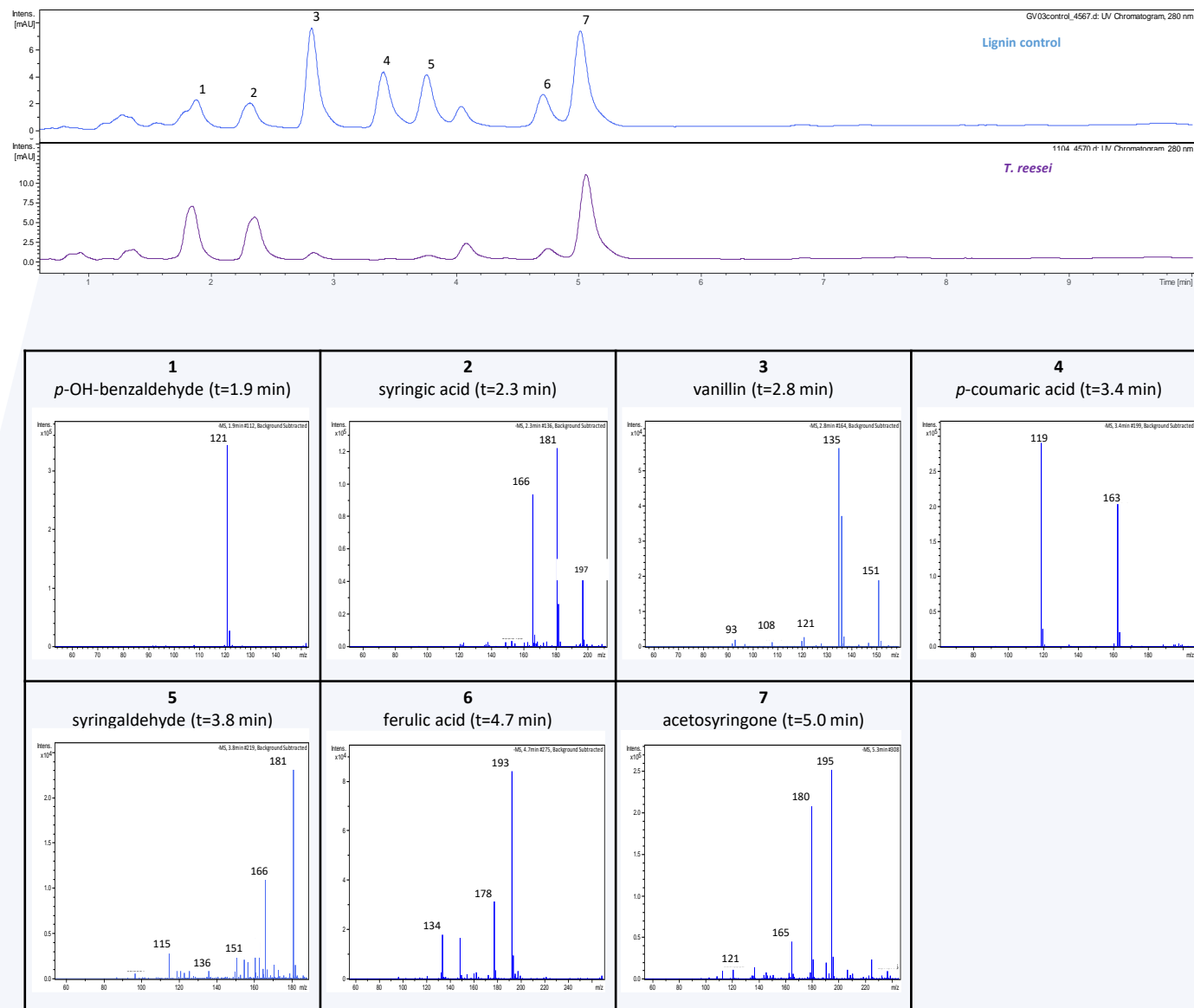


**Figure S1. *T. reesei* growth on technical soda lignin.** **A.** Growth rate of *T. reesei* on agar plates containing technical lignin in the presence and absence of 1 g/L glucose and **B.** mycelial appearance. **C.** Total mycelial dry weight of *T. reesei* during growth in liquid cultures on lignin in the presence and absence of 2.5 g/L maltose.



**Figure S2. LC-MS analysis on water-soluble lignin fraction.** The analysis is done on phenolic monomers extracted from the culture supernatant by ethyl acetate. Normalized chromatograms obtained with a C18 column (Highpurity, Thermo Electron Corporation, 2.7  $\mu$ m, 50 mm x 2 mm I.D.mm), a 5–100 vol.% aqueous acetonitrile, 1% HCOOH gradient (30min) and 0.4 ml/min flow rate, and with a 280 nm UV detection. ESI-MS spectra were obtained in the negative mode from scans acquired in a mass range of  $m/z$  120–2000.

**Table S1. Total phenolic and thioacidolysis yields of water-insoluble residual lignin after exposure to *T. reesei*.**

	PheOH (mmol g <sup>-1</sup> ) <sup>a</sup>	Thioacidolysis <sup>b</sup>	
		Total yield (μmol g <sup>-1</sup> )	S/G ratio
Control	2.90	132 ± 9	0.93 ± 0.05
<i>T. reesei</i>	2.80	89 ± 3	0.92 ± 0.00

<sup>a</sup> Data correspond to single determination  
<sup>b</sup> Data are mean values (± standard error) between duplicate analyses.

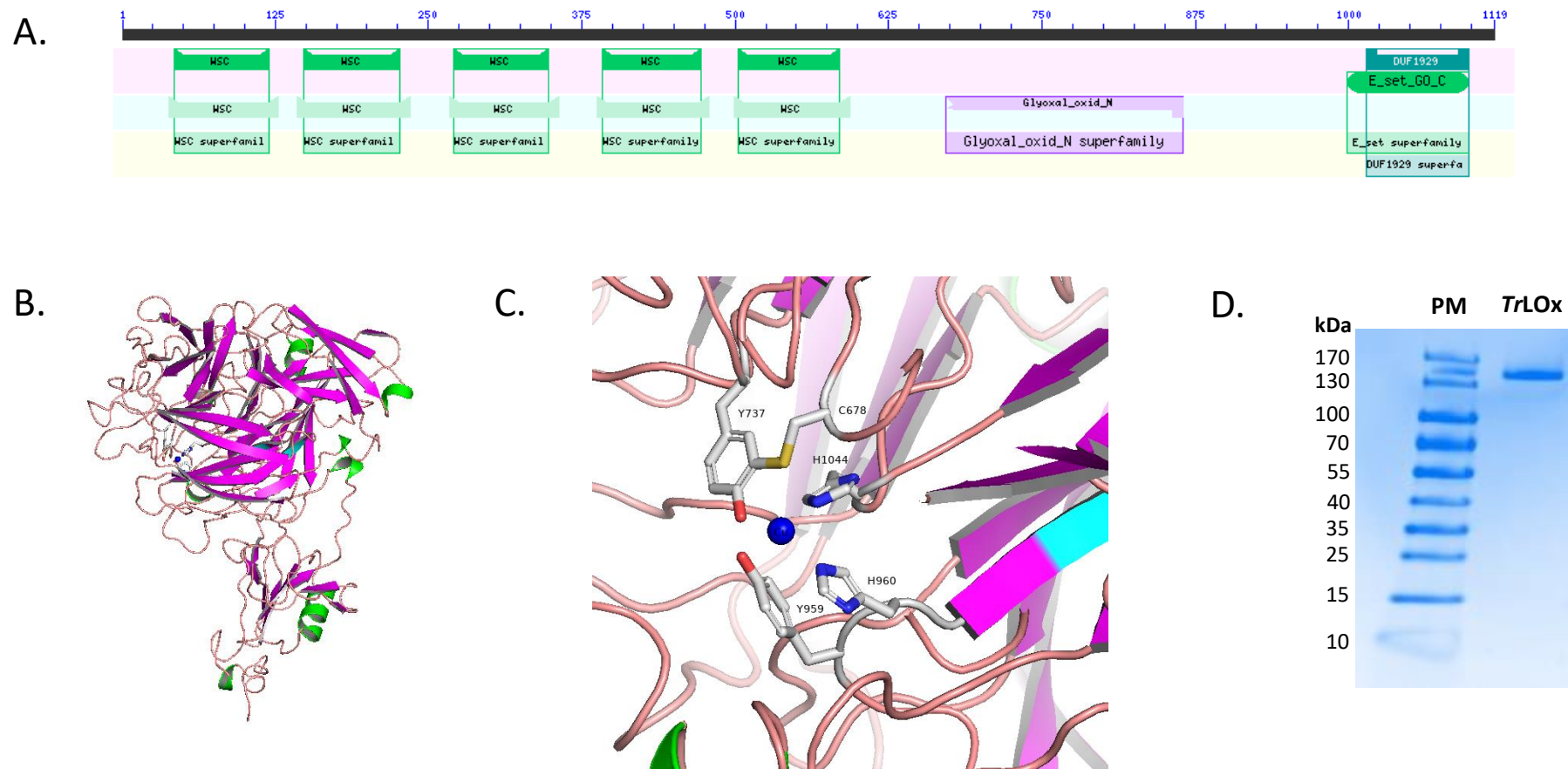
**Table S2.** Identified CAZymes in the secretomes during growth of *T. reesei* on technical lignin.

Protein ID	Cazy	Day of growth	Protein ID	Cazy	Day of growth
22915	AA3_2	7	69276	GH30_7	7 and 14
80659	AA3_3	14	82235	GH31	3, 7 and 14
<b>124282</b>	<b>AA5_1</b>	<b>3, 7 and 14</b>	80240	GH35	3, 7 and 14
78357	AA6	7 and 14	124016	GH36	3, 7 and 14
73643*	AA9-CBM1	7	123226	GH37	3, 7 and 14
77299	GH2	3, 7 and 14	3196	GH38	14
69245	GH2	3, 7 and 14	68064	GH43	7
62166*	GH2	7	45717	GH47	3, 7 and 14
58450	GH3	3, 7 and 14	55319	GH54-CBM42	3, 7 and 14
121735	GH3	3, 7 and 14	123283	GH54-CBM42	3, 7 and 14
121127	GH3	3, 7 and 14	121746	GH55	3, 7 and 14
47268	GH3	7	54242	GH55	3, 7 and 14
76672	GH3	7 and 14	70845	GH55	3, 7 and 14
104797	GH3	7 and 14	73248	GH55	3, 7 and 14
82616*	GH5_5	7	76210	GH62	3, 7 and 14
120312	CBM1-GH5_5	7	124175	GH64	3, 7 and 14
56996	GH5_7	7	123456	GH65-CBM32	3, 7 and 14
72567	CBM1-GH6	7 and 14	72526	GH67	3, 7 and 14
123989	GH7-CBM1	3, 7 and 14	71532	GH71	3, 7 and 14
122081	GH7-CBM1	7 and 14	108672	GH71-CBM24-CBM24	3, 7 and 14
120229*	GH10	7	120873	GH71-CBM24-CBM24	3, 7 and 14
74223	GH11	7 and 14	123538*	GH72	7
123818	GH11	3, 7 and 14	22914	GH72-CBM43	3, 7 and 14
123232*	GH12	7	82633	GH72-CBM43	3, 7 and 14
105956	GH13_1	3, 7 and 14	49081	GH74-CBM1	3, 7 and 14
1885	GH15-CBM20	3, 7 and 14	70341*	GH75	7
65406	GH16	3, 7 and 14	74807*	GH76	7 and 14
123726	GH16	3, 7 and 14	27395	GH76	7
49274	GH16	3, 7 and 14	67844*	GH76	7
39755	GH16	7	106575	GH79	3, 7 and 14
76266*	CBM18-GH16	7	71394	GH79	7
66792	GH17	3, 7 and 14	57098	GH92	3, 7 and 14
39942	GH17	3, 7 and 14	55733	GH92	3, 7 and 14
80833	GH18	3, 7 and 14	60635	GH92	7 and 14
119859	GH18	7 and 14	79921*	GH92	7
68347	GH18-CBM1	7 and 14	58802	GH95	3, 7 and 14
21725	GH20	3, 7 and 14	111138*	GH95	7
23346	GH20	7	5807*	GH95	7
103458	GH25	7	79606	GH115	7 and 14
72632	GH27	3, 7 and 14	70373	GH125	7 and 14
59391	GH27	3, 7 and 14	107850	CE1	7
27259*	GH27	7	44214	CE5	3, 7 and 14
122780	GH28	3, 7 and 14	73632*	CE5-CBM1	3 and 7
103049	GH28	7 and 14	58282	CE9	14
3094	GH30_3	3, 7 and 14	121418	CE16	3, 7 and 14
69736	GH30_3	7 and 14	103033	PL7_4	7
110894	GH30_5	3, 7 and 14	69189*	PL20	7
111849	GH30_7	3, 7 and 14			

\*proteins that were exclusively detected in the absence of maltose.

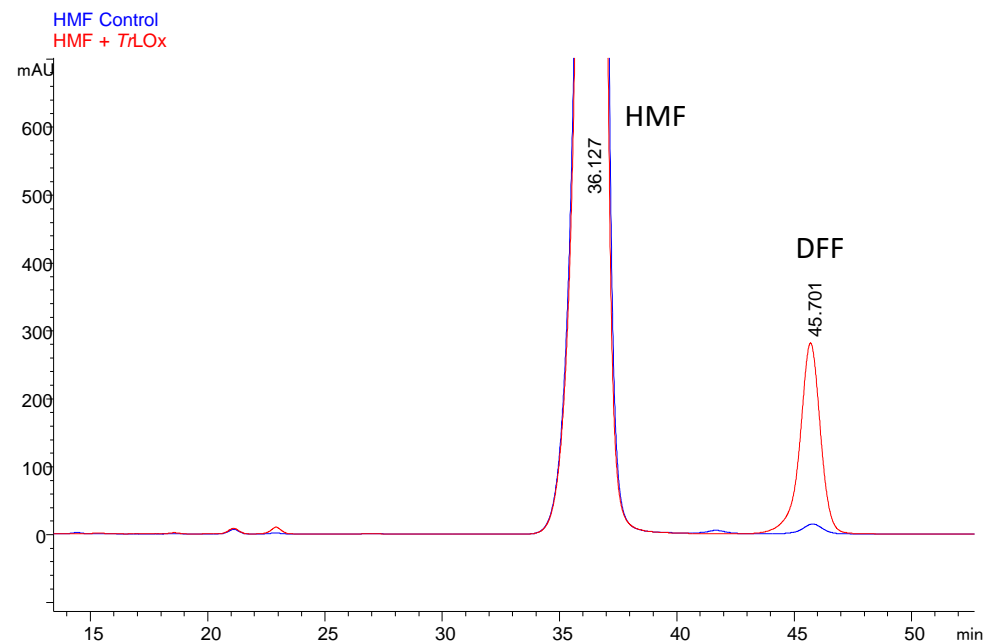
EFQ36699.1 CgrAAO/276-776	276	QNGQWSP I Q . . . . .	TLPLNP . . . . .	VAAYLVPAYPVVQDFLSFSFSPFT . . . . .	GGGPAYNTAFMRYNIKSSAASQFNVAETKDHMF . . . . .	PGMNHLDAGR . . . . .	LVINGGNTD . . . . .	AAV 373
CAP96757.1 PruROX/154-636	154	NGGVWGP T I . . . . .	DLPVVA . . . . .	VSQAV I PETN . . . . .	EVLV . . . . .	WSSWAKDD . . . . .	LHSRGYT . . . . .	LTAVWMNMDSNVSTQRKVQETHHDMF . . . . .
EFQ27661.1 CgrAAO/B4-574	84	VKGKWG D L I . . . . .	RLPVI P . . . . .	VAAIYVPSYPEPSRLFFSSWSNDA . . . . .	FSQASGMT . . . . .	QFGDYDFATGAI . . . . .	SQRTVTNTTHHDMF . . . . .	PG I SQLEDGR . . . . .
EFQ30446.1 CgrAlcOx/2-482	2	NVGKWG PMV . . . . .	KFPVVP . . . . .	VAVALVPETG . . . . .	NLLVWSGWPNRW . . . . .	TAGNGKT . . . . .	YTSLYNVNTGNI . . . . .	ISDAI VQNTQHDMF . . . . .
ELA25906.1 CfrAlcOx/2-482	2	LGQWSP L I . . . . .	KFPVVP . . . . .	VSVALPESG . . . . .	NLLVWSGWPNRW . . . . .	TAGNGKT . . . . .	YTSLYNVQGTGVNSDAV . . . . .	QNTQHDMF . . . . .
<i>Tr. reesei</i> L_Ox/154-693	154	GVGNWTSRLTTFYFWTGPFFYSWDFPAAGTAAAGSYEQLIGGVCILPMTTQSITGKVTFLEKWTGTE . . . . .	PNSTGAYE . . . . .	LDLSLVNQYLAWREMHV . . . . .	KTDIF . . . . .	AGGVTLDPDKAGRLTVGGWS . . . . .	GDSITYGV . . . . .	279
XP_013949912 TrGLOX/103-621	103	HVG . . . . .	GTFPYSWSFPEAGTAAAGSYEQLIGGVCILPMTTQSITGKVTFLEKWTGTE . . . . .	PNSTGAYE . . . . .	LDLSLVNQYLAWREMHV . . . . .	KTDIF . . . . .	AGGVTLDPDKAGRLTVGGWS . . . . .	GDSITYGV . . . . .
CAD79488.1 JmeaGLOX/1-605	1	. . . . .	ASKAG . . . . .	SYEVVNTNS . . . . .	L . . . . .	ASAMMLGLMBEDNVF . . . . .	ILDKAENNS . . . . .	ARLADGRH . . . . .
AAAR7595.1 JPhuGLOX/1-537	1	. . . . .	APGWRFLDKPNLS . . . . .	. . . . .	G . . . . .	IVALEAIVNSSLV . . . . .	IFDRATGDD . . . . .	PLKINGES . . . . .
ANJ20632.1 JTrGLOX/1-538	1	. . . . .	APSAPQWRF . . . . .	DLKKAERS . . . . .	G . . . . .	IVALESIVVSP . . . . .	TLVLFVDRAS . . . . .	DDQLINNH . . . . .
QCC62349.1 JTrGLOX/31-538	1	. . . . .	APSAPQWRF . . . . .	DLKKAERS . . . . .	G . . . . .	IVALESIVVSP . . . . .	TLVLFVDRAS . . . . .	DDQLINNH . . . . .
ANJ20633.1 JTrGLOX/21-539	1	. . . . .	APSTPTGQWFLNKAERS . . . . .	. . . . .	G . . . . .	IVALESIVVSP . . . . .	TLVLFVDRAT . . . . .	NDLQINNH . . . . .
EFQ36699.1 CgrAAO/276-776	374	TIDYDF . . . . .	ANTWTRAA . . . . .	MNMGRG . . . . .	YQSSVTLSDGRGFTIGGS . . . . .	TGGIGGQNGTPMKNGEYVDP . . . . .	KLNKWTALPGALVAPML . . . . .	TTYD . . . . .
CAP96757.1 PruROX/154-636	247	SIDPA . . . . .	SGKWTEGNT . . . . .	MIIITRGV . . . . .	QASATIDAGRVFIIGGS . . . . .	NGGNT . . . . .	YDKDGEIYDP . . . . .	DTEKYSTLKNALVRPMW . . . . .
EFQ27661.1 CgrAAO/B4-574	180	SIYDPA . . . . .	THEFTRGPN . . . . .	MTLARG . . . . .	YQTSCTLSNGKVFITIGGA . . . . .	SGE . . . . .	R . . . . .	VGNKGEYVDP . . . . .
EFQ30446.1 CgrAlcOx/2-482	96	SVLDFKKGESSFWTFLSN . . . . .	. . . . .	MQISLRG . . . . .	QSSCTTSEGIKFIIVIGGS . . . . .	SGA . . . . .	G . . . . .	TRNGEYVDP . . . . .
ELA25906.1 CfrAlcOx/2-482	96	SVLDFKKGESSFWTFLSN . . . . .	. . . . .	MQISLRG . . . . .	QSSCTTSEGIKFIIVIGGS . . . . .	SGA . . . . .	G . . . . .	TRNGEYVDP . . . . .
<i>Tr. reesei</i> L_Ox/154-693	280	RLYTFD . . . . .	GSAGVNGTNDWGENV . . . . .	ILKLGNGRW . . . . .	PTAMMNAKNSVLVIGGS . . . . .	SGS . . . . .	NSAPVPTLE . . . . .	ILPFTGTAPLY . . . . .
XP_013949912 TrGLOX/103-621	216	RLYTFD . . . . .	GSAGVNGTNDWGENV . . . . .	ILKLGNGRW . . . . .	PTAMMNAKNSVLVIGGS . . . . .	SGS . . . . .	NSAPVPTLE . . . . .	ILPFTGTAPLY . . . . .
CAD79488.1 JmeaGLOX/1-605	115	RLLEPN . . . . .	SQTWIDSPST . . . . .	TVAAQVMLOQPRW . . . . .	PIEVLDEGSDVIFIGGA . . . . .	SGGYI . . . . .	NRNPTPTDPLQNGQANPT . . . . .	TYEYF . . . . .
AAAR7595.1 JPhuGLOX/1-537	105	RIFEP . . . . .	ASPSGDDCTLF . . . . .	EDPATVHLLERW . . . . .	PSSVIRIFDQSLMIIIGGS . . . . .	HYL . . . . .	NPDPANSFEFF . . . . .	PPSKEQTPRP . . . . .
ANJ20632.1 JTrGLOX/1-538	107	RIFEP . . . . .	ASPSGDDCTVF . . . . .	EDPOTHLLEDRW . . . . .	PSSVIRIFDQSLMIIIGGS . . . . .	HYL . . . . .	NPDPANSFEFF . . . . .	PPSKEQTPRP . . . . .
QCC62349.1 JTrGLOX/31-538	107	RLFEP . . . . .	ASPSGDDCTLF . . . . .	EDPATVHLLERW . . . . .	PSSVIRIFDQSLMIIIGGS . . . . .	HYL . . . . .	NPDPANSFEFF . . . . .	PPSKEQTPRP . . . . .
ANJ20633.1 JTrGLOX/21-539	108	RLFEP . . . . .	ASPSGDDCTLF . . . . .	EDPVTLLHLEKRW . . . . .	PSSVIRIFDQSLMIIIGGS . . . . .	HYL . . . . .	NPDPANSFEFF . . . . .	PPSKEQTPRP . . . . .
EFQ36699.1 CgrAAO/276-776	488	AQR . . . . .	. . . . .	NTQNDGM . . . . .	GVTVMYDS . . . . .	GKIFAGGAGS . . . . .	YSD . . . . .	BDKALYA . . . . .
CAP96757.1 PruROX/154-636	396	TELYDPKTNQ . . . . .	. . . . .	ANEQSPNSIVR . . . . .	YHSGISLLLPDGRVFN . . . . .	GGSGGL . . . . .	. . . . .	GVSAPTNHFD . . . . .
EFQ27661.1 CgrAAO/B4-574	297	AATR . . . . .	. . . . .	RVYPASAAATMAL . . . . .	PLTPQNYQTITILF . . . . .	GGSVMSQDMQWNGYS . . . . .	SGPGGN . . . . .	ILGLQASDDCS . . . . .
EFQ30446.1 CgrAlcOx/2-482	212	AGLR . . . . .	. . . . .	GTDEDSMG . . . . .	GVSMYMDA . . . . .	AVAGKIF . . . . .	TYGGGKG . . . . .	YV . . . . .
ELA25906.1 CfrAlcOx/2-482	212	AGLR . . . . .	. . . . .	GTDEDSMG . . . . .	GVSMYMDA . . . . .	AVAGKIF . . . . .	TYGGGKG . . . . .	YV . . . . .
<i>Tr. reesei</i> L_Ox/154-693	402	GVANRDLGGRTYPLGTA . . . . .	AVLLPMAHPT . . . . .	TEPLNLVIL . . . . .	GGSSGEGAS . . . . .	. . . . .	. . . . .	NAIDNCVSTY . . . . .
XP_013949912 TrGLOX/103-621	338	GVNDPLGGRTYPLGTA . . . . .	AVLLPMAHPT . . . . .	TEPLNLVIL . . . . .	GGSSGEGAS . . . . .	. . . . .	. . . . .	NAIDNCVSTY . . . . .
CAD79488.1 JmeaGLOX/1-605	260	GVVV . . . . .	. . . . .	RVYPASAAATMAL . . . . .	PLTPQNYQTITILF . . . . .	GGSVMSQDMQWNGYS . . . . .	SGPGGN . . . . .	ILGLQASDDCS . . . . .
AAAR7595.1 JPhuGLOX/1-537	229	NGVR . . . . .	. . . . .	VTNPI . . . . .	DGSAILLPLSP . . . . .	DFIPEVLV . . . . .	GGSTADTSLP . . . . .	ST . . . . .
ANJ20632.1 JTrGLOX/1-538	231	NVNR . . . . .	. . . . .	VTNPI . . . . .	DGSAILLPLSP . . . . .	DFIPEVLV . . . . .	GGSTADTSLP . . . . .	ST . . . . .
QCC62349.1 JTrGLOX/31-538	231	NVNR . . . . .	. . . . .	VTNPI . . . . .	DGSAILLPLSP . . . . .	DFIPEVLV . . . . .	GGSTADTSLP . . . . .	ST . . . . .
ANJ20633.1 JTrGLOX/21-539	232	NVNR . . . . .	. . . . .	VTNPI . . . . .	DGSAILLPLSP . . . . .	DFIPEVLV . . . . .	GGSTADTSLP . . . . .	ST . . . . .
EFQ36699.1 CgrAAO/276-776	595	QAEVYDPVANT . . . . .	. . . . .	FTPVAAALAVPR . . . . .	YHSGTGLLLPDGRVFM . . . . .	GGGGLCVYGG . . . . .	. . . . .	GC . . . . .
CAP96757.1 PruROX/154-636	469	TELYDPKTNQ . . . . .	. . . . .	ANEQSPNSIVR . . . . .	YHSGTGLLLPDGRVFM . . . . .	GGGGLCVYGG . . . . .	. . . . .	GC . . . . .
EFQ27661.1 CgrAAO/B4-574	395	VAELFNPE . . . . .	TE . . . . .	WKQMAPMAVPR . . . . .	YHSGTGLLLPDGRVFM . . . . .	GGGGLCVYGG . . . . .	. . . . .	GC . . . . .
EFQ30446.1 CgrAlcOx/2-482	311	TELYDPKTNQ . . . . .	. . . . .	ANEQSPNSIVR . . . . .	YHSGTGLLLPDGRVFM . . . . .	GGGGLCVYGG . . . . .	. . . . .	GC . . . . .
ELA25906.1 CfrAlcOx/2-482	311	TELYDPKTNQ . . . . .	. . . . .	ANEQSPNSIVR . . . . .	YHSGTGLLLPDGRVFM . . . . .	GGGGLCVYGG . . . . .	. . . . .	GC . . . . .
<i>Tr. reesei</i> L_Ox/154-693	507	NALLYDPAKPLGSRITV . . . . .	. . . . .	MANITV . . . . .	YHSEAIJLLDGRVLYSG . . . . .	SDPPDDVN . . . . .	. . . . .	PEEYRVET . . . . .
XP_013949912 TrGLOX/103-621	443	NALLYDPAKPLGSRITV . . . . .	. . . . .	MANITV . . . . .	YHSEAIJLLDGRVLYSG . . . . .	SDPPDDVN . . . . .	. . . . .	PEEYRVET . . . . .
CAD79488.1 JmeaGLOX/1-605	360	VPVIYDPSKPRQRLS . . . . .	ANANKPSTIAR . . . . .	YHSSAILLPDGSVMV . . . . .	AGSNPHQDVALDMPT . . . . .	TGTPPO . . . . .	AFNTIYEV . . . . .	KEVWYPPY . . . . .
AAAR7595.1 JPhuGLOX/1-537	349	TPSYLTPDAPL . . . . .	LGKRI . . . . .	SNAGMPTTTP . . . . .	YHSSVTLT . . . . .	TQQGNFF . . . . .	IGGNNPNMFT . . . . .	TP . . . . .
ANJ20632.1 JTrGLOX/1-538	350	VPSYLTPDAPL . . . . .	LGKRI . . . . .	SNAGMPTTTP . . . . .	YHSSVTLT . . . . .	TQQGNFF . . . . .	IGGNNPNMFT . . . . .	TP . . . . .
QCC62349.1 JTrGLOX/31-538	350	VPSYLTPDAPL . . . . .	LGKRI . . . . .	SNAGMPTTTP . . . . .	YHSSVTLT . . . . .	TQQGNFF . . . . .	IGGNNPNMFT . . . . .	TP . . . . .
ANJ20633.1 JTrGLOX/21-539	350	VPSYLTPDAPL . . . . .	LGKRI . . . . .	SNAGMPTTTP . . . . .	YHSSVTLT . . . . .	TQQGNFF . . . . .	IGGNNPNMFT . . . . .	TP . . . . .
EFQ36699.1 CgrAAO/276-776	723	QRRILPTVYSTN . . . . .	. . . . .	QNTVALSI . . . . .	. . . . .	PNDNGV . . . . .	VPVPPGF . . . . .	WYFVAVAPSGVHS . . . . .
CAP96757.1 PruROX/154-636	584	QRRILPTVYSTN . . . . .	. . . . .	QNTVALSI . . . . .	. . . . .	PNDNGV . . . . .	VPVPPGF . . . . .	WYFVAVAPSGVHS . . . . .
EFQ27661.1 CgrAAO/B4-574	523	QRRVPLNVTVS . . . . .	. . . . .	QNEYSATL . . . . .	. . . . .	PDDYGL . . . . .	ILLPGYYL . . . . .	LVFVSTPQGT . . . . .
EFQ30446.1 CgrAlcOx/2-482	429	QRRILPTVYSTN . . . . .	. . . . .	QNTVALSI . . . . .	. . . . .	PNDNGV . . . . .	VPVPPGF . . . . .	WYFVAVAPSGVHS . . . . .
ELA25906.1 CfrAlcOx/2-482	429	QRRILPTVYSTN . . . . .	. . . . .	QNTVALSI . . . . .	. . . . .	PNDNGV . . . . .	VPVPPGF . . . . .	WYFVAVAPSGVHS . . . . .
<i>Tr. reesei</i> L_Ox/154-693	624	ARTLFPALSGAGT . . . . .	. . . . .	TCITL . . . . .	. . . . .	SPPSKPI . . . . .	APPGVYGLY . . . . .	ILLDGGI . . . . .
XP_013949912 TrGLOX/103-621	560	ARTLFPALSGAGT . . . . .	. . . . .	TCITL . . . . .	. . . . .	SPPSKPI . . . . .	APPGVYGLY . . . . .	ILLDGGI . . . . .
CAD79488.1 JmeaGLOX/1-605	527	QRAYVLDY . . . . .	TYTVNDQASVTY . . . . .	VMNPLPNT . . . . .	KAMNRL . . . . .	FPVPAF . . . . .	YV . . . . .	VTG6GVP . . . . .
AAAR7595.1 JPhuGLOX/1-537	477	ARLVFMES . . . . .	ISAD . . . . .	RKSLTFTT . . . . .	. . . . .	PPNGRV . . . . .	VPVPPGATV . . . . .	FT . . . . .
ANJ20632.1 JTrGLOX/1-538	478	ARLVFMES . . . . .	ISAD . . . . .	RKSLTFTT . . . . .	. . . . .	PPNGRV . . . . .	VPVPPGATV . . . . .	FT . . . . .
QCC62349.1 JTrGLOX/31-538	478	ARLVFMES . . . . .	ISAD . . . . .	RKSLTFTT . . . . .	. . . . .	PPNGRV . . . . .	VPVPPGATV . . . . .	FT . . . . .
ANJ20633.1 JTrGLOX/21-539	479	ARLVFMES . . . . .	ISAD . . . . .	RKSLTFTT . . . . .	. . . . .	PPNGRV . . . . .	VPVPPGATV . . . . .	FT . . . . .

**Figure S3. Sequence alignment of 11 functionally characterized AA5 enzymes with *Tr*LOx.** EFQ36699.1: aryl alcohol oxidase from *Colletotrichum graminicola*; CAP96757: Raffinose oxidase from *Penicillium rubens*; EFQ27661: aryl alcohol oxidase from *C. graminicola*; EFQ30446: Alcohol oxidase from *C. graminicola*; ELA25906: Alcohol oxidase from *C. fruticola*; XP\_013949912: Glyoxal oxidase from *T. virens*; CAD79488: Glyoxal oxidase from *Ustilago maydis*; AAAR87595: Glyoxal oxidase from *Phanerochaete chrysosporium*; ANJ20632: Glyoxal oxidase 1 from *Trametes cinnabarina*; QCC62349: Glyoxal oxidase 3 from *T. cinnabarina*; ANJ20633: Glyoxal oxidase 1 from *T. cinnabarina*. Residues involved in copper binding are highlighted in red and other residues involved in enzymatic activity and substrate preference are highlighted in green.

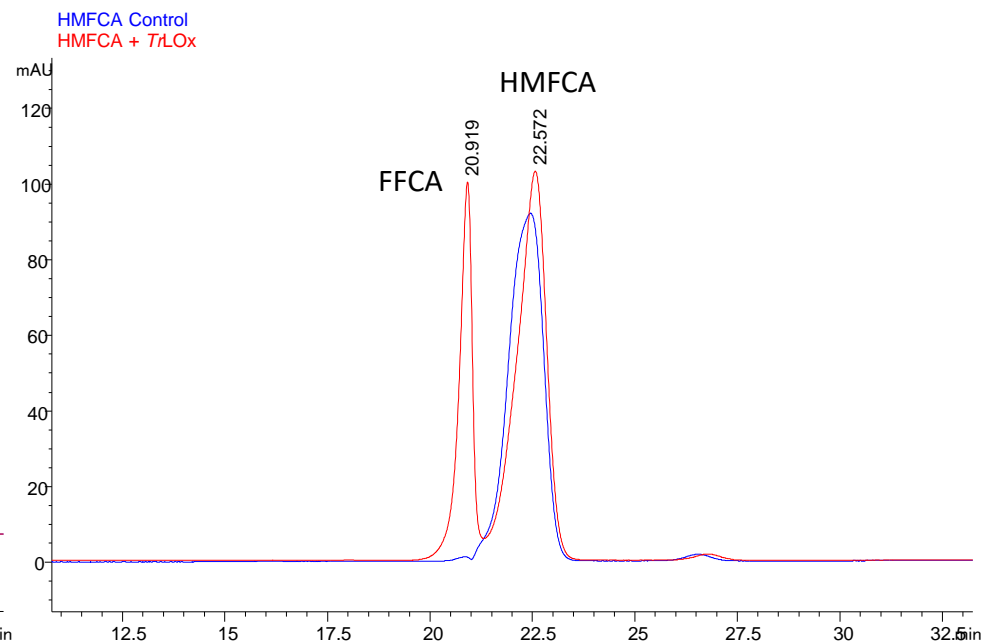


**Figure S4. Structural characteristics of *TrLOx*.** **A.** Predicted domain and functional sites in *TrLOx* encoding sequence. WSC domain: wall stress-responsive component; E\_set\_GO\_C: C-terminal Early set domain associated with the catalytic domain of galactose oxidase at the C-terminal end; DUF1929: Domain of unknown function. **B.** Predicted molecular structure of *TrLOx*. The model was generated using Phyre2 and with copper oxidase from *Colletotrichum graminicola* as template (PDB 6RYX). **C.** The active site of *TrLOx* containing the aromatic residues implicated in copper coordination. **D.** SDS-PAGE gel of purified *TrLOx* protein.

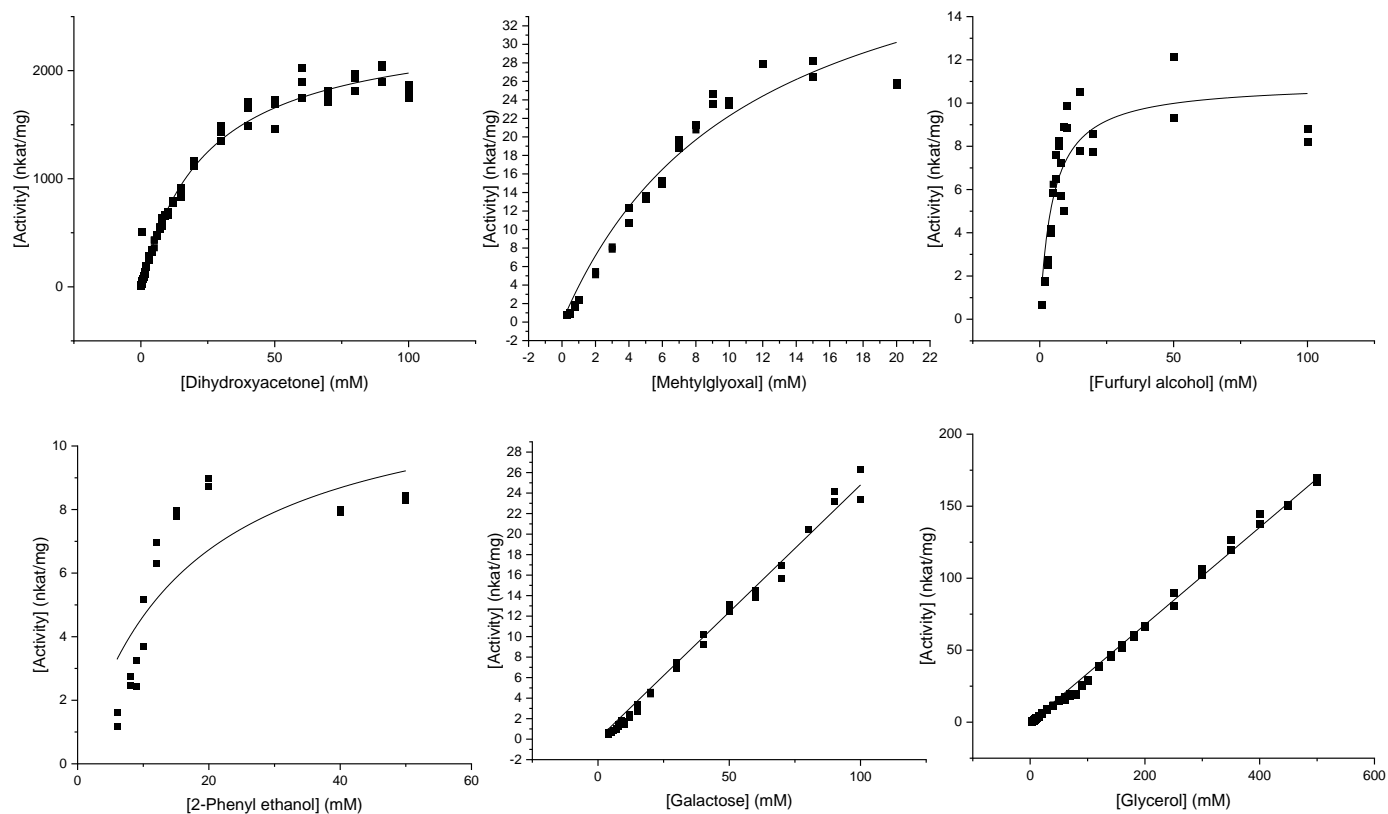
A.



B.

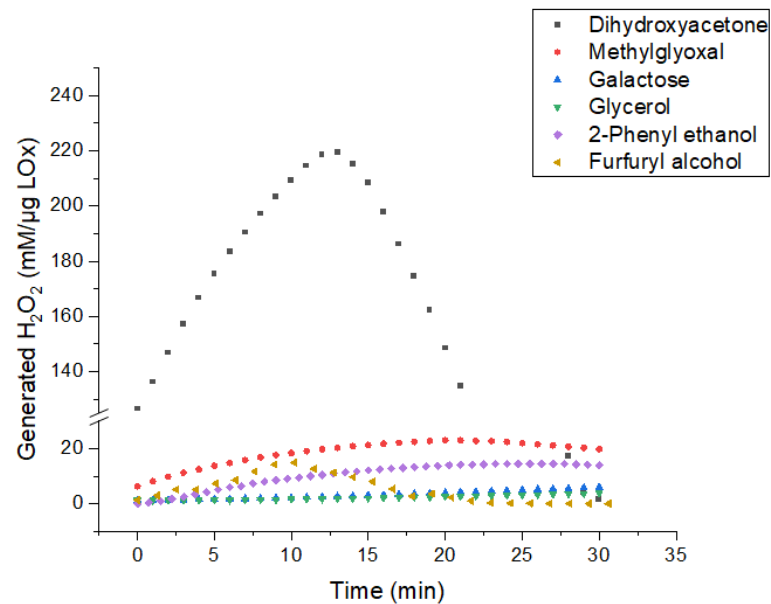


**Figure S5. HPLC analysis of *TrLOx* reactions on A. HMF and B. HMFCA.** The reactions were performed at 30 °C under agitation at 800 rpm over 24 h period in 50 mM tartrate buffer pH 6 and in the presence of 10 µg of enzyme and 5 mM of substrate. Reaction mixtures were separated on Aminex HPX87H column (300 × 7.8 mm) (BioRad) at 45 °C, with 2.5 mM sulfuric acid as the isocratic mobile phase with a flow rate of 0.6 mL/min. Eluted compounds were detected using a diode array detector at 280 nm. The reactions were stopped by incubating the mixture at 90 °C for 10 min and centrifuging at 15,000×g for 15 min. In blue are HMF and HMFCA standards and in red is the reaction with *TrLOx*.



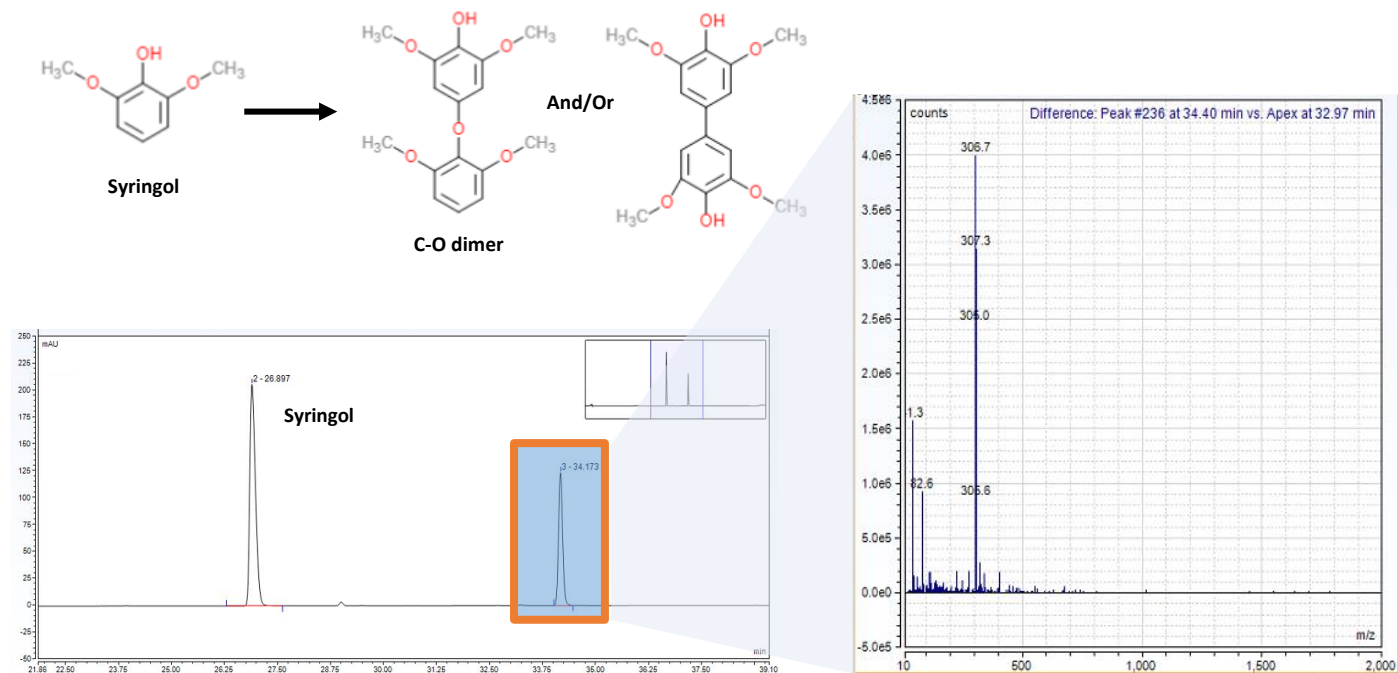
**Figure S6. Michaelis–Menten plots for the activity of *TrLOx* on the different tested substrates.**





**Figure S7. H<sub>2</sub>O<sub>2</sub> production overtime in the reaction of *TrLOx* on different substrates.** The fluorescence was followed at an excitation wavelength of 560 nm and an emission wavelength of 595 nm. The slope from the standard curve relating H<sub>2</sub>O<sub>2</sub> concentration and fluorescence was used to calculate the amount of generated H<sub>2</sub>O<sub>2</sub> over time (0-20 μM H<sub>2</sub>O<sub>2</sub>; 374.34 counts/μmol).

**A.**



**Figure S8. LC-MS chromatograms of the reaction of TrLOx on A. Syringol and B. Syringyl alcohol.** The molecular weight of the detected product with syringol (306.7) suggest the formation of C-C and/or C-O dimers.

**B.**

