SUPPORTING INFORMATION FOR

The maize pathogen *Ustilago maydis* secretes glycoside hydrolases and carbohydrate oxidases directed towards components of the fungal cell wall

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1. Supplementary Figures



Fig. S1. Time-course expression along *U. maydis* infection cycle on maize of the 11 genes coding for putative FCW-active CAZymes. (A) Schematic representation of *U. maydis* plant infection cycle (dpi, days post inoculation). (B) Differential gene expression (*vs* axenic condition). Original transcriptomic data were retrieved from (1) for the following genes (proteins) : UMAG_03551 (*Um*AA3_2-A), UMAG_03256 (*Um*AA3_2-B), UMAG_04044 (*Um*AA3_2-C), UMAG_10861 (*Um*AA7), UMAG_05550 (*Um*GH5_9-A), UMAG_00235 (*Um*GH5_9-B), UMAG_02134 (*Um*GH16_1-A), UMAG_06157 (*Um*GH135), UMAG_00638 (*Um*CE4_CDA1), UMAG_11922(*Um*CE4_CDA3) and UMAG_01788 (*Um*CE4_CDA4). Red arrows indicate the two enzymes characterized in the present study.



Fig. S2. SDS-PAGE analysis of *Um*GH16_1-A, with (A) and without (B) C-term extension. The enzymes were expressed in *P. pastoris* and purified by IMAC. The theoretical molecular weight of full length (*Um*GH16_1-A_FL) and truncated (*Um*GH16_1-A_cd) versions of *Um*GH16_1-A are 39.168 kDa and 33.439 kDa, respectively. Note that the higher molecular weight observed on SDS-PAGE is most likely due to protein *N*-glycosylation (7 putative glycosylation sites, exposed at the protein surface, are predicted by the online tool NetNGlyc -1.0).



Fig. S3. Comparison of structures of (A) *Um*GH16_1-A_FL (homology model, with AlphaFold) and (B) its closest structural homologue, the GH16_1-A from *Phanerochaete chrysosporium* (PDB 2W52; (2)).

Predicte	d cleavag	e sites of mu	Itiple protease families							
ATWS RNSV VHTN TTGK AGNS QSTQ	QSAVV RITSK TSCTI GVRVW YNQSG SSADS	KGNDF <mark>F</mark> NSYSDG PTT <mark>I</mark> SG YFPN <mark>N</mark> K CNAQYP LLKHSW	N D F D W F T D K D P T V <mark>V V L</mark> N V T H V P L G C Q S G T V A <mark>Y</mark> S N C S A Y P S A I P S D L S S S T S C S Y Q V G Y R G S S I I P S <mark>A</mark> A <mark>A</mark> L V G S F A	IGLVNYQSQ ATWPAFNT QPSNPGCR TLSTDAW NRSYWEVE AFYAAL	A N A R <mark>A Q N L</mark> V T E N I D S W V E T N G T S T T P N <mark>A</mark> Y L P I S L R L Y T K G	S F V D K Q G H F <mark>V M A V</mark> S P N G G E I D I M E N A N D P T W G R A L N R A G G G I S S C Y A D F E P H K I V F G D S A N A <mark>A</mark> I S P Q S <mark>V</mark> N	T T P V A L Q G Q Y P A N L V S I A M E R S F G D I T L C G D W S S R Q S S <mark>N</mark> K			
					C-tern	n extension				
Cleav	ed by Aspart	ic protease after	his residue (P1 position)							
_										
Cleav	ed by Cystei	ne protease after	this residue (P1 position)							
Cleav	ved by Metall	oprotease after th	is residue (P1 position)							
Cleav	ed by Serine	protease after thi	s residue (P1 position)							
Cleav	ved by differe	nt multiple prote	ase superfamilies after this positio	n (P1 position)						
RANK in ecretome	Score	OrthoDB	JGI ProtID	UMAG_ID	Enzyme class	Enzyme family	Protease Type			
13	75	254398	jgi Ustma2_2 9235	UMAG_11908	PROTEASE	Cathepsin D.	Aspartic peptidase			
20	39	254398	jgi Ustma2_2 9376	UMAG_02178	PROTEASE	Cathepsin D.	Aspartic peptidase			
42	18	226881	jgi Ustma2_2 13300	UMAG_06118	PROTEASE	Tripeptidyl-peptidase I.	Serine peptidase			
46	17	386362	jgi Ustma2_2 8783	UMAG_01888	PROTEASE	Carboxypeptidase D.	Serine peptidase			
80	5	138643	jgi Ustma2_2 8781	UMAG_01886	PROTEASE	Carboxypeptidase C.	Serine peptidase			
81	5	98560	jgi Ustma2_2 11242	UMAG_03947	PROTEASE	Carboxypeptidase D.	Serine peptidase			
91	3	254398	jgi Ustma2 2 6857	UMAG 00064	PROTEASE	Cathepsin D.	Aspartic peptidase			

Fig. S4. (A) Prediction of proteolytic cleavage sites in UmGH16_1-A and (B) proteases found in the TOP100 secreted proteins. Proteolytic cleavage sites were predicted with PROSPER (3).

PROTEASE

jgi|Ustma2_2|6857|



Fig. S5. Linkage analyses of Laminarin, yeast β-glucan and pachyman. (A) Total Ion Chromatograms (TIC) of partially methylated alditol acetates by GC-MS, **(B)** quantification and **(C)** reconstitution of sugar linkages.



Fig. S6. MALDI-ToF-MS analyses of soluble products released from Laminarin in the presence (A-B, red) or absence (C-D, blue) of UmGH16_1-A_cd. Full Spectra are presented in figure A and C. Representative zooms to highlight normal species, reduced species and species with a loss of water are shown figure B and D.



Fig. S7. UPLC-ESI-IT analyses of soluble products released from Laminarin by $UmGH16_1-A_cd$. (A) Base peak chromatography between 0 and 27 min. (red stars indicate a contaminant) and (B) ESI-MS spectra of each compounds revealed by the analysis. RT and nature of the oligosaccharides are indicated for each MS spectrum. For the sake of clarity, only the [M+Na]⁺ and the [M+K]⁺ ions are labelled with the corresponding m/z values.



Fig. S8. Control reactions of *Um*GH16_1-A on β -1,4 glucans, mixed β -1,3/1,4 glucan and β -1,3 glucooligosaccharides. The graphs show HPAEC-PAD chromatograms of reaction

products released from **(A-B)** laminari-oligosaccharides (DP2-DP6; 1 mM each) and **(C)** Avicel, α -chitin or Lichenan (10 mg.mL⁻¹ final concentration) by *Um*GH16_1-A_cd (10 nM). Panel B shows a zoom-in view of chromatograms displayed in panel A for reactions on Lam6 only (Lam2 to Lam 5 were not recognized as substrates by *Um*GH16_1-A_cd). On Lam6, very small amounts of products Lam2 and Lam 3 (at 12 and 16 min, red stars) were detected. In panel C, Avicel and α -chitin are linear polymers of β -1,4-linked D-glucose and *N*-acetylglucosamine units, respectively. Lichenan is a mixed β -1,3/1,4 glucan. All reactions were incubated during 4 h, in citrate phosphate buffer (50 mM, pH 5.5), in a thermomixer (30 °C, 1,000 rpm). All experiments were carried put in triplicate. However, for the sake of clarity, only one replicate is shown.



Fig. S9. Time-course release of short (Lam2-Lam5) and long (Lam6-Lam9) oligosaccharides from Laminarin by *Um*GH16_1-A_cd. The amount of oligosaccharides is expressed as (A) the absolute sum of peak areas and (B) the proportion of short and long oligosaccharides at each time point (the sum of short and long oligosaccharides is equal to 100% of released oligosaccharides at each time point). The reactions contained laminarin (10 mg.mL⁻¹), *Um*GH16_1-A_cd (10 nM) in citrate phosphate buffer (50 mM, pH 5.5) and incubated in a thermomixer (30 °C, 1,000 rpm). Data are presented as average values (n = 3, independent biological replicates) and error bars show s.d.



Fig. S10. Purity and molecular weight analyses of *Um***AA3_2-A. (A)** SDS-PAGE and **(B)** SEC analyses. The enzyme was expressed in *P. pastoris* and purified by IMAC followed by SEC. In panel A, the following samples were loaded on the SDS-PAGE gel: lane 1, IMAC-purified *Um*AA3_2-A; lane 2, heat-treated, IMAC-purified *Um*AA3_2-A; lane 3, SEC-purified *Um*AA3_2-A; lane 4, heat-treated, SEC-purified *Um*AA3_2-A. In panel B, using a calibrated SEC column, we determined an experimental MW of *Um*AA3_2-A of 48.2 kDa (average of n = 2 independent experiments) (theoretical MW = 64.9 kDa).



Fig. S11. Chemical structures of oligosaccharides tested in *Um*AA3_2-A substrate specificity screening.



Fig. S12. pH activity profile of *Um*AA3_2-A on (A) glucose and (B) G3G and (C) G6G. The graph shows the reduction rate of DCIP (400 μ M) by *Um*AA3_2-A (110 nM), in the presence of glucose (500 mM), G3G (5 mM) or G6G (5 mM) at different pH values (50 mM of tartrate or citrate-phosphate buffer). All reactions were carried out at 30 °C. Data points show average values and error bars show s.d. (n = 3 independent biological replicates).



Fig. S13. MALDI-ToF MS analysis of G3G, G3G3G and G6G oxidation by *Um*AA3_2-A. Spectra of G3G (A-D), G3G3G (B-E) and G6G (C-F) before (A-C) and after (D-F) treatment by *Um*AA3_2-A. The spectra show the detection of native oligosaccharides [M+Na] with m/z = 365 (for G3G and G6G) or 527 g.mol⁻¹ (for G3G3G). Upon addition of *Um*AA3_2-A oxidized species emerge: simple sodium adducts of oxidized form [M+16+Na] (m/z = 381 for G3G and G6G, or 543 for G3G3G) and double sodium adducts of oxidized form [M+16-H+2Na] (m/z = 403 for G3G and G6G, or 581 for G3G3G), which suggest the formation of aldonic acids (see Fig. S14 for validation).



Fig. S14. UPLC-MS analysis of G3G before (A) and after (B) oxidation by UmAA3_2-A.

Each panel shows the UPLC chromatogram (left) and MS spectra (right) using either positive (upper graph) or negative (lower graph) ionization mode.



Fig. S15. UPLC-MS analysis of G3G3G before (A) and after (B) oxidation by UmAA3_2-A. Each panel shows the UPLC chromatogram (left) and MS spectra (right) using either positive (upper graph) or negative (lower graph) ionization mode.



Fig. S16. UPLC-MS analysis of G6G before (A) and after (B) oxidation by UmAA3_2-A. Each panel shows the UPLC chromatogram (left) and MS spectra (right) using either positive (upper graph) or negative (lower graph) ionization mode.



Fig. S17. Structural comparison of *Pc***ODH (PDB id 6XUV), and** *Um***AA3_2-A (model). (A)** The structure of *Pc***ODH (in green) and** *Um***AA3_2-A (in blue) were superimposed in Pymol** and shown as cartoon. A global view facing the entrance active site is displayed on top of the figure. The additional loop of *Um*AA3_2-A is squared in black and the active site of both enzymes are subdivided in two parts: "left side" (squared in orange) and "right side" (squared in red). Zooms on these two parts are shown on the lower part of the figure, where the keys

amino acids for laminaribiose binding in *Pc*ODH and their structural equivalents in *Um*AA3_2-A are displayed as stick and annotated according to amino acid numbering in *Pc*ODH/*Um*AA3_2-A. These amino acids are annotated in black when conserved in both proteins or in red when substituted. The two catalytic histidines are labelled with an orange star. The Laminaribiose (colored in purple) and the Flavin adenine dinucleotide (FAD) co-factor (colored in yellow) are shown in sticks. **(B)** Comparison of the active site of laminaribiose active enzymes (*Pc*ODH and *Um*AA3_2-A) with glucose active enzymes (*Af*GDH and *An*GOX). The proteins structures are shown as surface and approximative perimeter of the active site entrance is delimited by a black dotted line.

Y64/Y61	
101/101	

AnGOX AfGDH PcODH UmAA3_2-A	I 1SNGIEASLLTDPKDVSGRTVDYIIAGGGLTGLTTAARLTENPNISVLVIESGSYESDRGPIIEDLNAGYGLIFGSSVD 1	77 62 80 70
AnGOX	78 HAYETVELAT - NNQTAL I RS <mark>G</mark> NG <mark>LGG</mark> STL VNGGTWT RPHKAQ VDSWETVFGNE GWNWDNVAAYSLQAE RARAP NAKQ 1	153
AfGDH	63 WQYQSI NQSYAGGKQQVL RAGKALGGTST I NGMAYTRAEDVQ I DVWQKL - GNE GWTWKDL L PYYLKSENL TAPTSSQ 1	138
PcODH	81 WAWEA DQGKVI HGGKTLGGSSSI NGAAWTRGL NAQ YDSWSSL L EPEEASVGWNWNNL FGYMKKAE AF SAPNDQQ 1	154
UmAA3_2-A	71 FKFNTVPQV GGRTKAPLG <mark>G</mark> RTLGG <mark>STSI NG</mark> AAWNRASRAQYDALGAL I NSADA <mark>GWNW</mark> NGLLGYMKKSENFVAPNQDQ 1	147
AnGOX	154 IAAGHYFNASCHGVNGTVHAGPRDTGDDYSPIVKALMSAV-EDRGVPTKKDFGCGDPHGVSM 2	214
AfGDH	139 VAAGAAYNPAVNGKEGPLKVGWSGSLASGNLSVALNRTF-QAAGVPWVEDVNGGKMRGFNI 1	198
PcODH	155 RAKGADSIASYHGTTGPVQATFPDEMYGGPQQPAFVNTVVNVTGMPHYKDLNGGTPNCVSI 2	215
UmAA3_2-A	148 RNLGAKWDPSVHGTSGPLEIGFTQIRNNNRRSTTNVGGASQQWKRMFTGPQQPAFIKAVGETLGVQQVDDQCSGQANSVAF 2	228
AnGOX	215 FPNTLHE DQVRSDAAREWLLPNY - QRPNLQVLTGQYVGKVLL - SQNGTTPR - AVGVEFG - TH - KGNTHNVYAKHEVLLA 20	88
AfGDH	199 YPSTLDV - DLNVREDAARAYYFPYD - DRKNLHLENTTANRLFWKNGSAEEAI - ADGVEITSAD GKVTRVHAKKEVIIS 22	74
PcODH	216 TPLSINWHDDDHRSSSIEAYYTPVENNRQGWTLLIDHMATKVLF - DGTNAPLT - AVGIEFGASDATGNRYKAFARKEVILA 20	94
UmAA3_2-A	229 TPNSIGV NGQRTSAASAYYTPVQ - NRDNLTILTGTMAKNLLWDAATSSNLLRSSGVVVQQGR - NGNQIRLVANKEVILA 30	05
	Q331/M349	
AnGOX	289 AG SAVSPTILEYSG IGMKSILEPLG I DTVVDL - PVGLNLQDQTTATVRSRITSA GAGQGQAAWFATFNETFGDYSE 30	63
AfGDH	275 AGALRSPLILELSGVGNPTILKKNNITPRVDLPTVGENLQDQFNNGMAGEGYGV L - AGASTVTYPSISDVFGNETD 30	49
PcODH	295 AGAIQTPALLQLSG IGDSDVLGPLG I STLSDLKTVGKNLQEQTQNA I GAKGNGF DPDGHGPTDA I AFPN I YQVFGSQAT 33	73
UmAA3_2-A	306 AGALNTPVLLQRSGVGAKTDLNS I GVDQR I ELAGVGKNLQDQTMTT I GSRANVNYA GGGPSAT I AMPN I QQIMSN - ST 30	82
	D418/D435 F428/D446 F416/F433 F421/F438 U W430/W448	
Angox	364 KAHELLNTKL EQWAEEAVARG - GFH - NTTALLIQYENYRDWI VNHNVAYSELFLDTAG VASFDVWDLLPFTRGYVHIL4:	39
Afgdh	350 SIVASLRSQLSDYAAATVKVSNGHM - KQEDLERLYQLQFDLIVKDKVPIAEILFHPGG - GNAVSSEFWGLLPFARGNIHIS4:	28
Pcodh	374 SAVQTIQSSLSAWAKTQ AAAGAL - SADALNTIYQTQADLIINHNAPVVELFFDSGFP - DDVGIVMWPLLPFSRGNVTIT4:	50
UmAA3_2-A	383 AVRSYITSNLDGWANQL LSQGHVASKEGVLAQWRSAISLIFDQKAPVVELFFDTGFPANSYGIDIWTLLPFSRGSIRAT4:	61
AnGOX	440 DKDPYLHHFAYDPQ <mark>YF</mark> LNELDLLGQAAATQLARNISNSGAMQTYFA-G <mark>ETIPG</mark> -DNLAYDADLSAWTEYIP5	08
AfGDH	429 SND-PTAPAAINPNYFMFEWDGKSQAGIAKYIRKILRSAPLNKLIA-KETKPGLSEIPATAADEKWVEWLK4	97
PcODH	451 SNN-PFAKPSVNVNYFSVDFDLTMHIAGARLSRKLLGSPPLSSLLV-GETVPGFKTVPNNGNGGTDADWKKWILKPGNS5	27
UmAA3_2-A	462 SQN-PYDGARIDPNYFGLPIDMDMQVASLRASRRVLQNSNLRSLTYNGETTPGFSLIPDGPNSGRYSRWRDWILGTLPNG55	41
	H528/H549 H571/H592	
AnGOX AfGDH PcODH UmAA3 2-A	I 509 YH F RPNYH GV GT CSMMPKEM GG VVD NAAR VY GV QG L RVI DG SIPPT QMSSHVMT VFYAMALKISDAILE DYASMQ 498 ANYRSNFH PV GT AAMMPRSI GG VVD NALRVY GT SNVR VVD ASVL PFQ VCGHLVST LYAVAERASDLI KEDAKSA- 528 AGF ASVAH PI GT AAMMKRSL GG VVD AQLK VY DT TNLRVVD ASMMPLQI SAHLSST LYGVAEKAAD LI KAAQ 542 SG FAAVSHQLGT AAMGSRSL GAVVD AKFK VY GT SNVR VVD ASVLPVQI SAHLSST LYGVAEKAAD TI LAR 61	33 71 98 11

Fig. S18. Multiple sequence alignment (MSA) of *An*GOX, *Af*GDH, *Pc*ODH and *Um*GH16_1-A. The MSA shows the presence of an extra loop in *Um*AA3_2-A (printed in green) and key residues involved in substrate binding (annotated according to amino acid numbering in *Pc*ODH/*Um*AA3_2-A; catalytic histidines are printed in red).



Fig. S19. Activity of *Um*GH16_1-A_cd and *Tsp*GH16_3 (from Megazyme) on polysaccharides extracts from *U. maydis*. The graphs show HPAEC-PAD chromatograms of reaction products released from (A) NaOH-extracted polysaccharides (5 mg.mL⁻¹ final concentration), and (B) from alkali insoluble material (approx. 1-5 mg.mL⁻¹), by the commercial *Tsp*GH16_3 (100 nM) and *Um*GH16_1-A_cd (100 nM). All reactions were incubated during 16 h, in citrate phosphate buffer (50 mM, pH 5.5), in a thermomixer (30 °C, 1,000 rpm). For the sake of clarity, only one chromatogram for each reaction condition is shown (each experiment was carried out at least in triplicate).



Fig. S20. *Um*GH16_1-A is not inhibited by G3G or G6G. The graphs show HPAEC-PAD chromatograms of reaction products released from laminarin (10 mg.mL⁻¹) by *Um*GH16_1-A_cd (10 nM) in the presence of various concentrations of (A) G3G (0-1 mM) or (B) G6G (0-10 mM). All reactions were incubated during 4 h, in citrate phosphate buffer (50 mM, pH 5.5), in a thermomixer (30 °C, 1,000 rpm), (n = 1). In the negative control reaction, Laminarin in the absence of enzyme was incubated in the same conditions as other reactions. Abbreviations: G3G, laminaribiose (also called Lam2); G6G, gentiobiose; Lam3 to Lam6, β -1,3-glucooligosaccharides of DP3 to 6.



Fig. S21. *Um***GH16_1-A is not inhibited by oxidized G3G or G6G.** The graphs show HPAEC-PAD chromatograms of reaction products released from laminarin (10 mg.mL⁻¹) by *Um*GH16_1-A_cd (10 nM) in the presence of oxidized G3G (G3G^{ox}; 1 mM) or oxidized G6G (G6G^{ox}, 1 mM). All reactions were incubated during 4 h, in citrate phosphate buffer (50 mM, pH 5.5), in a thermomixer (30 °C, 1,000 rpm), (n = 1). See the experimental section for the preparation of G3G^{ox} and G6G^{ox}. Abbreviations: G3G, laminaribiose (also called Lam2); G6G, gentiobiose; Lam3 to Lam6, β-1,3-glucooligosaccharides of DP3 to 6.

RANK in TOP 50 ^b	JGI Protid	Annotation in Re-Annotation 2012 in 2022		Score	Predicted Target (phylogeny- based) ^c	Biochemically characterized?	
1	jgi Ustma2_2 11640	GH27-CBM35	GH27-CBM35	586	PCW	NO	
2	jgi Ustma2_2 11831	GH62	GH62	556	PCW	NO	
3	jgi Ustma2_2 10689	GH10	GH10	429	PCW	NO	
5	jgi Ustma2_2 7673	GH51	GH51	281	PCW	NO	
6	jgi Ustma2_2 10518	CDH_2	AA3_2	233	ND	NO	
7	jgi Ustma2_2 11351	CDH_2	AA3_2	188	ND	NO	
8	jgi Ustma2_2 13127	FAD-Oxidase	AA7	180	FCW	NO	
9	jgi Ustma2_2 9924	CE4	CE4	119	FCW	Rizzi et al.	
11	jgi Ustma2_2 13337	UNK	GH135	90	FCW	NO	
12	jgi Ustma2_2 12578	GH5	GH5_16	88	PCW	NO	
16	jgi Ustma2_2 7458	CE4	CE4	58	FCW	Rizzi et al.	
17	jgi Ustma2_2 13257	GH3	GH3	56	PCW	NO	
24	jgi Ustma2_2 9413	GH37	GH37	32	PCW	NO	
27	jgi Ustma2_2 9206	GH26	GH26	27	PCW	NO	
29	jgi Ustma2_2 8673	CE4	CE4	26	FCW	Rizzi et al.	
35	jgi Ustma2_2 9331	GH16	GH16	22	FCW ?	This Study	
37	jgi Ustma2_2 8391	EXPN	EXPN	20	PCW	NO	
40	jgi Ustma2_2 9202	GH45	GH45	19	PCW	NO	
41	jgi Ustma2_2 12699	GH5	GH5_9	19	FCW	NO	
47	jgi Ustma2_2 7038	GH5	GH5_9	17	FCW	NO	
49	jgi Ustma2_2 10841	CDH_2	AA3_2	16	FCW ?	This Study	

Table. S1. The 21 CAZymes present in the TOP50 proteins secreted by *U. maydis* after re-analysis in 2022^a.

^a Initial secretomic data (*U. maydis* secretome harvested after 7 days of growth on maize bran) were published by (4).
^b Only CAZymes are shown in this Table.
^c Abbreviations: FCW, Fungal Cell Wall ; PCW, Plant Cell Wall ; ND, Not determined; UNK, Unknown.

	Ustilago maydis								Pycnoporus cinnabarinus				
		UmAA3_2-	A (This St	udy)	UmGDHIII (Wijayanti et al. (2021)) ^c				PcODH (Cerruti et al. (2021))				
	M-M model			initial slope	pe M-M model		el	initial slope	M-M model		el	initial slope	
	$k_{\rm cat}$	Км	$k_{\rm cat}/K_{\rm M}$	$k_{\rm cat}/K_{\rm M}$	$k_{\rm cat}$	Км	$k_{\rm cat}/K_{\rm M}$	$k_{\rm cat}/K_{\rm M}$	k_{cat}	Км	$k_{\rm cat}/K_{\rm M}$	$k_{\rm cat}/K_{\rm M}$	
	(s⁻¹)	(mM)	(s ⁻¹ .M ⁻¹)	(s ⁻¹ .M ⁻¹)	(S ⁻¹)	(mM)	(s ⁻¹ .M ⁻¹)	(s ⁻¹ .M ⁻¹)	(s-1)	(mM)	(s ⁻¹ .M ⁻¹)	(S ⁻¹ .M ⁻¹)	
Glc	9.2	454	20.2	18.0	0.2	12.5	18.4	-	50	755	67	47	
	± 0.6	± 52	± 3.7	± 2	±0.009	±0.4		-	± 3	±110	± 10	± 1	
G3G	21.5	36	600.00	636.00	1.0	250	4.0	-	71	77	917	777	
	± 1.4	± 4	± 105	± 76	±0.21	±20		-	± 4	± 10	± 129	± 21	
G6G	48.2	86	560	697.00	6.3	51	122.3	-	-	-	-	-	
	± 4.5	± 12	± 130	± 68	±0.3	<u>±</u> 4		-	-	-	-	-	

Table S2. Kinetic parameters of UmAA3_2-A and PcODH^{a,b}

^aKinetic parameters were calculated via non-linear regression fitting to the Michaelis-Menten equation. Catalytic efficiencies (k_{cat}/K_M) were also calculated by measurement of the slope of the linear phase (low [S] << K_M) of the Michaelis-Menten plot.

^bUmAA3_2-A and UmGDHIII are the same enzymes. PcODH was previously known as PcGDH.

^cWijayanti et al. used benzoquinone as electron acceptor, when dichlorophenolindophenol (DCIP) was used in the present study and by Cerruti et al.

2. Full abbreviations list

Enzymes/Microorganisms

AA: Auxiliary activities AR: AmplexRed® CE: Carbohydrate Esterases GH: Glycoside hydrolases HRP: Horseradish peroxidase Um : Ustilago maydis Tsp : Trichoderma spp.

Substrates/products

DCIP: 2,6-Dichlorophenolindophenol DMSO: dimethyl sulfoxide G3G: Laminaribiose G6G: Gentiobiose FCW: Fungal cell wall PCW: Plant cell wall YPD: Yeast Extract–Peptone–Dextrose

Methods

HPAEC-PAD: high-performance anion-exchange chromatography coupled with pulsed amperometric detection UPLC-MS: Ultra-performance liquid chromatography- mass spectrometry UPLC-ESI-MS: Ultra-performance liquid chromatography coupled to electrospray ionization and mass spectrometry UHPLC-ESI-IT: Ultra High-Performance Liquid Chromatography -Electrospray -Ion trap LC-MS: Liquid chromatography–mass spectrometry MALDI-ToF: Matrix-assisted laser desorption/ionization-Time-of-flight GC-MS: Gas chromatography–mass spectrometry SEC: Size-exclusion chromatography MSA: Multiple sequences alignment MW: Molecular weight SDS-PAGE: Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

3. Supplementary references list

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