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

Amos Musengi[1,2], Kim Durrell[1], Alaric Prins[1,3], Nuraan Khan[1], Mayowa Agunbiade[1], Tukayi Kudanga[1,4], Bronwyn Kirby-McCullough[3], Brett I. Pletschke[5], Stephanie G. Burton[6], Marilize Le Roes-Hill[1]*

[1]Applied Microbiology and Health Biotechnology Institute, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7535, South Africa
[2]Biotechnology Department, Harare Institute of Technology, P. O. Box BE 277, Belvedere, Harare, Zimbabwe
[3]Institute for Microbial Biotechnology and Metagenomics, Department of Biotechnology, University of the Western Cape, Bellville, 7535, South Africa
[4]Department of Biotechnology and Food Technology, Durban University of Technology, PO Box 1334, Durban, 4000, South Africa
[5]Department of Biochemistry and Microbiology, Faculty of Science, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa
[6]Vice-Principal: Research and Postgraduate Education and Department of Biochemistry, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa

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*Corresponding author: Dr Marilize Le Roes-Hill
E-mail: leroesm@cput.ac.za
Telephone: +27 21 953 8499

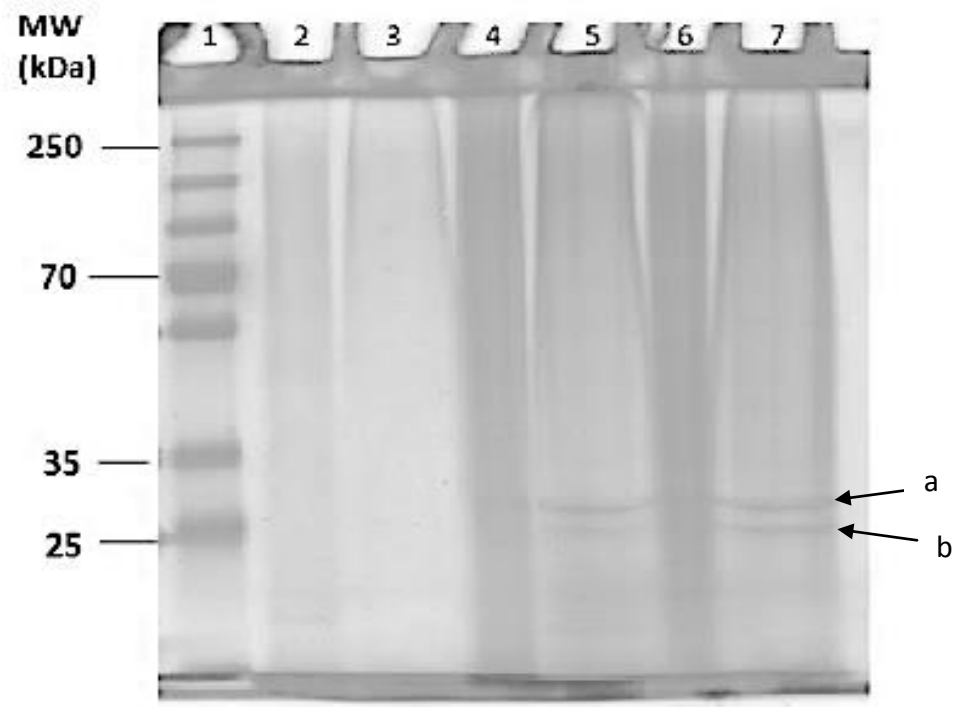


Figure S1: SDS-PAGE gel with samples from each purification step for the peroxidase from *Streptomyces albidoflavus* BSII#1. Lane 1, protein size marker (10 to 250 kDa). Lanes 2 and 3, crude enzyme boiled in DTT-containing and β-mercaptoethanol-containing loading buffer, respectively. Lanes 4 and 5, 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
Streptomyces albidoflavus BSII#1	0.95 mM	0.12 mmol min^{-1}	This study
Streptomyces avermitilis UAH30	1.45 mM	Not determined	[1]
Streptomyces sp. F6616	1.52 mM	Not determined	[2]
ALiP-P3 (*Streptomyces viridosporus* T7A)	0.37 mM	465.8 nmol mg $protein^{-1}$ min^{-1}	[3]
Streptomyces sp. AD 001	1.70 mM	529.7 nmol mg $protein^{-1}$ min^{-1}	[4]
HRP	0.84 mM	Not determined	[5]
P. chrysosporium	0.79 µM	Not determined	[6]
S. albidoflavus TN644	0.23 mM	Not determined	[7]

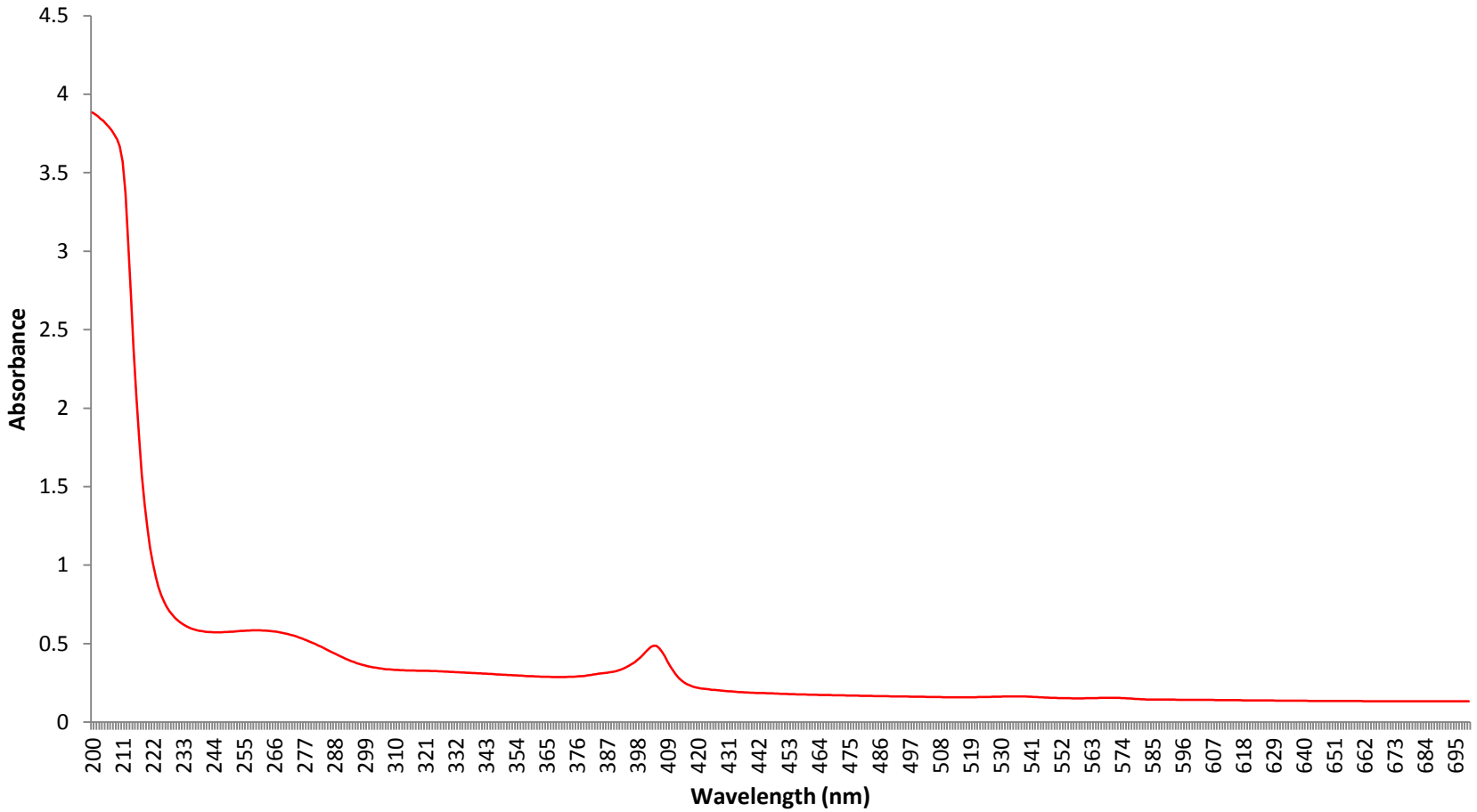


Figure S2: UV/Vis scan of the peroxidase. A peak corresponding to the Soret band characteristic of haem peroxidases is visible at approximately 400 nm.

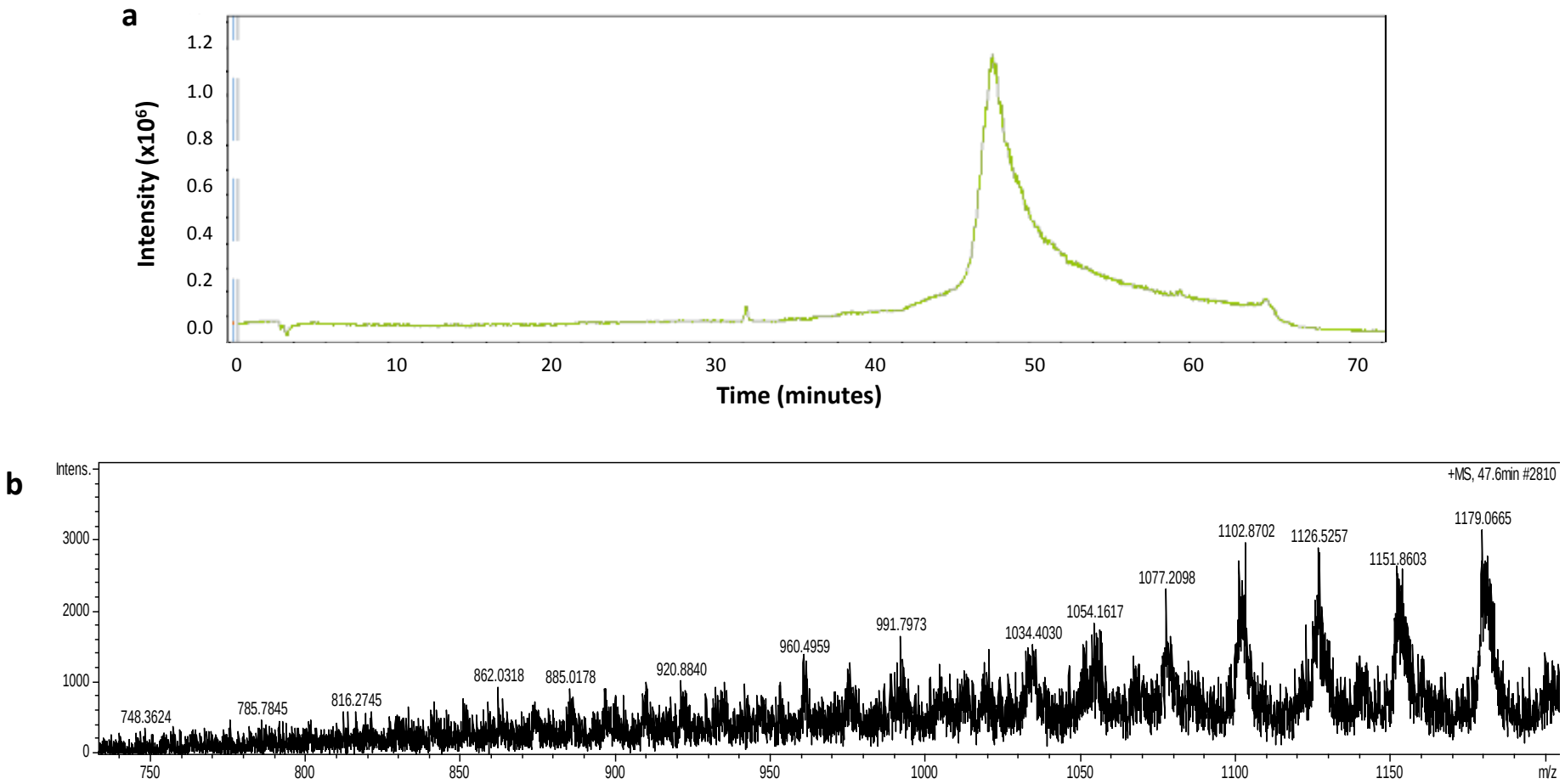
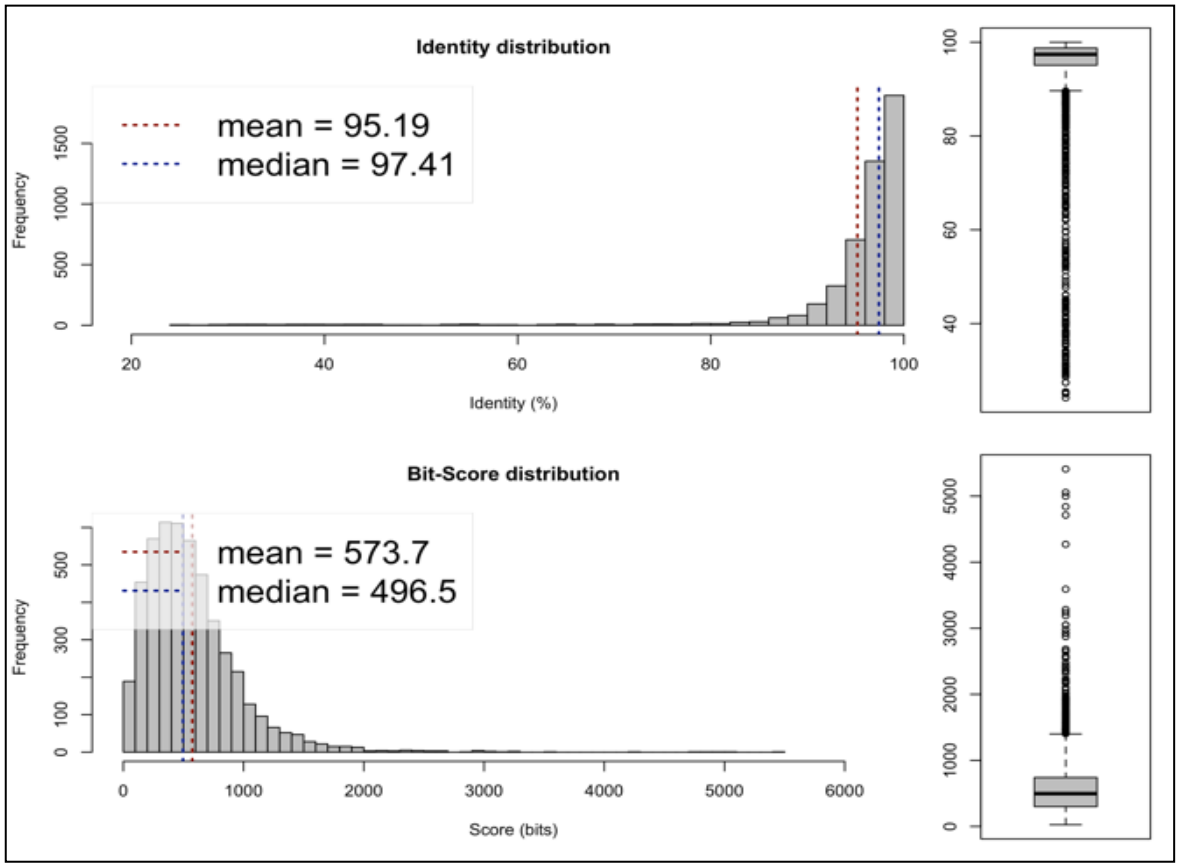


Figure S3: LC-MS analysis of the purified peroxidase from *Streptomyces albidoflavus* BSII#1. (a) HPLC chromatogram with major peak at 48 min (presumed to be the peroxidase of interest). (b) MS spectra of the peak at 48 min.

a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median = 97.41
Frequency
Identity (%)
Bit-Score distribution
mean = 573.7
median = 496.5
Frequency
Score (bits)

b

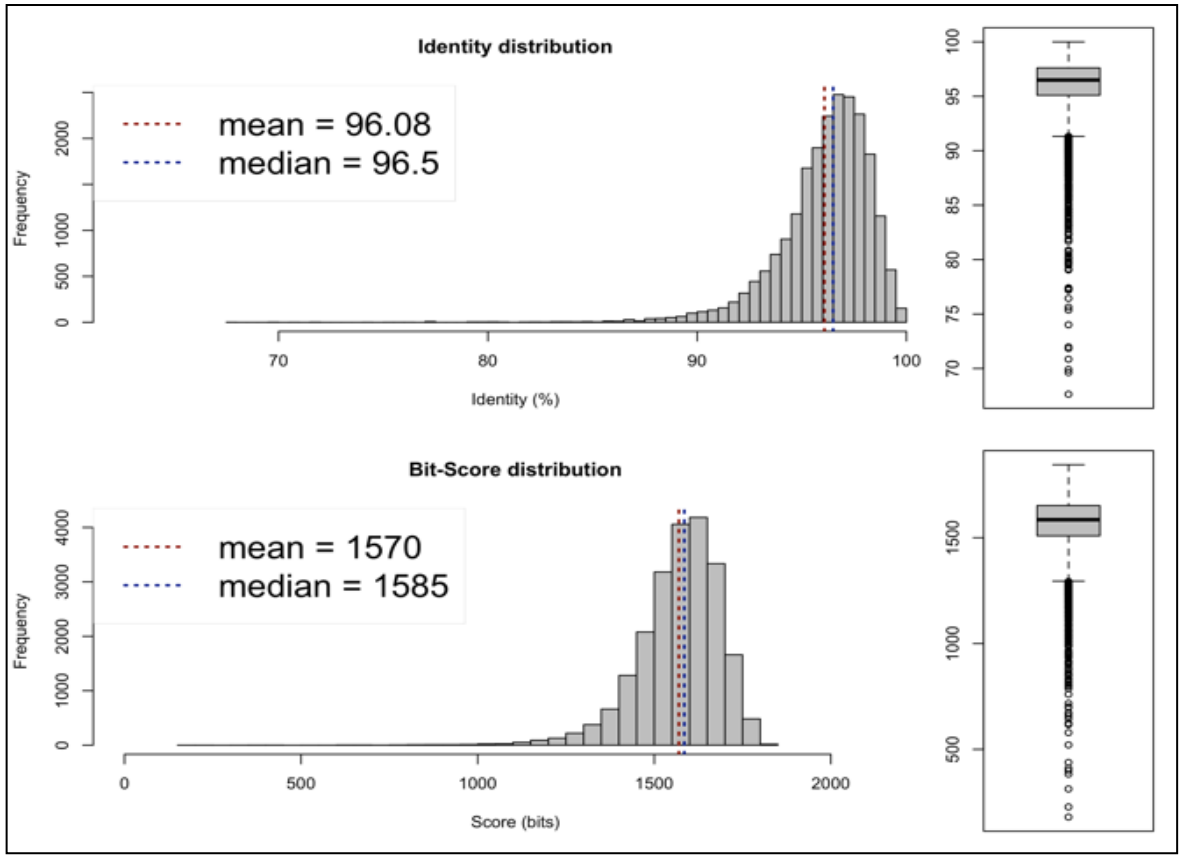


Figure S4: Results obtained for the AAI (a) and ANI (b) analysis between *Streptomyces albidoflavus* BSII#1 and *Streptomyces albidoflavus* NRRL B-1271^{T}.

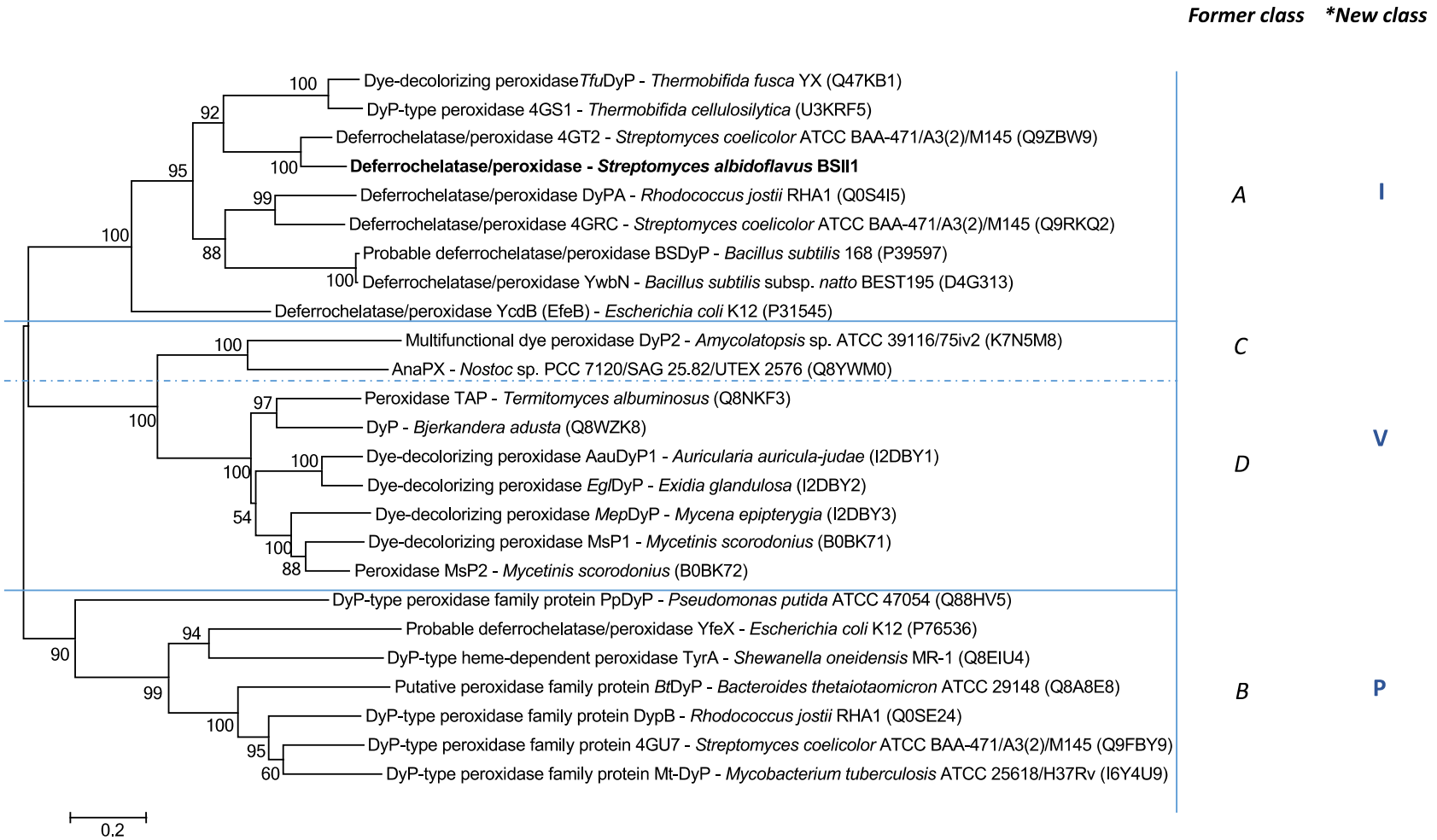
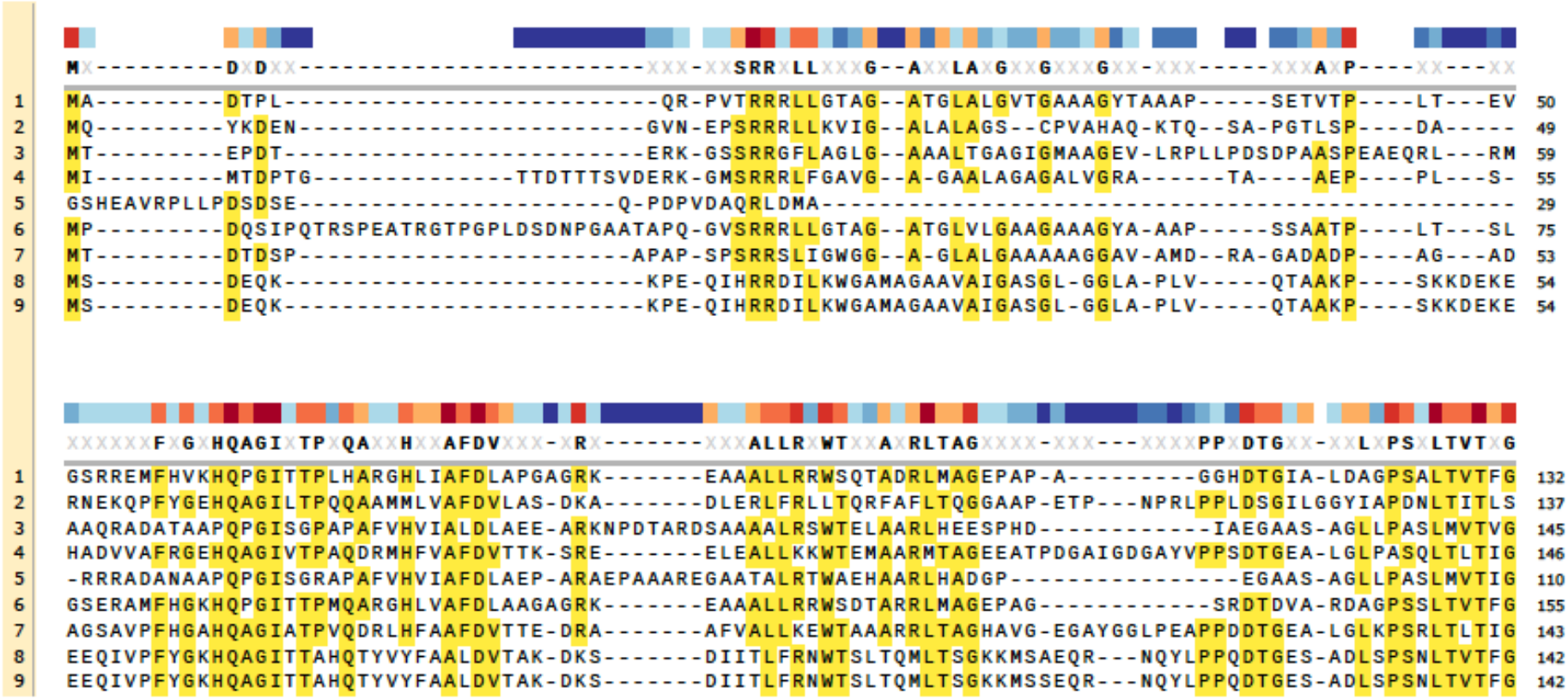
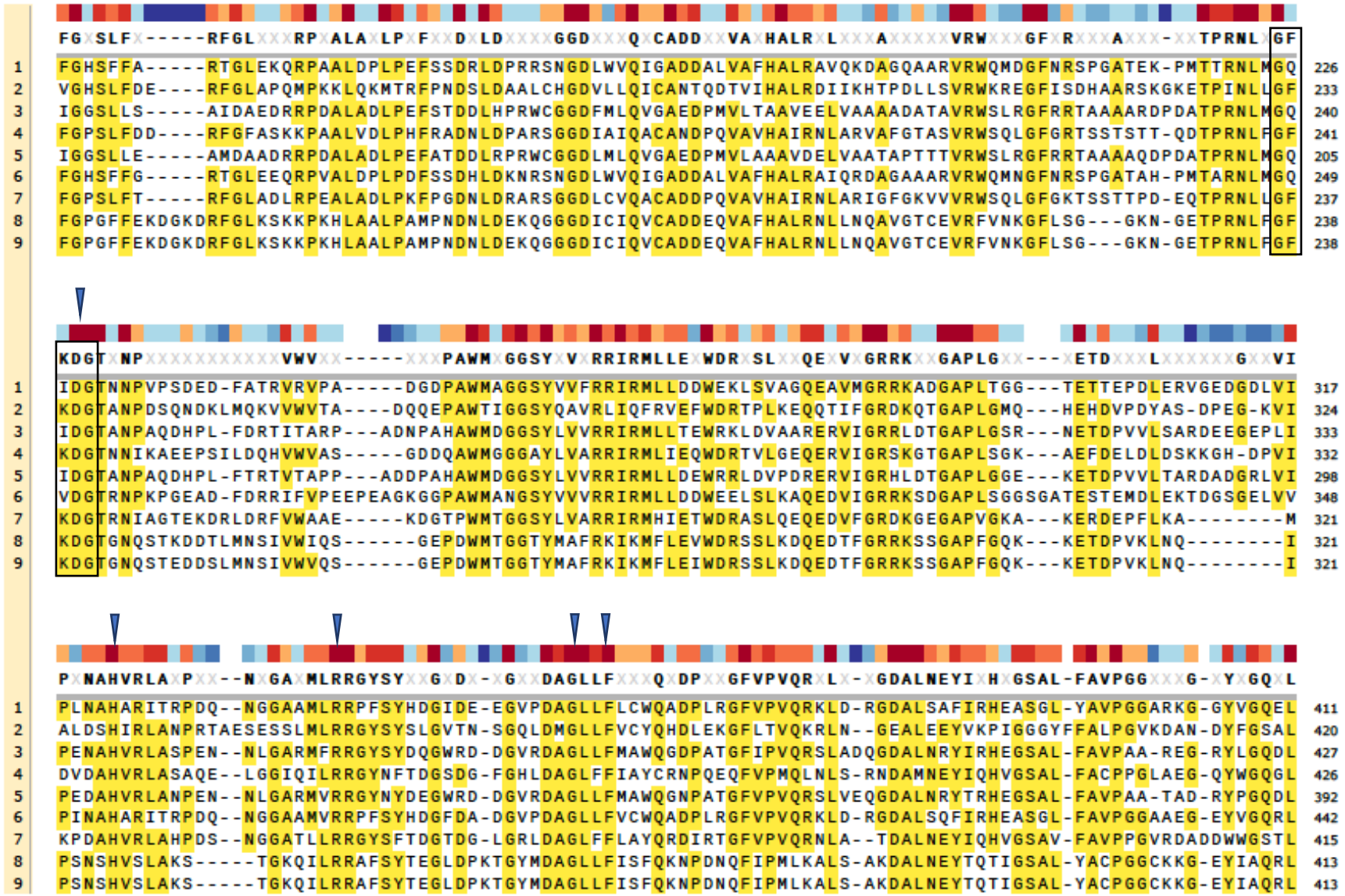


Figure S5: Neighbor-joining tree of DyP-type peroxidase sequences representative of the different DyP-type classes. The peroxidase from *Streptomyces albidoflavus* BSII#1 clusters with peroxidases within class I.

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_cellulosilytica_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





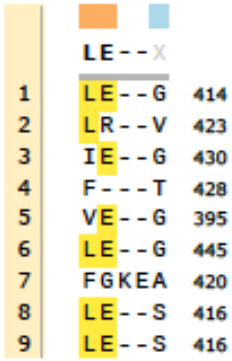


Figure S6: Amino acid sequence alignment of representative DyP-type peroxidases within class I of this protein family. The blue inverted triangles indicate the residues around the haem moiety (H = haem ligand; other four amino acids forms the hydrogen peroxide binding pocket). The black box shows the 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* BSII#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	*p*-coumaric acid	271.0613	272.0613
catechol	ferulic acid	301.0721	302.0721
catechin	syringaldazine	647.1469	648.1469
catechin	*p*-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

Atggcggacacccccttcagcgtccggtgacccggcggcgcctgctcggcaccgccggggccaccggactcgccctcggcgtgacgggagccgccgccggttacacggcggccgctccctccgagaccgtca
ctccgctgacggaggtgggctcccggcgcgagatgtttcacgtgaaacatcagcccggcatcaccacgccgctgcacgcccggggccatctgatcgccttcgacctggctcccggggcggggcgcaaggaggc
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gcggggcgcggaagggcgggtacgtggggcaggaactgctggaggggtag

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

MADTPLQRPVTRRRLLGTAGATGLALGVTGAAAGYTAAAPSETVTPLTEVGSRREMFHVKHQPGITTPLHARGHLIAFDLAPGAGRKEAAALLRRWSQTADRLMAG
EPAPAGGHDTGIALDAGPSALTVTFGFGHSFFARTGLEKQRPAALDPLPEFSSDRLDPRRSNGDLWVQIGADDALVAFHALRAVQKDAGQAARVRWQMDGFNRSP
GATEKPMTTRNLMGQIDGTNNPVPSDEDFATRVRVPADGDPAWMAGGSYVVFRRIRMLLDDWEKLSVAGQEAVMGRRKADGAPLTGGTETTEPDLERVGEDG
DLVIPLNAHARITRPDQNGGAAMLRRPFSYHDGIDEEGVPDAGLLFLCWQADPLRGFVPVQRKLDRGDALSAFIRHEASGLYAVPGGARKGGYVGQELLEG

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±2°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±0.9% and 63.8±1.2%, respectively. Optimum storage conditions included the addition of 7.5-10% glycerol and storage at -20°C (85.8±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