

Purification and characterisation of a DyP-type peroxidase produced by the native strain, *Streptomyces albidoflavus* BSII#1, and its application in coupling of phenolic monomers

Supplementary material

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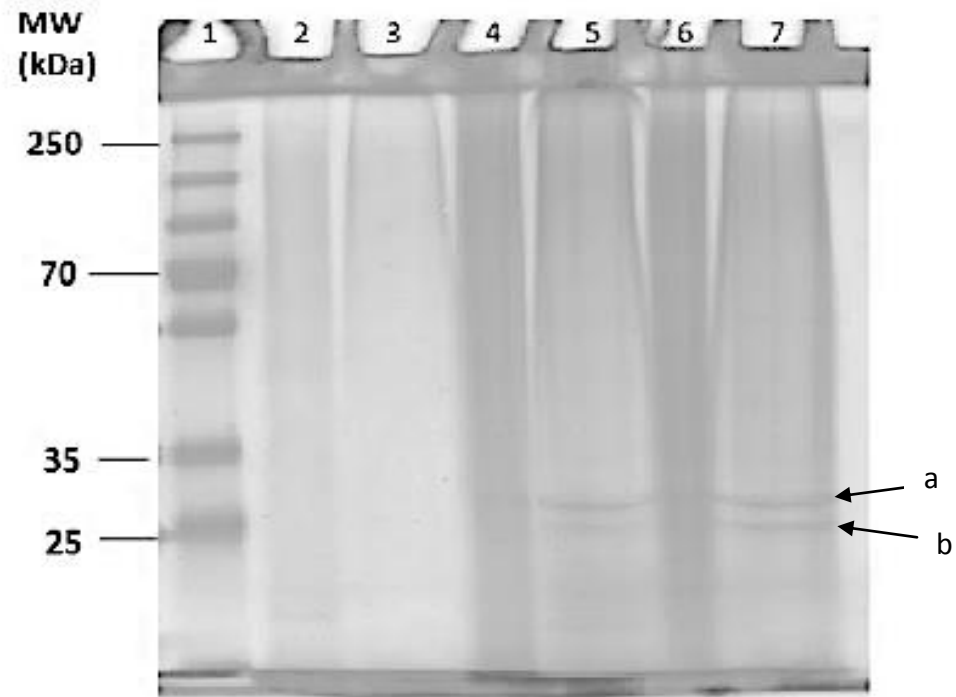


Figure S1: SDS-PAGE gel with samples from each purification step for the peroxidase from *Streptomyces albidoflavus* BSII#1. Lane 1, protein size marker (10 to 250 kDa). Lanes 2 and 3, crude enzyme boiled in DTT-containing and β -mercaptoethanol-containing loading buffer, respectively. Lanes 4 and 5, acid fraction boiled in DTT-containing loading buffer and β -mercaptoethanol-containing loading buffer, respectively. Lanes 6 and 7, the acetone fraction boiled in DTT-containing and β -mercaptoethanol-containing loading buffer, respectively. The arrows point to the most prominent protein bands with sizes estimated at (a) 26.6 kDa and (b) 24.4 kDa.

Table S1: Comparison of the kinetic constants of the peroxidase from *Streptomyces albidoflavus* BSII#1 with other peroxidases for the oxidation of 2,4-dichlorophenol.

Microorganism	K_m	V_{max}	Reference
<i>Streptomyces albidoflavus</i> BSII#1	0.95 mM	0.12 mmol min ⁻¹	This study
<i>Streptomyces avermitilis</i> UAH30	1.45 mM	Not determined	[1]
<i>Streptomyces</i> sp. F6616	1.52 mM	Not determined	[2]
ALiP-P3 (<i>Streptomyces viridosporus</i> T7A)	0.37 mM	465.8 nmol mg protein ⁻¹ min ⁻¹	[3]
<i>Streptomyces</i> sp. AD 001	1.70 mM	529.7 nmol mg protein ⁻¹ min ⁻¹	[4]
HRP	0.84 mM	Not determined	[5]
<i>P. chrysosporium</i>	0.79 μM	Not determined	[6]
<i>S. albidoflavus</i> TN644	0.23 mM	Not determined	[7]

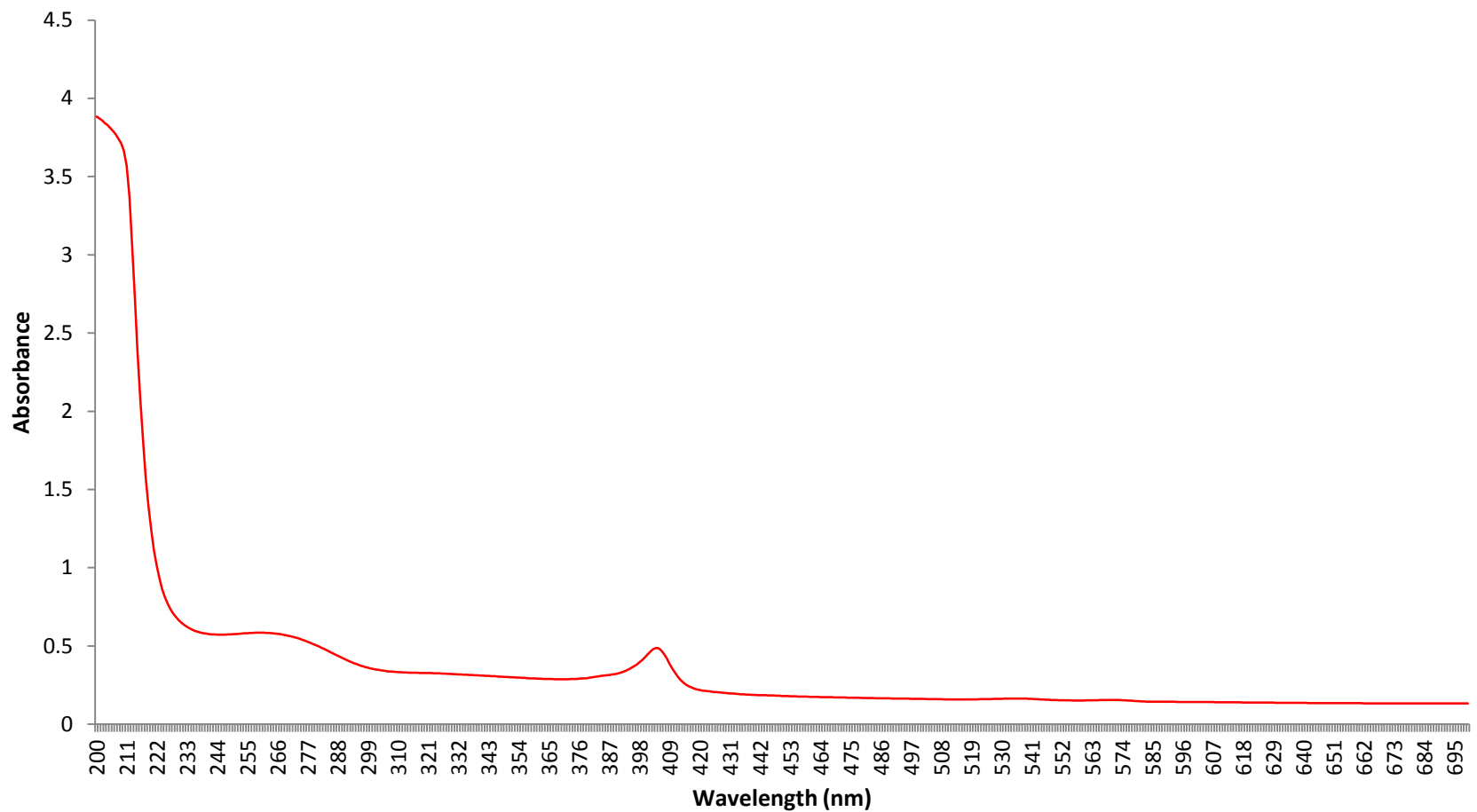


Figure S2: UV/Vis scan of the peroxidase. A peak corresponding to the Soret band characteristic of haem peroxidases is visible at approximately 400 nm.

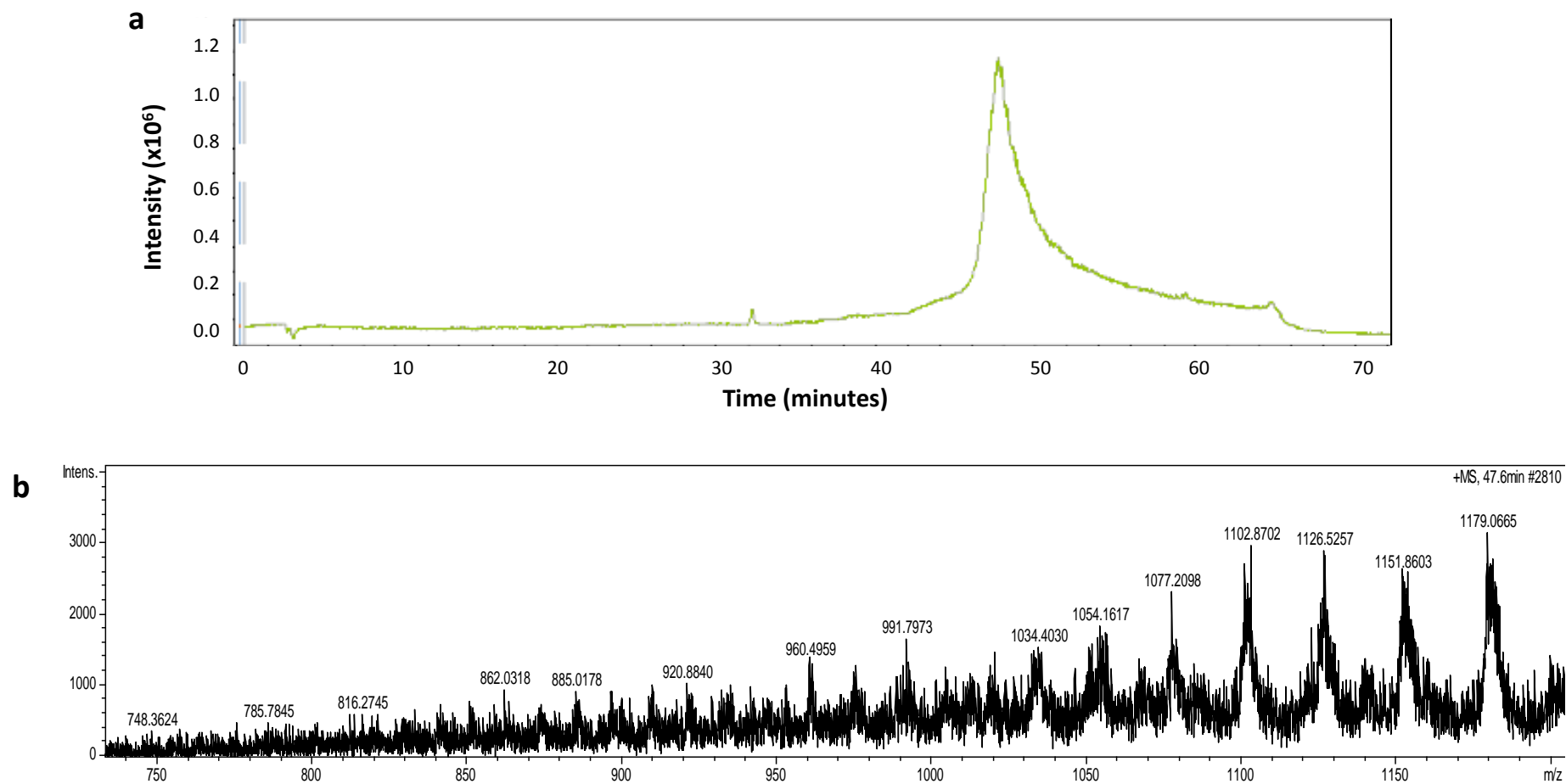
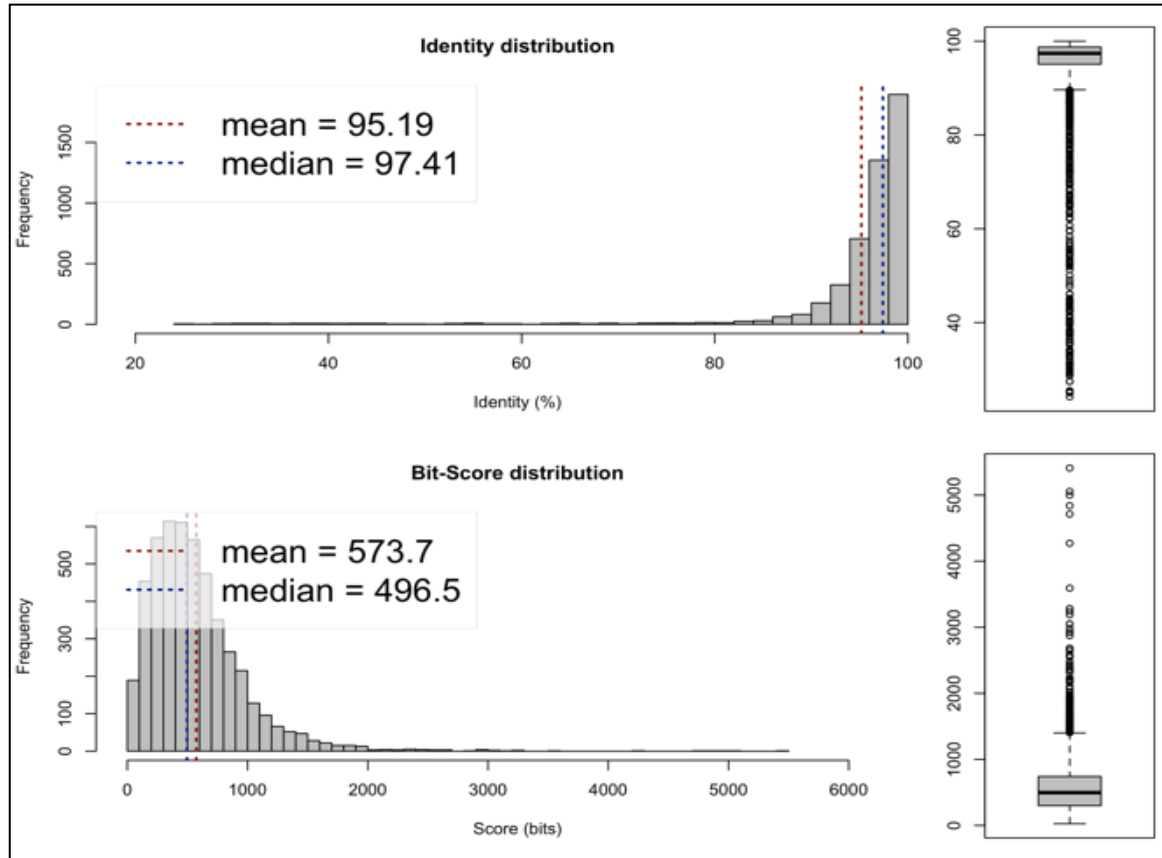


Figure S3: LC-MS analysis of the purified peroxidase from *Streptomyces albidoflavus* BSII#1. (a) HPLC chromatogram with major peak at 48 min (presumed to be the peroxidase of interest). (b) MS spectra of the peak at 48 min.

a



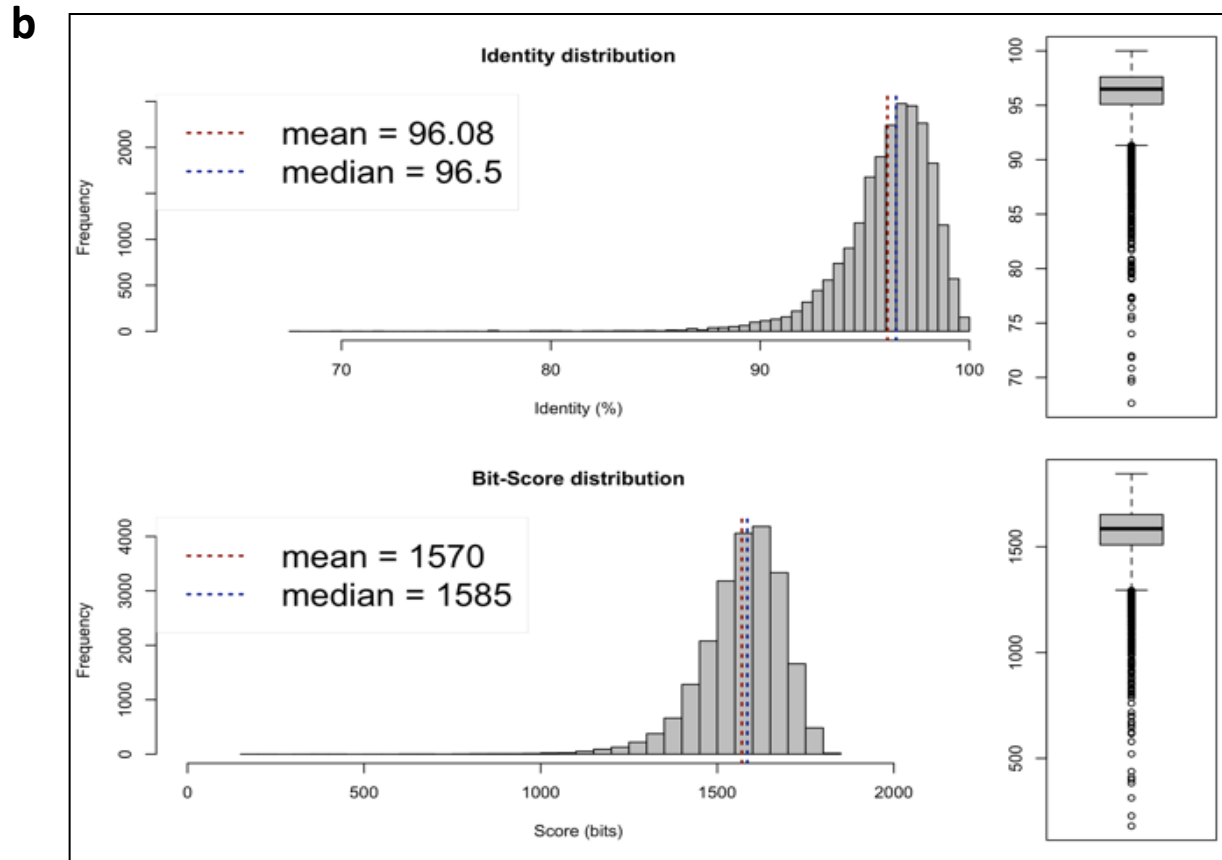


Figure S4: Results obtained for the AAI (a) and ANI (b) analysis between *Streptomyces albidoflavus* BSII#1 and *Streptomyces albidoflavus* NRRL B-1271^T.

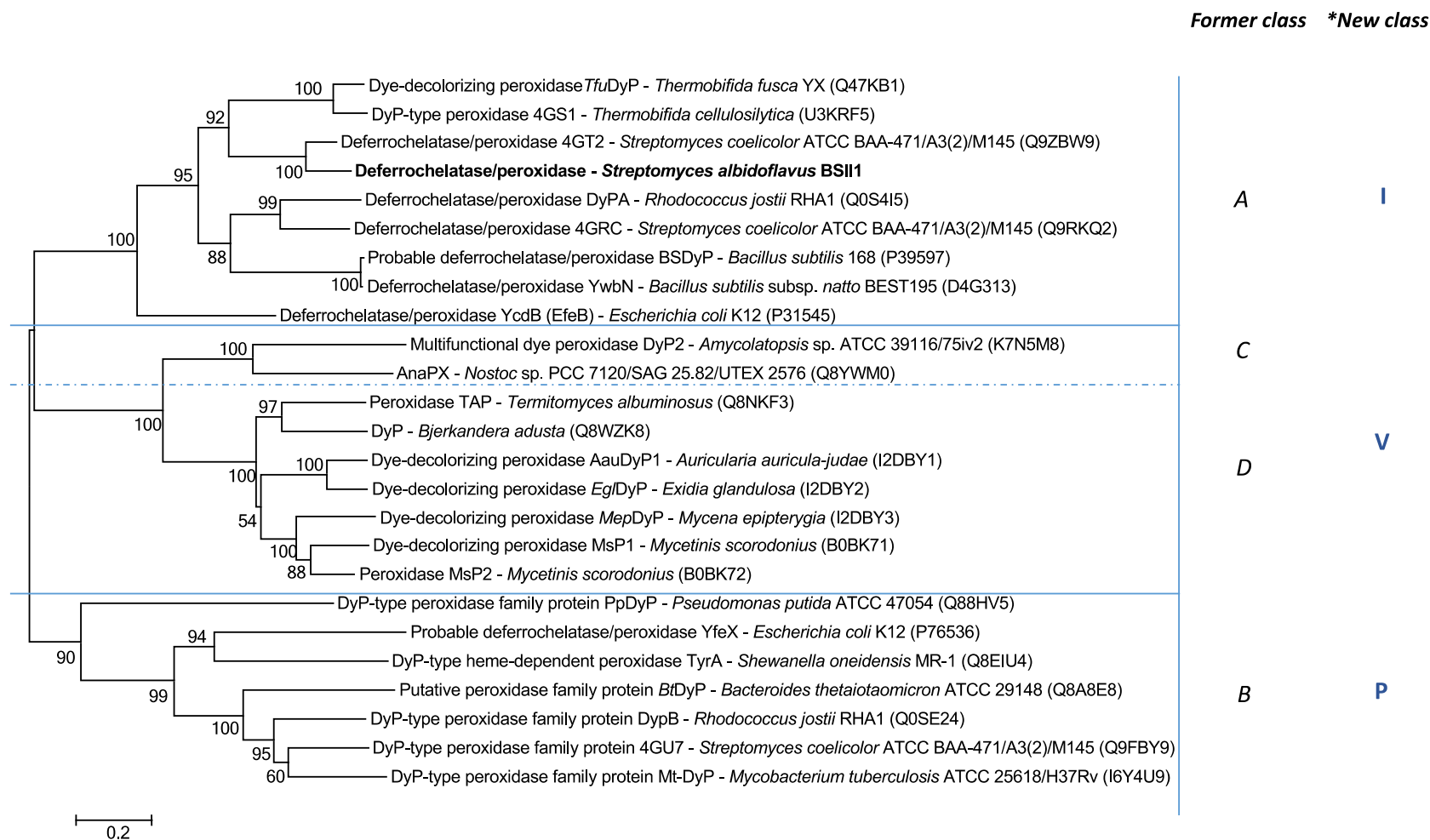
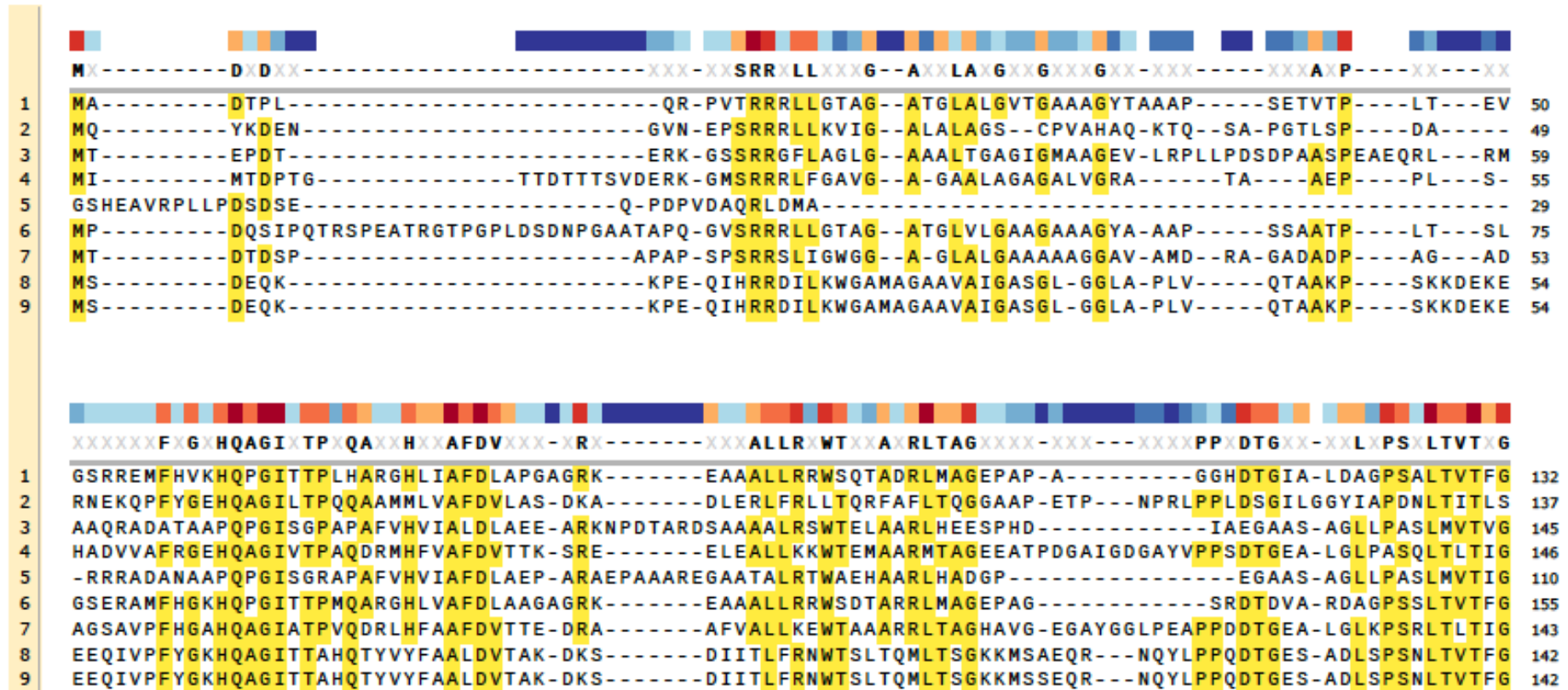



Figure S5: Neighbor-joining tree of DyP-type peroxidase sequences representative of the different DyP-type classes. The peroxidase from *Streptomyces albidoflavus* BSII#1 clusters with peroxidases within class I.


Consensus

1. *Streptomyces albidoflavus*_BSII_1
2. *Escherichia coli*_K12_Peroxidase_Ycdb_P31545
3. *Thermobifida fusca*_YX_Dye_decolorizing_peroxidase_TfuDyP_Q47KB1
4. *Rhodococcus jostii*_RHA1_Peroxidase_DyPA_Q0S4I5
5. *Thermobifida cellulositica*_DyP_type_peroxidase_4GS1_U3KRF5
6. *Streptomyces coelicolor*_M145_Peroxidase_4GT2_Q9ZBW9
7. *Streptomyces coelicolor*_M145_Peroxidase_4GRC_Q9RKQ2
8. *Bacillus subtilis*_168_Peroxidase_BsDyP_P39597
9. *Bacillus subtilis*_subsp_natto_BEST195_Peroxidase_YwbN_D4G313






FGXSLFX-----**RFGL**XXX**RPXALA**XP**FXDX**LDXXX**GGD**XXX**QXCADD**XX**VA**X**HALR**XLXXX**A**XXXX**VRW**XXX**GF**XXX**A**XXX-XX**TPRNL****GF**
 1 **FGHSFFA**-----**RTGLEKQRPAALDPLPEFSSDR**LD**PRRSNGD**L**WVQ**IG**ADDALVAFHALRAVQK**D**AGQAARVRWQMDGFN**RS**PGATEK**-**PMTTRNLM****GQ** 226
 2 **VGHSLFDE**-----**RFGLAPQMPKKLQKMTRFP**N**DSLDAALCHGDVLLQ**IC**ANTQD**TV**IHALRDI**IK**HTPDLLSVRWKREGF**IS**DHAARSKGK**ET**PINLL****GF** 233
 3 **IGGSLLS**-----**AIDAEDRRPDALADLPEFSTDD**L**HPRWCGGD**F**MLQVGAED**PM**VLTAAVEEL**V**AAAA**DATA**VRWSLRGFR**RT**AAAARDP**DAT**PRNLM****GQ** 240
 4 **FGPSLFDD**-----**RFGFASKKPAALVDLPHFRAD**N**LDPARSGGD**IA**IQCACANDPQVAVH**AIR**NLARVAFGTAS**VR**WSQLGFGRTS**ST**STT-QDTPRNL****FGF** 241
 5 **IGGSLLS**-----**AMDAADRRPDALADLPEFATDD**L**RPRWCGGD**L**MLQVGAED**PM**VLAAAVDEL**V**AATAP**TTT**VRWSLRGFR**RT**AAAARDP**DAT**PRNLM****GQ** 205
 6 **FGHSFFG**-----**RTGLEEQRPVALDPLPDFSSDH**L**DKNRSNGD**L**WVQ**IG**ADDALVAFHALRAIQ**RD**AGAAARVRWQ**M**NGFN**RS**PGATAH**-**PMTARNLM****GQ** 249
 7 **FGPSLFT**-----**RFGLADLRPEALADLPKFP**GD**NLDRARSGGD**LC**VQACADD**P**QVAVH**AIR**NLARIGFGKVV**VR**WSQLGFGKTS**ST**TPD-EQTPRNL****GF** 237
 8 **FGPGFFEKDGKDRFGLKSKKPKHLAALPAMP**ND**NLDEKQGGGD**IC**IQCACADDEQVAFHALRNL**LN**QAVGTCE**VR**RVNKGFL**SG---**GKN-GETPRNL****FGF** 238
 9 **FGPGFFEKDGKDRFGLKSKKPKHLAALPAMP**ND**NLDEKQGGGD**IC**IQCACADDEQVAFHALRNL**LN**QAVGTCE**VR**RVNKGFL**SG---**GKN-GETPRNL****FGF** 238



KDGTXX**NP**XXXXXXXXXXXX**VWV**XX-----**XXXPAWM**GG**GSY**V**RRIRMLLE**W**DR**X**SL**XX**QE**X**V**X**GRR**XX**GAP**L**G**XX---**ETD**XXX**L**XXXXXXXX**G**XX**VI**
 1 **IDGTNNP**VP**SD**ED-**FATRVRVPA**-----**DGDP**AW**MAGGSY**V**VRIRMLL**DD**WEKLSVAGQE**AV**MGRRKAD**G**AP**LT**G**---**TET**TE**PD**L**ERV**GED**GDLVI** 317
 2 **KDGTANP**DS**QNDKLMQKV**V**WVTA**-----**DQ**Q**EP**AW**TIGGSYQAVRLIQ**FR**VEFWDR**TP**LKEQQTIFGR**DK**QT**G**AP**L**GMQ**---**HEH**D**V**PD**YAS**-**D**PE**G**-**KVI** 324
 3 **IDGTANP**AQ**DHPL**-**FDR**T**ITARP**---**ADN**PA**HAWMDGGSYLV**VR**IRMLL**TE**WRKLDVA**ARE**VI**GR**RLDT**G**AP**L**GSR**---**NET**D**P**V**VLSAR**DE**E**GE**PLI** 333
 4 **KDGTNNI**KA**E**EPS**ILDQHV**V**AS**-----**GDD**Q**AWMGGGAYLV**ARR**IRMLIEQ**W**DR**TV**LGEQ**ER**VI**GR**SKGT**G**AP**L**SGK**---**A**EF**DEL**D**LD**SK**KGH**-**D**P**VI** 332
 5 **IDGTANP**AQ**DHPL**-**FTR**T**VTAPP**---**ADD**PA**HAWMDGGSYLV**VR**IRMLL**DE**WRRLD**VP**DR**ER**VI**GR**HLDT**G**AP**L**GGE**---**K**ET**D**P**V**LT**ARD**AD**GRLVI** 298
 6 **VDGTR**NP**K**PG**EAD**-**FDR**RI**FVPEE**PE**AGKGGPAWM**ANG**SYVV**VR**IRMLL**DD**WEELSLKA**Q**ED**VI**GRRKSD**G**AP**L**SGGSGATE**STEM**DLEKTDGSGEL**V**V** 348
 7 **KDGT**RNI**AGTEK**DR**LDRFV**W**AAE**-----**KD**GP**WMTGGSYLV**ARR**IRMH**IE**TWDR**AS**LQE**Q**ED**V**FR**DK**EG**AP**V**G**K**A---**K**ER**DE**P**FL**KA-----**M** 321
 8 **KDGT**GN**Q**ST**KDD**TL**MNSIV**W**I**Q**S**-----**G**EP**D**W**MTGGTYMAFRKIK**M**F**LE**V**W**DR**SS**LKDQ**ED**TF**GR**RKSSG**AP**FGQK**---**K**ET**D**P**V**KL**NQ**-----**I** 321
 9 **KDGT**GN**Q**ST**ED**DSL**MNSIV**W**V**Q**S**-----**G**EP**D**W**MTGGTYMAFRKIK**M**F**LE**I**W**DR**SS**LKDQ**ED**TF**GR**RKSSG**AP**FGQK**---**K**ET**D**P**V**KL**NQ**-----**I** 321



PXNAHVRLAXPXX--**N**X**GA**X**MLRRGYSY**XX**GDX**-X**G**XX**DAGLLF**XXX**Q**X**DP**XX**GFV**P**VQR**X**LX**-X**GDAL**NEY**I**X**H**X**GSAL**-**FAVP**GGXXX**G**-X**Y**X**GQ**X**L**
 1 **PLNAHAR**IT**R**PD**Q**--**NGGA**AM**LRRPFSY**H**D**G**I**DE-**E**GV**P**D**AGLLF**LC**WQ**AD**PLR**GF**V**P**VQR**K**L**D-**R**GD**AL**S**AF**IR**HEASGL**-**Y**AV**P**GG**A**R**K**G-**G**Y**V**G**Q**EL 411
 2 **ALDSHIR**LAN**P**RT**A**E**SS**LM**LRGYSY**SL**G**V**T**N-**S**Q**L**D**M**G**L**LF**V**C**Y**Q**H**LE**K**GF**L**T**V**Q**K**RL**N**--**G**E**A**LE**E**Y**V**K**P**I**G**GG**Y**F**F**AL**P**GV**K**D**A**N-**D**Y**F**GS**A**L 420
 3 **PENAHVRL**AS**P**EN--**N**L**G**AR**M**F**R**RG**YSY**D**Q**G**WRD**-**D**GV**R**D**AGLLF**MA**W**Q**GP**AT**GF**I**P**V**QR**S**LAD**Q**GD**AL**N**RY**IR**HE**GSAL**-**F**AV**P**AA-**R**EG-**R**Y**L**G**Q**DL 427
 4 **DVDAHVRL**AS**A**QE--**L**GG**I**Q**I**L**R**RG**YN**F**T**D**GS**D**G**-**F**GH**L**D**AGL**FF**I**AY**CR**NP**QE**Q**F**V**P**M**QL**N**L**S-**R**ND**A**M**NEYI**Q**H**V**GSAL**-**F**AC**P**P**G**LA**E**G-**Q**Y**W**G**Q**GL 426
 5 **PEDAHVRL**AN**P**EN--**N**L**G**AR**M**V**R**RG**YN**Y**D**E**G**WR**D**-**D**GV**R**D**AGLLF**MA**W**Q**GN**AT**GF**V**P**V**QR**S**LVE**Q**GD**AL**N**RY**TR**HE**GSAL**-**F**AV**P**AA-**T**AD-**R**Y**P**G**Q**DL 392
 6 **PINAHAR**IT**R**PD**Q**--**NGGA**AM**V**RR**P**F**S**Y**H**D**G**F**DA**-**D**GV**P**D**AGLLF**V**C**W**Q**AD**PLR**GF**V**P**VQR**K**L**D-**R**GD**AL**S**Q**F**IR**HE**ASGL**-**F**AV**P**GG**A**E**G**-**E**Y**V**G**Q**RL 442
 7 **KPD**AHV**R**LA**H**PD**S**--**N**GG**A**T**L**L**R**RG**YS**F**T**D**G**T**D**G-**L**GR**L**D**AGL**FF**L**AY**Q**R**D**IR**T**GF**V**P**VQR**N**L**A--**T**D**AL**NEY**I**Q**H**V**GS**AV-**F**AV**P**P**G**V**R**D**AD**D**W**W**G**ST**L** 415
 8 **PSNSHV**SL**A**KS-----**T**G**K**Q**I**L**R**RA**F**S**Y**TE**G**L**D**PK**T**GY**M**D**AGLLF**IS**F**Q**K**NP**DN**Q**F**I**P**ML**K**ALS-**A**K**D**AL**NEY**T**Q**T**I**GS**A**L-**Y**AC**P**GG**C**KK**G**-**E**Y**I**A**Q**RL 413
 9 **PSNSHV**SL**A**KS-----**T**G**K**Q**I**L**R**RA**F**S**Y**TE**G**L**D**PK**T**GY**M**D**AGLLF**IS**F**Q**K**NP**DN**Q**F**I**P**ML**K**ALS-**A**K**D**AL**NEY**T**Q**T**I**GS**A**L-**Y**AC**P**GG**C**KK**G**-**E**Y**I**A**Q**RL 413

	LE--X	
1	LE--G	414
2	LR--V	423
3	IE--G	430
4	F--T	428
5	VE--G	395
6	LE--G	445
7	FGKEA	420
8	LE--S	416
9	LE--S	416

Figure S6: Amino acid sequence alignment of representative DyP-type peroxidases within class I of this protein family. The blue inverted triangles indicate the residues around the haem moiety (H = haem ligand; other four amino acids forms the hydrogen peroxide binding pocket). The black box shows the highly conserved GXXDG motif found in all DyP-type peroxidases.

Table S2: Combinations of phenolic monomers that were coupled using the peroxidase from *Streptomyces albidoflavus* BSI#1.

Substrate 1	Substrate 2	Observed [M-1]	Expected [M]
guaiacol	chlorogenic acid	475.1257	476.1257
guaiacol	syringaldazine	481.1594	482.1594
guaiacol	caffeic acid	301.0733	302.0733
catechol	<i>p</i> -coumaric acid	271.0613	272.0613
catechol	ferulic acid	301.0721	302.0721
catechin	syringaldazine	647.1469	648.1469
catechin	<i>p</i> -coumaric acid	451.1377	452.1377
catechin	guaiacol	411.1087	412.1087
catechin	cinnamyl alcohol	421.1289	422.1289
catechin	caffeic acid	467.0988	468.0988
catechin	catechol	397.0902	398.0902

Nucleotide and amino acid sequences, DyP-type peroxidase, *Streptomyces albidoflavus* BSII#1

> Predicted dye-decolorizing peroxidase (DyP), YfeX-like subgroup, *Streptomyces albidoflavus* BSII#1

Atggcggacaccccccttcagcgtccggtgaccggcggcgctgctcggcaccgcccggggccaccggactcgcctcggcgtgacgggagccgcccggttacacggcggcgcctccctccgagaccgtca
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gagggacggggacctggtcatcctctcaacgcgcacggcggatcaccgccccgaccagaacggcggcgcggcgatgctccggcggccttctcataccacgacggcatcgcagaggaggcggtgccc
acggcgggctgcttctcctctgctggcaggccgaccgctcgcggcttctcctccagcgaagctcaccggggcgacgcgctcctcggccttcatccggcatgaggcgagcggcctgtacgccgtccc
gcggggcgcggaaggcgggtacgtggggcaggaactgctggagggttag

> Predicted dye-decolorizing peroxidase (DyP), YfeX-like subgroup, *Streptomyces albidoflavus* BSII#1

MADTPLQRPVTRRRLLGTAGATGLALGVTGAAAGYTAAAPSETVTPLETVGSRREMFHVKHQPGITTPHARGHLIAFDLAPGAGRKEAAALLRRWSQTADRLMAG
EPAPAGGHDTGIALDAGPSALTVTFGFHGSFFARTGLEKQRPAALDPLPEFSSDRLDPPRSNGDLWVQIGADDALVAFHALRAVQKDAGQAARVRWQMDGFNRSP
GATEKPMTTRNLMGQIDGTNNPVPSDEDFATRVRVPADGDPAWMAGGSYVVFRIRMLLDDWEKLSVAGQEAVMGRRKADGAPLTGGTETTEPDLERVGEDG
DLVIPLNAHARITRDPQNGGAAMLRRPFSYHDGIDEEGVDPDAGLLFLCWQADPLRGFVQVQRKLDKDRGDALSAFIRHEASGLYAVPGGARKGGYVQELLEG

Supplementary Methods and Materials

Storage of Purified Peroxidases

Aliquots of the ultrafiltrate fraction were mixed with glycerol to achieve final glycerol concentrations of between 2.5% and 25% (v/v) in 1 ml volumes in Eppendorf tubes. The tubes were divided into three sets which were incubated at ambient temperature ($25\pm 2^\circ\text{C}$), 4°C and at -20°C . Residual peroxidase activity for each glycerol concentration at each storage temperature was determined on a weekly basis for a period of six weeks.

Functional expression of an extracellular peroxidase

Streptomyces albidoflavus BSII#1 was cultivated under the optimal conditions for the production of extracellular peroxidase [8]. Total RNA was extracted using the ISOLATE II RNA mini kit (Bioline, London, United Kingdom) as per the manufacturer's instruction. RNA was converted to cDNA using the Maxima H minus first strand cDNA synthesis kit (Thermo Fischer Scientific, Bellfonte, USA) according to the manufacturer's instruction. cDNA was used as a template in PCR reactions with primers specific to the gene encoding for the dye decolourising peroxidase (s16.1_F 5'-GATCGACGGCACCAACAATC-3' and s16.1_R 5'-GTCGTGGTATGAGAAGGGGC-3'). PCR conditions were as follows: an initial denaturation (96°C for 2 min), 30 cycles of denaturation (96°C for 45 s), annealing (56°C for 30 s) and extension (72°C for 1 min), and a final extension (72°C for 5 min). The 358 bp amplicon was purified using the Stratec Molecular MSB® Spin PCRapace kit and sequenced. The sequence was submitted for BLASTn analysis and compared to the genome information. To ensure that the RNA was not contaminated with genomic DNA, a 16S rRNA gene PCR reaction was setup as previously described [9].

Supplementary Results

Stability of the Partially Purified Peroxidase in Storage

The stability of the partially purified peroxidase stored in different glycerol concentrations (up to 25% v/v glycerol) at three different storage temperatures (ambient, 4°C and -20°C) was assessed over a period of 6 weeks. Maximum activity retained in samples containing no glycerol and stored at ambient temperature and 4°C was $22.3\pm 0.9\%$ and $63.8\pm 1.2\%$, respectively. Optimum storage conditions included the addition of 7.5-10% glycerol and storage at -20°C ($85.8\pm 1.7\%$ activity retained after 6 weeks).

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Credit Author statement

Amos Musengi: Conceptualization, Methodology, Formal analysis, Writing – Original draft, Visualization **Kim Durrell:** Methodology, Formal analysis **Alaric Prins:** Methodology, Formal analysis, Writing – Review and editing **Nuraan Khan:** Conceptualization, Formal analysis, Writing – Review and editing **Mayowa Agunbiade:** Methodology, Formal analysis **Tukayi Kudanga:** Conceptualization, Methodology, Formal analysis, Writing – Review and editing **Bronwyn Kirby-McCullough:** Methodology, Writing – Review and editing **Brett I. Pletschke:** Conceptualization, Supervision, Writing – Review and editing **Stephanie G. Burton:** Conceptualization, Supervision, Funding acquisition **Marilize Le Roes-Hill:** Conceptualisation, Formal analysis, Resources, Writing – Review and editing, Supervision, Project administration, Funding acquisition