Exploring the biochemical and biocatalytic properties of bacterial DyP-type peroxidases

Colpa, Dana Irene

Chapter 1:

Introduction and outline of the thesis

DyP-type peroxidases: a promising and versatile class of enzymes

Dana I. Colpa, Marco W. Fraaije and Edwin van Bloois

This chapter is based on:
Journal of Industrial Microbiology and Biotechnology (2014) 41: 1–7.
DOI: 10.1007/s10295-013-1371-6

Introduction

Peroxidases (EC 1.11.1.x) represent a large family of oxidoreductases that typically use hydrogen peroxide as an electron acceptor to catalyze the oxidation of substrate molecules. The vast majority of these enzymes contain heme as a cofactor[1] and are ubiquitously present in prokaryotes and eukaryotes. Peroxidases take center stage in a variety of biochemical processes, ranging from the biosynthesis of cell wall material to immunological host-defense responses.[2,3] Heme-containing peroxidases were originally classified into two superfamilies: the plant peroxidases and the animal peroxidases.[4,a] Remarkably, some members of the peroxidase superfamily have been studied for more than a century like horseradish peroxidase (HRP), a prototype plant peroxidase.[5] In this respect, it was fascinating that the first member of a newly discovered peroxidase superfamily, the group of DyP-type peroxidases, was described in 1999.[6] In this chapter, we discuss the biochemical and structural features of DyP-type peroxidases as well as their promising biotechnological potential.

Phylogenetic and structural comparison

Dye-decolorizing (DyP-type) peroxidases were first discovered in fungi and named after their ability to degrade a wide range of dyes.[6] Subsequently, additional members were found in the proteomes of other fungi as well as in several bacteria.[7] This indicates that these enzymes are widespread like other peroxidases. Interestingly, recent genome sequence analysis revealed that these enzymes are prominent in bacteria, whereas only a small number is found in fungi and higher eukaryotes. Their occurrence in archaea is even more limited. The most comprehensive overview of the DyP-type peroxidase superfamily is offered by the InterPro database.[8] According to this database, the DyP superfamily currently (February 2018) comprises 12,670 enzymes of which 11,877 are found in bacteria, 741 in eukaryotes, and 52 in archaea. Additionally, DyP-type peroxidases are, according to PeroxiBase, further sub-classified into the phylogenetically distinct classes A, B, C, and D.[9] Alternative classifications

[a] More recently heme-containing peroxidases were reclassified in four independently evolved superfamilies: the peroxidase-catalase superfamily, the peroxidase-cyclooxygenase superfamily, the peroxidase-chlorite dismutase superfamily and the peroxidase-peroxygenase superfamily.[78] The peroxidase-catalase superfamily is formed by members of the previously called 'superfamily of bacterial, fungal and plant heme peroxidases'. The 'superfamily of the animal heme-dependent peroxidases' contains enzymes from all kingdoms of life and was renamed to the peroxidase-cyclooxygenase superfamily. The peroxidase-chlorite dismutase superfamily consists of three protein families that share a common fold: DyP-type peroxidases, chlorite dismutases and EfeB, previously called the CDE-superfamily[79]. In this chapter the original classification will be used.

with three and five classes have also been proposed.[10,11] Subfamilies C and D are grouped together in the classification with three classes. An overview of the DyP-type peroxidases characterized thus far is shown in Table 1 and a phylogenetic tree is shown in Figure 1. Many of the potential bacterial enzymes are putative cytoplasmic enzymes (class B and C), indicating that they are involved in intracellular metabolism. In contrast, enzymes belonging to class A contain a Tat-dependent signal sequence, which suggests that they function outside of the cytoplasm or extracellularly as previously confirmed by us and others.[12–14] Class D contains primarily fungal variants. For some of these peroxidases, it has been shown that they are involved in dye decolorization.[7] Nevertheless, the physiological function of the majority of DyP-type peroxidases is at present unclear, although evidence is accumulating that some bacterial variants are involved in the degradation of lignin.[15–19] This suggests that these enzymes can be regarded as the bacterial equivalents of the fungal lignin degrading peroxidases.

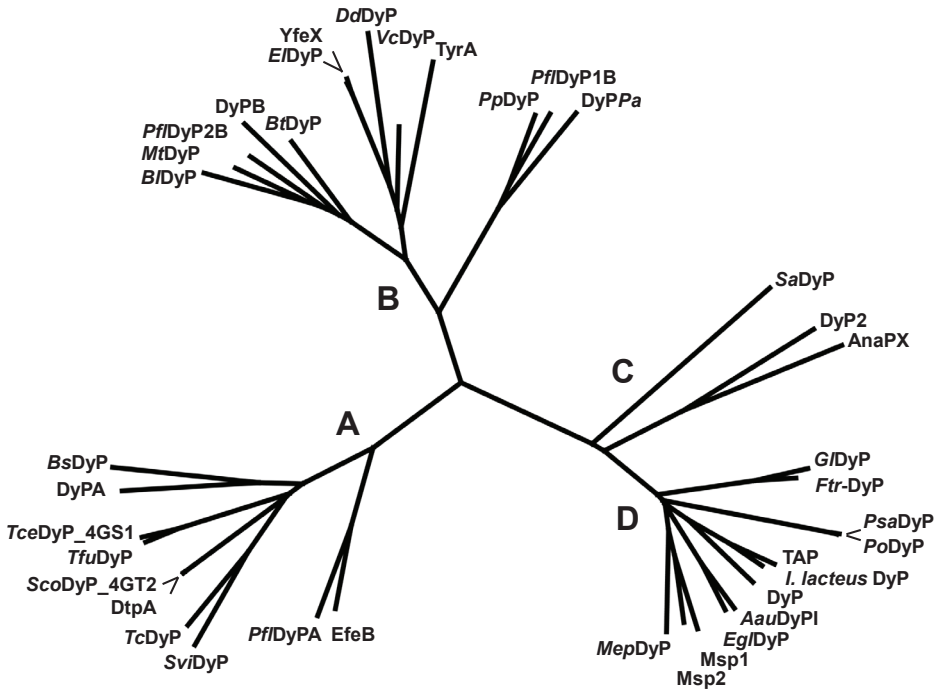

Figure 1. Phylogenetic tree of the DyP-type peroxidases characterized thus far (February 2018). The sequence alignment and phylogenetic tree were made by Geneious version 8.1.9 using ClustalW alignment with GONNET as cost matrix for the sequence alignment and neighbor-joining as tree build method.

Table 1. DyP-type peroxidases characterized thus far (February 2018).

Class	Protein name	Organism	PDB	UniProtKB code	Ref.
A	*Bs*DyP/YwbN	*Bacillus subtilis*		P39597	[12,20]
	DtpA	*Streptomyces lividans* TK24	5MJH	A0A076MAJ9	[21,22]
	DypA	*Pseudomonas fluorescens Pf-5*		Q4KBM1	[17]
	DypA	*Rhodococcus jostii* RHA1		Q0S4I5	[16]
	EfeB/YcdB	*Escherichia coli* O157	2Y4F	P31545	[23]
	*Svi*DyP	*Saccharomonospora viridis* DSM 43017		C7MS11	[24]
	*Tc*DyP	*Thermomonospora curvata*	5JXU	D1A807	[25]
	*Tfu*DyP	*Thermobifida fusca* YX	5FW4	Q47KB1	[14,26]
	(*Sco*DyP)	*Streptomyces coelicolor* ATCC BAA-471	4GT2	Q9ZBW9	-
	(*Tce*DyP)	*Thermobifida cellulosilityica*	4GS1	U3KRF5	-
B	*Bl*DyP	*Brevibacterium linens* M18		NCBI ref: WP_101555111.1	[27]
	*Bt*DyP	*Bacteriodes thetaiotaomicron*	2GVK	Q8A8E8	[28]
	*Dd*DyP	*Dictyostelium discoideum*		Q556V8	[29]
	DypB	*Rhodococcus jostii* RHA1	3QNR	Q0SE24	[16,30]
	Dyp1B	*Pseudomonas fluorescens Pf-5*		Q4KAC6	[17]
	Dyp2B	*Pseudomonas fluorescens Pf-5*		Q4KA97	[17]
	DyP*Pa*	*Pseudomonas aeruginosa* PKE117		D5LRR6	[31]
	*El*DyP	*Enterobacter lignolyticus*	5VJ0	E3G9I4	[32]
	*Mt*DyP	*Mycobacterium tuberculosis* H37Rv		I6Y4U9	[33]
	*Pp*DyP	*Pseudomonas putida*		Q88HV5	[34]
	TyrA	*Shewanella oneidensis*	2HAG	Q8EIU4	[28,35]
	*Vc*DyP	*Vibrio cholerae*	5DE0	Q9KQ59	[36]
	YfeX	*Escherichia coli* O157:H7 str. Sakai	5GT2	P76536	[37]
C	AnaPX	*Anabaena sp.* PCC 7120	5C2I	Q8YWM0	[38]
	DyP2	*Amycolatopsis sp.* 75iv2	4G2C	K7N5M8	[39]
	*Sa*DyP2	*Streptomyces avermitilis*		Q82HB1	[40]
D	*Aau*DyPI/AjPI	*Auricularia auricula-judae*	4AU9	I2DBY1	[41,42]
	DyP/*Bad*DyP	*Bjerkandera adusta* Dec 1	2D3Q	Q8WZK8	[6,43]
	*Egl*DyP	*Exidia glandulosa*		I2DBY2	[44]
	Ftr-DyP	*Funalia trogii (Coriolopsis trogii)*		GenBank: AUW34346.1	[45]
	*Gl*DyP	*Ganoderma lucidum*		G0X8C9	[46]
	I. lacteus DyP	*Irpex lacteus*		A0A1R7T0P5	[47]
	*Mep*DyP	*Mycena epipterygia*		I2DBY3	[44]
	MsP1/*Msc*DyP1	*Mycetinis scorodonius*		B0BK71	[48]
	MsP2/*Msc*DyP2	*Mycetinis scorodonius*		B0BK72	[48]
	*Po*DyP	*Pleurotus ostreatus*		Q0VTU1	[49]
	(r)*Psa*DyP	*Pleurotus sapidus*		A0A0F7VJ89	[50]
	TAP	*Termitomyces albuminosus*		Q8NKF3	[51]

DyP-type peroxidases are unrelated at the primary sequence level to peroxidases of the plant and animal superfamilies. They also lack the typical heme-binding motif of plant peroxidases, comprising one proximal histidine, one distal histidine, and one crucial arginine (Fig. 2).[3,5,7] Moreover, DyP-type peroxidases and plant peroxidases both bind the heme cofactor non-covalently, unlike animal peroxidases, which bind the heme cofactor covalently (Fig. 2).[52] All DyP-type peroxidases contain the so-called GXXDG-motif in their primary sequence, which is part of the heme-binding region. This motif is important for peroxidase activity because replacement of the conserved aspartate by an alanine or asparagine inactivates the enzyme, while heme-binding is not affected.[14,43] Based on these results, it was proposed that the conserved aspartate of the GXXDG-motif is functionally similar to the distal histidine of plant peroxidases.[1,3] However, the catalytic role of this conserved aspartate was put into question by a recent study. It was shown that substitution of the aspartate of the GXXDG-motif of *Escherichia coli* EfeB/YcdB by an asparagine only marginally affected the peroxidase activity of this enzyme.[23]

A limited number of fungal and bacterial DyP-type peroxidases have been characterized in some detail, including elucidation of their crystal structures, see Table 1. While DyP-type peroxidases from the different subclasses often exhibit a remarkable low sequence similarity, their overall structural topology is highly conserved. Structurally, DyP-type peroxidases comprise two domains that contain α-helices and anti-parallel β-sheets, unlike plant and mammalian peroxidases that are primarily α-helical proteins (Fig. 2).[5,52] Both domains in DyP-type peroxidases adopt a unique ferredoxin-like fold and form an active site crevice with the heme cofactor sandwiched in between. The heme-binding motif contains a highly conserved histidine in the C-terminal domain of the enzyme (Fig. 2), which seems to be an important heme ligand and is therefore functionally similar to the proximal histidine of plant peroxidases.[23,28,35,43] To test the role of the proximal histidine of DyP-type peroxidases as a heme ligand, we replaced this residue by an alanine in *Tfu*DyP from *Thermobifida fusca*. This resulted in a loss of heme, which demonstrates that this residue is indeed an important heme ligand of DyP-type peroxidases.[14] In addition, fungal DyP-type peroxidases also contain a conserved histidine in the N-terminal domain of the enzyme, which was previously assigned as heme ligand.[53] However, this residue does not contribute to heme binding according to the available structures.[43] Clearly, more structural studies are required to unveil the molecular details by which DyP-type peroxidases catalyze oxidations.

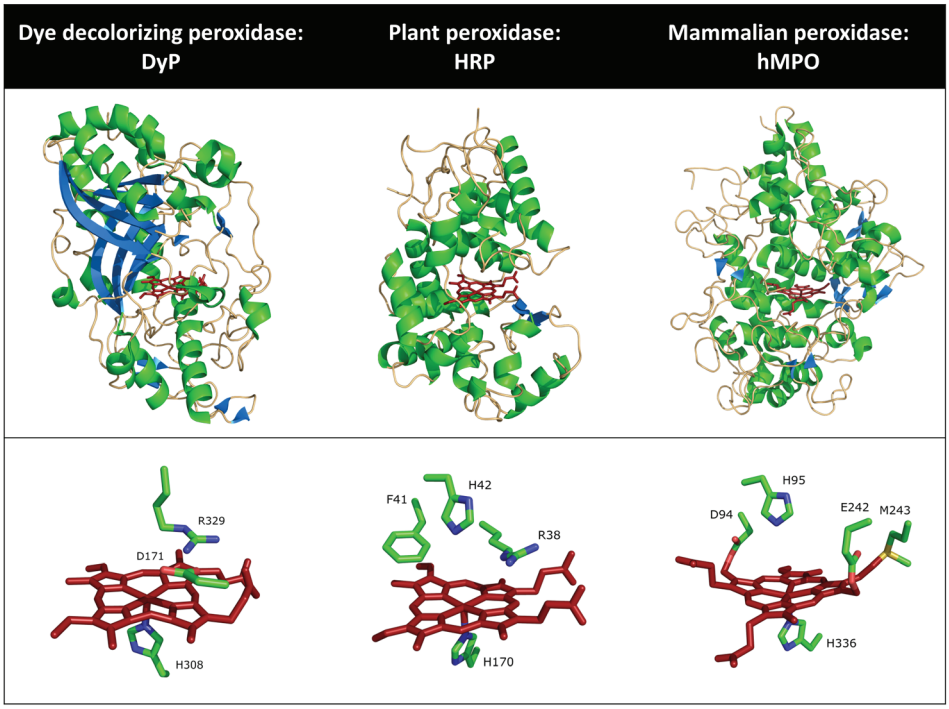

Figure 2. Structural comparison of DyP from *Bjerkandera adusta* Dec1 (a), HRP from *Armoracia rusticana* (b) and human myeloperoxidase (hMPO) from *Homo sapiens* (c). α-helices are shown in green, β sheets are in blue, and the heme cofactor is in red. Close-up of key amino acids in the heme-surrounding region of DyP (d), HRP (e) and human myeloperoxidase (f). The proximal histidine of DyP (His308) and both the distal and proximal histidines of HRP (His42 and His170) and human myeloperoxidase (His95 and His336) are indicated, as well as catalytically important residues of DyP (e.g., Asp171 and Arg329) and HRP (e.g., Arg38 and Phe41). Heme is covalently bound by Asp94, Glu242, and Met243 in human myeloperoxidase. PDB files used: DyP, 2D3Q; HRP, 1ATJ; hMPO, 1CXP.

Biochemical properties

The biochemical properties of about forty DyP-type peroxidases of fungal and bacterial origin have been analyzed thus far. These enzymes are typically 50-60 kDa, while several bacterial variants are somewhat smaller (about 40 kDa). All characterized DyP-type peroxidases contain non-covalently bound heme (protoheme IX) as cofactor.[13,14,28,31,35,43] In addition, several oligomeric states have been reported, ranging from monomers to hexamers.[6,13,14,23,28,35,38] It has been well established that the catalytic mechanism of plant and animal peroxidases proceeds via formation of compound I. This is the first (high-oxidation) intermediate in the reaction cycle of peroxidases and is formed by a reaction between H_2O_2 and the Fe(III) resting state of the enzyme. It is therefore generally

assumed that this is also the case for DyP-type peroxidases. Although the exact details about their catalytic cycle are still unclear, several recent studies point towards major differences between the catalytic mechanism of DyP-type peroxidases and other peroxidases. Based on four novel structures of a fungal DyP, it was proposed that the aspartate of the GXXDG-motif functions as acid-base catalyst and swings into a proper position that is optimal for interaction with H_2O_2.[54] The aspartate catalyzes compound I formation through the transfer of a proton from the proximal to the distal oxygen atom of H_2O_2, hence facilitating heterolytic cleavage of the O-O bond of H_2O_2 (Fig. 3). Compound I, an oxoferryl iron with a porphyrin-based cation radical, reacts consecutively with two equivalents of substrate to return via compound II (Fe(IV)) back to the resting state of the heme (compound 0, Fe(III)). This crucial role of the conserved aspartate as a catalytic residue agrees well with the results of the mutagenesis studies on a fungal and a bacterial DyP as discussed above. However, it is in contrast to, for example, plant peroxidases where the distal histidine functions as an acid-base catalyst and compound I formation is assisted by an essential arginine (Fig. 2).[5] Furthermore, analysis of the peroxidative cycle of DypB from *Rhodococcus jostii* RHA1 established that its conserved aspartate is not required for peroxidase activity because replacement of this residue by alanine had a marginal effect on the reactivity towards H_2O_2 and the formation of compound I. Rather, a conserved arginine of DypB was found to be essential for peroxidase activity.[55] It therefore appears that DyP-type peroxidases employ different residues as acid-base catalyst(s) during their catalytic cycle.

Remarkably, DyP-type peroxidases are able to oxidize substrates that are too large to fit in the active site. DypB, for instance, shows saturation kinetics towards the large molecules of Kraft lignin.[30] Long-range electron transfer (LRET) between the heme cofactor and the surface of DypB was suggested as a potential mechanism. More recently, an LRET pathway to the surface of *Aau*DyPI of *Auricularia auricula-judae* was identified.[42,56] Residues Tyr337 and Leu357 facilitate electron transfer from the heme cofactor of *Aau*DyPI to the surface of this fungal DyP, forming a surface-exposed oxidation site that might react with bulky substrates. Tyr337 is conserved in fungal DyPs. A comparable, but not identical, long-range electron transfer pathway is present in lignin peroxidases (LiP) from the plant superfamily of peroxidases (Fig. 4).[42,57] For instance, in LiP from *Phanerochaete chrysosporium,* a surface-exposed tryptophan was shown to be the interaction site of veratryl alcohol.[58]

The most distinguishing feature of DyP-type peroxidases is their unparalleled catalytic properties. Firstly, these enzymes are active at low pH, which is most likely dictated by the aspartate of the GXXDG-motif that

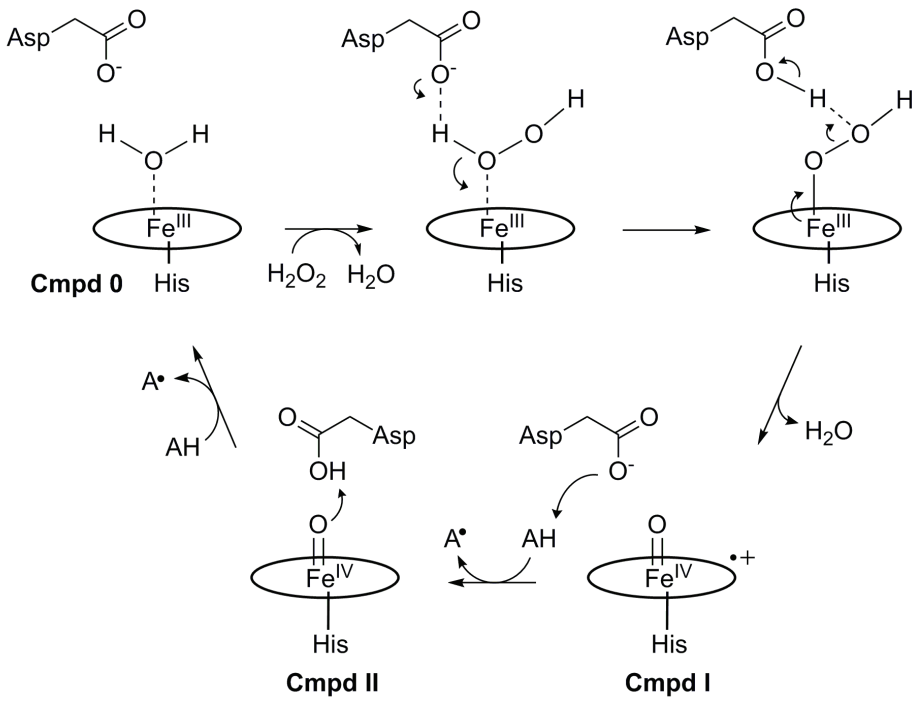

Figure 3. Schematic representation of the proposed mechanism of DyP-type peroxidases in case substrate AH reacts with the heme directly. Aspartate is shown as acid-base catalyst for the formation of compound I, oxoferryl iron with a porphyrin-based cation radical. Compound I will be reduced in two one-electron reductions back to the resting state, thereby forming two substrate radicals.

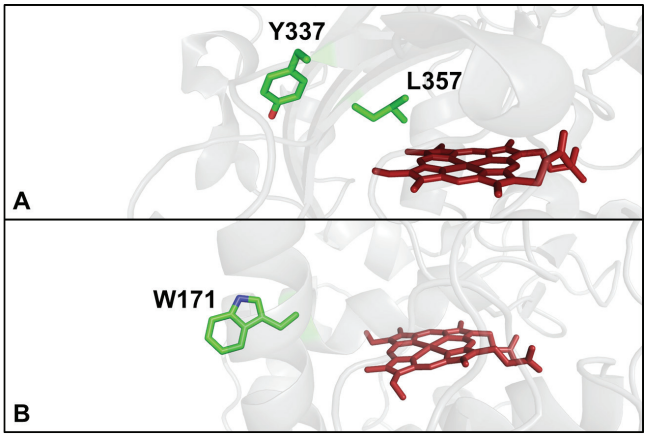

Figure 4. Structural comparison of the residues involved in long-range electron transfer of *Aau*DyPI from *Auricularia auricula-judae* (a) and LiP from *Phanerochaete chrysosporium* (b). Residues Tyr337 and Leu357 of AauDyP and Trp171 of LiP are involved in long-range electron transfer. PDB files used: *Aau*DyP, 4AU9 and LiP, 1LLP.

functions as an acid-base catalyst at low pH for at least a subset of DyP-type peroxidases.[14,43] Secondly, DyP-type peroxidases exhibit a unique substrate acceptance profile. These enzymes are able to degrade various dyes efficiently and in particular anthraquinone dyes, which are poorly accepted by plant and animal peroxidases. Furthermore, DyP-type peroxidases display poor activity towards azo dyes and small non-phenolic compounds unlike plant and animal peroxidases.[6,13,61–63,14,16,23,28,35,38,59,60] Moreover, we have relatively recently established that *Tfu*DyP is able to oxidize aromatic sulfides enantioselectively, similar to plant peroxidases, thereby expanding their biocatalytic scope.[5,14,64,65] Intriguingly, DyP-type peroxidases appear to be multifunctional enzymes displaying not only oxidative activity but also hydrolytic activity.[7,48]

Biotechnological potential

Plant peroxidases are attractive biocatalysts because of their broad substrate range, neutral pH optimum, and ability to catalyze reactions such as halogenations, epoxidations, hydroxylations, and enantioselective oxidations, often accompanied with good yields.[66] However, the exploitation of these enzymes is hampered by their notoriously difficult heterologous expression and limited stability. With regards to the latter, it is interesting to note that DyP-type peroxidases appear remarkable robust, as shown by us and others.[14,67,68] Furthermore, our characterization of *Tfu*DyP showed that this enzyme is expressed well heterologously in *E. coli.*[14] Combined, this shows that the bacterial enzymes are a promising alternative for known peroxidases of fungal origin because of the difficulties in fungal genetics and protein expression. The potential of DyP-type peroxidases as useful biocatalysts for industrial applications is further emphasized by their ability to degrade a variety of synthetic dyes, indicating that these enzymes can be used for the bioremediation of dye-contaminated waste water. Moreover, several recent studies showed that DyP-type peroxidases are involved in the biodegradation of lignocellulosic material, which is highly resistant to (bio)chemical degradation. For example, DypB from *Rhodococcus jostii* RHA 1 showed activity towards polymeric lignin as well as lignin model compounds.[16] Additionally, the hydrolytic degradation of wheat straw was increased by external addition of DyP from *Irpex lacteus.*[47] Together, these studies show that DyP-type peroxidases act as ligninolytic enzymes, thereby pointing towards a major role of these enzymes in the microbial degradation of lignin.[19,69] Moreover, it was reported that two fungal DyP-type peroxidases are able to degrade β-carotene.[48] The degradation of β-carotene is of interest for the food industry, enabling the enzymatic whitening of whey-containing foods and beverages. This specific application was patented and the respective fungal DyP-

type peroxidase is marketed under the name MaxiBright by DSM. The discovery of novel antimicrobial targets has become a pressing matter due to the vast increase of antibiotic-resistant, pathogenic bacteria.[70] With regards to this issue, it is important to emphasize that, as noted earlier, DyP-type peroxidases are remarkably abundant in the proteomes of bacteria, including many pathogenic bacteria, while these enzymes are absent in mammals. This indicates that DyP-type peroxidases could be promising, novel anti-microbial (pro)drug targets. This notion is supported by a recent study that showed that a DyP-type peroxidase from *Pseudomonas fluorescens* GcM5-1A is toxic to cells of the Japanese black pine.[71]

Conclusions

The group of DyP-type peroxidases comprises a newly identified superfamily of peroxidases, which are unrelated in sequence and structure to well-known peroxidases belonging to the plant or animal superfamilies. DyP-type peroxidases exhibit unique reaction features by displaying novel substrate specificities and reactivities. Additionally, DyP-type peroxidases can be remarkably robust and combined this unveils their potential use as biocatalysts in a variety of biotechnological applications. However, these enzymes are only active under acidic conditions, which severely restrict their number of applications. It is therefore desirable to alter their pH optimum by enzyme redesign to broaden their applicability. Despite the promising biocatalytic potential of DyP-type peroxidases, much more work is needed to fully characterize the catalytic mechanism of DyP-type peroxidases, their heme biochemistry, as well as the exact role of the catalytic residues and in particular the function of the conserved aspartate. Additional high-resolution structures of DyP-type peroxidases from all the various subclasses are therefore required, preferably in combination with different ligands. The limited number of DyP-type peroxidases characterized so far has established that these enzymes exhibit a vastly different substrate scope than plant and animal peroxidases, using, however, a restricted set of diagnostic substrates. It is therefore desirable that more and diverse substrates should be tested in order to fully understand their biocatalytic scope. Lastly, future studies should be aimed at investigating the potential of DyP-type peroxidases in the biodegradation of lignocellulosic material and as novel microbial (pro)drug targets. In conclusion, it can be expected that the growing number of DyP-type peroxidases biochemically and structurally characterized will fully delineate their biotechnological potential. This will also provide new leads for the construction of improved variants suitable for biotechnological applications.

Aim and outline of the thesis

The research in this thesis was financed by the Netherlands Organization for Scientific Research (NWO) under the graduate program: synthetic biology for advanced metabolic engineering, project number 022.004.006.

The aim of the research presented in this thesis was to broaden the knowledge on class A DyP-type peroxidases. Two DyPs were selected and used as model enzymes: *Tfu*DyP from *Thermobifida fusca* YX and *Svi*DyP from *Saccharomonospora viridis* DSM 43017.[14,24] *Tfu*DyP was isolated and characterized by van Bloois et al as a thermostable, Tat-dependently secreted peroxidase which was easily overexpressed in *Escherichia coli*. The substrate scope of this enzyme covers dyes and monophenolic compounds while it also shows peroxygenase activity in the enantioselective sulfoxidation of aromatic sulfides. *Svi*DyP is homologous to *Tfu*DyP (42% sequence identity) and has the advantage over *Tfu*DyP that it is active at a slightly higher pH range.

DyP-type peroxidases are named after their ability to degraded recalcitrant dyes.[6] Previous studies focused predominantly on anthraquinone and azo dyes.[6,20,38] To study the biotechnological potential of DyP-type peroxidases further, an extensive substrate profiling study was performed with *Tfu*DyP as model enzyme. **Chapter 2** presents the activity of *Tfu*DyP on thirty dyes from seven distinct classes, three natural carotenoids and various lignin model compounds.

For their activity DyP-type peroxidases rely on a tightly bound heme cofactor. On the proximal side of the heme a histidine functions as the fifth ligand of the heme iron, while on the distal side two catalytically important residues are found: an aspartate and an arginine. The oxidation site(s) of small and large substrates are presumably different and not fully understood. Some small compounds are known to enter the heme pocket and react with the heme cofactor directly. Large compounds, e.g. bulky dyes and lignin model compounds, are however too large to enter the active site. **Chapter 3** describes a mutagenesis study on *Tfu*DyP with the aim to identify more catalytically important residues or residues that determine the pH optimum of the enzyme. This chapter presents the effect of mutations in the heme pocket, in the predicted hydrogen peroxide tunnel and close to the surface exposed heme propionate. Some (DyP-type) peroxidases depend on long-range electron transfer for the activity of substrates that are too large to enter the active site. To explore whether *Tfu*DyP is dependent on such a mechanism, mutagenesis was performed on the surface exposed tyrosines and tryptophans. For the biotechnological applicability of DyP-type peroxidases, it would be beneficial to shift the pH optimum for activity

to a more neutral pH range. Mutations that were proven beneficial in shifting the pH optimum of *Pp*DyP and *Bs*DyP were studied in *Tfu*DyP.[72,73]

For the industrial applicability of DyP-type peroxidases a high protein overexpression level is desired. Many DyP-type peroxidases are from bacterial origin and are therefore relatively easily heterologously overexpressed in a bacterial host. This is in stark contrast to peroxidases from eukaryotes. Even though *Tfu*DyP originates from *T. fusca* and is easily overexpressed in *E. coli,* increasing the overexpression level to 200 mg per liter culture broth led to an almost inactive enzyme. **Chapter 4** discusses the correlation between the activity of *Tfu*DyP and the expression level in *E. coli*. Analysis of the protein by UV-vis absorbance spectroscopy and high-resolution mass spectroscopy on the extracted heme cofactor revealed the reason for the inactivity of the enzyme.

Most enzymes in nature are involved in metabolic pathways in which the product of one enzyme is the substrate of another enzyme. In some cases this even led to the fusion of enzymes to bi/multifunctional protein complexes. Peroxidases and oxidases form catalytically logical combinations and are often coexpressed in nature: peroxidases require hydrogen peroxide for their activity, a by-product of the oxidases. **Chapter 5** describes the first successful recombinant expression of artificial oxidase-peroxidase fusion enzymes. We fused *Svi*DyP to four distinct oxidases: alditol oxidase (HotAldO), chitooligosaccharide oxidase (ChitO), eugenol oxidase (EugO) and 5-hydroxymethylfurfural oxidase (HMFO).[74–77] Special attention was paid to exploring the potential applicability of the designed oxidase-peroxidase fusions. Two fusion enzymes were used in one-pot two-step cascade reactions while the other two fusion enzymes could be applied as biosensor for the detection of various sugars.

References

1 Banci L (1997) Structural properties of peroxidases. *J. Biotechnol.* **53**, 253–263.

2 Davies MJ, Hawkins CL, Pattison DI, and Rees MD (2008) Mammalian heme peroxidases: from molecular mechanisms to health implications. *Antioxid. Redox Signal.* **10**, 1199–1234.

3 Passardi F, Cosio C, Penel C, and Dunand C (2005) Peroxidases have more functions than a Swiss army knife. *Plant Cell Rep.* **24**, 255–265.

4 Welinder KG, Mauro JM, and Norskov-Lauritsen L (1992) Structure of plant and fungal peroxidases. *Biochem. Soc. Trans.* **20**, 377–340.

5 Veitch NC (2004) Horseradish peroxidase: A modern view of a classic enzyme. *Phytochemistry* **65**, 249–259.

6 Kim SJ and Shoda M (1999) Purification and characterization of a novel peroxidase from *Geotrichum candidum* Dec 1 involved in decolorization of dyes. *Appl. Environ. Microbiol.* **65**, 1029–1035.

7 Sugano Y (2009) DyP-type peroxidases comprise a novel heme peroxidase family. *Cell. Mol. Life Sci.* **66**, 1387–1403.

8 Finn RD, Attwood TK, Babbitt PC, Bateman A, Bork P, Bridge AJ, Chang H-Y, Dosztányi Z, El-Gebali S, Fraser M, Gough J, Haft D, Holliday GL, Huang H, Huang X, Letunic I, Lopez R, Lu S, Marchler-Bauer A, Mi H, Mistry J, Natale DA, Necci M, Nuka G, Orengo CA, Park Y, Pesseat S, Piovesan D, Potter SC, Rawlings ND, Redaschi N, Richardson L, Rivoire C, Sangrador-Vegas A, Sigrist C, Sillitoe I, Smithers B, Squizzato S, Sutton G, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Xenarios I, Yeh L-S, Young S-Y, and Mitchell AL (2017) InterPro in 2017 - beyond protein family and domain annotations. *Nucleic Acids Res.* **45**, D190–D199.

9 Fawal N, Li Q, Savelli B, Brette M, Passaia G, Fabre M, Mathé C, and Dunand C (2013) PeroxiBase: a database for large-scale evolutionary analysis of peroxidases. *Nucleic Acids Res.* **41**, D441–D444.

10 Yoshida T and Sugano Y (2015) A structural and functional perspective of DyP-type peroxidase family. *Arch. Biochem. Biophys.* **574**, 49–55.

11 Singh R and Eltis LD (2015) The multihued palette of dye-decolorizing peroxidases. *Arch. Biochem. Biophys.* **574**, 56–65.

12 Jongbloed JDH, Grieger U, Antelmann H, Hecker M, Nijland R, Bron S, and van Dijl JM (2004) Two minimal Tat translocases in *Bacillus*. *Mol. Microbiol.* **54**, 1319–1325.

13 Sturm A, Schierhorn A, Lindenstrauss U, Lilie H, and Brüser T (2006) YcdB from *Escherichia coli* reveals a novel class of Tat-dependently translocated hemoproteins. *J. Biol. Chem.* **281**, 13972–13978.

14 van Bloois E, Torres Pazmiño DE, Winter RT, and Fraaije MW (2010) A robust and extracellular heme-containing peroxidase from *Thermobifida fusca* as prototype of a bacterial peroxidase superfamily. *Appl. Microbiol. Biotechnol.* **86**, 1419–1430.

15 Adav SS, Ng CS, Arulmani M, and Sze SK (2010) Quantitative iTRAQ secretome analysis of cellulolytic *Thermobifida fusca*. *J. Proteome Res.* **9**, 3016–3024.

16 Ahmad M, Roberts JN, Hardiman EM, Singh R, Eltis LD, and Bugg TDH (2011) Identification of DypB from *Rhodococcus jostii* RHA1 as a lignin peroxidase. *Biochemistry* **50**, 5096–5107.

17 Rahmanpour R and Bugg TDH (2015) Characterisation of Dyp-type peroxidases from *Pseudomonas fluorescens* Pf-5: Oxidation of Mn(II) and polymeric lignin by Dyp1B. *Arch. Biochem. Biophys.* **574**, 93–98.

18 Rahmanpour R, Rea D, Jamshidi S, Fülöp V, and Bugg TDH (2016) Structure of *Thermobifida fusca* DyP-type peroxidase and activity towards Kraft lignin and lignin model compounds. *Arch. Biochem. Biophys.* **594**, 54–60.

19 de Gonzalo G, Colpa DI, Habib MHM, and Fraaije MW (2016) Bacterial enzymes involved in lignin degradation. *J. Biotechnol.* **236**, 110–119.

20 Santos A, Mendes S, Brissos V, and Martins LO (2014) New dye-decolorizing peroxidases from

Bacillus subtilis and *Pseudomonas putida* MET94: towards biotechnological applications. *Appl. Microbiol. Biotechnol.* **98**, 2053–2065.

21 Kekilli D, Moreno-Chicano T, Chaplin AK, Horrell S, Dworkowski FSN, Worrall JAR, Strange RW, and Hough MA (2017) Photoreduction and validation of haem-ligand intermediate states in protein crystals by *in situ* single-crystal spectroscopy and diffraction. *IUCrJ* **4**, 263–270.

22 Petrus MLC, Vijgenboom E, Chaplin AK, Worrall JAR, van Wezel GP, and Claessen D (2016) The DyP-type peroxidase DtpA is a Tat-substrate required for GlxA maturation and morphogenesis in *Streptomyces*. *Open Biol.* **6**, 150149.

23 Liu X, Du Q, Wang Z, Zhu D, Huang Y, Li N, Wei T, Xu S, and Gu L (2011) Crystal structure and biochemical features of EfeB/YcdB from *Escherichia coli* O157: Asp235 plays divergent roles in different enzyme-catalyzed processes. *J. Biol. Chem.* **286**, 14922–14931.

24 Yu W, Liu W, Huang H, Zheng F, Wang X, Wu Y, Li K, Xie X, and Jin Y (2014) Application of a novel alkali-tolerant thermostable DyP-type peroxidase from *Saccharomonospora viridis* DSM 43017 in biobleaching of Eucalyptus Kraft pulp. *PLoS One* **9**, e110319.

25 Shrestha R, Chen X, Ramyar KX, Hayati Z, Carlson EA, Bossmann SH, Song L, Geisbrecht B V, and Li P (2016) Identification of surface-exposed protein radicals and a substrate oxidation site in A-class dye-decolorizing peroxidase from *Thermomonospora curvata*. *ACS Catal.* **6**, 8036–8047.

26 Rahmanpour R, Rea D, Jamshidi S, Fülöp V, and Bugg TDH (2016) Structure of *Thermobifida fusca* DyP-type peroxidase and activity towards Kraft lignin and lignin model compounds. *Arch. Biochem. Biophys.* **594**, 54–60.

27 Sutter M, Boehringer D, Gutmann S, Günther S, Prangishvili D, Loessner MJ, Stetter KO, Weber-Ban E, and Ban N (2008) Structural basis of enzyme encapsulation into a bacterial nanocompartment. *Nat. Struct. Mol. Biol.* **15**, 939–947.

28 Zubieta C, Krishna SS, Kapoor M, Kozbial P, McMullan D, Axelrod HL, Miller MD, Abdubek P, Ambing E, Astakhova T, Carlton D, Chiu H-J, Clayton T, Deller MC, Duan L, Elsliger M-A, Feuerhelm J, Grzechnik SK, Hale J, Hampton E, Han GW, Jaroszewski L, Jin KK, Klock HE, Knuth MW, Kumar A, Marciano D, Morse AT, Nigoghossian E, Okach L, Oommachen S, Reyes R, Rife CL, Schimmel P, van den Bedem H, Weekes D, White A, Xu Q, Hodgson KO, Wooley J, Deacon AM, Godzik A, Lesley SA, and Wilson IA (2007) Crystal structure of two novel dye-decolorizing peroxidases revel a β-barrel fold with a conserved heme-binding motif. *Proteins* **69**, 223–233.

29 Rai A, Fedorov R, and Manstein DJ (2014) Expression, purification and crystallization of a dye-decolourizing peroxidase from *Dictyostelium discoideum*. *Acta Crystallogr. Sect. FStructural Biol. Commun.* **70**, 252–255.

30 Roberts JN, Singh R, Grigg JC, Murphy MEP, Bugg TDH, and Eltis LD (2011) Characterization of dye-decolorizing peroxidases from *Rhodococcus jostii* RHA1. *Biochemistry* **50**, 5108–5119.

31 Li J, Liu C, Li B, Yuan H, Yang J, and Zheng B (2012) Identification and molecular characterization of a novel DyP-type peroxidase from *Pseudomonas aeruginosa* PKE117. *Appl. Biochem. Biotechnol.* **166**, 774–785.

32 Shrestha R, Huang G, Meekins DA, Geisbrecht B V, and Li P (2017) Mechanistic insights into dye-decolorizing peroxidase revealed by solvent isotope and viscosity effects. *ACS Catal.* **7**, 6352–6364.

33 Contreras H, Joens MS, McMath LM, Le VP, Tullius M V, Kimmey JM, Bionghi N, Horwitz MA, Fitzpatrick JAJ, and Goulding CW (2014) Characterization of a *Mycobacterium tuberculosis* nanocompartment and its potential cargo proteins. *J. Biol. Chem.* **289**, 18279–18289.

34 Sezer M, Santos A, Kielb P, Pinto T, Martins LO, and Todorovic S (2013) Distinct structural and redox properties of the heme active site in bacterial dye decolorizing peroxidase-type peroxidases from two subfamilies: Resonance raman and electrochemical study. *Biochemistry* **52**, 3074–3084.

35 Zubieta C, Joseph R, Krishna SS, McMullan D, Kapoor M, Axelrod HL, Miller MD, Abdubek P, Acosta C, Astakhova T, Carlton D, Chiu H-J, Clayton T, Deller MC, Duan L, Elias Y, Elsliger M-A, Feuerhelm J, Grzechnik SK, Hale J, Han GW, Jaroszewski L, Jin KK, Klock HE, Knuth MW, Kozbial P, Kumar A, Marciano D, Morse AT, Murphy KD, Nigoghossian E, Okach L, Oommachen S, Reyes R, Rife CL, Schimmel P, Trout C V., van den Bedem H, Weekes D, White A, Xu Q, Hodgson KO, Wooley J, Deacon AM, Godzik A, Lesley SA, and Wilson IA (2007) Identification and structural characterization of heme binding in a novel dye-decolorizing peroxidase, TyrA. *Proteins* **69**, 234–243.

36 Uchida T, Sasaki M, Tanaka Y, and Ishimori K (2015) A dye-decolorizing peroxidase from *Vibrio cholerae*. *Biochemistry* **54**, 6610–6621.

37 Liu X, Yuan Z, Wang J, Cui Y, Liu S, Ma Y, Gu L, and Xu S (2017) Crystal structure and biochemical features of dye-decolorizing peroxidase YfeX from *Escherichia coli* O157 Asp^{143} and Arg^{232} play divergent roles toward different substrates. *Biochem. Biophys. Res. Commun.* **484**, 40–44.

38 Ogola HJO, Kamiike T, Hashimoto N, Ashida H, Ishikawa T, Shibata H, and Sawa Y (2009) Molecular characterization of a novel peroxidase from the cyanobacterium *Anabaena* sp. strain PCC 7120. *Appl. Environ. Microbiol.* **75**, 7509–7518.

39 Brown ME, Barros T, and Chang MC (2012) Identification and characterization of a multifunctional dye peroxidase from a lignin-reactive bacterium. *ACS Chem. Biol.* **7**, 2074–2081.

40 Sugawara K, Nishihashi Y, Narioka T, Yoshida T, Morita M, and Sugano Y (2017) Characterization of a novel DyP-type peroxidase from *Streptomyces avermitilis*. *J. Biosci. Bioeng.* **123**, 425–430.

41 Liers C, Bobeth C, Pecyna M, Ullrich R, and Hofrichter M (2010) DyP-like peroxidases of the jelly fungus *Auricularia auricula-judae* oxidize nonphenolic lignin model compounds and high-redox potential dyes. *Appl. Microbiol. Biotechnol.* **85**, 1869–1879.

42 Strittmatter E, Liers C, Ullrich R, Wachter S, Hofrichter M, Plattner DA, and Piontek K (2013) First crystal structure of a fungal high-redox potential dye-decolorizing peroxidase substrate interaction sites and long-range electron transfer. *J. Biol. Chem.* **288**, 4095–4102.

43 Sugano Y, Muramatsu R, Ichiyanagi A, Sato T, and Shoda M (2007) DyP, a unique dye-decolorizing peroxidase, represents a novel heme peroxidase family: ASP171 replaces the distal histidine of classical peroxidases. *J. Biol. Chem.* **282**, 36652–36658.

44 Liers C, Pecyna MJ, Kellner H, Worrich A, Zorn H, Steffen KT, Hofrichter M, and Ullrich R (2013) Substrate oxidation by dye-decolorizing peroxidases (DyPs) from wood- and litter-degrading agaricomycetes compared to other fungal and plant heme-peroxidases. *Appl. Microbiol. Biotechnol.* **97**, 5839–5849.

45 Kolwek J, Behrens C, Linke D, Krings U, and Berger RG (2018) Cell-free one-pot conversion of (+)-valencene to (+)-nootkatone by a unique dye-decolorizing peroxidase combined with a laccase from *Funalia trogii*. *J. Ind. Microbiol. Biotechnol.* **45**, 89–101.

46 Kung CP, Wu YR, and Chuang HW (2014) Expression of a dye-decolorizing peroxidase results in hypersensitive response to cadmium stress through reducing the ROS signal in Arabidopsis. *Environ. Exp. Bot.* **101**, 47–55.

47 Salvachúa D, Prieto A, Martínez ÁT, and Martínez MJ (2013) Characterization of a novel dye-decolorizing peroxidase (DyP)-type enzyme from *Irpex lacteus* and its application in enzymatic hydrolysis of wheat straw. *Appl. Environ. Microbiol.* **79**, 4316–4324.

48 Scheibner M, Hülsdau B, Zelena K, Nimtz M, de Boer L, Berger RG, and Zorn H (2008) Novel peroxidases of *Marasmius scorodonius* degrade β-carotene. *Appl. Microbiol. Biotechnol.* **77**, 1241–1250.

49 Faraco V, Piscitelli A, Sannia G, and Giardina P (2007) Identification of a new member of the dye-decolorizing peroxidase family from *Pleurotus ostreatus*. *World J. Microbiol. Biotechnol.* **23**, 889–893.

50 Lauber C, Schwarz T, Nguyen QK, Lorenz P, and Lochnit G (2017) Identification, heterologous expression and characterization of a dye-decolorizing peroxidase of *Pleurotus sapidus*. *AMB Express* **7**, 164.

51 Johjima T, Ohkuma M, and Kudo T (2003) Isolation and cDNA cloning of novel hydrogen peroxide-dependent phenol oxidase from the basidiomycete *Termitomyces albuminosus*. *Appl. Microbiol. Biotechnol.* **61**, 220–225.

52 Zamocky M, Jakopitsch C, Furtmüller PG, Dunand C, and Obinger C (2008) The peroxidase-cyclooxygenase superfamily: reconstructed evolution of critical enzymes of the innate immune system. *Proteins* **72**, 589–605.

53 Sugano Y, Ishii Y, and Shoda M (2004) Role of H164 in a unique dye-decolorizing heme peroxidase DyP. *Biochem. Biophys. Res. Commun.* **322**, 126–132.

54 Yoshida T, Tsuge H, Konno H, Hisabori T, and Sugano Y (2011) The catalytic mechanism of dye-decolorizing peroxidase DyP may require the swinging movement of an aspartic acid residue. *FEBS J.* **278**, 2387–2394.

55 Singh R, Grigg JC, Armstrong Z, Murphy MEP, and Eltis LD (2012) Distal heme pocket residues of B-type dye-decolorizing peroxidase: Arginine but not aspartate is essential for peroxidase activity. *J. Biol. Chem.* **287**, 10623–10630.

56 Strittmatter E, Wachter S, Liers C, Ullrich R, Hofrichter M, Plattner DA, and Piontek K (2013) Radical formation on a conserved tyrosine residue is crucial for DyP activity. *Arch. Biochem. Biophys.* **537**, 161–167.

57 Choinowski T, Blodig W, Winterhalter KH, and Piontek K (1999) The crystal structure of lignin peroxidase at 1.70 A resolution reveals a hydroxy group on the Cβ of tryptophan 171: a novel radical site formed during the redox cycle. *J. Mol. Biol.* **286**, 809–827.

58 Doyle WA, Blodig W, Veitch NC, Piontek K, and Smith AT (1998) Two substrate interaction sites in lignin peroxidase revealed by site-directed mutagenesis. *Biochemistry* **37**, 15097–15105.

59 Burner U, Krapfenbauer G, Furtmüller PG, Regelsberger G, and Obinger C (2000) Oxidation of hydroquinone, 2,3-dimethylhydroquinone and 2,3,5-trimethylhydroquinone by human myeloperoxidase. *Redox Rep.* **5**, 185–190.

60 Reszka KJ, McCormick ML, and Britigan BE (2001) Peroxidase- and nitrite-dependent metabolism of the anthracycline anticancer agents daunorubicin and doxorubicin. *Biochemistry* **40**, 15349–15361.

61 Reszka KJ, Wagner BA, Burns CP, and Britigan BE (2005) Effects of peroxidase substrates on the Amplex red/peroxidase assay: Antioxidant properties of anthracyclines. *Anal. Biochem.* **342**, 327–337.

62 Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. *Appl. Microbiol. Biotechnol.* **56**, 69–80.

63 Chen H (2006) Recent advances in azo dye degrading enzyme research. *Curr. Protein Pept. Sci.* **7**, 101–111.

64 Klibanov AM (2003) Asymmetric enzymatic oxidoreductions in organic solvents. *Curr. Opin. Biotechnol.* **14**, 427–431.

65 van Rantwijk F and Sheldon RA (2000) Selective oxygen transfer catalysed by heme peroxidases: synthetic and mechanistic aspects. *Curr. Opin. Biotechnol.* **11**, 554–564.

66 Regalado C, García-Almendárez BE, and Duarte-Vázquez MA (2004) Biotechnological applications of peroxidases. *Phytochem. Rev.* **3**, 243–256.

67 Liers C, Ullrich R, Hofrichter M, Minibayeva F V, and Beckett RP (2011) A heme peroxidase of the ascomyceteous lichen *Leptogium saturninum* oxidizes high-redox potential substrates. *Fungal Genet. Biol.* **48**, 1139–1145.

68 Pühse M, Szweda RT, Ma Y, Jeworrek C, Winter R, and Zorn H (2009) *Marasmius scorodonius* extracellular dimeric peroxidase - Exploring its temperature and pressure stability. *Biochim. Biophys. Acta* **1794**, 1091–1098.

69 Bugg TDH, Ahmad M, Hardiman EM, and Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. *Curr. Opin. Biotechnol.* **22**, 394–400.
70 Amini S and Tavazoie S (2011) Antibiotics and the post-genome revolution. *Curr. Opin. Microbiol.* **14**, 513–518.
71 Kong L, Guo D, Zhou S, Yu X, Hou G, Li R, and Zhao B (2010) Cloning and expression of a toxin gene from *Pseudomonas fluorescens* GcM5-1A. *Arch. Microbiol.* **192**, 585–593.
72 Mendes S, Catarino T, Silveira C, Todorovic S, and Martins LO (2015) The catalytic mechanism of A-type dye-decolourising peroxidase BsDyP: neither aspartate nor arginine is individually essential for peroxidase activity. *Catal. Sci. Technol.* **5**, 5196–5207.
73 Mendes S, Brissos V, Gabriel A, Catarino T, Turner DL, Todorovic S, and Martins LO (2015) An integrated view of redox and catalytic properties of B-type PpDyP from *Pseudomonas putida* MET94 and its distal variants. *Arch. Biochem. Biophys.* **574**, 99–107.
74 Winter RT, Heuts DPHM, Rijpkema EMA, van Bloois E, Wijma HJ, and Fraaije MW (2012) Hot or not? Discovery and characterization of a thermostable alditol oxidase from *Acidothermus cellulolyticus* 11B. *Appl. Microbiol. Biotechnol.* **95**, 389–403.
75 Ferrari AR, Lee M, and Fraaije MW (2015) Expanding the substrate scope of chitooligosaccharide oxidase from *Fusarium graminearum* by structure-inspired mutagenesis. *Biotechnol. Bioeng.* **112**, 1074–1080.
76 Dijkman WP and Fraaije MW (2014) Discovery and characterization of a 5-hydroxymethylfurfural oxidase from *Methylovorus* sp. strain MP688. *Appl. Environ. Microbiol.* **80**, 1082–1090.
77 Jin J, Mazon H, van den Heuvel RHH, Janssen DB, and Fraaije MW (2007) Discovery of a eugenol oxidase from *Rhodococcus* sp. strain RHA1. *FEBS J.* **274**, 2311–2321.
78 Zámocký M, Hofbauer S, Schaffner I, Gasselhuber B, Nicolussi A, Soudi M, Pirker KF, Furtmüller PG, and Obinger C (2015) Independent evolution of four heme peroxidase superfamilies. *Arch. Biochem. Biophys.* **574**, 108–119.
79 Goblirsch B, Kurker RC, Streit BR, Wilmot CM, and Dubois JL (2011) Chlorite dismutases, DyPs and EfeB: 3 microbial heme enzyme families comprise the CDE structural superfamily. *J. Mol. Biol.* **408**, 379–398.