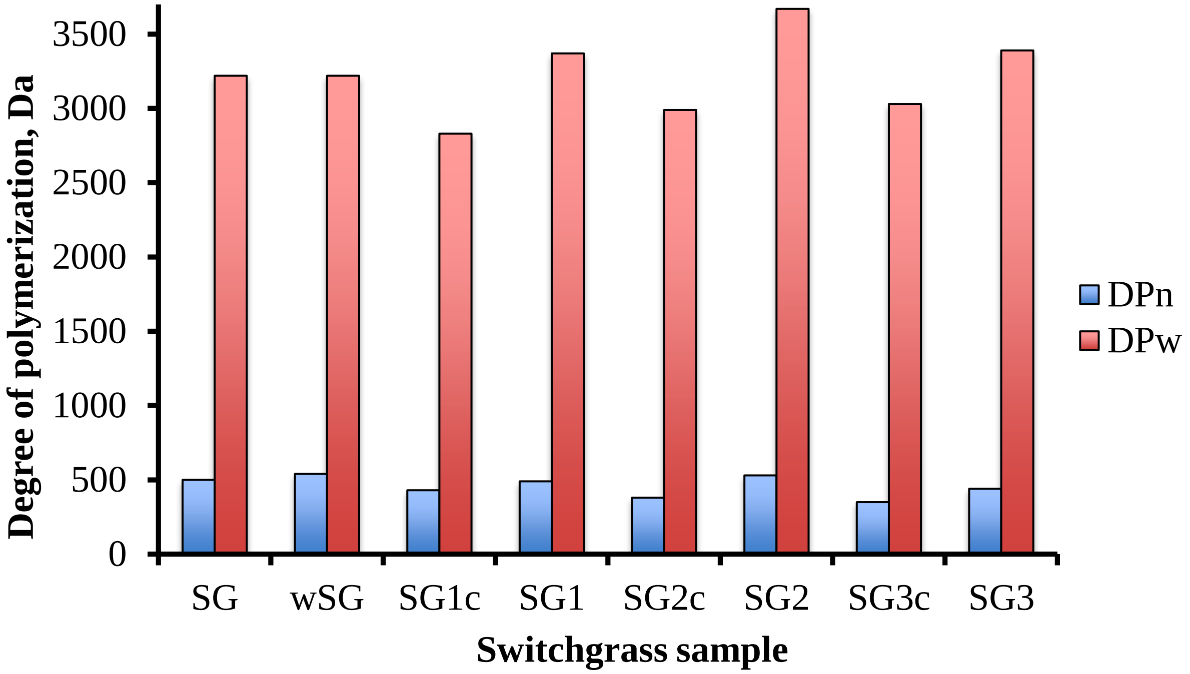


Figure S8. ^{13}C CP/MAS spectra of switchgrass samples. All spectra were recorded on pre-wet samples (30-60% water content), and the line-fitting analysis of spectra was performed using NUTS NMR Data Processing software (Acorn NMR, Inc). Error analysis was conducted by performing three individual isolations, NMR acquisitions and line-fit data processing indicating percent crystallinity can be determined within ± 1.6%. The samples are: (**1**) Hemicellulose and lignin: -COO-R, CH_3-COO-, (**2**) Lignin: G-units $C_{1,4}$ or/and S-units $C_{3,5}$; (**3**) Lignin: H-units $C_{1,2,6}$ or/and S-units $C_{1,4}$; (**4**) Carbohydrates: crystalline C_4; (**5**) Carbohydrates: amorphous C_4; (**6**) Hemicellulose: CH_3-COO. See Table S1 for additional details.

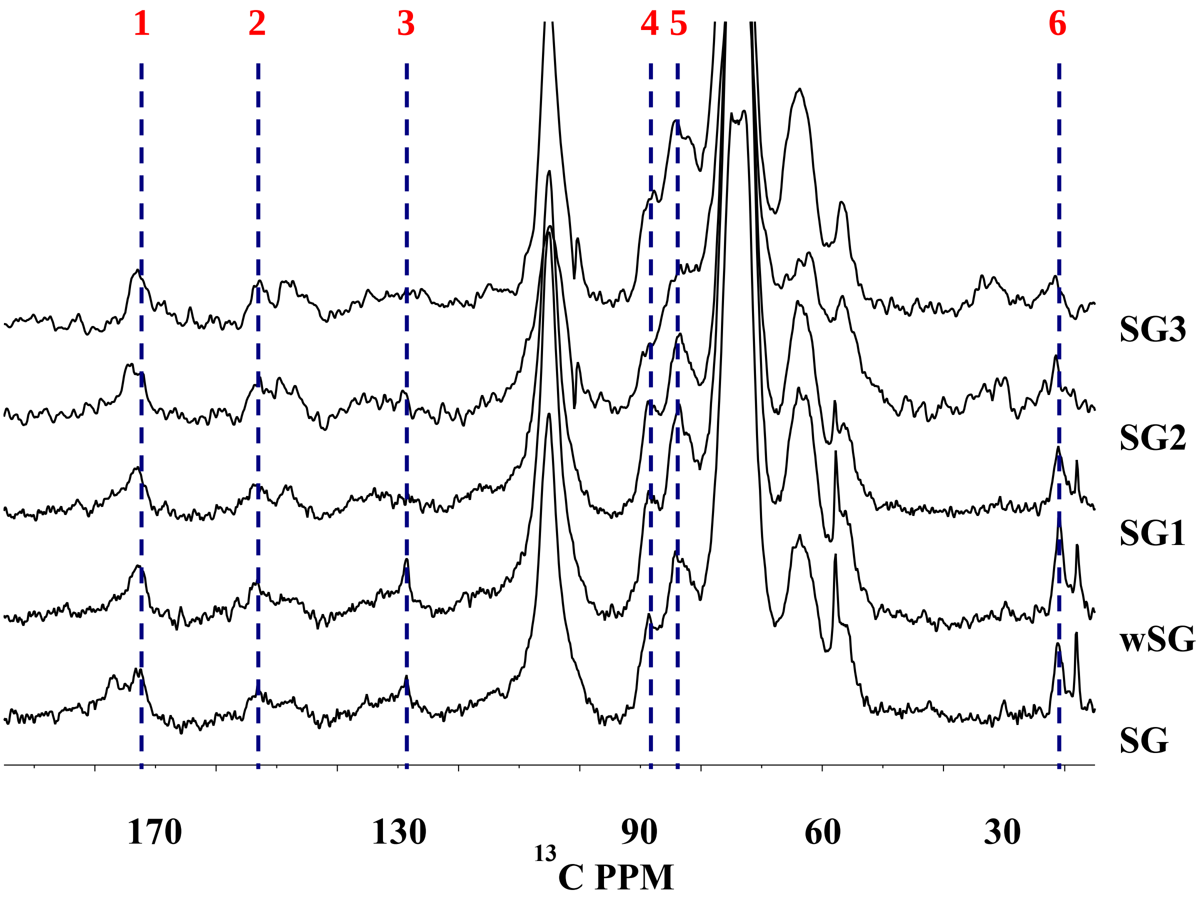


Figure S9. Contents (%, w/w) of structural sugars and lignin in SG, wSG and after three successive treatments of wSG with (SG1-3) and without (SG1c-3c) *C. bescii*. Samples for carbohydrate and acid-insoluble lignin (Klason lignin) analysis were prepared using a two-stage acid hydrolysis protocol based on TAPPI methods T-222 om-88 as described in the Methods section.

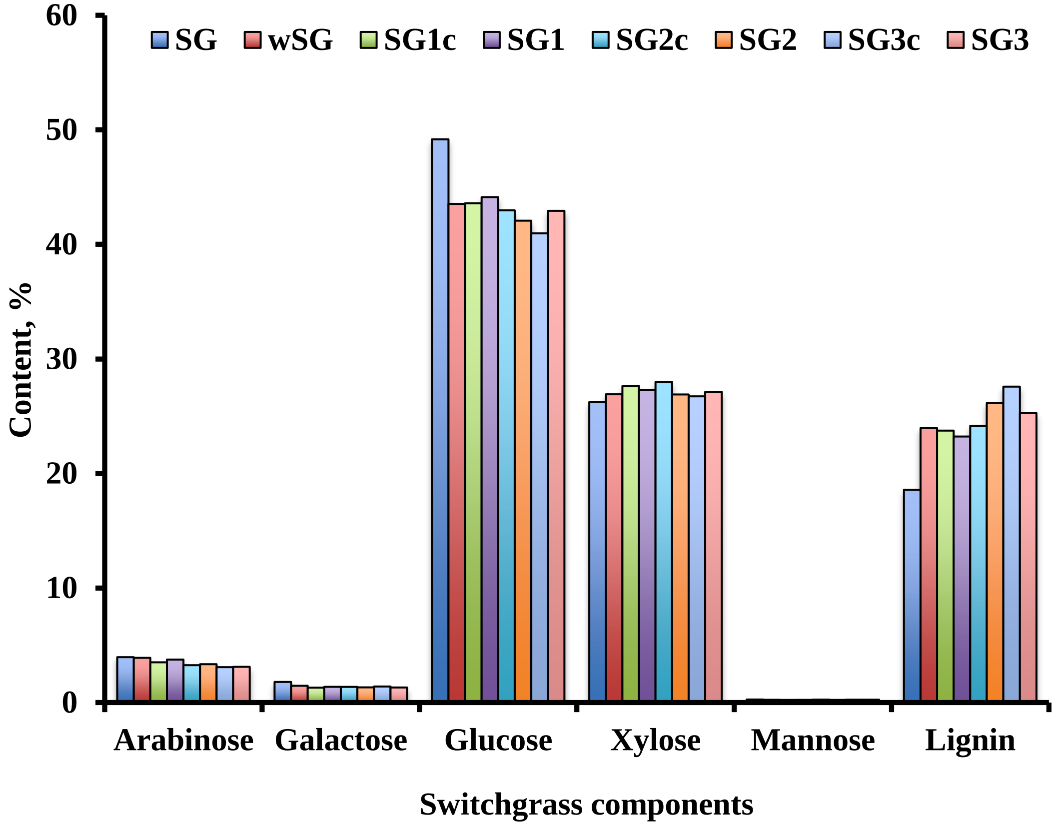


Figure S10. Glycosyl residue composition of (**A**) total switchgrass alcohol insoluble residue AIR cell walls, and after their sequential extractions with: (**B**) ammonium oxalate, (**C**) sodium carbonate, (**D**) 1 M KOH, (**E**) 4 M KOH, (**F**) chlorite and (**G**) post chlorite 4 M KOH. Switchgrass samples were hydrolyzed in 1 M methanolic-HCl for 18 h at 80ºC. The trimethylsilyl (TMS) derivatives were analyzed by GC-MS for glycosyl residue composition. The monosaccharides are abbreviated as arabinose (Ara), rhamnose (Rha), fucose (Fuc), xylose (Xyl), galacturonic acid (GalA), glucuronic acids (GlcA), mannose (Man), galactose (Gal), and glucose (Glc). Each data point is the average of 4 replicates and the error bar represents the standard deviation of the mean, n=4.