

A novel starch binding laccase from the wheat pathogen *Zymoseptoria tritici* highlights the functional diversity of ascomycete laccases

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Table S1 List of *Zymoseptoria tritici* (ZtrLac1A) and 6 other homologues with bimodular organizations

	Locus number	Length	Identity (Catalytic domain)	CBM	Linker length
<i>Zymoseptoria tritici</i> , ZtrLac1A	XP003852363	723	100%	110	12
<i>Zymoseptoria brevis</i> , ZbrLac1A	KJX94131	723	99%	110	12
<i>Sphaerulina musiva</i> , SmuLac1A	XP_016758816	742	69%	108	32
<i>Pseudocercospora musae</i> , PmuLac1A	KXT09849	746	68%	107	32
<i>Mycosphaerella eumusae</i> , MeuLac1A	KXS98121	719	69%	107	17
<i>Pseudocercospora fijiensis</i> , PfiLac1A	XP_007930185	587	68%	--	--
<i>Dothistroma septosporum</i> , DseLac1A	EME38505	729	68%	107	12
<i>Daldinia eschscholtzii</i> , DesLac1A	AEX97872	418	60%	--	--
<i>Baudoinia panamericana</i> , BpaLac1A	XP_007679364	703	62%	108	17

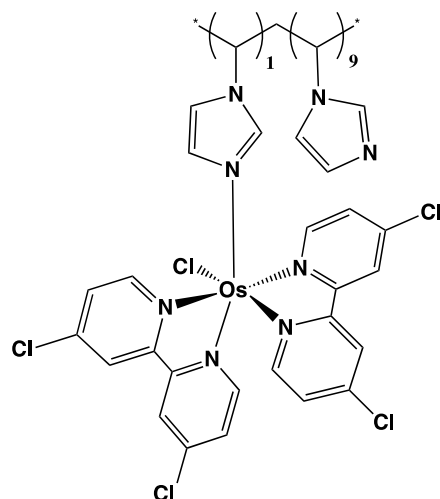


Fig. S1 Chemical structure of the osmium polymer [Os(4,4'-dichloro-2,2'-bipyridine)₂(poly vinylimidazole)₁₀Cl]·Cl with formal potentials $E^{\circ} = 0.549$ V vs. SHE

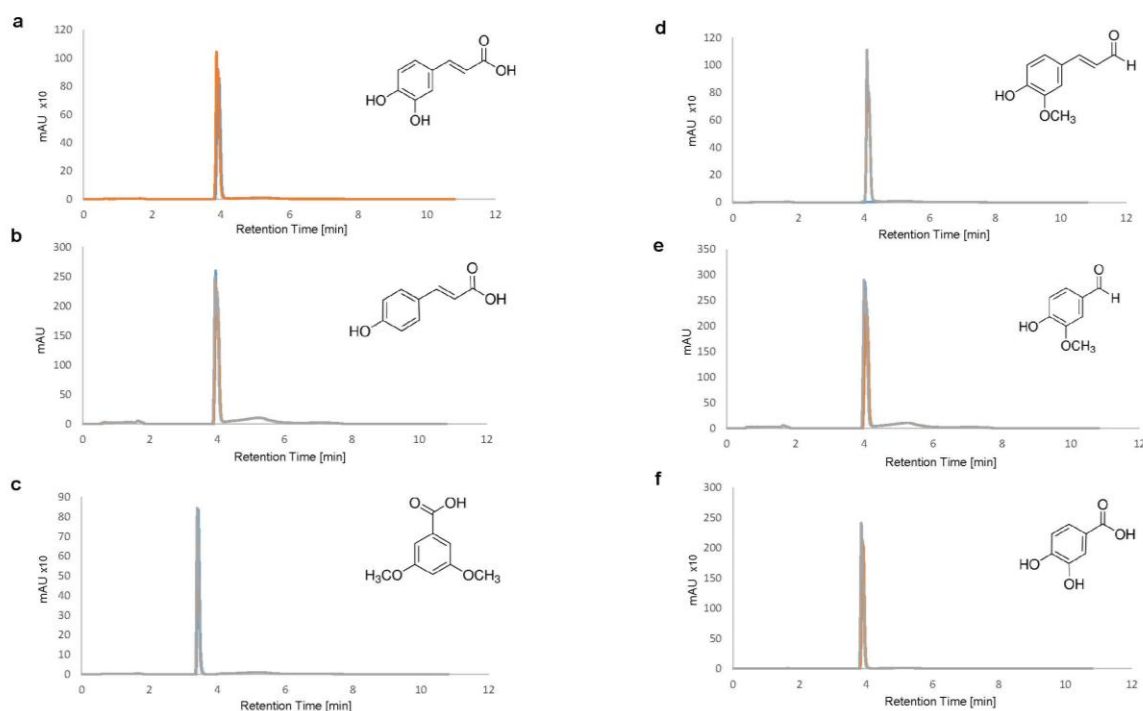
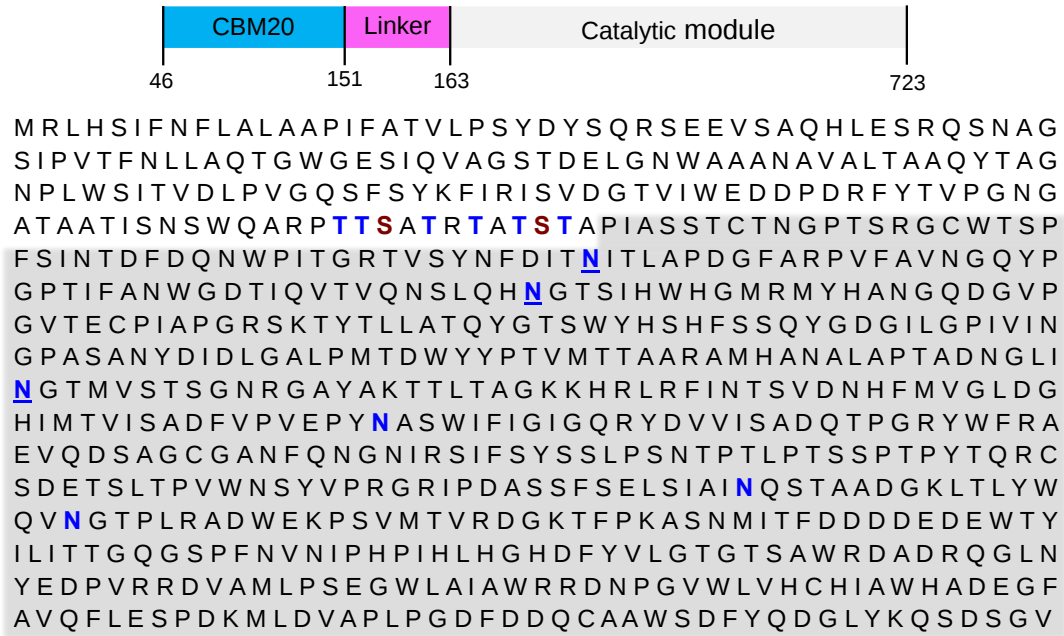


Fig. S2 HPLC ultimate profiles of different aromatic substrates assayed for *ZtrLac1A* activity screening. The substrates are: (a) caffeic acid, (b) *p*-coumaric acid, (c) 3,5 dimethoxybenzoic acid, (d) coniferyl aldehyde, (e) vanillin and (f) 3,4 dihydroxybenzoic acid. All reactions were conducted in 0.5 ml for 45 and 90 min depicted in orange and blue respectively and controls are shown in grey. The reaction mixtures were analyzed by high performance liquid chromatography (HPLC3000), using reversed phase column C-18 (Kinetex LC column 100 mm x 4.6 mm, particle size 2.6 μ m). The elution was performed using a gradient of solvent A: water/TFA (100:0.01; v/v), solvent B: acetonitrile/TFA (100:0.01; v/v) and detection was conducted at 285 and 322 nm. The

steps of gradient were linear from 0-4% of B for 5 min, 4-30% of B for 2 min and 30-100% of B for 2 min eluted at flowrate of 1.0 ml/min at room temperature

a



b

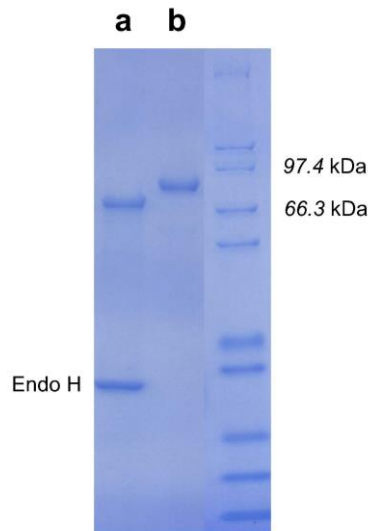


Fig. S3 (a) Laccase catalytic module, carbohydrate binding module and linker are colored in grey, blue and pink, respectively. Asparagine residues predicted to be *N*-glycosylated are colored in blue and those with more than 0.6 potential indicated with underline. **(b)** Purified *ZtrLac1A* analyzed using SDS-PAGE after EndoH treatment A and before EndoH treatment B. The Mark 12 protein standard (Invitrogen) is in right lane

b

Loop B	
ZtrLacIa	429
CDD50266	426
APAI4269	426
ALR81978	425
AGZ90172	430
CCT61140	419
AHZ58328	420
AHZ58333	419
AHZ58327	419
AHZ58330	427
MallLacIa	422
AEO58496	418
ADA41449	422
AEO68473	418
TarLacIa	421
CAA70061	422
EAP61995	422
EAA27703	417
AAA33105	421
XP003711640	423
CDP29442	433
EAA27863	436
BAB32575	424
AFK76451	426
XP003715087	432
CDP31732	448
ABY89704	419
AHN82367	429
BacLacIa	421
AAK77953	422
ABM21605	421
ABM21603	421
ABM21604	422
APAO7533	422
CAM12502	426
AGZ62514	421
AAK77952	407
EAA30359	425
XP003718807	437
CAP64719	438
EAA61461	421
EAA62557	426
CAP98129	420
BAE54583	424
CCT67757	419

Loop B

ABS19940	419
CEF76058	419
AFG30950	413
XP003712139	422
AEO64417	420
APA07387	418
AQA29307	418
EAQ71537	423
CAK48347	419
CCT64283	419
CEF85471	419
AHZ65139	419
CAK44895	412
AA999671	419
CAK40046	425
ACR16059	417
CAP65571	424
BAA08486	449
AGG59042	417
CCT66683	456
CEF71993	461
CAK46289	459
CCT71494	461
AH265141	460
CCT72796	460
ABS19939	460
SCB65047	461
AEO64812	454
EAA29734	500
CAP64870	484
AHC30245	444
CCT64574	441
ABS19938	441
CEF84713	441
AGT80114	441
AHD26939	441
ANH22780	441
ACS45199	441
EAA34842	419
CAP67656	444
AEO54598	444
AEO65600	444
APA07098	447
XP003720855	446
APA14467	454
AGG59052	404

c

Loop C	
ZtrLacIa	378
CDD50266	372
APAI4269	372
ALR81978	371
AGZ90172	380
CCT61140	368
AHZ58328	369
AHZ58333	368
AHZ58327	368
AHZ58330	379
MallLacIa	371
AEO58496	367
ADA41449	371
AEO68473	367
TarLacIa	370
CAA70061	371
EAP61995	371
EAA27703	366
AAA33105	370
XP003711640	374
CDP29442	381
EAA27863	385
BAB32575	373
AFK76451	375
XP003715087	381
CDP31732	397
ABY89704	369
AHN82367	376
BacLacIa	367
AAK77953	368
ABM21605	367
ABM21603	367
ABM21604	367
APAO7533	367
CAM12502	372
AGZ62514	369
AAK77952	353
EAA30359	371
XP003718807	380
CAP64719	383
EAA61461	370
EAA62557	377
CAP98129	369
BAE54583	375
CCT67757	370

Loop C

ABS19940	370
CEF76058	370
AFG30950	364
XP003712139	372
AEO64417	371
APA07387	369
AQA29307	369
EAQ71537	374
CAK48347	370
CCT64283	371
CEF85471	371
AHZ65139	371
CAK44895	363
AA999671	370
CAK40046	374
ACR16059	367
CAP65571	371
BAA08486	390
AGG59042	358
CCT66683	385
CEF71993	392
CAK46289	395
CCT71494	405
AH265141	404
CCT72796	402
ABS19939	402
SCB65047	403
AEO64812	405
EAA29734	427
CAP64870	413
AHC30245	419
ABS19938	391
CEF84713	391
AGT80114	391
AHD26939	391
ANH22780	391
ACS45199	391
EAA34842	369
CAP67656	394
AEO54598	394
AEO65600	394
APA07098	397
XP003720855	396
APA14467	402
AGG59052	352

d

Cu1 site		Cu1 site	
ZtrLac1A	498	ABSI19940_1	481
CCD50266_1	496	CEP76058_1	481
APA14269_1	496	AFG30950_1	476
ALR81978_1	495	XP003712139_1	483
AG290172_1	493	ABO64417_1	483
CCT61140_1	484	APA07387_1	480
AH258328_1	481	AQA29307_1	480
AH258333_1	480	EAQ71537_1	480
AH258327_1	480	CAK48347_1	481
AH258330_1	491	CCT62493_1	481
MalLac1A	498	CEP85471_1	481
AEO58496_1	494	AH265139_1	481
ADA41449_1	498	CAK44895_1	476
AEO68473_1	494	AA99671_1	482
TarLac1A	498	CAK40046_1	495
CAP70061_1	499	ACR16059_1	482
CAP61995_1	499	CAP65571_1	487
EAA27703_1	493	BAA08486_1	515
AAA33105_1	497	AG059042_1	487
XP003711640_1	499	CCT76683_1	524
CDP29442_1	508	CEP71993_1	529
EAA27863_1	512	CAK46289_1	537
BAB32575_1	512	CCT71494_1	531
AFK76451_1	502	AH265141_1	528
XP003715087_1	496	CCT72796_1	529
CDP31732_1	511	ABSI19939_1	529
ABY89704_1	498	SCB65047_1	530
AHN82367_1	495	CDP31240_1	570
BacLac1A	486	AE064812_1	519
AAK77953_1	487	EAA29734_1	570
ABM21605_1	486	CAP64870_1	552
ABM21603_1	486	AHC30245_1	505
ABM21604_1	487	CCT64574_1	501
APA07533_1	487	ABSI19938_1	501
CAM12502_1	489	CEP84713_1	501
AG262514_1	484	AGT80114_1	501
AAK77952_1	467	AH26939_1	501
EAA30359_2	490	AH22780_1	498
XP003718807_1	500	ACS45199_1	502
CAP64719_1	502	EAA34842_1	479
EAA61461_1	502	CAP67656_1	502
EAA62557_1	486	AE054598_1	502
CAP98129_1	480	AE065600_1	502
BAE54583_1	534	APA7098_1	507
CCT67757_1	481	XP003720855_1	502
		AFAL4467_1	514
		AG059052_1	463

e

C-terminus		C-terminus	
ZtrLac1A	546	ABSI19940_1	529
CCD50266_1	545	CEP76058_1	529
APA14269_1	545	AFG30950_1	524
ALR81978_1	544	XP003712139_1	533
AG290172_1	542	ABO64417_1	531
CCT61140_1	542	APA07387_1	527
AH258328_1	530	AQA29307_1	528
AH258333_1	529	EAQ71537_1	537
AH258327_1	530	CAK48347_1	529
AH258330_1	540	CCT62493_1	532
MalLac1A	549	CEP85471_1	532
AEO58496_1	545	AH265139_1	532
ADA41449_1	549	CAK44895_1	522
AEO68473_1	549	AA99671_1	530
TarLac1A	553	CAK40046_1	543
CAP70061_1	550	ACR16059_1	532
CAP61995_1	550	CEP65571_1	537
EAA27703_1	544	BAA08486_1	567
AAA33105_1	548	AG059042_1	539
XP003711640_1	541	CCT76683_1	576
CDP29442_1	556	CEP71993_1	581
EAA27863_1	563	CAK46289_1	594
BAB32575_1	546	CCT71494_1	582
AFK76451_1	550	AH265141_1	582
XP003715087_1	544	CCT72796_1	582
CDP31732_1	559	ABSI19939_1	564
ABY89704_1	536	SCB65047_1	581
AHN82367_1	543	CAK33200_1	615
BacLac1A	539	CDP31240_1	622
AAK77953_1	536	AE064812_1	570
ABM21605_1	535	EAA29734_1	623
ABM21603_1	535	CAP64870_1	552
ABM21604_1	536	AHC30245_1	553
APA07533_1	536	CCT64574_1	549
CAM12502_1	538	ABSI19938_1	549
AG262514_1	533	CEP84713_1	549
AAK77952_1	518	AGT80114_1	549
EAA30359_2	541	AH26939_1	549
XP003718807_1	549	AH22780_1	536
CAP64719_1	553	ACS45199_1	550
EAA61461_1	533	CAP67656_1	550
EAA62557_1	534	AE054598_1	550
CAP98129_1	528	AE065600_1	550
BAE54583_1	534	APA7098_1	556
CCT67757_1	539	XP003720855_1	554
		AFAL4467_1	551
		AG059052_1	511

Fig. S5 Sequence alignment of *ZtrLac1A* (catalytic module) along representative members of AA1 subfamily 3 deposited in CAZy database. All three loops A, B, C, Cu1 site ligands as well as the C-terminus regions are depicted in boxes. Fully conserved residues are in red letters, semi-conserved in blue and non-conserved in black. Accession numbers are shown except *ZtrLac1A*, *MalLac1A*, *TarLac1A* and *BacLac1A*

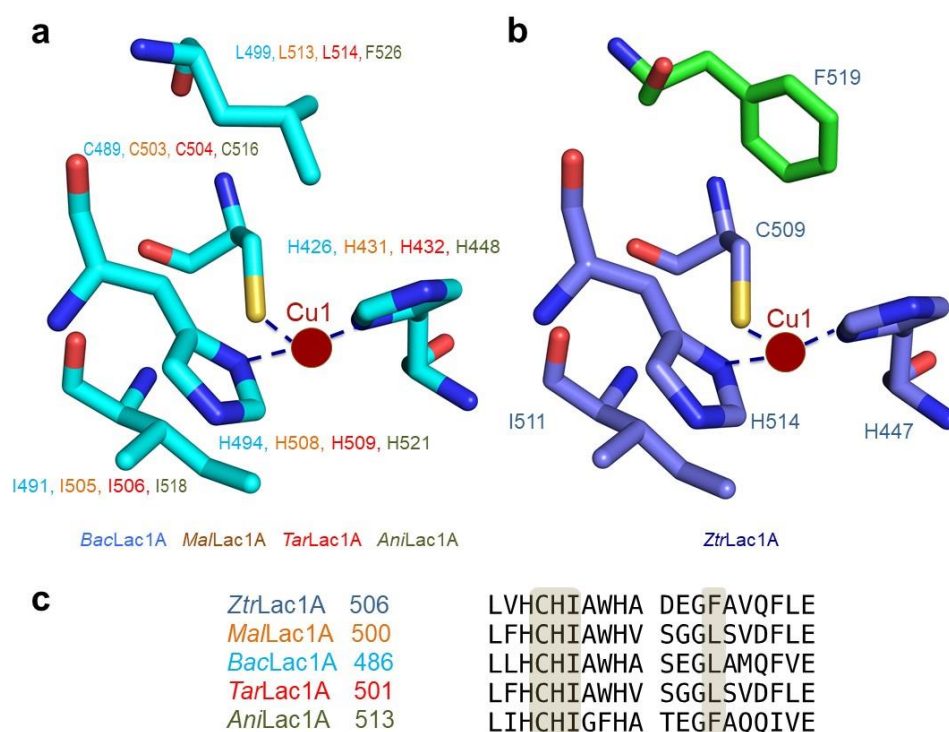


Fig. S6 Architecture of Cu1 copper site in *BacLac1A*, *MalLac1A*, *TarLac1A* and *AniLac1A* (**a**) and *ZtrLac1A* (**b**). The Cu1 copper ions are depicted as brown spheres and their coordination bonds are shown in black dashed lines. The structure of *BacLac1A* PDB entry 3SQR was used [41]. (**c**) Structural sequence alignment of *ZtrLac1A* with *MalLac1A*, *TarLac1A*, *BacLac1A* and *AniLac1A*

References

- Osipov E, Polyakov K, Kittl R, Shleev S, Dorovatovsky P, Tikhonova T, Hann S, Ludwig R, Popov V. Effect of the L499M mutation of the ascomycetous *Botrytis aclada* laccase on redox potential and catalytic properties. *Acta Crystallogr D Biol Crystallogr.* 2014;70:2913–2923.