

Figure 1. Structural homology model of PsalPOX. The model was generated with the SWISS-MODEL server using the X-ray crystal structure of *Pleos-DyP4* (PDB-ID 6fsk).

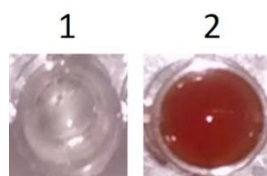


Figure 2. Detection of hydrogen peroxide in the IEX fraction with *o*-dianisidine and HRP in the presence of *trans*-anethole after 1 h of incubation at pH 6.0 and RT. 1: blank (buffer instead of IEX fraction); 2: IEX fraction. *o*-Dianisidine was oxidized to a red-brown colorant by HRP in the presence of H₂O₂.

KDQ29984.1	mlhqsyypyltshlawlptslasafvywrgidplifprsrlnrhnlragrrmihtdnrvvgqgdwr	65
KDQ29972.1	-----mhlflr---lliacv	12
ESK85927.1	-----mfsiyp	6
KLO09144.1	-----mk-ail---s	6
THU81532.1	-----mt---vk-flvaacv	11
KDQ29984.1	--glrssiatssstydyviigggtaglavanrlsedplvsiaievieagnaeahlrevfipglf---g	125
KDQ29972.1	--glrssiatssaydyviigggtaglavasrlsedpsvsivvieagnaeahlepevfipglf---g	72
ESK85927.1	avivilslhsafsdydvviigggtagltvasrlaeddsqvavieaganaehrpdvfipgmkgqig	71
KLO09144.1	yflvlqlalrsfavfdyvivvggtagltvasrlsedpsvtvavieagnaeahnlepevfvpqli---	68
THU81532.1	aalapvanvlassydyiiigggtgltvasrlaedksnqvlvlaegnpaeglqevfvpgli---g *: :*: *: *:*:*:*:* *: :*: * *: :*: :	73
KDQ29984.1	sgqaalttlwnaykttppmlnnrslvnagr vlggsttinamlfpragraqydawaelnr dawg	190
KDQ29972.1	sggaftttlwnaynttpqmllnnsrlvnagr vlggsttinamlfpragraqdawaelnr dawg	137
ESK85927.1	vgtkppelnwayktvpqthlngrvltvnagralggstvinamifprakkeqydawgilnndtswt	136
KLO09144.1	tqgsfttlwnayrtvpqvnlnsnrltnvagkalggstiinsimifpraekedydwgalnndsswt	133
THU81532.1	tqgsfttlwnayrtapgenlnnrtltnvagkalggstiinsimifpraekaagydwagtlnndsswt * *****.* ** *.***.*** *: :*: * *: :*: *	138
KDQ29984.1	wdallpfrrservtppdafqnagsvtyvypevhgsa-sdgrkvkgypnfiffegstlwraqaavav	254
KDQ29972.1	wdallpfrrservtppdafqneggityvevhgsa-sdgrkvkgypnfiffegstlwraqaavav	201
ESK85927.1	weallpfkrsesftvpndfqasn-garydpqghfgd---gslkvgfpnfyfpqemlwqntsigt	197
KLO09144.1	wdallpfknseiftppnafqsas-grvrdpsvhgfs---grvhvgfnpfffeqsqlwktsesgl	194
THU81532.1	wdallpfkkseiftppnafqrnd-gatfrvdvhgfnesegvrkvgnfnffpqstlwrtzsmj *: :*: *.** . * *: * . * *: :*: * * . * :: :	202
KDQ29984.1	gfpgerdlasgdpqhgvsvpesidvtnytrcsaacayftpfasrpfnvitnatvtrrivwdtpsp	319
KDQ29972.1	gfpgerdlasgdpqhgvsvpesidvtnytrcsaacayftpfasrpfnvitnatvtrvvwdtpss	266
ESK85927.1	gfpaatlslngephavgppsfidaingtrcsacaftytfadpnpftitnanvtriwseen	262
KLO09144.1	gfpaaspdlangphavgvapdslnaantrcsaacayftpfaerpntfvlnatvstriiwsnst	259
THU81532.1	gfpaaspdlangsphavgvapdsldaanntrcsaacyftpfaeqnpftvlnatvtriwtasaq ***. *: :*.**.*** : * :*: . * *****.:****.:***.: :*.**: **:*	267
KDQ29984.1	gshsnegqrnlraiaavefvragrnsr--tesvtvrrevvvsagtigtprvfelsgignssiiraagv	383
KDQ29972.1	gshsdksqrnlraiaavefvragrnsr--tesvtvrrevvvsagtigtpkelfelsgignssiiraagv	330
ESK85927.1	n-----sslatagevyytnnetm---tlevtgevilsgigtisgskilelsignstilaaagv	317
KLO09144.1	an-----agstkaiaveyasngtllga---sverevivsagigtispkvlslsgvgnatiltaagv	317
THU81532.1	nd-----stltasgevuidslnqtscrailsrkgevivsagigtisgskilelsgvgnstilqaagi . * .*: : *: :*: :*: :*: :*: :*: :* : *	326
KDQ29984.1	npvlelpvsgenlag-----lltsfpgslgl	409
KDQ29972.1	npvlelpvsgenlagef-----clltsfpgslgl	358
ESK85927.1	epllldiptvgenla-----gmayaavsktligi	342
KLO09144.1	qpqvnlstvgenladhvhnwvnsftsfnltkdl-lnnatflaeqlwlwnktrtgllsaaprslgi	381
THU81532.1	spaldiptvgenladhvwswanafnisltkdalllntdfaqqglidlwfknrtglysaaprslgi . * : * : * : **** : : : *: :	391
KDQ29984.1	pgplnifndttrilptllqeakanlhfhalfangnallegkiragehiveirlyaddvalpens-	473
KDQ29972.1	pgplnifndtgitptllqeakanlhfhalfangnallegkiragehiveirlyaddvalpensl	424
ESK85927.1	tapldvvsqsl-ldtlltqaesnlsnyasqfsgnnpdlargleaqhrlafslsqenqqlcveln-	406
KLO09144.1	aapsnvfnqsg-ltalvtqsrantlataisfsgnadlakgiaaqldhalslyaedkelplemn-	444
THU81532.1	aapsndvfeese-fnrllinqeeslsrfasefsgnnpklakgiesqyralslrqnelplemn- * : :*: : : : ln:nq. * * : *** * * : * * : * * : *	454
KDQ29984.1	----lfpgyaga-aadrpqrnyttimnvlyaplrsgrthitssdirtapvvdpayymhplda	530
KDQ29972.1	caallsyfpyyaga-aadrpqrnyttimnvlyaplrsgrthitssdirtapvvdpayymhplda	487
ESK85927.1	----ldagyvgptplesrplrnfittvstilyaplarsgrhisssdpftvplvdpaywahpmdv	465
KLO09144.1	----lepysgptplavrpnrtfftintvlyaplrsgrthitsspsvppadvpnpyyahpmdi	503
THU81532.1	----lepysgptaadfardpynrtainslvlyplsgrthitsspslapvvdpaywahpldv * * * * .*: : : *: :*: :*: :*: :* . * * * * : *: *	513
KDQ29984.1	athaaginlaraktlitppmdsiylgefepgkdvtspqdissvlraaivssdnhvtgmtammpek	595
KDQ29972.1	athaaginlarartlitppmdsiylgefepgkdvtspqdissvlraaivssdnhvtgmtammqel	552
ESK85927.1	aiahvgigialakmlrsppldsiydefefegve--sdkeinwlrgr-atstdnhvtgtlsmmprel	527
KLO09144.1	aahiggirlarmltspplgdsflgefepgaekevdaqigswlrn-atsdnhetgtlaamprel	567
THU81532.1	aihqvagiklarkmlqssplsstyegefegtcketdaeiewlrgv-vasdnhevgsilaamlpkdm * : .*: .*: : * : :*: : * : * . . : **** . * : :*: : :	577
KDQ29984.1	ggvvdtelrvygignvr vadasiiipvpsha ststvymigeraadlikhrspl-----	649
KDQ29972.1	ggvvdtelrvygieenvr vadasiiipvpsha tvplrillithladlqpvdchldwraresilwv	622
ESK85927.1	ggvvdteqlkyvigtmnrvvdasvipfpvashtaeststvymigeraadiirksgn-----	580
KLO09144.1	ggvvdtnlkvygtanrvrvdasiiipfisahasistvyaigekevrgsd-----	614
THU81532.1	ggvvdteillygtsnrvrvdasvipfisaahlsssvymigeraadiikrn----- *****.* ** ****.**: :*: :* : : : : :	627
KDQ29984.1	-----	649
KDQ29972.1	lllaafltrvlrlqissisivdihcnhl	644
ESK85927.1	-----	580
KLO09144.1	-----	614
THU81532.1	-----	627

Figure 3. Alignment of the hypothetical protein (KDQ29984.1) from *P. ostreatus*, which was the best hit for the 75 kDa band of the IEX fraction by a homology search against the public database NCBI, and other members of the GMC oxidoreductase family. KDQ29972.1: hypothetical protein from *P. ostreatus* (92% identity to KDQ29984.1); ESK85927.1: glucose oxidase from *Moniliophthora roreri* (57% identity to KDQ29984.1); KLO09144.1: alcohol oxidase from *Schizopora paradoxa* (56% identity to KDQ29984.1); THU81532.1: alcohol oxidase from *Dendrothele bispora* (55% identity to KDQ29984.1). Asterisks indicate conserved residues, colons equivalent residues and dots partial residue conservation. Peptides identified by protein sequencing are underlined. Alignment was performed with Clustal Omega (European Bioinformatics Institute, Hinxton, UK).

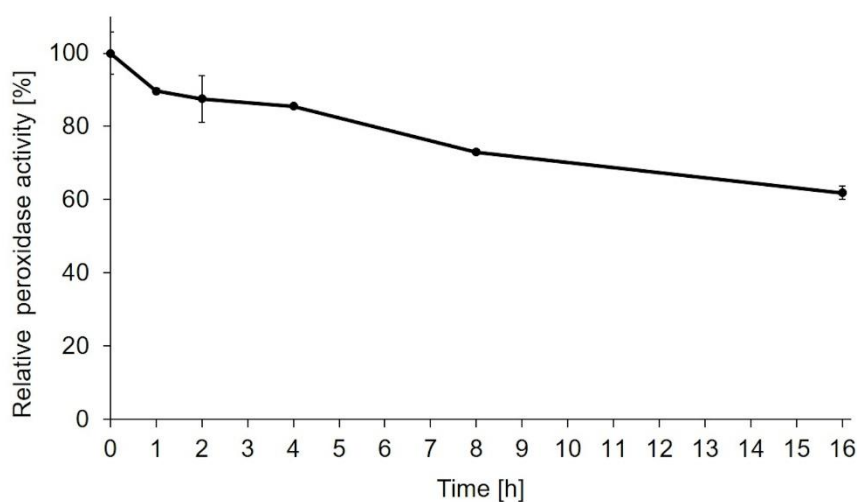


Figure 4. Stability of PsaPOX during biotransformation of *trans*-anethole over 16 h. The reaction was performed in the presence of 100 μ M H_2O_2 and 25 mM $MnSO_4$ in 100 mM sodium acetate buffer pH 3.5 at RT. Relative peroxidase activity [%] was defined as the percentage of activity detected with respect to the starting activity. Values are the average of triplicate experiments with standard deviations shown as error bars.

Table 1. *p*-Anisaldehyde concentration after biotransformation of *trans*-anethole with different basidiomycetes.

strain	<i>p</i> -anisaldehyde concentration [mM]	origin
<i>Pleurotus sapidus</i>	5.36	DSMZ, strain no. 2866
<i>Pleurotus eryngii</i>	4.07	CBS, strain no. 613.91
<i>Pleurotus ostreatus</i>	3.74	DSMZ, strain no. 1020
<i>Pleurotus ostreatus</i> var. <i>florida</i>	2.00	Laboratory collection
<i>Pleurotus sajor-caju</i>	1.14	DSMZ, strain no. 5339
<i>Pleurotus pulmonarius</i>	1.04	DSMZ, strain no. 5331
<i>Trametes versicolor</i> *	0.96	Laboratory collection
<i>Trametes suaveolens</i>	0.62	DSMZ, strain no. 5237
<i>Pleurotus lampas</i>	0.79	CBS, strain no. 323.49
<i>Lentinus lepideus</i>	0.29	CBS, strain no. 450.79
<i>Wolfiporia cocos</i>	0.18	CBS, strain no. 279.55
<i>Gloeophyllum odoratum</i>	0.18	CBS, strain no. 444.61
<i>Piptoporus soleniensis</i>	0.17	CBS, strain no. 492.76
<i>Schizophyllum commune</i>	0.10	DSMZ, strain no. 1024
<i>Gloeophyllum trabeum</i>	0.00	DSMZ, strain no. 3087
<i>Phanerochaete chrysosporium</i>	0.00	DSMZ, strain no. 1547

* Two variants of the strain were tested for the bioconversion. The results for the strain with higher *p*-anisaldehyde concentrations are presented.

DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, CBS: Westerdijk Fungal Biodiversity Institute

Table 2. *p*-Anisaldehyde concentration after biotransformation of *trans*-anethole with the active IEX fraction in the presence or absence of Mn²⁺ and/or H₂O₂ for 16 h at RT. The *p*-anisaldehyde concentrations are the average of duplicate experiments with indication of standard deviations.

MnSO ₄ concentration [mM]	H ₂ O ₂ concentration [μM]	<i>p</i> -anisaldehyde concentration [μM]
0	0	30 ± 1
1	0	450 ± 17
1	50	600 ± 12

Table 3. *p*-Anisaldehyde concentration after bioconversion of *trans*-anethole by recombinant PsaPOX with and without addition of H₂O₂ and Mn²⁺ for 16 h at RT. The *p*-anisaldehyde concentrations are the average of duplicate experiments with indication of standard deviations.

MnSO ₄ concentration [mM]	H ₂ O ₂ concentration [μM]	<i>p</i> -anisaldehyde concentration [μM]
0	0	0 ± 0
0	100	189 ± 6
25	100	320 ± 20

Table 4. *p*-Anisaldehyde concentration after biotransformation of *trans*-anethole, (*E*)-methyl isoeugenol, and α -methylstyrene by recombinant PsaPOX (1 U/mL) in the presence of 100 μM H₂O₂ and 25 mM MnSO₄ for 16 h at pH 3.5 and RT. The values are the average of duplicate experiments with indication of standard deviations.

Substrate	Product	Product concentration active enzyme [μM]	Product concentration inactivated enzyme (control) [μM]
<i>trans</i> -anethole	<i>p</i> -anisaldehyde	342 ± 20	22 ± 7
(<i>E</i>)-methyl isoeugenol	veratraldehyde	114 ± 12	48 ± 9
α -methylstyrene	acetophenone	24 ± 7	0 ± 0