Supplementary information

Mechanisms involved in xyloglucan catabolism by the cellulosome-producing bacterium *Ruminiclostridium cellulolyticum*

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Supplementary Table S1: Kinetic parameters of the hydrolytic activities of Cel9U, Cel9X, Cel44O and Xgh74A on tamarind xyloglucan

Enzyme	k_{cat}[a]	K_m[b]	k_{cat}/K_m[c]
Cel9U[d]	2,302 ± 157	7.5 ± 0.15	307
Cel9X[d]	6,560 ± 231	8.4 ± 0.4	781
Cel44O	970 ± 38	0.21 ± 0.06	4,619
Xgh74A	2,304 ± 78	0.37 ± 0.01	6,227

[a]values are given in µmol of products released per µmol of enzyme x min^{-1}.
[b]values are given in g x L^{-1} of xyloglucan.
[c]values are given in µmol of products released per µmol of enzyme x min^{-1}L x g^{-1}.
[d]data are from reference[19].

Supplementary Table S2: Specific activity of the β-glucosidase GH3A on 1 mM cellooligosaccharides.	
Substrate	Specific activity[a]
Cellobiose	9.0 ± 0.20[b]
Cellotriose	12.4 ± 1.3
Cellotetraose	11.6 ± 1.3
Cellopentaose	14.0 ± 2.3
Cellohexaose	10.7 ± 0.6

[a]values are given in µmol of products released per µmol of enzyme x min^{-1}.
[b]the data show the mean and standard deviation of two replicates.

Supplementary Table S3: MALDI-TOF analyses of the products released during the sequential degradation of XXXG by successive rounds of incubation with either the α-xylosidase or the β-glucosidase.

Corresponding steps in Figures 9c or 9d	Composition	Expected m/z $[M+Na]^+$	Observed m/z $[M+Na]^+$
Substrate	XXXG[a]	1,085.338	1,085.306
9c step 1 (+ α-xylosidase)	GXXG	953.296	953.322
9c step 2 (+ β-glucosidase)	XXG	791.243	791.237
9c step 3 (+ α-xylosidase)	GXG	659.201	659.137
9c step 4 (+ β-glucosidase)	XG	497.148	497.131
Substrate	XXXG	1,085.338	1,085.306
9d step 1 (+β-glucosidase)	XXXG	1,085.338	1,085.290
9d step 2 (+α-xylosidase)	GXXG	953.296	953.209
9d step 3 (+β-glucosidase)	XXG	791.243	791.198
9d step 4 (+α-xylosidase)	GXG	659.201	659.139
9d step 5 (+β-glucosidase)	XG	497.148	497.081

[a]see Fig. 1 for xyloglucan motif abbreviations.

Supplementary Table S4: list of primers used

Primers used for overexpression in *E. coli* of selected genes

Protein (gene locus)	Primer name	5'-3' primer sequence
Cel44O	44Of	AAAAAACCATGGCTGCCAGTGACGCAATAAACG
(Ccel_0429)	44Or	AAAAAACTCGAGCCAATCAATTGAATCAACATACATT
Xgh74A	Ccel_1207f	TTTTTTCCATGGCAAATGGCCCCGTAAGCGCCCCGTATAATTGGAA
(Ccel_1207)	Ccel_1207r	GGGGGGCTCGAGAAATGAGGTAATTATTCCCAATAAATAAC
Gal42A	2451ncof	TTTTTTCCATGGATAAATATATGCCTATTGGTATG
	2451mnf	CTAAAGGATTTCCACGGCGGACGTC
(Ccel_2451)	2451mnr	GACGTCCGCC*G* TGGAAATCCTTTAG
	2451xhor	TTTTTCTCGAGTATTTTAGCTTTTAGCACTTTTACGTCA
Glu3A	2454Ncof	AAAAACCATGGAATACGATCAGATAGATAAAAAAATTGATGAAC
(Ccel_2454)	2454Xhor	AAAAACTCGAGCAGAGCAAGAGCTATAGCTATCG
Xyl31A	2455ncof	TATATACCATGGCTAAATTTCTAAATGGATACTGGATGAGT
(Ccel_2455)	2455xhor	TTTTTCTCGAGTAGAATTCTTACTGTATAACTGCCAGTAGA
SBP_{2458}	SBPf	AATTCATATGCACCACCACCACCACCACGCTTGTACCAAACCGGGAACCGACAGC
(Ccel_2458)	SBPrev	AATTCTCGAGTTATTTACTGTTTTCTGCAGCCTGCTTGCGGG
Cel9Xt	XdokeTf	GCATTCGCCAACAGTAATTTGTCAGGTACTCCTTCTACTAAATTATACGGC
	XdokeTR	GCCGTATAATTTAGTAGAAGGAGTACCTGACAAATTACTGTTGGCGAATGC
	X830f	CCCCCAGGTTCAAGATAGGA
9X-44O	9X44Of	CAGTAATTTGTCAGGTGAAAACTCCATAGCTGCCAGTGACGCAATAAACG
	9X44Or	CGTTTATTGCGTCACTGGCAGCTATGGAGTTTTCACCTGACAAATTACTG
	pETrev	ATGTTTGACAGCTTATCATCGA

Primers used for qPCR experiments designed with the Primer-BLAST tool

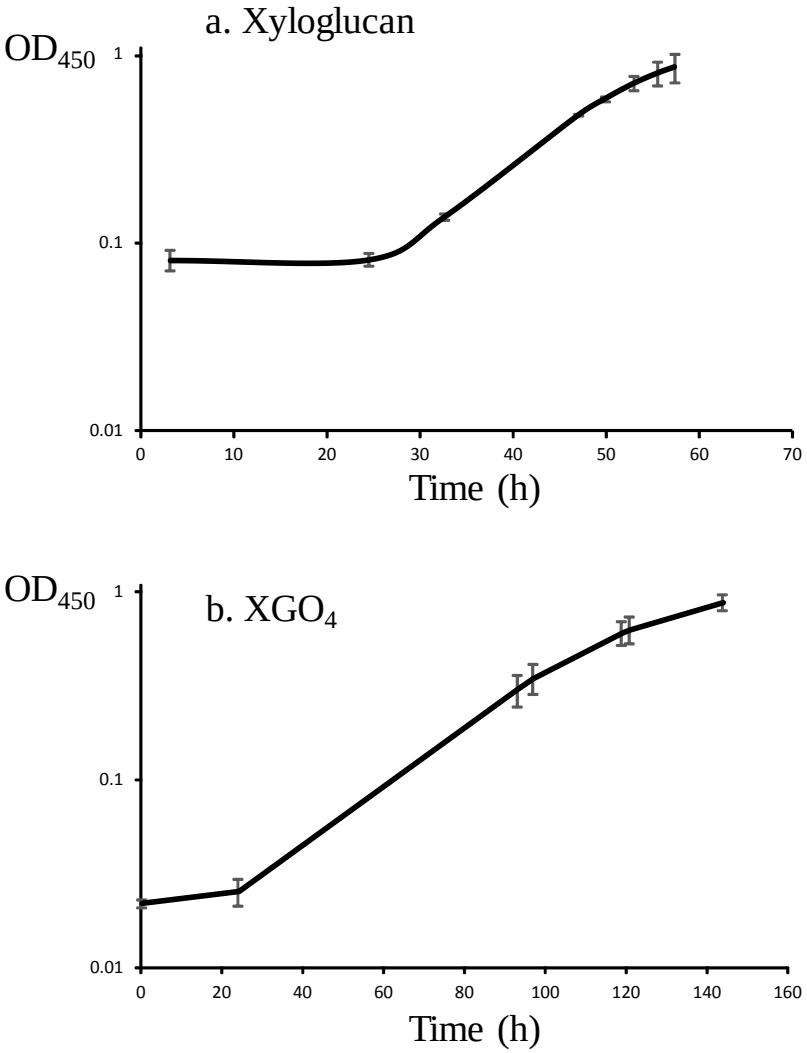
Protein (gene locus)	Primer name	5'-3' primer sequence
Gal42A	2451f	CTCGTGGATGTCGCAAAAGT
(Ccel_2451)	2451r	CACCACTCATGGTTAAGCCG
Regulator	2452f	CGGCAAGCTCGGTAAATCTT
(Ccel_2452)	2452r	CGTCCCACAGAAGCAAACAA
Sensor	2453f	CAGCACCATGTGGGATAACG
(Ccel_2453)	2453r	CGTATACGGCGTACCAAAGC
Glu3A	2454f	TGCTGCCAATAACCAGGAGA
(Ccel_2454)	2454r	TCCGTAATCAACTGACGGCT
Xyl31A	2455f	TACGGTTTGGGAGAGCGATT
(Ccel_2455)	2455r	TCCAACGATTCGCCTTCAAC
TMD_{2456}	2456f	GAAGTCAAGCGTGGTTGGTT
(Ccel_2456)	2456r	CCACAAACTCCAAAGCGTCA
TMD_{2457}	2457f	TGCTTAATCTGGGGCTTCGT
(Ccel_2457)	2457r	CAAGAATGGGAACGCAGAGG
SBP_{2458}	2458f	TGGCAAAGCACGGAGATAAC
(Ccel_2458)	2458r	CCTCGGAAGGAGTAATGGGA
Cel44O	0429f	AGCACCGTCAGAAAGATGGA
(Ccel_0429)	0429r	AGAGAGCCGGTTCATTGTCA
Cel9U	0755f	CGGTAAAGACGGAGGGAAGT
(Ccel_0755)	0755r	ATTGTAATCCCCGGCATCGT
Xgh74A	1207f	GAAGCGGTAACGGACTTTGG
(Ccel_1207)	1207r	GGGTTCCATGTTTCTCCTGC
Cel9X	2621f	TACTGAGTGGCAGCACGTAA
(Ccel_2621)	2621r	AGCCGGATATGCTCTTTCCT
RpoD	RpoDdir	AAACATAGTCAAGAAAGTAGAAAAG
(Ccel_0541)	RpoDrev	CTATACTAACAACCAGCCTTAAG
16S rRNA	16s235F	CTATGTTTCTTGAGTGCCGG
(Ccel_R0018)	16s235R	ATACTTATTGTGTTAACTCCGG

Primers used for construction of *R. cellulolyticum* mutant strain MTL2458

Protein (gene locus)	Primer name	5'-3' primer sequence
	IBS-SBP	AAAAAAGCTTATAATTATCCTTACATGCCGCCGTCGTGCGCCCAGATAGGGTG
	EBS1d-SBP	CAGATTGTACAAATGTGGTGATAACAGATAAGTCGCCGTCCCTAACTTACCTTTCTTTGT
	EBS2-SBP	TGAACGCAAGTTTCTAATTTCGGTTGCATGTCGATAGAGGAAAGTGTCT
	EBS	CGAAATTAGAAACTTGCGTTCAGTAAAC
SBP_{2458} (Ccel_2458)	SBPf	GGGTGGATTTATTCACTGTTTGA
	SBPr	GGTTGGCAAGAGCGACGGTATGGTG
Em^R	ramF	ACGCGTTATATTGATAAAAATAATAATAGTGGG
	ramR	ACGCGTGCGACTCATAGAATTATTTCCTCCCG

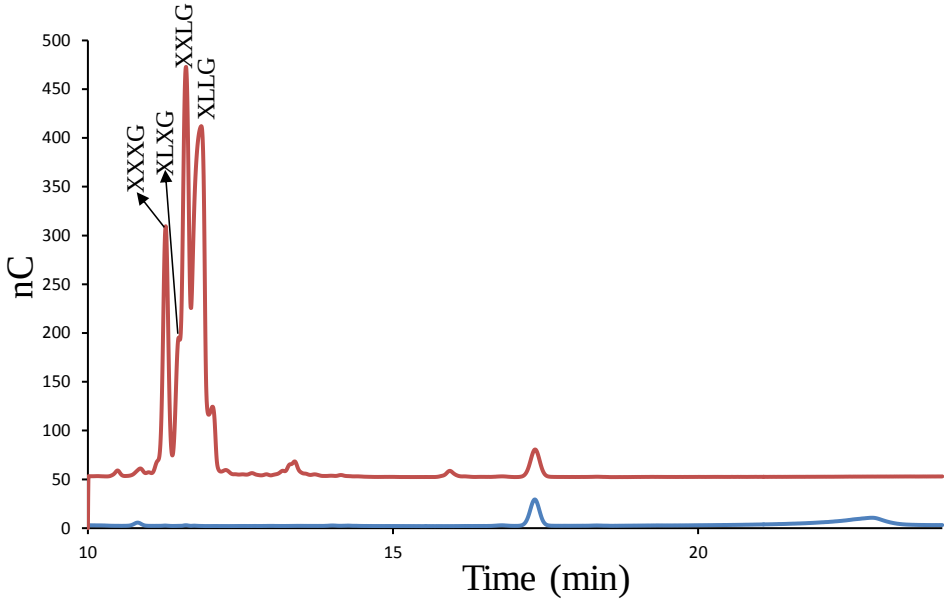
Supplementary Figure S1: Growth on minimal medium supplemented with a) tamarind xyloglucan or b) XGO_4 at 3.5 g/L.

The data show the mean of three independent experiments and the bars represent the standard deviation. Cultures on minimal medium supplemented with tamarind xyloglucan (**a**) or XGO_4 (**b**) were used as inoculum (1/20).



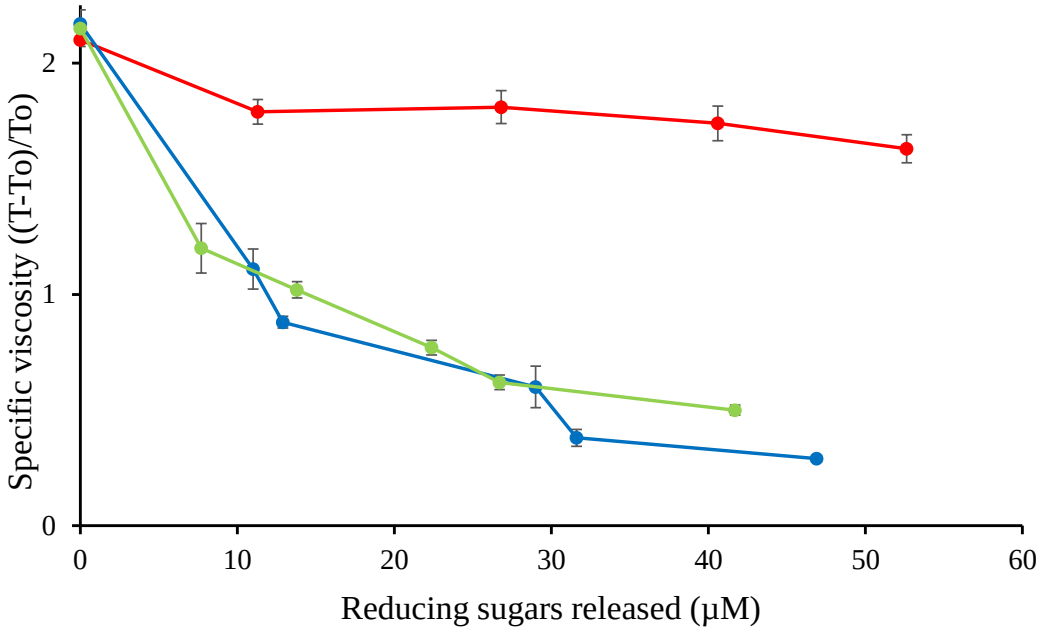
Supplementary Figure S2: Complete degradation of 3.5 g/L xyloglucan by Cel9X.

Xyloglucan was incubated for 24 h at 37°C with no (blue) or 0.5 µM of Cel9X (red). The obtained sugars are indicated on top of each peak. The mixture of XGO_4 oligosaccharides served to prepare the XGO_4-based minimal medium used for growth of *R. cellulolyticum* (Figure S1) and mRNA extraction subsequently used for reverse transcription and quantitative PCR experiments (Figure 7).



Supplementary Figure S3: Changes in relative xyloglucan viscosity versus reducing sugars released by Cel9X, Cel44O and Xgh74A.

Green line, Cel9X; blue line, Cel44O; red line, Xgh74A. The data show the mean and standard deviation of three independent experiments. The specific viscosity was determined as $((T-T_0)/T_0)$ where T and T_0 correspond to the flow time of xyloglucan with enzyme and the flow time of buffer, respectively.



Supplementary Figure S4: HPAEC-PAD analysis of the action of the β-glucosidase Glu3A on the end products generated by a) Cel44O and b) Cel9X on xyloglucan.

Xyloglucan (3.5 g/L) was incubated for 24 h at 37°C with 0.5 µM of Cel44O (**a**) or 0.5 µM of Cel9X (**b**), prior heating at 100 °C for 5 min. The xyloglucan oligosaccharides obtained in each case (black lines, **a** and **b**) were then incubated with 1 µM of Glu3A for 30 min (red lines, **a** and **b**) or 2 h (blue lines, **a** and **b**). The obtained sugars are indicated on top of each peak.

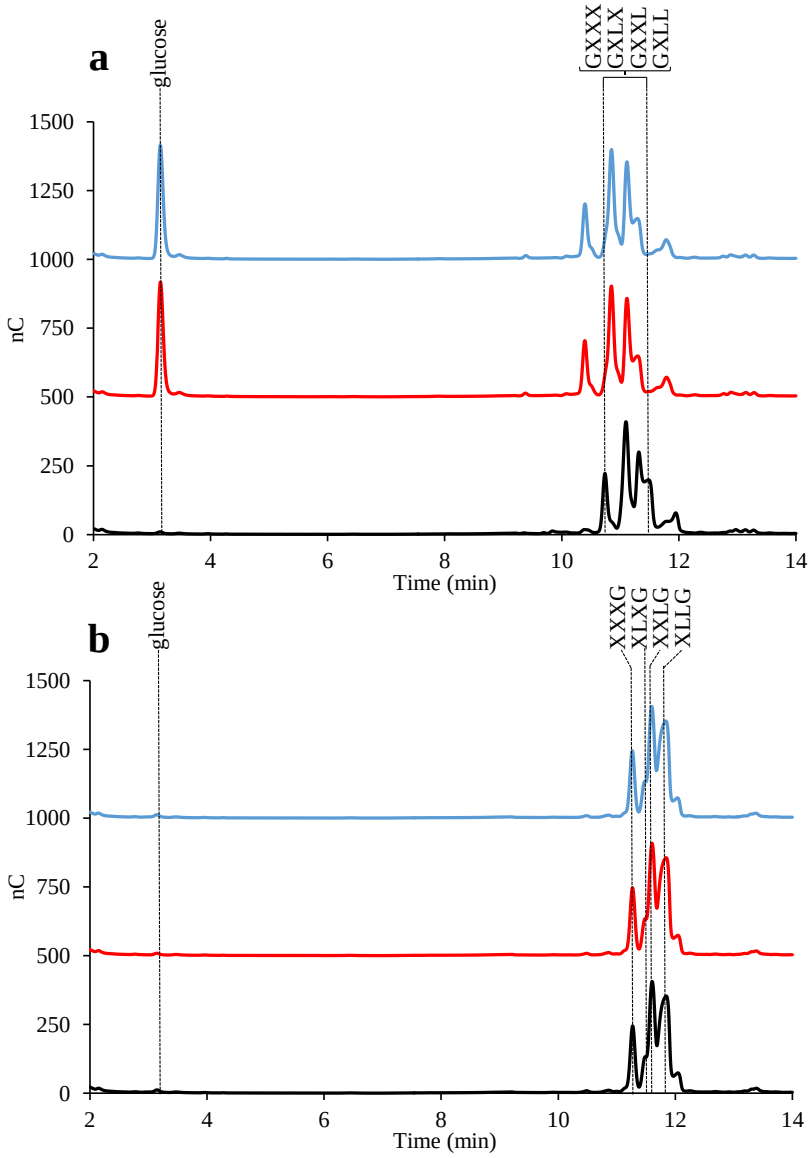


Figure S5: Lack of interaction between the SBP_{2458} and isoprimeverose observed by ITC. The upper part of each panel shows the raw binding heats and the lower part shows the integrated binding heats minus the dilution control heats. The SBP was at 40 µM and the ligand at 4 mM. Similar ITC data were obtained for cellotriose, cellotetraose, glucose, xylose and galactose.

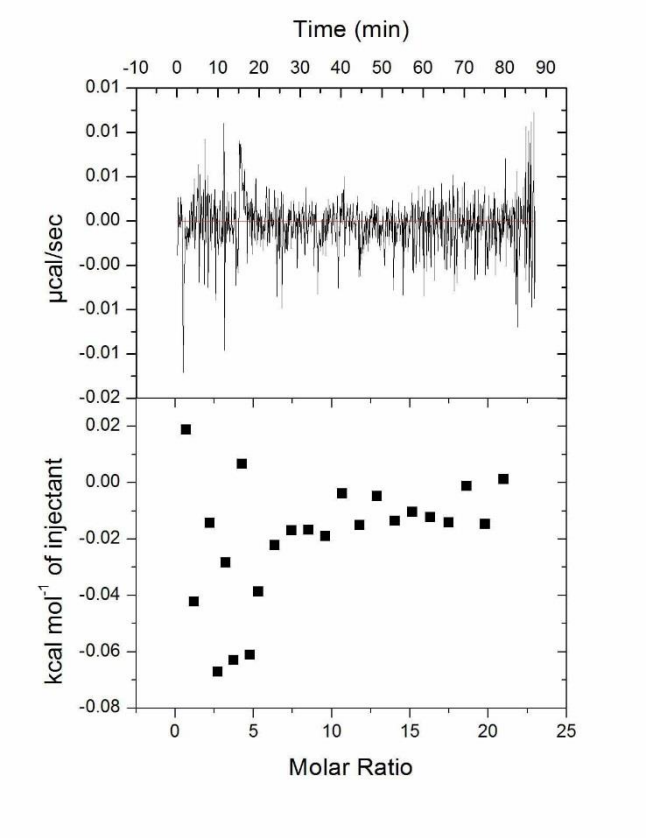


Figure S6: Growth of wild-type and mutant MTL2458 strains of *R. cellulolyticum* on minimal medium supplemented with a) cellobiose (2 g/L) or b) tamarind xyloglucan (3.5 g/L).

The cultures were inoculated (1/33) with cellobiose (2 g/L) grown precultures. The data show the mean of three independent experiments for wild type (black squares) and MTL2458 (open squares) strains, respectively. Bars represent the standard deviations.