Supporting Information

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Plasmid Construction and Assessment of Enzymatic Activities Against Soluble Substrates

The pET22b plasmid was engineered to produce fusion protein constructs. A 48-bp oligonucleotide adaptor encoding NdeI, KpnI, BamHI, HindIII, EcoRI, SacI, SalI, and XhoI was ligated into NdeI- and XhoI-digested pET22b to give a modified pFV1 vector, leaving the C-terminal His_6-tag sequence untouched. A DNA fragment encoding a ~15-amino acid proline- and threonine-rich linker was cloned into HindIII- and EcoRI-digested pFV1 to generate pFV1-PT. DNA sequences encoding the catalytic modules of Xyl10A, Xyl11A, Pel10A, Abf51, and the various CBMs were amplified by PCR using thermostable KOD Hot start DNA polymerase (Novagen). Primers used are listed in Table S2. All catalytic modules were cloned into EcoRI- and XhoI-digested pFV1-PT vector. The amplified DNA fragments encoding CBM2b-1-2, CBM15, and CBM2a were then cloned into BamHI- and HindIII-restricted pFV1-PT containing the catalytic module DNA sequences to generate fusions separated by the P/T linker and distinct CBMs and catalytic modules. Amplified DNA encoding CBM3a was cloned into NdeI- and KpnI-digested pFV1-PT to generate fusions with specific catalytic domains. A CBM2b-1-2:GFP construct was used to allow direct imaging of CBM2b-1-2 binding. The sequence encoding the GFP fragment was amplified by PCR using standard reaction conditions and primers containing XhoI restriction sites. The amplified DNA was cloned in frame into XhoI-digested pFV1 containing the BamHI/HindIII-ligated CBM2b-1-2 DNA sequence to generate a protein in which GFP is fused to the C terminus. Soluble proteins were produced in *Escherichia coli* strain BL21 using standard procedures for all constructs generated. The construct was purified by immobilized metal affinity chromatography. All recombinant proteins used in this study contained a C-terminal His_6 tag. The specific activities of all constructs against soluble substrates were measured using a polygalacturonic acid assay for Pel10A fusion proteins (1), a 4-nitrophenyl-α-arabinofuranoside assay for GH51 fusion proteins (2), and the 3,5-dinitrosalicylic acid assay with birchwood xylan as a substrate for GH10 and GH11fusion proteins, as shown in Table S1.

1. Brown IE, Mallen MH, Charnock SJ, Davies GJ, Black GW (2001) Pectate lyase 10A from Pseudomonas cellulosa is a modular enzyme containing a family 2a carbohydrate-binding module. *Biochem J* 355:155–165.

2. Beylot MH, McKie VA, Voragen AG, Doeswijk-Voragen CH, Gilbert HJ (2001) The Pseudomonas cellulosa glycoside hydrolase family 51 arabinofuranosidase exhibits wide substrate specificity. *Biochem J* 358:607–614.

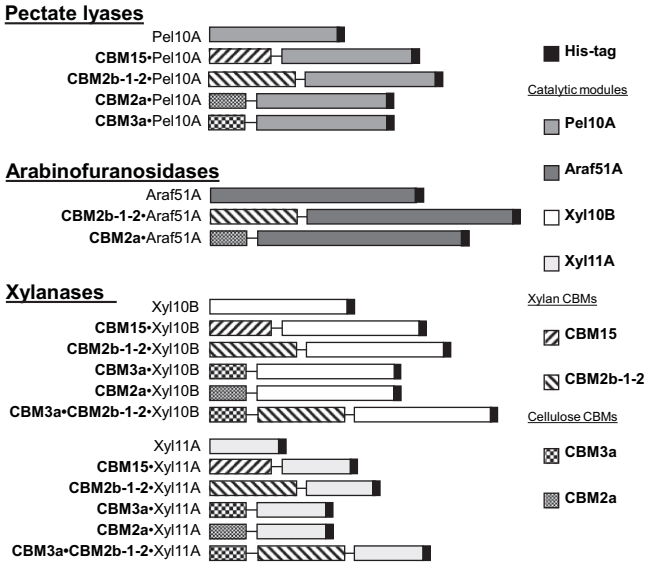

Fig. S1. Molecular architectures of the hybrid enzymes containing catalytic domains fused to CBMs used in this study. Pel10A, *C. japonicus* pectate lyase 10A; Abf51A, *C. japonicus* arabinofuranosidase 51A; Xyl10B, *C. mixtus* xylanase 10B; Xyl11A, *Neocallimastix patricarium* xylanase 11.

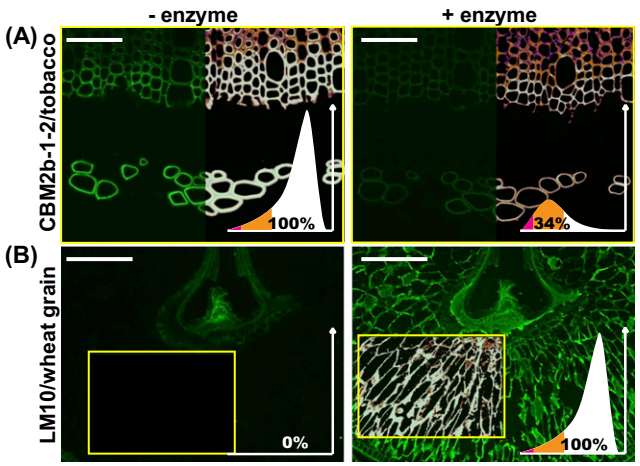

Fig. S2. Quantification of probe-based immunofluorescence in relation to enzymatic treatments. Fluorescence is shown as observed with the left panels (*A*) or the inserts (*B*) showing the same image with overlaid colors reflecting the different fluorescence intensities. The schematic curves laid out as inserts show the fluorescence quantification and the corresponding distribution of intensities, as described previously in Hervé et al. (1). (*A*) Detection of the xylan-probe binding CBM2b-1-2:GFP to secondary cell walls of tobacco stem sections before and after xylanase treatment. In this case, all fluorescence in equivalent micrographs of equivalent regions of the sections was quantified. CBM2b-1-2:GFP fluorescence is reduced by treatments with xylanases. (Scale bar: 100 μm.) (*B*) Indirect immunofluorescence detection of LM10 in the endosperm of wheat grain sections before and after treatment with arabinofuranosidase. The signal is maximal after the enzymatic treatment. In this case, equivalent regions of the micrographs containing only endosperm cells were selected for quantification. (Scale bar: 500 μm.)

Table S1. In vitro catalytic activities against soluble substrates of the hybrid enzymes used in the study

Pectate lyases: substrate: polygalacturonic acid

	$K_{CAT,\ min}{}^{-1}$	K_m, M	K_{CAT}, SE	K_m, SE
Pel10A	671	2.255	17.9	0.244
CBM15_Pel10A	706	2.257	43.2	0.559
CBM2b12_Pel10A	738	1.847	21.4	0.240
CBM3A_Pel10A	736	2.172	24.0	0.293
CBM2A_Pel10A	681	1.722	20.1	0.236

Arabinofuranosidases: substrate: 4-nitrophenyl-α-arabinofuranoside

	$K_{CAT,\ min}{}^{-1}$	K_m, M	K_{CAT}, SE	K_m, SE
Abf51A	62.2	73.3	1.8	6.2
CBM2b-1-2·Abf51A	42.2	99.3	1.3	8.4
CBM2a·Abf51A	52.4	82.3	1.0	4.6

Xylanases: substrate: birchwood xylan

	$K_{CAT,\ min}{}^{-1}$	K_m, mg/mL	K_{CAT}, SE	K_m, SE
Xyl10B	1,431	0.423	110	0.137
CBM15·Xyl10B	1,132	0.324	109	0.152
CBM2b-1-2·Xyl10B	1,238	0.318	66	0.083
CBM3a·Xyl10B	1,372	0.495	121	0.170
CBM2a·Xyl10B	1,433	0.550	115	0.164
CBM3a·CBM2b-1-2·Xyl10B	1,120	0.295	62	0.084
	$K_{CAT,\ s}{}^{-1}$	K_m, mg/mL	K_{CAT}, SE	K_m, SE
Xyl11A	1,056	1.614	63	0.280
Xyl11A·CBM15	998	2.111	60	0.335
CBM2b-1-2·Xyl11A	1,178	1.655	48	0.194
CBM3a·Xyl11A	994	1.327	59	0.245
CBM2a·Xyl11A	1,175	1.480	46	0.173
CBM3a·CBM2b-1-2·Xyl11A	1,226	1.568	63	0.235

Table S2. Primers used in construction of fusion proteins with restriction sites underlined

PEL10A Eco	5′-CTCCCGGAATTCACAGGCCGGATGCTAACCCTGGATG-3′
PEL10A Xho	5′-CTCCCGCTCGAGCAGGTAACCCACTTTCTGGGCGAA-3′
XYN10 Eco	5′-CTCGAATTCGGTTTGGCCAGCCTGGCAGATTTCC-3′
XYN10 Xho	5′-CTCGCTCGAGACGACCACTCAATGCCTCGACC-3′
XYN11 Eco	5′-CTCGAATTCAAGTTTACTGTCGGTAATGG-3′
XYN11 Xho	5′-CTCGCTCGAGAGTTGTGTAAACATCTAATAAGG-3′
GH51 Eco	5′-GTAGGAATTCATGCGCCGTTTGAAACCCCTCATCG-3′
GH51 Xho	5′-TTATCTCGAGTTCCACCGCAACCACAACGACCGAT-3′
CBM15 Bam	5′-CTCGGATCCGTCGCTGCCAGCGAGG-3′
CBM15 Hind	5′-CTCGTGAAGCTTGGCTTCCTGTGCGAGTG-3′
CBM2b Bam	5′-CTCGGATCCAGCACCGGCTGCTCGGTCACCG-3′
CBM2b Hind	5′-CTCGTGAAGCTTGCCCGTGGCGCACGTAGCGC-3′
CBM2a Bam	5′-CACAAGGGATCC*ATG*GCAACTTGCAGTTATAACATTACCAA-3′
CBM2a Hind	5′-CACAAGAAGCTTCACAG ATCCCGAGCA GATACTGCCA-3′
CBM3a Nde	5′-CCGGACAAGCATATGAATTTGAAGGTTGAATTCTACAACAGCAATC-3′
CBM3a Kpn	5′-CACAAGGGTACCGGGTTCTTTACCCCATACAAGAACA-3′