Supplemental Table 1. Primers used for cloning genes in this study

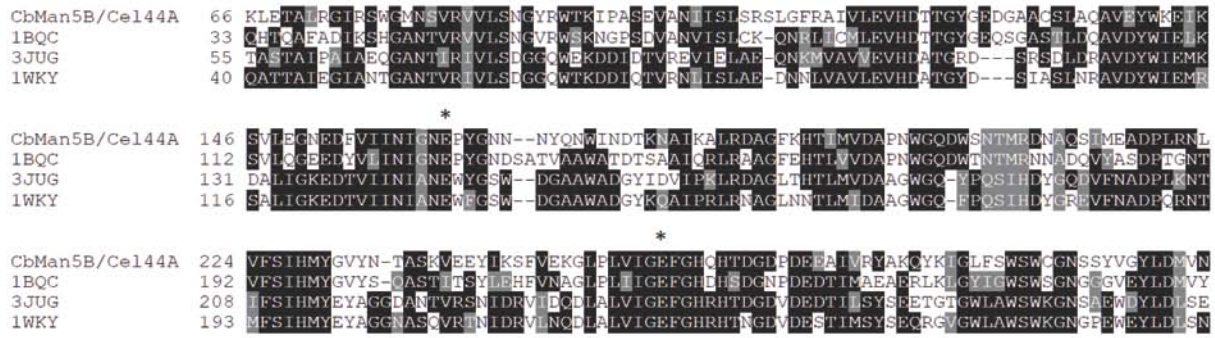
Primer	Sequence
CbMan5B/Cel44A-WT-F	5’-GAC GAC GAC AAG ATG GCT ACA TCT AAT GAT GGA GTA GTG AAG -3’
CbMan5B/Cel44A-WT-R	5’-GAG GAG AAG CCC GGT TAA TTT AGT TTG TAC TGA GGT TGA ATA TAA AAC GAT ATG G -3’
CbMan5B/Cel44A-TM1-F	5’-GAC GAC GAC AAG ATG GCT ACA TCT AAT GAT GGA GTA GTG AAG -3’
CbMan5B/Cel44A-TM1-R	5’-GAG GAG AAG CCC GGT TAG TTA AAC CTT ATC TGT ATC TCC CCT GTG TC -3’
CbMan5B/Cel44A-TM2-F	5’-GAC GAC GAC AAG ATG GTA GGG TAC TTG GAC ATG GTA AAC AAT TGG GA -3’
CbMan5B/Cel44A-TM2-R	5’-GAG GAG AAG CCC GGT TAA TTT AGT TTG TAC TGA GGT TGA ATA TAA AAC GAT ATG G -3’
CbMan5B/Cel44A-TM3-F	5’-GAC GAC GAC AAG ATG GGA CAG ATA AAG GTA CTG TAT GCT AAC AAG GAG ACA AAT -3’
CbMan5B/Cel44A-TM3-R	5’-GAG GAG AAG CCC GGT TAA TTT AGT TTG TAC TGA GGT TGA ATA TAA AAC GAT ATG G -3’
CbMan5B/Cel44A-TM4-F	5’-GAC GAC GAC AAG ATG GGA CAG GAG CCG AGT GGA GCG-3’
CbMan5B/Cel44A-TM4-R	5’-GAG GAG AAG CCC GGT TAA TTT AGT TTG TAC TGA GGT TG -3’
CbMan5B/Cel44A-TM4Δ1βf	5’-GAC GAC GAC AAG ATG GGA CAG GAG CCG AGT GGA GCG-3’
CbMan5B/Cel44A-TM4Δ1βr	5’-GAG GAG AAG CCC GGT TAA GGT ACC TCA AGA GTT AAA ACA TTG-3’
CbMan5B/Cel44A-TM4Δ2βf	5’-GAC GAC GAC AAG ATG GGA CAG GAG CCG AGT GGA GCG-3’
CbMan5B/Cel44A-TM4Δ2βr	5’-GAG GAG AAG CCC GGT TAT CCC ATT TTT CTA ACA GTA GGA CTA TTG-3’
CbMan5B/Cel44A-TM4Δ3βf	5’-GAC GAC GAC AAG ATG GGA CAG GAG CCG AGT GGA GCG-3’
CbMan5B/Cel44A-TM4Δ3βr	5’-GAG GAG AAG CCC GGT TAG CTA TCA AAA CCA TAA ATT TCT GC-3’
CbMan5B/Cel44A-TM4Δ9βf	5’-GAC GAC GAC AAG ATG GGA CAG GAG CCG AGT GGA GCG-3’
CbMan5B/Cel44A-TM4Δ9βr	5’-GAG GAG AAG CCC GGT TAA TAT TTT GAG CCT TTT CCA TCA TAA TTA AG-3’

Supplemental Table 2. Amounts of products released from cellopentaose or PASC by CbMan5B/Cel44A-TM2, CbCel9B/Man5B-TM1, CbCel9B/Man5B-TM1, and CbCel5B-TM1

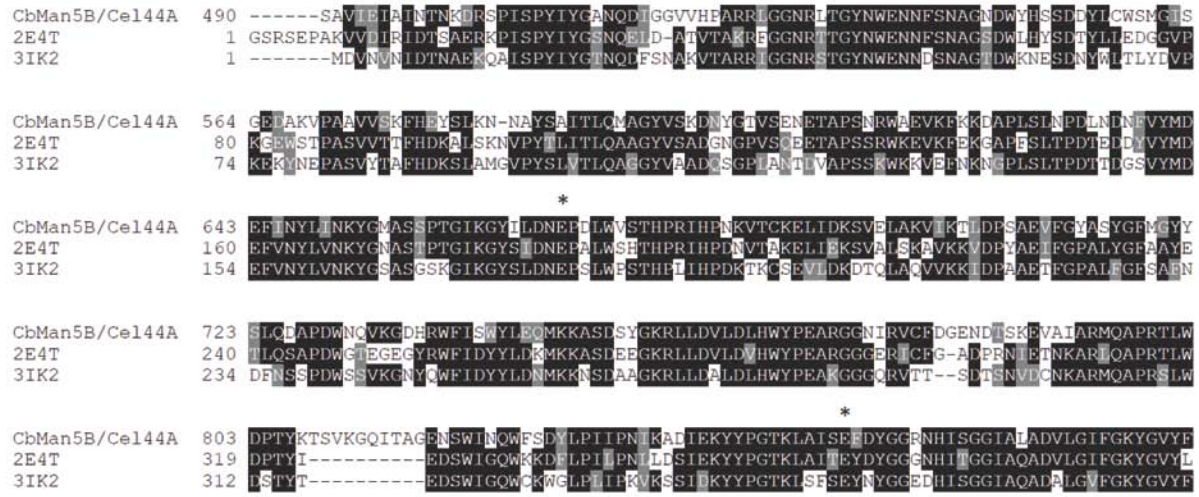
Substrate: G5	Reaction time						
	Released sugar	0 min	2 min	10 min	30 min	4 h	24 h
CbMan5B/ Cel44A-TM2	G1	N.D	0.2±0.1	0.8±0.2	2.1±0.3	4.0±0.6	6.7±0.9
	G2	N.D	N.D	N.D	<0.1	1.4±0.3	1.9±0.3
	G3	N.D	N.D	N.D	<0.1	1.7±0.1	2.7±0.3
	G4	N.D	<0.1	0.9±0.1	2.4±0.5	4.2±0.4	2.3±0.3
	G5	6.0±0.1	5.8±0.1	4.8±0.5	2.6±0.5	N.D	N.D
CbCel9B/ Man5B-TM1	G1	N.D	3.8±0.5	5.3±0.2	5.9±0.7	6.1±0.5	6.5±0.8
	G2	N.D	9.9±0.9	11.3±0.5	11.7±0.1	12.2±0.9	12.0±0.5
	G3	N.D	2.1±0.3	0.5±0.1	N.D	N.D	N.D
	G4	N.D	N.D	N.D	N.D	N.D	N.D
	G5	6.0±0.1	N.D	N.D	N.D	N.D	N.D
CbCel5C/ Cel5A-TM2	G1	N.D	N.D	N.D	1.3±0.1	2.3±0.1	4.6±1.0
	G2	N.D	5.7±0.3	6.7±0.6	7.4±0.3	8.8±0.8	10.9±0.6
	G3	N.D	6.6±0.9	5.2±0.1	4.8±0.4	3.9±0.6	N.D
	G4	N.D	N.D	N.D	N.D	N.D	N.D
	G5	6.0±0.1	N.D	N.D	N.D	N.D	N.D
Cbcel5B-TM1	G1	N.D	N.D	1.4±0.0	2.2±0.1	5.8±1.4	6.3±0.1
	G2	N.D	6.1±0.9	7.5±0.6	8.8±0.7	11.8±0.6	12.0±0.6
	G3	N.D	5.9±0.1	4.8±0.0	4.0±0.3	N.D	N.D
	G4	N.D	N.D	N.D	N.D	N.D	N.D
	G5	6.0±0.1	N.D	N.D	N.D	N.D	N.D
Substrate: PASC	**Reaction time**						
	Released sugar	**0 min**	**2 min**	**10 min**	**30 min**	**4 h**	**24 h**
CbMan5B/ Cel44A-TM2	G1	N.D	0.2±0.0	0.4±0.0	1.0±0.1	1.6±0.2	2.8±0.2
	G2	N.D	0.7±0.1	0.8±0.2	1.9±0.3	2.0±0.2	2.1±0.3
	G3	N.D	0.7±0.1	0.8±0.2	2.0±0.1	3.7±0.3	5.1±0.5
	G4	N.D	0.7±0.1	1.1±0.2	4.2±0.2	2.6±0.1	1.4±0.6
	G5	N.D	N.D	N.D	N.D	N.D	N.D
CbCel9B/ Man5B-TM1	G1	N.D	1.2±0.2	2.5±0.2	4.2±0.3	12.5±0.7	16.0±1.2
	G2	N.D	1.7±0.3	4.9±0.6	5.2±0.5	16.1±0.8	18.7±1.0
	G3	N.D	0.2±0.0	1.1±0.2	1.2±0.2	N.D	N.D
	G4	N.D	0.4±0.0	0.4±0.0	0.2±0.0	N.D	N.D
	G5	N.D	N.D	N.D	N.D	N.D	N.D
CbCel5C/ Cel5A-TM2	G1	N.D	N.D	1.2±0.3	2.1±0.2	3.4±0.7	6.6±1.0
	G2	N.D	13.3±0.3	20.6±0.7	21.6±1.1	24.8±0.1	26.8±1.1
	G3	N.D	5.7±0.6	6.8±0.5	9.7±0.7	7.3±2.1	4.9±1.2
	G4	N.D	N.D	N.D	N.D	N.D	N.D
	G5	N.D	N.D	N.D	N.D	N.D	N.D
Cbcel5B-TM1	G1	N.D	N.D	2.6±0.2	4.0±0.2	8.3±0.7	12.3±0.5
	G2	N.D	7.6±0.0	10.9±0.6	15.0±0.5	18.1±1.3	20.0±0.7
	G3	N.D	3.6±0.6	2.9±0.3	2.5±0.5	N.D	N.D
	G4	N.D	N.D	N.D	N.D	N.D	N.D
	G5	N.D	N.D	N.D	N.D	N.D	N.D

Note: G1, G2, G3, G4 and G5 stand for glucose, cellobiose, cellotriose, cellotetraose, and cellopentaose, respectively. “0 min” represents a common standard in which no enzyme was added. The concentration unit is mM. N.D means “non-detected”.

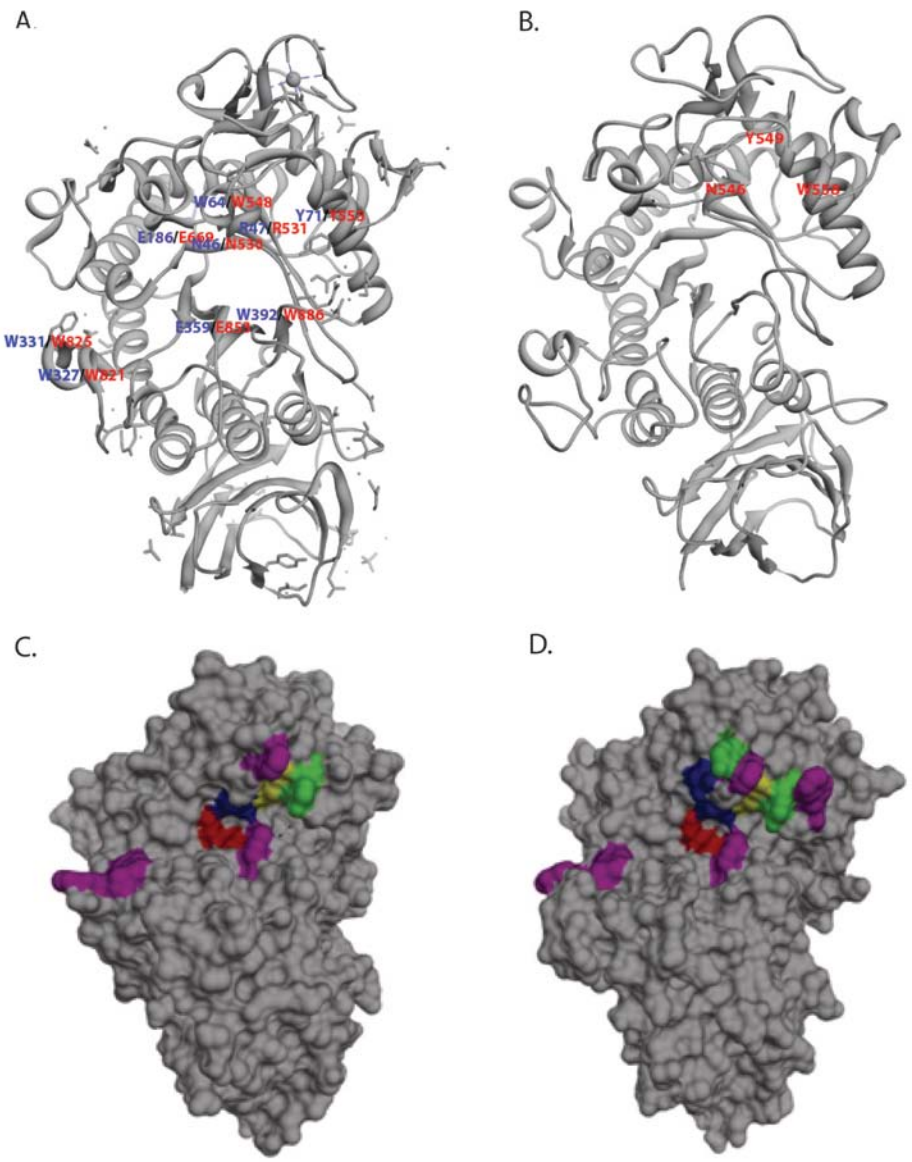
A. Alignment of the GH5 catalytic module of CbMan5B/Cel44A with those of its homologs



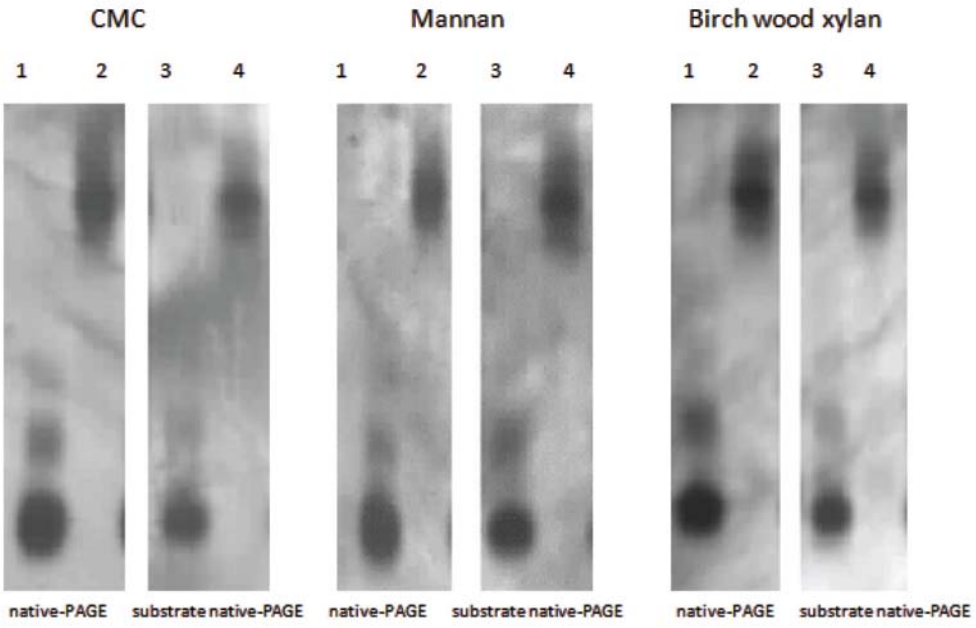
B. Alignment of the GH44 catalytic module of CbMan5B/Cel44A with those of its homologs



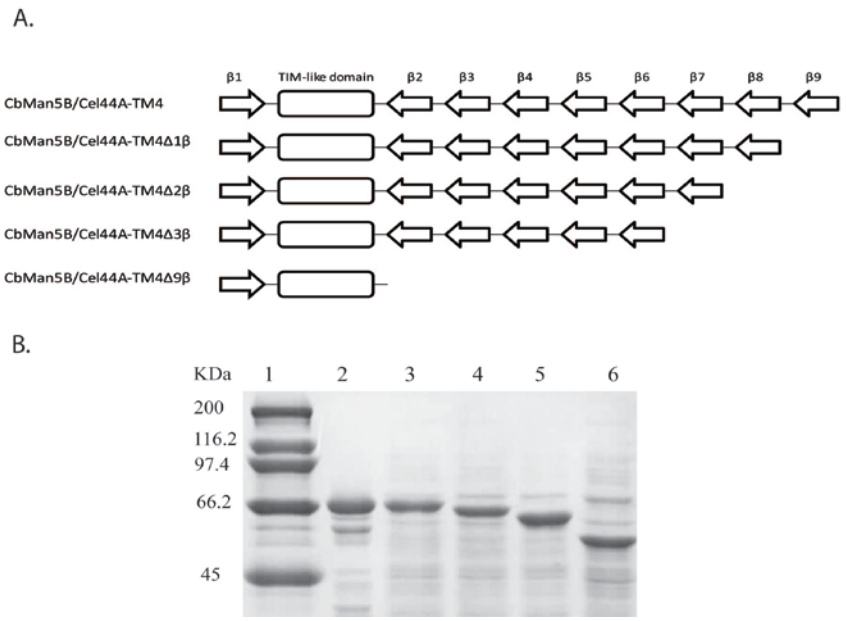
Supplemental Figure 1. Alignments of the GH5 and GH44 catalytic modules of CbMan5B/Cel44A with their homologs. The homologs are from crystallized proteins in the protein 3-dimensional structure database. A: Alignment of the GH5 catalytic module of CbMan5B/Cel44A with those of its homologs; B: Alignment of the GH44 catalytic module of CbMan5B/Cel44A with those of its homologs. Note: The asterisks (*) indicate the conserved catalytic residues. The PDB entries are given in the alignments and the sources of the proteins are: 1BQC, *Thermobifida fusca* KW3 β-mannanase (GenBank accession number: CAA06924); 3JUG, *Bacillus* sp. N16-5 β–mannanase (GenBank accession number: AAT06599); 1WKY, *Bacillus* sp. JAMB-602 β–mannanase (GenBank accession number: AAT06599); 2E4T, *Clostridium thermocellum* F1 Cel9D-Cel44A (GenBank accession number: BAA12070); 3IK2: *Clostridium acetobutylicum* ATCC 824 endoglucanase (GenBank accession number: AAK78891).



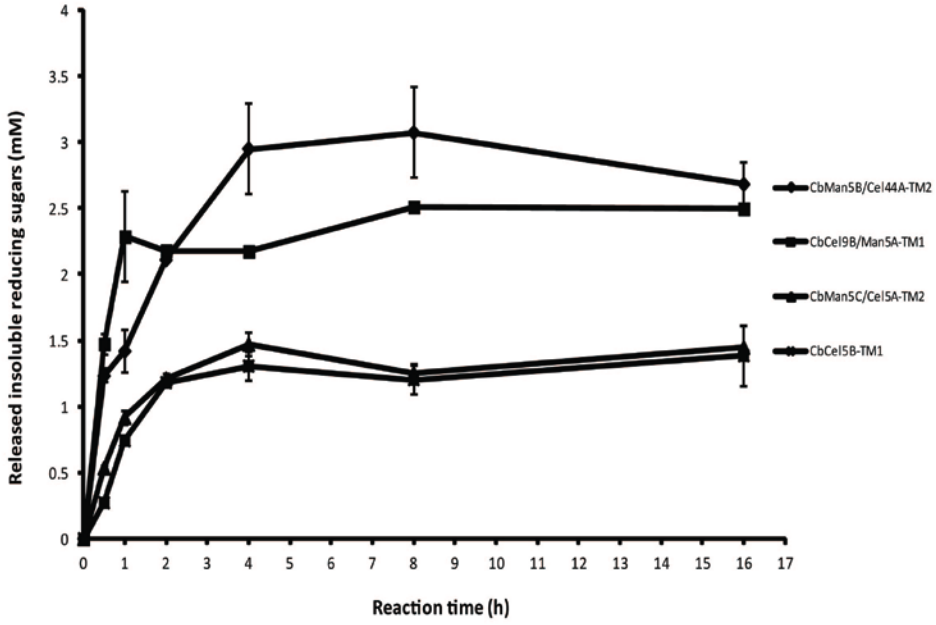
Supplemental Figure 2. Structural alignments of the GH44 modules from *C. thermocellum* and *C. bescii*. A: Alignment of cartoon representations of *C. thermocellum* and *C. bescii* GH44 modules. Ligand-binding and catalytic residues of the *C. thermocellum* GH44 module are labeled in blue, whereas those in the *C. bescii* GH44 homolog are labeled in red. B: Cartoon alignment of the *C. thermocellum* and *C. bescii* GH44 modules. The residues labeled in red are found in the *C. bescii* GH44 but not in the *C. thermocellum* GH44. *C:* A surface representation for ligand binding and catalytic residues of the *C. thermocellum* GH44 module. D: A modeled surface representation for ligand binding and catalytic residues of the *C. bescii* GH44. Note: Glu residues shown in red, Trp residues shown in magenta, Arg residues shown in yellow, Tyr residue shown in green, and Asn residues shown in blue.



Supplemental Figure 3. Polysaccharide binding ability of Man5B/Cel44A-TM4 with CMC (glucose-configured), Mannan (mannose-configured) and Birchwood xylan (xylose-configured) as substrates. Five microliters of 0.8 mg/ml protein samples were applied to native gel (5% acrylamide) and substrate infused native gel (5% acrylamide, 0.1% substrate), and electrophoresis was carried out for 2 h at 80 V using BSA as a standard. Lane1, BSA in native gel; Lane 2, Man5B/Cel44A-TM4 in native gel; Lane 3, BSA in substrate infused native gel; Lane 4, Man5B/Cel44A-TM4 in substrate infused native gel.

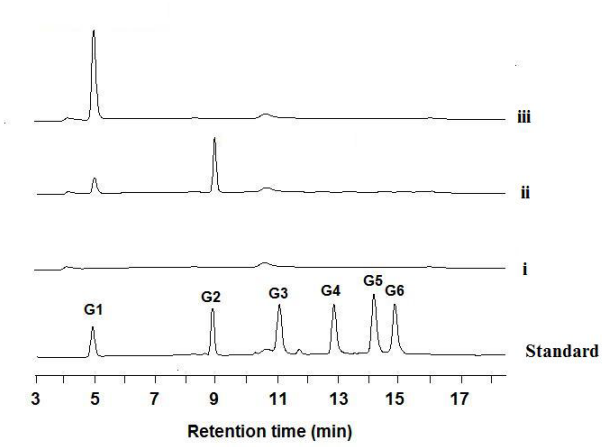


Supplemental Figure 4. Schematic structure (A) and SDS-PAGE (B) analysis of CbMan5B/Cel44A-TM4 and its truncated mutants (CbMan5B/Cel44A-TM4Δ1β, CbMan5B/Cel44A-TM4Δ2β, CbMan5B/Cel44A-TM4Δ3β, and CbMan5B/Cel44A-TM4Δ9β) of *C. bescii*. CbMan5B/Cel44A-TM4 and its truncated mutants were analyzed on a 12% SDS polyacrylamide gel. Lane 1: protein molecular mass marker; lane 2: CbMan5B/Cel44A-TM4 (soluble protein); lane 3: CbMan5B/Cel44A-TM4Δ1β (insoluble protein); lane 4: CbMan5B/Cel44A-TM4Δ2β (insoluble protein); lane 5: CbMan5B/Cel44A-TM4Δ3β (insoluble protein); lane 6: CbMan5B/Cel44A-TM4Δ9β (insoluble protein).

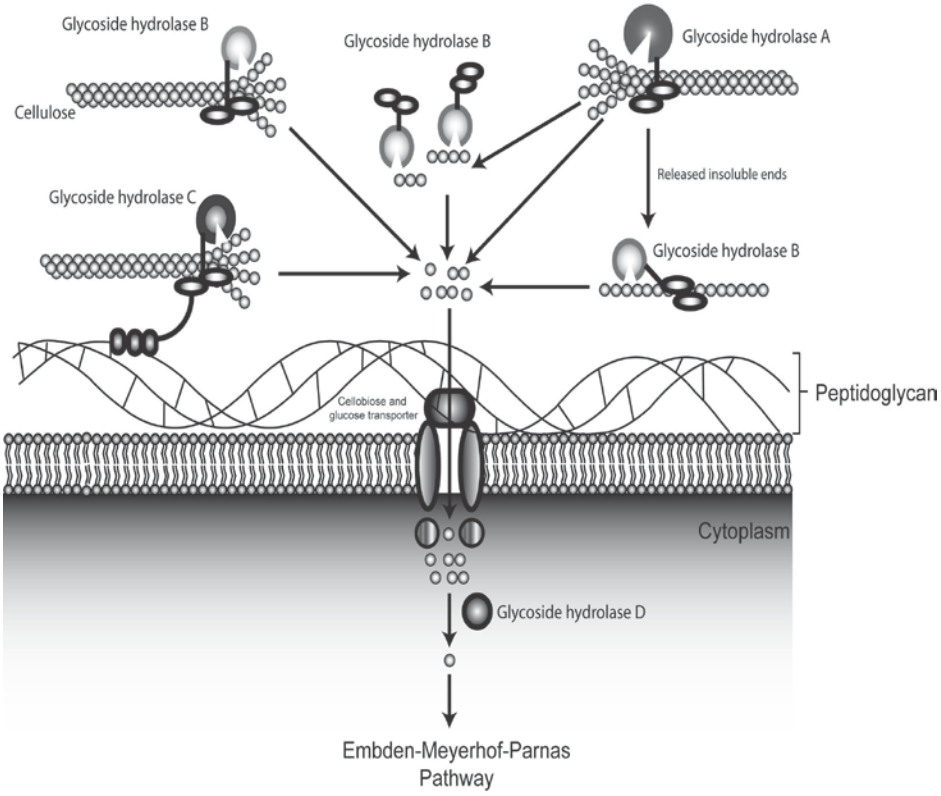


Supplemental Figure 5. Amounts of insoluble reducing ends released from Avicel hydrolyzed by CbMan5B/Cel44A-TM2, CbCel9B/Man5A-TM1, CbMan5C/Cel5A-TM2 and CbCel5B-TM1. Avicel (5 mg/ml) was incubated with 2 μM of each enzyme in 50 mM citrate buffer (pH 5.5) at 70°C. At different time points, a sample was removed and heat-inactivated. After the reaction, the Avicel was pelleted at 15,871 × *g* for 15 min, and the precipitate was washed twice with 1ml of 6 M guanidine-HCl, and then soaked in 6 M guanidine-HCl for 20min, and washed four times with distilled water and twice with 50 mM sodium acetate buffer (pH 5.5) to remove bound protein. The washed insoluble sample was re-suspended in 0.2 ml of citrate buffer (pH5.5) and reducing ends present were determined by the BCA method using glucose as a standard.

A.


iii
ii
i
G1
G2
G3
G4
G5
G6
Standard
3
5
7
9
11
13
15
17
Retention time (min)

B.


Glycoside hydrolase B
Cellulose
Glycoside hydrolase B
Glycoside hydrolase A
Released insoluble ends
Glycoside hydrolase C
Glycoside hydrolase B
Peptidoglycan
Cellobiose and glucose transporter
Cytoplasm
Glycoside hydrolase D
Embden-Meyerhof-Parnas Pathway

Supplemental Figure 6. Cellulose hydrolysis and utilization by *C. bescii*. (A) HPAEC-PAD analysis of end products released from PASC by a mixture of *C. bescii* endoglucanases (CbMan5B/Cel44A-TM2, CbCel9B/Man5A-TM1, CbMan5C/Cel5A-TM2, CbCel5B-TM1 and CbCdx1A) i, PASC (5 mg/ml) was incubated at 70°C for 32 h as control. ii, PASC (5 mg/ml) was incubated with a combination of the four enzymes (0.5 µM CbMan5B/Cel44A-TM2, 0.5 µM CbCel9B/Man5A-TM1, 0.5 µM CbMan5C/Cel5A-TM2 and 0.5 µM CbCel5B-TM1) for 16 h. The enzymes were inactivated at 100 °C for 10 min, and the reaction was divided into two parts (sample A and sample B). Incubation of sample A was continued at 70 °C for 16 h. The reaction mixtures were removed and analyzed by HPAEC-PAD. iii, The reaction mixture of sample B was incubated after addition of 0.5 µM CbCdx1A for another 16 h at 70 °C and analyzed by HPAEC-PAD for end products. (B) Schematic representation for a proposed mechanism for nutrient acquisition from cellulose by *C. bescii*. Glycoside hydrolase A and Glycoside hydrolase B have signal peptides that allow their transport outside of the cell. Glycoside hydrolase A represents endoglucanases (*e.g*. CbMan5B/Cel44A-TM2) that hydrolyze cellulose into large products, and Glycoside hydrolase B represents endoglucanases (*e.g*. CbCel9B/Man5A-TM1, CbMan5C/Cel5A-TM2, CbCel5B-TM1) that act on the ends and end-products available to produce mainly glucose and cellobiose. The cellobiose and glucose are transported into the cell by sugar-transporters, and the cellobiose is digested into glucose by CbCdx1A, located intracellularly. The glucose is then metabolized through the Embden-Meyerhof-Parnas pathway by *C. bescii*.