Appl Biochem Biotechnol (2011) 164:681–713
DOI 10.1007/s12010-011-9167-5

Actinobacterial Peroxidases: an Unexplored Resource for Biocatalysis

Marilize le Roes-Hill · Nuraan Khan · Stephanie Gail Burton

Received: 4 October 2010 / Accepted: 10 January 2011 /
Published online: 30 January 2011

Abstract Peroxidases are redox enzymes that can be found in all forms of life where they play diverse roles. It is therefore not surprising that they can also be applied in a wide range of industrial applications. Peroxidases have been extensively studied with particular emphasis on those isolated from fungi and plants. In general, peroxidases can be grouped into haem-containing and non-haem-containing peroxidases, each containing protein families that share sequence similarity. The order *Actinomycetales* comprises a large group of bacteria that are often exploited for their diverse metabolic capabilities, and with recent increases in the number of sequenced genomes, it has become clear that this metabolically diverse group of organisms also represents a large resource for redox enzymes. It is therefore surprising that, to date, no review article has been written on the wide range of peroxidases found within the actinobacteria. In this review article, we focus on the different types of peroxidases found in actinobacteria, their natural role in these organisms and how they compare with the more well-described peroxidases. Finally, we also focus on work remaining to be done in this research field in order for peroxidases from actinobacteria to be applied in industrial processes.

Keywords Actinobacteria · Biocatalysis · Peroxidases

Introduction

The wide range of peroxidase applications in industrial processes and in the biomedical field is currently dominated by plant and fungal peroxidases (many of which are also commercially available). However, these peroxidases represent only a fraction of the peroxidases found in nature; a large group of peroxidases, those produced by bacteria, have hardly been explored. Relatively little is known about the potential applications and redox

M. le Roes-Hill · N. Khan · S. G. Burton (✉)
Biocatalysis and Technical Biology Research Group, Cape Peninsula University of Technology, Bellville Campus, P.O. Box 1906, Bellville 7535 Cape Town, South Africa
e-mail: burtons@cput.ac.za

potentials of bacterial peroxidases, except for a few descriptive papers and sequences found in databases [1, 2].

The order *Actinomycetales* covers a broad group of microorganisms that are well-known for their applications in the pharmaceutical industry (production of antibiotics and antioxidants), the biocatalysis industry (production of numerous enzymes), in natural processes (carbon cycling) and as pathogens (plant, animal and human, e.g. tuberculosis) [3, 4]. Information on the roles of peroxidases in these organisms is largely speculative, and therefore, their potential for exploitation in industrial processes is, at most, only suggested and requires further study. Some peroxidases have, however, been extensively researched and may be applied in processes such as antibiotic production, organic and polymer synthesis, lignocellulosics degradation and the degradation of xenobiotics. In this review, we explore the different types of peroxidases found in members of the order *Actinomycetales*, with a focus on the genera which have been extensively studied, the natural roles of the peroxidases and their inferred potential biotechnological applications.

Classes and Roles of Peroxidases in Actinobacteria

Classification of Peroxidases

Peroxidases encompass a broad group of enzymes represented in the traditional Enzyme Commission classification system as the group EC 1.11.1.X. Fifteen EC numbers have been assigned to this group (EC 1.11.1.1–EC 1.11.1.16; EC 1.11.1.4 has been removed), but some enzymes that exhibit a dual function, one of which is a peroxidase activity, have also been classified as peroxidases (linoleate diol synthase (EC 1.13.11.44), prostaglandin endoperoxide synthase (EC 1.14.99.1), NAD(P)H oxidase (EC 1.6.3.1) and 4-carboxymuconolactone decarboxylase (EC 4.1.1.44)). Some peroxidase groups, e.g. DyP-type peroxidases, have not yet been assigned an EC number and are generally grouped within the EC 1.11.1.7 group [2]. Peroxidases can also be divided into haem- and non-haem-containing enzymes; both groups are widely distributed in nature and have been isolated and described in animals, plants and microorganisms.

Information regarding the distribution of peroxidases within the order *Actinomycetales* is presented in Table 2 and has been compiled from data collected from literature, the peroxidase database, PeroxiBase [2, 5]; the comprehensive enzyme information system, BRENDA [6] and from genome mining searches of the genome sequences from the NCBI database [7]. A total of 58 searchable genome sequences from the order *Actinomycetales* have been published at the time of writing of this review. These genomes were searched using “catalase” and “perox” as keyword searches. The peroxidase family groups which have been isolated from members of the order *Actinomycetales*, or detected in genome sequences from these organisms, are represented in Fig. 1.

Haem Peroxidases

Haem peroxidases contain a ferriprotoporphyrin IX prosthetic group in their active site (resting state) where the fifth ligand of the iron atom is typically coordinated with a histidine (with the exception of the chloroperoxidase (CPO) of the marine fungus *Calderiomyces fumago*, where the coordination is to a cysteine [8]). The haem acts as a reservoir for electron pooling and provides the necessary redox potential to allow a variety of reactions such as

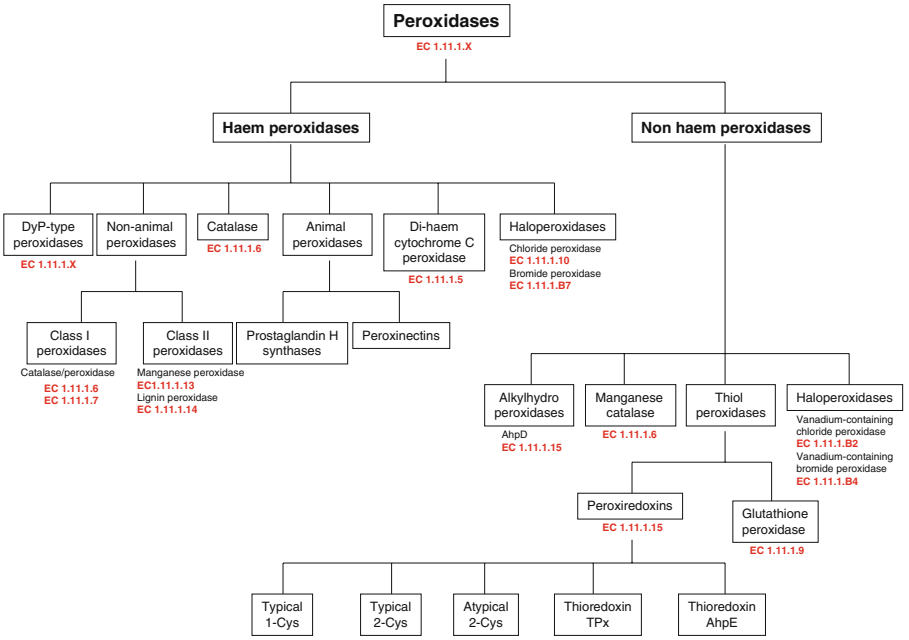


Fig. 1 Known peroxidase families isolated from or detected in the genome sequences of members of the order *Actinomycetales*

oxidation or peroxidation to occur [9]. Haem peroxidases share a low sequence homology (often <20%) but share conserved folding and secondary structure: the haem is creviced in two antiparallel α-helices [10]. Although haem peroxidases have different substrate specificities, they all share the same catalytic cycle (Table 1), and they all exhibit the typical displacement of the Soret band (which typically absorbs at 406–409 nm) when reduced [11].

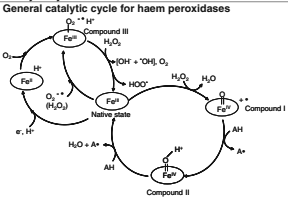
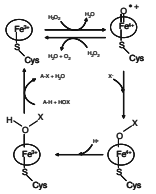
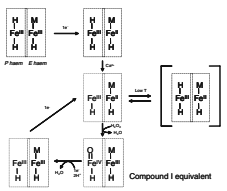
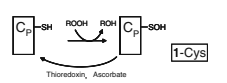
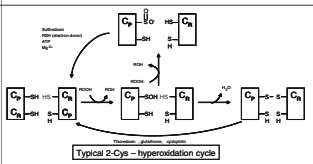
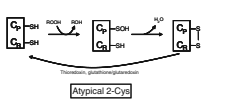
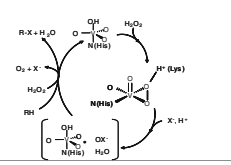
The majority of haem-containing peroxidases undergo a catalytic cycle during which electron transfer results in the reduction of H_2O_2 to water. The native enzyme is converted into an intermediate form (compound I) which holds a Fe^{4+} oxoferryl centre and a porphyrin-based cation radical (oxidised by two electrons from H_2O_2). Compound II is formed when one electron is removed from a substrate by compound I. The subsequent reaction of the native enzyme with the radical converts compound II back to the native enzyme (Table 1 [12]):

$$\text{Resting peroxidase} + H_2O_2 \rightarrow \text{Compound I} + H_2O$$
$$\text{Compound I} + AH_2 \rightarrow \text{Compound II} + AH\bullet$$
$$\text{Compound II} + AH_2 \rightarrow \text{Resting peroxidase} + AH\bullet + H_2O$$

If an excess of H_2O_2 is present, the resting enzyme converts to the compound III form, which is an inactivated form with reduced peroxidase activity [12]. The catalytic cycles of peroxidases are annotated in Table 1.

Based on sequence similarities, haem peroxidases are grouped into two superfamilies: The first consists of bacterial, fungal and plant peroxidases (not found in animals), while the second group consists of peroxidases found in animals, fungi and bacteria [2]. The first group can further be divided into class I (ascorbate peroxidase, cytochrome *c* peroxidase and catalase peroxidase), class II (lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase), class III and a group of other non-animal peroxidases. In addition to the two superfamilies, some smaller protein families fall within the general grouping of haem

Table 1 Catalytic cycles of peroxidases and their role in *Actinomycetales*

Enzyme type	Catalytic cycle	Role in *Actinomycetales*	References
Haem peroxidases • Catalase • Catalase-peroxidase • Lignin peroxidase • Manganese peroxidase • Haloperoxidase • DyP-type peroxidase • Animal peroxidase • Di-haem cytochrome *c* peroxidase	**General catalytic cycle for haem peroxidases**  **Catalytic cycle for haem haloperoxidases** 	**Catalase (EC 1.11.1.6)** • Defence against radical oxygen species generated within their growth environment or by infected hosts (mostly defence against H_2O_2) **Catalase-Aperoxidase (EC 1.11.1.6 a nd EC 1.11.1.7)** • Same function as catalase; essential for host survival in the genus *Mycobacterium* **Lignin peroxidase (EC 1.11.1.14)** • Lignin degradation as part of carbon cycling **Manganese peroxidase (EC 1.11.1.13)** • It is speculated that this enzyme is present in selected actinobacteria and that its role is similar to that of fungal manganese peroxidases, which play an essential role in lignin degradation. Possibly involved in diuron (pesticide) degradation. **Haloperoxidases (EC 1.11.1.10 and EC 1.11.1.B7)** • The role of this peroxidase is inconclusive.	[55–59] [55, 64–66, 68, 70–72] [31, 37–39] [33, 42] [48]
	Catalytic cycle for di-haem cytochrome *c* peroxidases 	**DyP-type peroxidase (EC 1.11.1.X)** • The role in actinobacteria is unknown, but can be used to degrade anthraquinone dyes. **Di-haem cytochrome *c* peroxidase (EC 1.11.1.5)** • The role of this peroxidase is unclear, but may play a role in the removal of H_2O_2 under hypoxia conditions.	[73–75, 77] [78, 79]
Non-haem peroxidases • Peroxiredoxins • Haloperoxidases • Glutathione peroxidase • Manganese catalase • Alkylhydroperoxidases (AhpD)	**Catalytic cycles of peroxiredoxins**    **Catalytic cycle of vanadium haloperoxidases** 	**Peroxiredoxins (EC 1.11.1.15)** • Consists of different types: 1-cysteine, typical 2-cysteine and the atypical 2-cysteine. They play a key role in oxidative stress response, e.g. removal of reactive oxygen and reactive nitrogen species. In conjunction with catalases and glutathione peroxidases, these enzymes are essential for the survival of pathogens within their infected hosts. **Haloperoxidases (EC 1.11.1.B2 and EC 1.11.1.B4)** • The activity of non-haem chloroperoxidases in streptomycetes are linked to proteosome activity, but the general function is unknown. Vanadium-containing haloperoxidases in streptomycetes are speculated to play a role in antibiotic production, but needs to be confirmed. **Glutathione peroxidase (EC 1.11.1.9)** • Similar function to peroxiredoxins. **Manganese catalase (EC 1.11.1.6)** • Same role as classical catalases, but require the presence of manganese to catalyse reactions. **Alkylhydroperoxidase D (EC 1.11.1.15)** • Form part of an antioxidant defence mechanism.	[83, 84, 87–89] [93–95] [59,83,96] [43, 45, 50] [97, 98]

peroxidases: catalase, di-haem cytochrome *c* peroxidase, DyP-type peroxidase, haloperoxidase and NADH peroxidase [2].

Lignin Peroxidase (EC 1.11.1.14)

LiP (non-animal class II haem peroxidase) are among the various enzymes involved in the mineralisation of lignin and catalyse the first step in lignin degradation (depolymerisation). Under nutrient-limited conditions, LiP is secreted along with H_2O_2 to catalyse the depolymerization of lignin. It has been proposed that the enzyme attacks lignin directly by binding to the lignin matrix [13]. To date, this group of peroxidases formally contains only fungal extracellular lignin peroxidases, but there have been several reports on the detection of lignin peroxidases in actinobacteria (see below).

Most actinobacteria that have been found to produce extracellular peroxidases belong to the family *Streptomycetaceae*, and most of the peroxidases produced have been detected in experiments focusing on phenol and/or lignin degradation. A study by Crawford [14] presented the first conclusive evidence that streptomycetes can decompose lignin (with complete mineralisation of lignin to CO_2). Selective isolations have shown that the most active lignocellulose-degrading actinobacteria all belong to the family *Streptomycetaceae*.

Properties of Lignin Peroxidase

Initial oxidation of lignin by actinobacteria appears to involve a peroxidase-based mechanism where peroxidase plays a role in lignin transformation by generating water-soluble polymeric products called acid-precipitable polymeric lignins (APPL) [15–17]. In addition to APPL, several single-ring phenolic compounds are also released during the degradation of lignin by streptomycetes [18]. It is suggested that actinobacteria initially cleave the C_α–C_β and β-O-4 ether linkages within the lignin polymer, increasing the C_α-carbonyl content [16, 19]. The degradation of lignin by actinobacteria occurs during primary growth, in contrast with lignin mineralisation by fungi, which occurs during nutrient limitation (secondary growth). Studies on the growth of actinobacteria on wheat straw showed that the amount of APPLs decreased over time, suggesting that further degradation or modification of the APPLs occurs [20]. Furthermore, all enzymes were found in association with or bound to the APPLs suggesting that, in nature, actinobacterial enzymes are secreted into the soil environment and may be found in association with humic substances. Kirby [21] also noted that while many enzymes from actinobacteria have the ability to degrade lignin, it is still unknown whether this is their primary role in nature.

One of the most extensively studied lignin-degrading actinobacterial peroxidases is produced by *Streptomyces viridosporus* T7A [15, 22–27]. This peroxidase oxidatively cleaves chimeric lignin substructure model compounds into single-ring products [15]. It was noted that APPL-overproducing strains also overproduced peroxidases and cellulases indicating that these enzymes together were involved in lignin degradation [26]. The lignin peroxidase from *S. viridosporus* T7A is an extracellular, haem-containing enzyme of which four isoforms have been identified, with ALiP-P3 designated as the dominant haem protein [15, 22]. ALiP-P3 has been shown to be immunologically related to an extracellular peroxidase from *Streptomyces chromofuscus* A11, but different from the well-known horseradish peroxidase or lignin peroxidase from the fungus *Phanerochaete chrysosporium* [23]. *Thermomonospora mesophila* has a lignin degradation mechanism similar to ALiP-P3 [28], and Adhi et al. [29] found that *Streptomyces badius* produced a LiP similar to ALiP-P3, but with higher peroxidase activity. The lignin-degrading peroxidase from *S. viridosporus* T7A had similar

substrate specificity to fungal manganese peroxidases rather than lignin peroxidase, but did not require exogenous manganese [30]. The N-terminal sequence of this protein shared an 81% identity to 66–76 amino acids of the manganese peroxidase of the ascomycete, *Arthromyces ramosus* [30]. HPLC analysis and inhibition studies confirmed ALiP-P3 as a haem protein [15].

Lignin degradation by other actinobacteria has also been reported. For example, *T. mesophila* and *S. badius* were also found to oxidatively cleave lignins. They were able to use a β-aryl ether dimer as a carbon and energy source to produce monomeric products, and this was suggested to be due to the extracellular peroxidase and catalase activities detected in both strains [31]. Mercer et al. [32] detected extracellular peroxidases in *Streptomyces thermoviolaceus* and *Streptomyces* strain EC22. The study showed that while extracellular peroxidase activity was common among actinobacteria, the highest peroxidase producers were those strains for which there was evidence of lignin degradation. While peroxide-dependent oxidation of veratryl alcohol is frequently used for the detection of LiP in white rot fungi, the extracellular peroxidase produced by actinobacteria is more readily detected using 2,4-dichlorophenol as a substrate. In a recent publication, Niladevi and Prema [33] reported on the rich diversity of actinobacteria in mangrove environments, where 20 strains were screened for their ability to produce lignin-degrading enzymes, but only six isolates were shown to produce lignin peroxidase, as determined by the 2,4-dichlorophenol assay. One strain, *Streptomyces psammoticus*, was reported to produce all three of the major lignin-degrading enzymes (lignin peroxidase, manganese peroxidase and laccase).

Interestingly, several lignin peroxidases have been found in the genome sequences of various *Mycobacterium* species (Table 2), but none has been characterised. Whether these are expressed and what their potential role would be is unclear.

A Case of Mistaken Identity?

Mason et al. [34] investigated whether the peroxidases produced by actinobacteria are indeed extracellular haem peroxidases. There have been extensive studies into the production of peroxidases by actinobacteria which may be involved in lignin degradation/solubilisation: *S. thermoviolaceus*, *S. viridosporus* T7A and *Thermomonospora fusca* BD25 (reclassified as *Thermobifida fusca* [35]). The peroxidases of *S. thermoviolaceus* and *S. viridosporus* were previously classified as haem peroxidases based on their spectral features, but when compared to the lignin peroxidase of *P. chrysosporium*, it was evident that the enzymes do not have the spectral features associated with the changes in the redox state of the haem iron. Furthermore, electron paramagnetic resonance spectroscopy of the peroxidase from *T. fusca* BD25 showed that a non-haem iron and copper are present at the catalytic site [36]. Mason et al. [34] also showed that *T. fusca* secretes a porphyrin which can be mistaken for haem and that the peroxidase activity detected was due to the presence of copper ions. A similar metalloporphyrin has also been detected in the growth medium of *S. viridosporus*, and it was therefore concluded that streptomycetes do not secrete lignin-degrading haem peroxidases. While some research groups have isolated and purified the lignin-degrading proteins in question, especially ALiP-P3, the nature of these enzymes has yet to be clarified.

Role of Lignin Peroxidase

Borgmeyer and Crawford [37] first reported the ability of *S. viridosporus* T7A and *S. badius* 252 to grow on hardwood, softwood and grass lignins, indicating the possible role

Table 2 Distribution of peroxidases within the order *Actinomycetales*

Enzyme	Strain	Information obtained from
Peroxidase (EC 1.11.1.7)	*Streptomyces thermoviolaceus* NCIMB 10076	BRENDA
	Mycobacterium tuberculosis	BRENDA
Catalase (EC 1.11.1.6)	*Acidothermus cellulolyticus* 11B	Genome mining
	Arthrobacter aurescens TC1	Genome mining
	Arthrobacter chlorophenolicus A6	Genome mining
	Beutenbergia cavernae DSM 12333	Genome mining
	Clavibacter michiganensis subsp. *michiganensis*	Genome mining
	Clavibacter michiganensis subsp. *sepedonicus*	Genome mining
	Corynebacterium aurimucosum ATCC 700975	Genome mining
	Corynebacterium diphtheriae NCTC 13129	Genome mining
	Corynebacterium efficiens YS-314	Genome mining
	Corynebacterium glutamicum ATCC 13032	Genome mining
	Corynebacterium jeikeium K411	Genome mining
	Corynebacterium kroppenstedtii DSM 44385	Genome mining
	Corynebacterium urealyticum DSM 7109	Genome mining
	Frankia alni ACN14a	Genome mining
	Frankia sp.	PeroxiBase
	Frankia sp. R43	BRENDA
	Kineococcus radiotolerans SRS30216	Genome mining
	Kocuria rhizophila DC2201	Genome mining
	Leifsonia xyli subsp. *xyli* CTCB07	Genome mining
	Micrococcus luteus	BRENDA
	Micrococcus lysodeikticus	BRENDA
	Mycobacterium avium subsp. *paratuberculosis* K-10	Genome mining; PeroxiBase
	Mycobacterium bovis BCG Pasteur 1173P2	Genome mining

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Mycobacterium bovis subsp. *bovis* AF2122/97	Genome mining
	Mycobacterium gilvum PYR-GCK	Genome mining
	Mycobacterium marinum M	Genome mining
	Mycobacterium smegmatis MC2 155	Genome mining
	Mycobacterium spp. KMS and JLS	Genome mining
	Mycobacterium sp. JC1 DSM 3803	BRENDA
	Mycobacterium tuberculosis	BRENDA
	Mycobacterium ulcerans Agy99	Genome mining
	Mycobacterium vanbaalenii PYR-1	Genome mining
	Nocardia farcinica IFM 10152	Genome mining; PeroxiBase
	Nocardioides sp. JS614	Genome mining
	Propionibacterium acnes KPA171202	Genome mining
	Renibacterium salmoninarum ATCC 33209	Genome mining
	Rhodococcus erythropolis PR4	Genome mining
	Rhodococcus jostii RHA1	Genome mining
	Rhodococcus opacus B4	Genome mining
	Saccharopolyspora erythraea	PeroxiBase
	Streptomyces avermitilis MA-4680	Genome mining
	Streptomyces coelicolor A3(2)	Genome mining; BRENDA
	Streptomyces griseus subsp. *griseus* NBRC 13350	Genome mining
	Thermobifida fusca YX	Genome mining
Catalase peroxidase (EC 1.11.1.6 and 1.11.1.7)	*Arthrobacter chlorophenolicus* A6	Genome mining
	Brevibacterium linens	PeroxiBase
	Frankia sp.	PeroxiBase
	Frankia sp. R43	BRENDA
	Janibacter sp. HTCC 2649	PeroxiBase

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Mycobacterium avium subsp. *Paratuberculosis*	PeroxiBase
	Mycobacterium bovis	PeroxiBase
	Mycobacterium bovis BCG	PeroxiBase
	Mycobacterium flavescens	PeroxiBase
	Mycobacterium fortuitum	PeroxiBase
	Mycobacterium gilvum PYR-GCK	Genome mining
	Mycobacterium intracellulare	PeroxiBase
	Mycobacterium leprae Br4923	Genome mining
	Mycobacterium marinum M	Genome mining
	Mycobacterium smegmatis	PeroxiBase
	Mycobacterium sp.	PeroxiBase
	Mycobacterium sp. strain JC1 DSM 3803	BRENDA
	Mycobacterium spp. KMS, JLS and MCS	Genome mining
	Mycobacterium tuberculosis	PeroxiBase; BRENDA
	Mycobacterium ulcerans Agy99	Genome mining
	Mycobacterium vanbaalenii	PeroxiBase
	Nocardia farcinica	PeroxiBase
	Nocardioides sp.	PeroxiBase
	Rhodococcus erythropolis PR4	Genome mining
	Rhodococcus opacus B4	Genome mining
	Saccharopolyspora erythraea	PeroxiBase
	Salinispora arenicola CNS-205	Genome mining
	Salinispora tropica CNB-440	Genome mining
	Streptomyces coelicolor	PeroxiBase
	Streptomyces griseus subsp. *griseus* NBRC 13350	Genome mining
	Streptomyces lividans	PeroxiBase
	Streptomyces reticuli	PeroxiBase
	Symbiobacterium thermophilum	PeroxiBase
Lignin peroxidase (EC 1.11.1.14)	*Mycobacterium bovis* BCG Pasteur 1173P2 (LipJ)	Genome mining
	Mycobacterium bovis BCG strain Tokyo 172	Genome mining

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Mycobacterium bovis subsp. *bovis* AF2122/97 (LipJ)	Genome mining
	Mycobacterium marinum M (LipJ)	Genome mining
	Mycobacterium sp. MCS (LipJ)	Genome mining
	Mycobacterium tuberculosis F11 (LipJ)	Genome mining
	Mycobacterium tuberculosis H37Ra (LipJ)	Genome mining
	Mycobacterium tuberculosis H37Rv (LipJ)	Genome mining
	Mycobacterium ulcerans Agy99 (LipJ)	Genome mining
	Streptomyces viridosporus strain T7A	BRENDA
Manganese peroxidase (EC 1.11.1.13)	None	
Chloride peroxidase (EC 1.11.1.10)	*Streptomyces toyocaensis*	BRENDA
Bromide peroxidase (haem-containing) (EC 1.11.1.B7)	*Streptomyces phaeochromogenes* (bromoperoxidase/catalase)	BRENDA
DyP-type peroxidase (EC 1.11.1.X)	*Acidothermus cellulolyticus* 11B	Genome mining
	Clavibacter michiganensis subsp. *michiganensis* NCPPB382	Genome mining
	Frankia alni ACN14a	Genome mining
	Frankia sp. Ccl3	Genome mining
	Frankia sp. EAN1pec	Genome mining
	Kineococcus radiotolerans SRS30216	Genome mining
	Kocuria rhizophila DC2201	Genome mining
	Mycobacterium avium 104	Genome mining
	Mycobacterium avium subsp. *paratuberculosis* (type B&C)	Genome mining
	Mycobacterium gilvum PYR-GCK	PeroxiBase
	Mycobacterium marinum M	Genome mining
	Mycobacterium smegmatis (type A)	Genome mining
	Mycobacterium spp. KMS, JLS and MCS	PeroxiBase

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Mycobacterium sp. (type A&C)	Genome mining
	Mycobacterium ulcerans Agy99	PeroxiBase
	Mycobacterium vanbaalenii (type A&C)	Genome mining
	Nocardia farcinica (type A)	PeroxiBase
	Nocardioides sp. JS614	PeroxiBase
	Renibacterium salmoninarum ATCC 33209	Genome mining
	Salinispora arenicola (type A)	Genome mining
	Salinispora tropica (type A)	PeroxiBase
	Streptomyces avermitilis (type C)	PeroxiBase
	Streptomyces griseus subsp. *griseus* NBRC 13350	PeroxiBase
	Streptomyces sviceus ATCC 29083 (type C)	Genome mining
	Thermobifida fusca YX	Genome mining; PeroxiBase
Di-haem cytochrome c peroxidase (EC 1.11.1.5)	*Symbiobacterium thermophilum*	PeroxiBase
No haem, no metal haloperoxidase No haem, vanadium chloroperoxidase (EC 1.11.1.B2—chloride peroxidase and EC 1.11.1.B4—bromide peroxidase)	*Arthrobacter aurescens* TC1—Haloperoxidase	Genome mining
	Arthrobacter chlorophenolicus A6—Haloperoxidase	Genome mining
	Clavibacter michiganensis subsp. *sepedonicus*—non-haem CPO	Genome mining
	Frankia alni ACN14a—non-haem CPO, BPO	Genome mining
	Frankia sp. EAN1pec—non-haem haloperoxidase	Genome mining
	Kineococcus radiotolerans SRS30216—non-haem CPO	Genome mining
	Mycobacterium avium 104—non-haem BPO-A2	Genome mining
	Mycobacterium bovis BCG Pasteur 1173P2—non-haem haloperoxidase, BPOA	Genome mining
	Mycobacterium bovis BCG strain Tokyo 172—non-haem BPOC	Genome mining
	Mycobacterium bovis subsp. *bovis* AF2122/97—non-haem BPO-A1, CPO, haloperoxidase	Genome mining

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Mycobacterium gilvum PYR-GCK—non-haem CPO	Genome mining
	Mycobacterium leprae Br4923—non-haem BPO, haloperoxidase	Genome mining
	Mycobacterium leprae TN—non-haem BPO, haloperoxidase	Genome mining
	Mycobacterium marinum M—non-haem BPO, haloperoxidase	Genome mining
	Mycobacterium smegmatis MC2 155—non-haem BPO-A2	Genome mining
	Mycobacterium sp. KMS—non-haem CPO	Genome mining
	Mycobacterium sp. MCS—non-haem BPO, CPO	Genome mining
	Mycobacterium tuberculosis CDC1551—BPO	Genome mining
	Mycobacterium tuberculosis F11—non-haem BPOA, haloperoxidase	Genome mining
	Mycobacterium tuberculosis H37Ra—non-haem haloperoxidase, BPOA	Genome mining
	Mycobacterium tuberculosis H37Rv—non-haem BPOA	Genome mining
	Mycobacterium ulcerans Agy99—non-haem BPO, haloperoxidase	Genome mining
	Mycobacterium vanbaalenii PYR-1—non-haem CPO	Genome mining; PeroxiBase
	Rhodococcus erythropolis PR4—non-haem haloperoxidase	Genome mining; PeroxiBase
	Rhodococcus jostii RHA1—chloroperoxidase	Genome mining
	Rhodococcus opacus B4—non-haem haloperoxidase	Genome mining
	Saccharopolyspora erythraea NRRL 2338—non-haem CPO	Genome mining
	Salinispora tropica CNB-440—non-haem CPO	Genome mining; PeroxiBase

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Streptomyces aureofaciens—non-haem, no metal	PeroxiBase
	Streptomyces aureofaciens ATCC10762 (vanadium-containing bromide peroxidase)	BRENDA
	Streptomyces aureofaciens Tü24 (vanadium-containing chloride peroxidase)	BRENDA
	Streptomyces avermitilis MA-4680—non-haem CPO	Genome mining
	Streptomyces coelicolor A3(2)—non-haem CPO, haloperoxidase	Genome mining; PeroxiBase
	Streptomyces griseus Tü6—vanadium-containing bromide peroxidase	BRENDA
	Streptomyces lividans—non-haem, no metal	PeroxiBase
	Streptomyces lividans TK64—vanadium-containing chloride peroxidase	BRENDA
	Streptomyces venezuelae—bromoperoxidase/catalase	BRENDA
Glutathione peroxidase (EC 1.11.1.9)	*Arthrobacter aurescens* TC1	Genome mining
	Arthrobacter chlorophenolicus A6	Genome mining
	Clavibacter michiganensis subsp. *michaganensis* NCPPB382	Genome mining
	Clavibacter michiganensis subsp. *sepedonicus*	Genome mining
	Corynebacterium diphtheriae NCTC 13129	Genome mining
	Corynebacterium efficiens YS-314	Genome mining
	Corynebacterium glutamicum ATCC 13032	Genome mining
	Corynebacterium kroppenstedtii DSM 44385	Genome mining
	Frankia alni ACN14a	Genome mining
	Frankia sp. Ccl3	Genome mining
	Frankia sp. EAN1pec	Genome mining
	Kineococcus radiotolerans SRS30216	Genome mining

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Leifsonia xyli subsp. *xyli* CTCB07	Genome mining
	Mycobacterium avium 104	Genome mining
	Mycobacterium gilvum PYR-GCK	Genome mining
	Mycobacterium smegmatis MC2 155	Genome mining
	Mycobacterium spp. KMS, JLS, MCS	Genome mining
	Nocardia farcinica IFM 10152	Genome mining
	Nocardiodes sp. JS614	Genome mining
	Renibacterium salmoninarum ATCC 33209	Genome mining
	Rhodococcus erythropolis PR4	Genome mining
	Rhodococcus jostii RHA1	Genome mining
	Rhodococcus opacus B4	Genome mining
	Streptomyces avermitilis MA-4680	Genome mining
	Streptomyces coelicolor A3(2)	Genome mining
	Streptomyces griseus subsp. *griseus* NBRC 13350	Genome mining
	Thermobifida fusca YX	Genome mining
Manganese catalase (EC 1.11.1.6)	*Arthrobacter aurescens*	PeroxiBase
	Clavibacter michiganensis	PeroxiBase
	Clavibacter michiganensis subsp. *sepedonicus*	Genome mining
	Clavibacter michiganensis subsp. *michiganensis.*	Genome mining
	Kineococcus radiotolerans	PeroxiBase
	Mycobacterium smegmatis	PeroxiBase
	Nocardia farcinica	PeroxiBase
	Rubrobacter xylanophilus	PeroxiBase
	Saccharopolyspora erythraea	PeroxiBase
	Thermobifida fusca	PeroxiBase
Alkylhydroperoxidase D (EC 1.11.1.15)	*Acidothermus cellulolyticus* 11B	Genome mining
	Beutenbergia cavernae DSM 12333	Genome mining

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Clavibacter michiganensis subsp. *michiganensis* NCPPB382	Genome mining
	Corynebacterium diphtheriae	PeroxiBase
	Corynebacterium glutamicum ATCC 13032	Genome mining
	Corynebacterium kroppenstedtii DSM 44385	Genome mining
	Frankia sp.	PeroxiBase
	Frankia alni ACN14a	Genome mining; PeroxiBase
	Kineococcus radiotolerans SRS30216	Genome mining
	Mycobacterium avium	PeroxiBase
	Mycobacterium avium subsp. *paratuberculosis*	PeroxiBase
	Mycobacterium bovis	PeroxiBase
	Mycobacterium bovis BCG	PeroxiBase
	Mycobacterium bovis subsp. *bovis* AF2122/97	Genome mining
	Mycobacterium gilvum PYR-GCK	Genome mining
	Mycobacterium leprae (theoretical translation/pseudogene)	PeroxiBase
	Mycobacterium marinum M	Genome mining
	Mycobacterium smegmatis	PeroxiBase
	Mycobacterium spp. KMS, JLS, MCS	Genome mining
	Mycobacterium tuberculosis	PeroxiBase; BRENDA
	Mycobacterium ulcerans	PeroxiBase
	Mycobacterium vanbaalenii PYR-1	Genome mining
	Nocardia farcinica	PeroxiBase
	Nocardioides sp. JS614	Genome mining
	Rhodococcus erythropolis PR4	Genome mining
	Rubrobacter xylanophilus DSM 9941	Genome mining
	Saccaharopolyspora erythraea NRRL 2338	Genome mining
	Salinispora arenicola CNS-205	Genome mining

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Salinispora tropica CNB-440	Genome mining
	Streptomyces avermitilis	PeroxiBase
	Streptomyces coelicolor	PeroxiBase
	Streptomyces viridosporus	PeroxiBase
Peroxiredoxin (EC 1.11.1.15)	*Arthrobacter aurescens*—AhpE-like Prx	PeroxiBase
	Corynebacterium jeikeium—typical 2-Cys	PeroxiBase
	Frankia alni—1-Cys, AhpE-like	PeroxiBase
	Mycobacterium avium—atypical 2-Cys, AhpE-like	PeroxiBase
	Mycobacterium avium subsp. *paratuberculosis*—typical 2-Cys	PeroxiBase
	Mycobacterium bovis—AhpE-like	PeroxiBase
	Mycobacterium gilvum—atypical 2-Cys, AhpE-like	PeroxiBase
	Mycobacterium smegmatis—AhpE-like	PeroxiBase
	Mycobacterium tuberculosis—thioredoxin, AhpE-like	PeroxiBase
	Mycobacterium ulcerans—AhpE-like	PeroxiBase
	Mycobacterium vanbaalenii—AhpE-like	PeroxiBase
	Nocardia farcinica—AhpE-like	PeroxiBase
	Propionibacterium acnes—typical 2-Cys, thioredoxin	PeroxiBase
	Rhodococcus sp.—1-Cys, typical 2-Cys, AhpE-like	PeroxiBase
	Saccharopolyspora erythraea—atypical 2-Cys, AhpE-like	PeroxiBase
	Salinispora arenicola—AhpE-like	PeroxiBase
	Salinispora tropica—AhpE-like	PeroxiBase
	Streptomyces albus—atypical 2-Cys	PeroxiBase
	Streptomyces avermitilis—typical 2-Cys, atypical 2-Cys, AhpE-like	PeroxiBase

Table 2 (continued)

Enzyme	Strain	Information obtained from
	Streptomyces coelicolor—atypical 2-Cys, AhpE-like	PeroxiBase
	Streptomyces viridosporus—typical 2-Cys	PeroxiBase

Peroxiredoxin is found in almost all the genome sequences screened

played by actinobacteria in carbon cycling. Godden et al. [31] proposed a scheme for the degradation of lignin by actinobacteria and suggested that their filamentous nature is essential for the colonization and penetration of plant materials. The role of ALiP-P3 was conclusively shown to be involved in lignin degradation through mutation studies performed by Magnuson and Crawford [38]. It is speculated that actinobacteria produce humic acid-like complexes during lignocarbohydrate solubilisation, playing an important role in humification in soil and compost, and that the key enzyme involved is a peroxidase [31, 39].

Manganese Peroxidase (EC 1.11.1.13)

MnP are classified as class II non-animal peroxidases, and thus far, all classified manganese peroxidases have been produced by fungi. They are among the key enzymes produced by white rot fungi during lignin mineralisation [40]. These haem-containing glycoproteins are often present in multiple isoforms. For example, 11 different isoforms of manganese peroxidase have been isolated from *Ceriporiopsis subvermispora* [40]. Their catalytic cycle resembles that of other haem peroxidases, but they use Mn^{2+} as the preferred electron donor. The formation of Mn^{3+} results in the oxidation of various phenolic compounds (including lignin) due to the oxidative potential of Mn^{3+}. This oxidation process is also dependent on the presence of organic acids [41].

The presence of MnP in actinobacteria was not reported in PeroxiBase [5], and none was detected from genome mining. However, Esposito et al. [42] reported on the detection of MnP activity in the degradation of a pesticide, Diuron, by a consortium of actinobacteria using the non-specific phenol red assay, while Niladevi and Prema [33] used a more specific assay and showed MnP activity in *S. psammoticus*. Further studies are underway to determine the properties of the enzyme [33]. Lucana et al. [43, 44] reported the presence of a mycelium-associated, haem-containing enzyme (CpeB) in *Streptomyces reticuli* that has catalase/peroxidase and MnP activity. The oxidation of substrates by this enzyme is either haem-dependent or it could be haem-independent which is coupled to Mn^{2+}/Mn^{3+} peroxidation [43, 44]. The fact that the latter is haem-independent could mean that it is probably a manganese catalase (MnCat; see section on manganese catalases).

Haloperoxidase (EC 1.11.1.10 and EC 1.11.1.B7)

Haloperoxidases oxidise halide ions (X^-) in the presence of H_2O_2 resulting in an electrophilic reaction with organic substrates: $X^- + H_2O_2 + H^+ + R_2C{-}H \rightarrow R_2C{-}X + 2H_2O$.

Haem-containing chloroperoxidases are found in diverse environments, and the structure of the chloroperoxidase from the marine fungus, *C. fumago*, has been well-studied [45].

Properties of Haloperoxidase

Marshall and Wright [46] described a haem-containing chloroperoxidase (EC 1.11.1.10) from *Streptomyces toyocaensis* NRRL 15009 as well as a haem catalase–bromoperoxidase (EC 1.11.1.B7). The N-terminal sequence of this protein showed 100% identity with an unpublished putative catalase/bromoperoxidase from a chloramphenicol producer, *Streptomyces venezuelae*. Knoch et al. [47] described this catalase–bromoperoxidase from *S. venezuelae*, detecting brominating activity (1.8 U/mg protein) and catalase activity (963 U/mg protein) and suggested that this enzyme may be involved in the chlorination of chloramphenicol. However, the study by Facey et al. [48] disproved this and suggested that its key role in *S. venezuelae* would be a catalase function.

Van Pée and Lingens [49] described the catalytic properties of a catalase–bromoperoxidase produced by *Streptomyces phaeochromogenes*. The enzyme is very similar to algal bromoperoxidases and chloroperoxidases. Bromination activity was difficult to detect in crude extracts since the catalase function effectively removed any H_2O_2 added to the system, but upon purification, the enzyme catalysed the formation of carbon–bromide bonds.

Role of Haloperoxidase

While some haloperoxidases have been detected in actinobacteria, the role of this enzyme in nature is still undetermined.

Catalase (EC 1.11.1.6)

Catalases, also called hydroperoxidases, are classified into the catalase superfamily and are one of the most extensively studied enzyme classes [50]. Although the gene encoding for the classical monofunctional catalase is quite widespread in the genome sequences screened (Table 2), very few have been described.

Properties of Catalases

The assay for catalase is based on the catalytic breakdown of hydrogen peroxide and typically defines catalase activity:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Catalase activity has been detected in three classes of proteins which are structurally different and have different primary sequences: monofunctional catalases (haem-containing, classical catalase), catalase–peroxidases (haem-containing, more closely related to plant peroxidases) and manganese catalases (non-haem proteins) [50]. These enzymes can further be divided into two groups based on the size of their subunits: large, >75 kDa or small, <60 kDa. Typical catalases are very similar (irrespective of the source of isolation) in their properties. They consist of four subunits of equal size, 2.5–4 haem prosthetic groups per tetramer, ranging from 225 to 270 kDa in size, and they have broad pH optima of 5–10. Furthermore, catalases are notably resistant to treatment with ethanol or chloroform, and they are inhibited by 3-amino-1,2,4-triazole [51]. More detailed information on catalases can be found in Chelikani et al. [50].

Few actinobacterial catalases have been reported; the catalase produced by *Streptomyces coelicolor* ATCC 10147 is the best studied example [51–53]. It was shown that upon the exposure of *S. coelicolor* ATCC 10147 to various concentrations of H_2O_2, catalase activity was upregulated and the strain became resistant to killing by H_2O_2 when pre-treated with low concentrations of H_2O_2 [52]. A similar antioxidant response was observed in *Streptomyces* sp. strain M3004 upon exposure to H_2O_2 and paraquat [54]. Further studies on the catalase from *S. coelicolor* ATCC 10147 showed this enzyme to be consistent with other monofunctional catalases, and no peroxidase activity was detected [51, 53].

Role of Catalases

The expression of catalases in bacteria usually occurs in response to oxidative stress due to the presence of H_2O_2 or other reactive oxygen species (ROS) that are either generated within the natural environment or as a defence mechanism utilised by the hosts they are infecting. The key role played by monofunctional catalase in actinobacteria is protection against high levels of H_2O_2. Tavares et al. [55], for example, described a catalase produced by *Frankia* sp. strain R43, an organism which is involved in the formation of nitrogen-fixing nodules in the roots of dicotyledonous plant species. For the strain to survive, it requires an effective defence system against the ROS produced by the host plant during infection. In addition to a cytoplasmic catalase (intracellular), the strain also produces a catalase–peroxidase to deal with the high amounts of H_2O_2 generated. The occurrence of catalases in other *Frankia* spp. has also been reported in other studies [56, 57].

Vera-Cabrera et al. [58] investigated the distribution of a *Nocardia brasiliensis* catalase gene fragment among various actinobacterial genera. *N. brasiliensis* is a pathogenic actinobacterium known to cause mycetoma, a subcutaneous disease. Patients infected with this organism typically have antibodies in their sera which cross-react with a unique catalase, designated P61, produced by *N. brasiliensis*. A similar gene sequence was found in members of the genera *Nocardia*, *Gordonia*, *Rhodococcus* and *Streptomyces*. No peroxidase activity was detected, indicating that the enzyme was a classical monofunctional catalase. Wu et al. [59] also reported the occurrence and role of a catalase (KatG) in the opportunistic pathogen, *Nocardia farcinica*. This enzyme, along with superoxide dismutase, alkylhydroperoxide reductase, glutathione peroxidase, peptide methionine sulphoxide reductase, thioredoxin and thioredoxin reductase, is expressed in response to the production of ROS by the host's phagocytes, indicating the importance of these enzymes in ensuring the survival of *N. farcinica* within the host.

Catalase–Peroxidase (EC 1.11.1.6 and EC 1.11.1.7)

Catalase–peroxidases are classified as non-animal peroxidases within the class I peroxidase group. They have the ability to remove H_2O_2 both catalytically and peroxidatively [60]:

$$\text{Catalytic} : 2H_2O_2 \rightarrow 2H_2O + O_2(\text{dismutation})$$
$$\text{Peroxidatic} : H_2O_2 + 2AH_2 \rightarrow 2H_2O + 2AH\bullet\,(\text{reduction})$$

It is proposed that this enzyme evolved through the fusion of a primordial peroxidase gene with a catalase gene [60]. Under conditions of low H_2O_2 and the presence of a suitable substrate, the peroxidase activity is the dominant activity and vice versa [60]. The natural substrate of the peroxidase function is unknown, but it is speculated that the enzyme

predominantly acts as a catalase since substrates for the peroxidase are not typically found in the cytoplasm [61]. While the monofunctional catalase is widespread in nature, catalase–peroxidases seem to be limited to bacteria and some fungi [62].

Properties of Catalase–Peroxidase

The properties of these bifunctional enzymes differ from those of classical catalases in a number of ways: Their catalytic activity is pH dependent with a pH optimum of 6–6.5, and they are more sensitive to temperature, ethanol/chloroform and H_2O_2 than typical catalases. Furthermore, they are insensitive to 3-amino-1,2,4-triazole but are reduced by dithionite (characteristic of peroxidases) [51, 61]. Microbial catalase–peroxidases are typically dimeric or tetrameric with 0.5–1 molecules of the proto-haem IX per subunit and exhibit the typical displacement of the Soret band when reduced [11].

A haem-containing catalase–peroxidase from *Streptomyces cyaneus* was isolated and characterised during a study of the distribution of peroxidases from actinobacteria [63]. An early paper on the catalase–peroxidase from *S. reticuli* [11] showed that the enzyme (CpeB) has broad substrate specificity: It oxidises ascorbate, guaiacol and veratryl alcohol. Most known catalase–peroxidases (except KatP from *Escherichia coli*) are cytoplasmic, but CpeB is mycelium-associated. The 160-kDa dimeric CpeB shares a high amino acid sequence identity with KatG from *Caulobacter crescentus*, *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*, KatP from *E. coli* and PerA from *Archaeoglobus fulgidis* and *Bacillus stearothermophilus*. The authors also noted the possibility that the expression of the catalase–peroxidase gene may be regulated by FurS, a ferric uptake regulator. Hahn et al. [62] showed that the expression of the catalase–peroxidases is under the regulation of FurS and that the genes encoding for *fur* and the catalase–peroxidase are on the same operon, an organisation or feature also observed in *S. coelicolor*, *S. reticuli* and *Mycobacterium* spp.

Hahn et al. [62] reported two isoforms of catalase–peroxidase produced in *S. coelicolor* during aerial mycelium formation. This transient production of catalase–peroxidases also occurs in other streptomycetes. For example, in *Streptomyces seoulensis*, two isoforms are present in the substrate mycelium, and a third is present in aerial and sporulated mycelia [62]. Lucana et al. [43, 44] showed that the expression of the catalase–peroxidase of *S. reticuli* is under the control of a two-component regulatory system, SenS (sensor histidine kinase)/SenR (response regulator), which negatively regulates the catalase–peroxidase/*fur* expression as well as the expression of HbpS, an extracellular enzyme that is responsible for the binding of haem.

Various mycobacterial catalase–peroxidases (KatG) have been described: Menéndez et al. [64] described the two catalase–peroxidases (KatG I and KatG II) produced by *Mycobacterium fortuitum*, Rafii et al. [65] isolated and purified the catalase–peroxidase of a non-pathogenic fast-growing *Mycobacterium* sp. Pyr-1 and Ro et al. [66] purified a catalase–peroxidase from *Mycobacterium* sp. strain JC1 DSM 3803 which produced the enzyme at high levels when grown in the presence of carbon monoxide and methanol as sole carbon and energy sources. The crystal structure of the *M. tuberculosis* KatG has been elucidated and has provided insight into the bi-functionality of the enzyme [67, 68]. Interestingly, KatGs have a high sequence homology to other peroxidases, including the fungal cytochrome *c* peroxidase, prokaryotic peroxidases and plant ascorbate peroxidase but show very little sequence homology to monofunctional catalases [67]. In addition, KatGs also exhibit Mn^{2+}-dependent peroxidase, cytochrome P450 oxygenase and peroxynitritase activities [67].

Role of Catalase–Peroxidase

The role of catalase–peroxidases in the actinobacteria resembles that of classical catalase, i.e. the removal of H_2O_2 generated within their immediate environment. Tavares et al. [55] identified a catalase–peroxidase from *Frankia* sp. strain R43 but found no evidence to suggest that this enzyme plays any role in dealing with the oxidative stress produced by the plant host infected by this strain. However, the role of catalase–peroxidase in host survival in the genus *Mycobacterium* has been extensively studied. These catalase–peroxidases are grouped with the heat-labile mycobacterial T-catalases (as opposed to the classical catalases which are grouped within the heat stable M-catalases) [65, 69].

In mycobacteria, catalase–peroxidases provide protection against ROS produced by macrophages upon infection of their human hosts. Catalase–peroxidase reduces the levels of ROS and inhibits nitric oxide production and is therefore sometimes also referred to as catalase–peroxidase–peroxynitritase. Ironically, catalase–peroxidases produced by mycobacteria are responsible for the activation of isoniazid, a first-line anti-mycobacterial antibiotic, to a toxic oxidised form which inhibits mycolic acid production in the pathogen, leading to the killing of the mycobacterium. The correlation between mutations, insertions or the lack of KatG and an increased incidence of isoniazid resistance in *Mycobacterium* spp. have been reported by various studies [64–66, 68, 70–72].

DyP-Type Peroxidase (EC 1.11.1.X)

The DyP-type peroxidases have not been allocated a specific Enzyme Commission numbering but are considered to belong to the general group, EC 1.11.1.X. However, this group of dye-decolourizing peroxidases have recently been proposed as a new group of peroxidases. They show a higher degree of specificity towards the degradation of anthraquinone dyes than azo dyes and are able to oxidise phenolic compounds, but not non-phenolic compounds [73, 74]. They also have the ability to degrade hydroxyl-free anthraquinone dyes which are not substrates for other peroxidases [75]. The first DyP-type peroxidase was isolated from the basidiomycete, *Thanatephorus cucumeris* Dec 1, and these peroxidases are not considered to be part of the plant or animal peroxidase superfamilies [74, 76].

Properties of DyP-Type Peroxidase

The DyP-type peroxidases are glycoproteins (17% saccharides) with different primary and tertiary structures compared to other peroxidases and unique reaction characteristics [74, 76]. The enzymes exhibit the classical Soret band at 406 nm, and the reduced form shows a peak at 556 nm, indicating the presence of a proto-haem prosthetic group [73, 74]. The tertiary structure of DyP-type peroxidases differs from other haem peroxidases, e.g. the H_2O_2 binding site contains an aspartic acid, as compared with histidine in other haem peroxidases. Furthermore, the binding site resembles that of chloroperoxidases (which contain glutamic acid). Chloroperoxidases resemble peroxygenase $P450_{BS\beta}$, and a possible relationship among the three types of enzymes may exist. DyP-type peroxidases also have hydrolase activity and therefore have potential application in the degradation of xenobiotics.

The natural role of DyP-type peroxidases is still unknown [74, 76]. A search of the InterPro Database showed a wide abundance of genes (in various bacterial genomes) potentially encoding for this enzyme. Van Bloois et al. [77] therefore recommended that DyP-type peroxidases should be grouped into a bacterial peroxidase superfamily (similar to the plant and animal superfamilies). PeroxiBase [5] further grouped the DyP-type peroxidases into three groups: A (those containing a Tat-signal sequence for transportation across the cytoplasmic membrane) and B and C (cytosolic enzymes with a potential role in intracellular metabolism [77]).

Recently, Van Bloois et al. [77] described a DyP-type peroxidase produced by *T. fusca*. This peroxidase is similar to plant peroxidases in its ability to oxidise aromatic sulphides (only with low efficiency), a reaction not typically catalysed by other DyP-type peroxidases. The new DyP-type peroxidase from *T. fusca* falls within group A and is easily expressed in *E. coli*, robust and oxidizes a range of substrates, making this a promising alternative to well-established peroxidases such as horse radish peroxidases [77].

Di-haem Cytochrome *c* Peroxidase (EC 1.11.1.5; Di-haem Peroxidase Superfamily)

These enzymes are found in the periplasm of various bacteria and are reported to be ubiquitous in genome sequences [78]. Their role is still unclear; they are thought to remove H_2O_2 generated under hypoxia conditions (lack of oxygen), but whether the enzyme can compete for available electrons with terminal oxidases is still questionable [78]. They share 40–50% level of identity, and their function is the reduction of H_2O_2 to water by two electrons delivered singly from a small redox protein (SRP): 2 SRP (reduced)$+H_2O_2+2H^+ \rightarrow$ 2SRP (oxidised) $+2H_2O$. Their catalytic cycle is therefore very different from other peroxidases (see Table 1).

PeroxiBase [5] reports the presence of a di-haem cytochrome *c* peroxidase in the actinobacterium *Symbiobacterium thermophilum*, but no other sequences were detected via genome mining. The study by Niebisch and Bott [79] suggested that a di-haem cytochrome *c* peroxidase forms part of the respiratory chain in the soil bacterium *Corynebacterium glutamicum* as part of the cytochrome bc_1 complex, but further studies are required for confirmation.

Animal Peroxidases or the Peroxidase–Cyclooxygenase Superfamily

These peroxidases have only been detected in animal cells, and while there is no real explanation as to why they should be found in actinobacteria, some animal peroxidases have been predicted from actinobacterial genome sequences. However, it is possible that the presence of animal peroxidase sequences can be attributed to divergent evolution, horizontal gene transfer or errors made in the interpretation of the genome data [80].

Within the peroxinectin peroxidase group, a bacterial peroxicin has been detected in the genome sequence of an *Arthrobacter* sp. [5], and a short peroxidockerin with a type I dockerin domain has been detected in the genome sequence of the marine actinobacterium, *Salinispora arenicola* [5]. Prostaglandin H synthases (EC 1.14.99.1) are known to exhibit both cyclooxygenase and peroxidase activity. In the cells of mammalian organs, they play a key role in the production of prostaglandins, prostacyclines and thromboxanes, with polyunsaturated fatty acids as the substrate [81]. Prostaglandin H synthase (cyclo-oxygenase) sequences have been detected in the genome sequences of a *Frankia* sp., *M. smegmatis*, *Mycobacterium vanbaalenii* and *Streptomyces avermitilis* [5] (Table 2).

In addition, genome mining has detected a predicted ovoperoxidase sequence in the genome of a *Frankia* sp. This enzyme is known to play a key role in catalysing dityrosine cross-linkages between polypeptides (H_2O_2 dependent) during the formation of the fertilization envelope upon egg fertilization in mammals, amphibians, insects, molluscs and crustaceans [82]. Phylogenetic analysis performed by LaFleur et al. [82] showed that ovoperoxidases share sequence identity with other haem-dependent peroxidases such as horseradish peroxidase, yeast cytochrome *c* peroxidase and human peroxisomal catalase.

Non-haem Peroxidases

Non-haem peroxidases consist of four main protein families: alkylhydroperoxidases, haloperoxidases, manganese catalases and thiol peroxidases, the latter being grouped into glutathione peroxidases and peroxiredoxins (Prx) [2]. These peroxidases either have no metal associated with the active site or have a manganese or vanadium associated with the active site.

Peroxiredoxin (EC 1.11.1.15)

Prx are thiol peroxidases or thiol-specific antioxidants that are involved in the enzymatic degradation of H_2O_2 (to water), organic hydroperoxides (to corresponding alcohol) and peroxynitrite (to nitrite) [83, 84].

Properties of Peroxiredoxins

Peroxiredoxins are classified into three major subclasses: typical 2-cysteine peroxiredoxin (Prx I–IV), atypical 2-cysteine peroxiredoxin (Prx V) and 1-cysteine peroxiredoxin (Prx VI), based on the number and position of cysteine residues involved in the catalytic reaction through the formation of disulphide bonds [85, 86] (Table 1). The redox activity is dependent on a conserved cysteine at the N terminus, which is analogous to the selenocysteine active site found in glutathione peroxidases [87].

Role of Peroxiredoxins

Peroxiredoxins are found in microorganisms, plants and mammals where, in conjunction with glutathione peroxidases and catalases, they play a key role in the removal of or protection against ROS and reactive nitrogen species [83, 87]. As with catalases and catalase–peroxidases, peroxiredoxins play a role in the survival of pathogenic bacteria within their hosts. In mycobacteria, thioredoxin peroxidase is considered essential for the survival of mycobacteria in phagocytes of human hosts [88]. This enzyme is therefore of importance in the pharmaceutical industry since the gene can be targeted in gene therapy. Sherman et al. [89] reported a link between AhpC and drug resistance in *M. tuberculosis* where the expression of AhpC is thought to be involved in isoniazid resistance. Wu et al. [59] identified the key antioxidant defence system in *N. farcinica* which consists of: AhpC, KatG (catalase), superoxide dismutase, thioredoxin and glutathione peroxidase. This is similar to mechanisms in other bacteria which, for example, allow for aerobic growth of *Staphylococcus aureus* and are involved in resistance to oxidative stress, heat, salt or ethanol stress in *Bacillus subtilis* [59].

Haloperoxidase (EC 1.11.1.B2 and EC 1.11.1.B4)

This group of peroxidases includes the non-haem, non-metal haloperoxidases and the non-haem, vanadium-containing chloroperoxidases.

Non-haem, Non-metal Haloperoxidase

Non-haem haloperoxidases halogenate various substrates in the presence of halides and H_2O_2; they differ from the well-characterised haem-type haloperoxidases and form a distinct family [90]. The esterase of *Pseudomonas fluorescens* shares a 40–50% amino acid sequence similarity to non-haem haloperoxidases and has the ability to catalyse a brominating reaction. Bromoperoxidases are also able to catalyse ester hydrolysis, and this bi-functionality led to a detailed analysis of the structural and functional relationship of esterases and non-haem haloperoxidases: A comparison of serine-hydrolases to haloperoxidases showed the conservation of the α/β-hydrolase fold across both groups and a Ser-His-Asp catalytic triad [45, 90]. As with lipases and esterases, the presence of short aliphatic acids is required for the reaction to occur, and it has been proposed that peroxoacids are involved as reaction intermediates [45]. This group of haloperoxidases has therefore been reclassified as α/β-hydrolases [91]. While the non-haem chloroperoxidase in *S. coelicolor* is known to be linked to proteosome activity in the organism [92], the general function of actinobacterial haloperoxidases in nature is unknown.

Vanadium-Containing Haloperoxidases

Vanadium-containing haloperoxidases have been detected mainly in marine algae. The reaction mechanism (Table 1) and active site are described in detail in Butler [93]. The acidic solution of *cis*-dioxovanadium (V) catalyses the oxidation of halides by H_2O_2 resulting in the halogenation of organic substrates and the halide-assisted disproportionation of H_2O_2 [93]. Vanadium bromoperoxidases are sought-after in industry because of their ability to introduce bromine groups into aromatic compounds which often have antibacterial or anticancer properties [94]. Littlechild et al. [94] definitively showed that the enzyme is directly responsible for the bromination of organic compounds. The vanadium bromoperoxidases are found in the marine environment (marine algae) whereas vanadium chloroperoxidases have only been identified in terrestrial fungi and two streptomycetes—detailed reaction mechanisms are described in Neumann et al. [95]. This is the first report of a vanadium chloroperoxidase in bacteria, and it has been placed in the context of a dedicated biosynthetic gene cluster: napyradiomycin biosynthesis in *Streptomyces aculeolatus* NRRL 18422 and *Streptomyces* sp. CNQ-525 [95].

Glutathione Peroxidase (EC 1.11.1.9)

Glutathione peroxidase is similar to the peroxiredoxins in activity, but differs at the sequence level [83]. Glutathione peroxidase reduces H_2O_2 or organic hydroperoxides to water or the corresponding alcohol respectively using reduced glutathione [96]. In mammals, this enzyme plays a key role in protecting erythrocytes against ROS and ensures their recycling. Their activity is dependent on the presence of a highly conserved seleno-cysteine within the active site [96]. The production of glutathione in prokaryotes

is mainly limited to the cyanobacteria and purple bacteria; mycothiol is the major thiol produced in actinobacteria [59]. It is thought that the detection of a putative glutathione peroxidase within the genome sequence of *N. farcinica* is not necessarily an indication of glutathione production, but the enzyme may be produced at high levels to convert glutathione produced by the infected host [59]. Interestingly, 26 of the available actinobacterial genomes screened have the putative sequence for glutathione peroxidase, and this peroxidase may be more widespread in actinobacteria than previously thought.

Manganese Catalase (EC 1.11.1.6)

Manganese catalases are often referred to as pseudocatalases because they do not have a haem protoporphyrin present at their catalytic core but have two manganese ions present instead [50]. They catalyse the following reaction:

$$H_2O_2 + Mn^{2+} - Mn^{2+}(2H^+) \rightarrow Mn^{3+} - Mn^{3+} + 2H_2O$$
$$H_2O_2 + Mn^{3+} - Mn^{3+} \rightarrow Mn^{2+} - Mn^{2+}(2H^+) + O_2$$

MnCat is not as widespread in nature as the haem-containing catalases and is mostly found in bacteria [50]. The manganese peroxidase reported for *S. reticuli* may be a MnCat [43, 44].

Alkylhydroperoxidase D-Like Superfamily

Members of this superfamily are often referred to as carboxymuconolactone decarboxylases (CMD), and they are known to be involved in protocatechuate catabolism. They have no sequence similarity to AhpC, but they have alkylhydroperoxidase activity due to the presence of a thioredoxin-like active site and therefore play a key role in the antioxidant defence mechanism in various organisms [97, 98]. The various subclasses are composed of proteins with different CMD domains. For example, alkylhydroperoxidase D (AhpD; EC 1.11.1.15) contains two CMD domains; in some bacteria, a gene fusion of CMD and hydrolase results in the expression of both enzymes, which are always required within the same pathway [98]. Of this group, only the AhpD exhibits peroxidase activity. AhpD has been detected in a number of actinobacteria (Table 2), and it is thought that these enzymes play a similar role in the antioxidant defence mechanism in these organisms [98].

Potential for Industrial Application of Actinobacterial Peroxidases

Certain peroxidases are able to oxidise substrates of high redox potential in a non-specific manner, and hence, there is a wide range of potential uses for these enzymes. Peroxidases are known to catalyse a wide range of reactions, including enantioselective reduction of hydroperoxides to their corresponding alcohols, hydroxylation of arenes, oxidation of aromatic amines and phenols, epoxidation of olefins, halogenation, *N*-oxidation, and sulphoxidation (extensive discussion on these reactions in [99]). While relatively few actinobacterial peroxidases have previously been used in such applications, the potential for use of actinobacterial peroxidases in these applications is clear.

Degradation of Major Pollutants

Aromatic compounds (including phenols and aromatic amines) constitute one of the major classes of pollutants and are found in effluents from a wide range of industries.

The majority of reports on detoxification of wastewater contaminated with phenols, cresols and chlorinated phenols utilise horseradish peroxidase (HRP), and other sources of peroxidases are also under investigation such as soybean peroxidase [100], turnip peroxidase [101–103] and a peroxidase from *Coprinus macrorhizus* [104]. While the use of actinobacterial peroxidases in the degradation of pollutants is not as extensive, peroxidases from actinobacteria are increasingly being investigated for this purpose. Antonopoulos et al. [105] investigated the dechlorination of chlorophenols using an actinobacterial strain (*Streptomyces albus* ATCC 3005), where crude concentrated peroxidase from *S. albus* successfully oxidised all chlorophenols tested except pentachlorophenol. Winter et al. [106] showed that soil actinobacterial isolates adapted to, degraded and partly dehalogenated, the chlorinated compounds in spent sulphite bleach effluent. Lignin peroxidases have been widely investigated for application in the pulp and paper industry where numerous recalcitrant lignosulphonated compounds are formed [107]. The lignin peroxidase, ALiP-P3, from *S. viridosporus* T7A was shown to be induced in the presence of lignosulphonated compounds, the activity being dependent on the type of lignosulphonated compound used.

Several actinobacteria produce lignin-degrading peroxidases [15–17] which may have the potential to degrade lignin-like substances as those reported for fungal lignin and manganese peroxidase, e.g. coal. Pizzul et al. [108, 109] found that *Rhodococcus wratislaviensis* was able to degrade phenanthrene and anthracene (polyaromatic hydrocarbons; PAH), and *Rhodococcus* sp. DSM 44126 was able to degrade phenanthrene in liquid medium. While these studies did not show that peroxidases were responsible for degradation, a strong correlation between degradation of PAHs and ionisation potential in manganese peroxidase–lipid peroxidation systems was indicated [110]. Furthermore, Lee et al. [111] compared the ability of *P. chrysosporium* with that of *Streptomyces* spp. (*S. viridosporus* T7A, *S. badius* 252 and *Streptomyces setonii* 75Vi2) to degrade degradable plastic polyethylene. The bacterial strains were more successful in degrading the plastic than the fungal strain, and this was the first report of bacterial degradation of oxidised polyethylenes in pure culture [111].

Peroxidases have shown potential for use in the degradation of various environmentally persistent herbicides, which cause concern over their long-term accumulation and toxicity. Three actinobacterial strains isolated from contaminated soils have shown potential for degradation of complex herbicides, with one strain degrading up to 37% of the herbicide Diuron in in vitro assays [42]. It is suggested that this degradation is due to the oxidation of the aromatic structure of the herbicide by MnP, as has been reported for the fungus *P. chrysosporium* [112].

Lignin and Lignocellulose Degradation

Lignin is a highly complex, stable and irregular polymer consisting of non-repeating phenyl propanoid units linked by various carbon–carbon and ether bonds [113, 114]. The role of LiP in lignin depolymerisation is well-known, and its mechanism of action is very similar to HRP [115]. The study by Crawford [14] was the first to show conclusive evidence that streptomycetes can decompose lignin with complete mineralisation of lignin to CO_2. Selectively isolated lignocellulose-degrading actinobacteria and the top three degraders all belonged to the family *Streptomycetaceae*. Initial oxidation of lignin appears to involve a peroxidase-based mechanism where peroxidase plays a role in lignin transformation by generating water-soluble polymeric products called APPL [15–17].

In many cases, the direct involvement of LiP and/or MnP (fungal) in biopulping and biobleaching processes has been demonstrated. Extracellular peroxidase activity

has been shown to be responsible for lignin depolymerisation by *P. chrysosporium*, and similar activity has been described in ligninolytic streptomycetes [15] and other actinobacteria [20]. Purified LiP and MnP decolourise Kraft effluents [116] and oxidise a broad range of xenobiotic compounds (summarised in [117]). Tuncer et al. [118] optimised the production of an extracellular lignocellulolytic enzyme from *T. fusca* BD25 as this strain is able to solubilise lignin at high temperatures more efficiently than any other bacteria or fungi. Pasti et al. [17] investigated the lignin-solubilizing ability of actinomycete symbionts isolated from the guts of termites. The bioconversion of the lignocellulose was followed through ^{14}C studies, lignocellulose, lignin and carbohydrate loss from growth on maize stalks and the production of APPLs. This shows that, although currently there is little evidence that actinobacteria can depolymerise lignin on a scale comparable to fungi, there is still a significant potential for the discovery of novel actinomycetes with greater lignin-degrading potential and different active parameters.

One of the major challenges to the conversion of cellulose to simple sugars and subsequent conversion of sugars to useable fuel is the presence of lignin. Removal of lignin before saccharification is imperative for optimal biological conversion of biomass to biofuel. Lignin is chemically difficult to degrade due to free radical coupling [119] and due to its variable structure [120]. The lignin depolymerisation products formed using LiP are phenolic compounds which are prone to re-polymerise, but MnP or laccases can convert the phenolic products to form quinones and finally reduction of the quinones to the corresponding hydroquinones [121]. Several actinobacterial peroxidases have been shown to depolymerise lignin (see section on lignin peroxidases).

Dye Degradation

Synthetic dyes with complex aromatic molecular structures are used in many industrial processes from paper printing to textile dyeing, and a significant portion (10–15%) of these recalcitrant, toxic dyes are released in industrial effluents where they cause environmental damage [122]. Physicochemical methods such as chemical oxidation, reverse osmosis and adsorption can be used to remove these pollutants. However, the high costs, limited applicability, high energy input and the possibility for the production of toxic by-products make these options undesirable. Microbial degradation is viewed as a less expensive, less intrusive alternative [123].

MnP has been shown to catalyse the oxidation of several monomeric phenols and aromatic dyes. Reactions are dependent of the presence of both divalent manganese and certain types of buffers [124]. HRP is effective at degradation of industrially important dyes, e.g. Remazol blue [125]. HRP in its free form has been found to effectively decolourize textile dyes and effluents and reduce effluent toxicity [123]. However, inactivation of HRP by the dyes is a major limitation to commercialisation of this process. The use of actinobacteria in dye degradation is well researched. Dyes such as poly B-411, poly-R418 and RBBR are often used as substrates to monitor ligninolytic activity of *Streptomyces* species [126]. *T. mesophila*, *S. badius* and *S. viridosporus* T7A are among the most active Poly R decolourising actinobacterial strains [127]. While it is suggested that the dye-degrading action is due to the action of lignin peroxidase, the study shows no evidence that this is the case [127]. The recently described DyP-type peroxidase from *T. bifida* has been recommended as an alternative for the degradation of anthraquinone dyes, based on the fact that the enzyme is robust, can be expressed in a suitable host and degrades anthraquinone dyes with a high efficiency [77].

S. chromofuscus and *P. chrysosporium* have been found to display very similar routes for the degradation of recalcitrant man-made sulphonated azo dyes [128]. During oxidation of dyes and sulphanilic acid using crude peroxidases from *P. chrysosporium* (both MnP and LiP detected in the crude extract) and *S. chromofuscus*, there was no consumption or release of oxygen which suggested that water or H_2O_2 was the hydroxylation agent acting on the dyes [128]. Analysis of intermediates and mechanisms of azo dye degradation by ligninolytic peroxidases of *P. chrysosporium* and *S. chromofuscus* have led to the conclusion that the ligninolytic peroxidases convert the azo dye to a cation radical [126, 128] that is susceptible to the nucleophilic attack of water or H_2O_2 molecules.

Other Applications

CPO has generated interest in the fine chemicals industry due to its wide substrate range and selectivity of reactions. CPO has a natural halogenating activity and catalyses synthetically useful (enantiose-selective) oxygen transfer reactions, e.g. asymmetric epoxidation of olefins, allylic, benzylic and propargylic hydroxylation, asymmetric sulphoxidation and regioselective oxidation of indoles (reviewed in [129]). CPO oxidises several phenolic compounds. In addition, oxidation of ethanol to aldehyde and oxidation of chloride ions occurs. Immobilised CPO and glucose on an electrode can be used for measurement of chlorophenol congeners [130]. Actinobacteria (e.g. *S. toyocaensis*, Table 2) produce chloroperoxidases which may have similar application potentials [95].

Peroxidases can be used in analytical systems for the detection and quantification of various compounds [131]. Peroxidases co-immobilised with a hydrogen peroxide-producing enzyme such as glucose oxidase, in a biosensor configuration, could be used for the determination of glucose, alcohols, glutamate and choline or phenols or aromatic amines in the nanomolar range [132]. Peroxidases yield stable chromogenic products at low concentrations which is an excellent property for use in diagnostic kits and the preparation of enzyme-conjugated antibodies [133]. Enzyme-linked immunosorbent assays, otherwise called enzyme immunoassays, are tests designed to detect antigens or antibodies by producing an enzyme-triggered change of colour. Also, peroxidase isoenzymes from turnip have been used in a diagnostic test kit for the determination of uric acid with results comparable to a commercially available uric acid test kit [134]. A colorimetric-based total cholesterol test was developed using cholesterol oxidase, cholesterol esterase and peroxidase which was accurate, precise and rapid [135]. However, the low enzyme stability and high costs have restricted application of this method for routine use. The properties of actinobacterial peroxidases, as discussed in this review, make these enzymes a viable alternative in terms of enzyme stability and production costs. Furthermore, ease of engineering of prokaryotic genes and enzymes (as compared to their eukaryotic counterparts) make them ideally suited for meeting the needs of industrial processes.

Conclusions and Future Perspectives

While many of the applications stated have been tested using fungal or plant peroxidases (i.e. mainly HRP), it is clear that there is a need for investigation into other sources of peroxidases. These enzymes catalyse a wide range of reactions, and peroxidases from different sources may be able to catalyse different, useful reactions. While HRP is most often used for the majority of analytical applications employing peroxidase (as it is most readily available), peroxidases from

other sources could prove to be a good substitute for HRP. Actinobacteria could prove to be an excellent source of new peroxidases, with novel, innovative applications.

The oxidative capacity of peroxidases is typically determined by active site topology, substrate accessibility and their redox potential [136]. Active site topology and substrate accessibility are becoming clearer through the increased number of crystal structures available. Atomic detailed information on the prosthetic groups can show exact stereochemical properties. This can be achieved through the use of new generation synchrotron radiation coupled with cryogenic techniques, which in turn allows for the determination of the exact location of hydrogen bonds essential for the catalytic mechanism [113]. The crystal structure of the catalase–peroxidase from *M. tuberculosis* has been resolved, providing insight into the bi-functionality of the enzyme [67, 68]. It is essential to increase the amount of information available on actinobacterial peroxidase crystal structures to gain more insight into how these enzymes differ from their fungal and plant counterparts and how their functionality can be manipulated. Combining structural and functional information can provide useful information for the successful engineering of a biocatalyst [1, 9].

The presence of distal and proximal amino acids around the prosthetic groups in peroxidases influences the redox potential of the enzyme. Various studies on the mutation of these amino acids have given variable results in different peroxidase groups [10]. High redox potential of a peroxidase is essential for the application of peroxidases in industrial applications [137]. HRP and fungal lignin peroxidases have among the highest redox potentials of the well-described peroxidases [137]. The only redox potential information available for actinobacterial peroxidases is for catalase–peroxidases from mycobacteria [136]. One of the first steps in determining the suitability of actinobacterial peroxidases for industrial applications would therefore be the determination of their redox potentials, especially for those peroxidases that clearly show potential for application. For the improvement or adaptation of an enzyme for the application in an industrial process, various methods can be used: enzyme engineering (change the reaction environment, e.g. the use of organic solvents/non-aqueous media) and protein engineering (genetic alterations to change structure, function and activity) [138]. All these methods could help us to achieve the goal of creating new biocatalysts with required properties to target specific bioprocesses.

References

1. Gumiero, A., Murphy, E. J., Metcalfe, C. L., Moody, P. C. E., & Raven, E. M. (2010). *Archives of Biochemistry and Biophysics, 500*, 13–20.
2. Passardi, F., Theiler, G., Zámocký, M., Cosio, C., Rouhier, N., Teixera, F., et al. (2007). *Phytochemistry, 68*, 1605–1611.
3. Arenskötter, M., Bröker, D., & Steinbuchel, A. (2004). *Applied and Environmental Microbiology, 70* (6), 3195–3204.
4. Bull, A. T., Ward, A. C., & Goodfellow, M. (2000). *Microbiology and Molecular Biology Reviews, 64* (3), 573–606.
5. PeroxiBase. (2010). PeroxiBase—peroxidase database. Available at http://peroxibase.isb-sib.ch.
6. Chang, A., Scheer, M., Grote, A., Schomburg, I., & Schomburg, D. (2009). *Nucleic Acids Research, 37*, D588–D592. Database issue.
7. NCBI. (2010). NCBI database. Available at http://www.ncbi.nlm.nih.gov.
8. Van Rantwijk, F., & Sheldon, R. A. (2000). *Current Opinion in Biotechnology, 11*(6), 554–564.
9. Matsunaga, I., & Shiro, Y. (2004). *Current Opinion in Chemical Biology, 8*(2), 127–132.
10. Battistuzzi, G., Bellei, M., Bortolotti, C. A., & Sola, M. (2010). *Archives of Biochemistry and Biophysics, 500*, 21–36.
11. Zou, P.-J., Borovok, I., Lucana, D., Müller, D., & Schrempf, H. (1999). *Microbiology, 145*, 549–559.
12. Van Deurzen, M. P. J., Van Rantwijk, F., & Sheldon, R. A. (1997). *Tetrahedron, 53*(39), 13183–13220.

13. Morgenstern, I., Klopman, S., & Hibbett, D. S. (2008). *Journal of Molecular Evolution, 66*, 243–257.
14. Crawford, D. L. (1978). *Applied and Environmental Microbiology, 35*(6), 1041–1045.
15. Ramachandra, M., Crawford, D., & Hertel, G. (1988). *Applied and Environmental Microbiology, 54* (12), 3057–3063.
16. Crawford, D. L., Pometto, A. L., 3rd, & Crawford, R. L. (1983). *Applied and Environmental Microbiology, 45*(3), 898–904.
17. Pasti, M. B., Pometto, A. L., III, Nuti, M. P., & Crawford, D. L. (1990). *Applied and Environmental Microbiology, 56*(7), 2213–2218.
18. Crawford, D., Doyle, J., Wang, Z., Hendricks, C., Bentjen, S., Bolton, H., Jr., et al. (1993). *Applied and Environmental Microbiology, 59*(2), 508–518.
19. McCarthy, A. J. (1987). *FEMS Microbiology Reviews, 46*(2), 145–163.
20. Ball, A. S., Godden, B., Helvenstein, P., Penninckx, M. J., & McCarthy, A. J. (1990). *Applied and Environmental Microbiology, 56*(10), 3017–3022.
21. Kirby, R. (2006). *Advances in Applied Microbiology, 58*, 125–168.
22. Bon, E. P. S., Nascimento, H. J., Macedo, J. M. B., & Silva, J. G., Jr. (1999). *Biotechnology Techniques, 13*, 289–293.
23. Burke, N. S., & Crawford, D. L. (1998). *Applied Microbiology and Biotechnology, 49*, 523–530.
24. Macedo, J. M. B., Gottschalk, L. M. F., & Bon, E. P. S. (1999). *Applied Biochemistry and Biotechnology, 77–79*, 735–778.
25. McMullen, G., Meehan, C., Conneely, A., Kirby, N., Robinson, T., Nigam, P., et al. (2001). *Applied Microbiology and Biotechnology, 56*, 81–87.
26. Ramachandra, M., Crawford, D. L., & Pometto, A. L., III. (1987). *Applied and Environmental Microbiology, 53*(12), 2754–2760.
27. Yee, D. C., Jahng, D., & Wood, T. K. (1996). *Biotechnology Progress, 12*, 40–46.
28. McCarthy, A. J., Paterson, A., & Broda, P. (1986). *Applied Microbiology and Biotechnology, 24*(4), 347–352.
29. Adhi, T. P., Korus, R. A., & Crawford, D. L. (1989). *Applied and Environmental Microbiology, 55*(5), 1165–1168.
30. Spiker, J. K., Crawford, D. L., & Thiel, E. C. (1992). *Applied Microbiology and Biotechnology, 37*, 518–523.
31. Godden, B., Ball, A. S., Helvenstein, P., McCarthy, A. J., & Penninckx, M. J. (1992). *Journal of General Microbiology, 138*, 2441–2448.
32. Mercer, D. K., Iqbal, M., Miller, P. G. G., & McCarthy, A. J. (1996). *Applied and Environmental Microbiology, 62*(6), 2186–2190.
33. Niladevi, K. N., & Prema, P. (2005). *Actinomycetologica, 19*(2), 40–47.
34. Mason, M. G., Ball, A. S., Reeder, B. J., Silkstone, G., Nicholls, P., & Wilson, M. T. (2001). *Applied and Environmental Microbiology, 67*(10), 4512–4519.
35. LPSN. (2010). List of prokaryotic names with standing in nomenclature. Available at http://www.bacterio.cict.fr/.
36. Svistunenko, D. A., Rob, A., Ball, A., Torres, J., Symons, M. C. R., Wilson, M. T., et al. (1999). *Biochimica et Biophysica Acta, 1434*, 74–85.
37. Borgmeyer, J. F., & Crawford, D. L. (1985). *Applied and Environmental Microbiology, 49*(2), 273–278.
38. Magnuson, T. S., & Crawford, D. L. (1992). *Applied and Environmental Microbiology, 58*(3), 1070–1072.
39. Trigo, C., & Ball, A. S. (1994). *Microbiology, 140*, 3145–3152.
40. Hofrichter, M. (2002). *Enzyme and Microbial Technology, 30*, 454–466.
41. Kuan, I.-C., Johnson, K. A., & Tien, M. (1993). *The Journal of Biological Chemistry, 268*(27), 20064–20070.
42. Esposito, E., Paulilo, S. M., & Manfio, G. P. (1998). *Chemosphere, 37*(3), 541–548.
43. Lucana, D. O. D. O., Schaa, T., & Schrempf, H. (2004). *Microbiology, 150*, 2575–2585.
44. Lucana, D. O. D. O., Zou, P., Nierhaus, M., & Schrempf, H. (2005). *Microbiology, 151*, 3603–3614.
45. Hofmann, B., Tölzer, S., Pelletier, I., Altenbuchner, J., Van Pée, K.-H., & Hecht, H. J. (1998). *Journal of Molecular Biology, 279*(4), 889–900.
46. Marshall, G. C., & Wright, G. D. (1996). *Biochemical and Biophysical Research Communications, 219* (2), 580–583.
47. Knoch, M., Van Pée, K.-H., Vining, L. C., & Lingens, F. (1989). *Journal of General Microbiology, 135*, 2493–2502.
48. Facey, S. J., Groß, F., Vining, L. C., Yang, K., & Van Pée, K.-H. (1996). *Microbiology, 142*, 657–665.
49. Van Pée, K.-H., & Lingens, G. D. (1985). *Journal of General Microbiology, 131*, 1911–1916.
50. Chelikani, P., Fita, I., & Loewen, P. C. (2004). *Cellular and Molecular Life Sciences, 61*(2), 192–208.

51. Kim, H.-P., Lee, J.-S., Hah, Y. C., & Roe, J.-H. (1994). *Microbiology, 140*, 3391–3397.
52. Lee, J.-S., Hah, Y.-C., & Roe, J.-H. (1993). *Journal of General Microbiology, 139*, 1013–1018.
53. Walker, G. E., Dunbar, B., Hunter, I. S., Nimmo, H. G., & Coggins, J. R. (1995). *Microbiology, 141*, 1377–1383.
54. Işik, K., Kayali, H. A., Şahin, N., Göndoğda, E. O., & Tarhan, L. (2007). *Process Biochemistry, 42*(2), 235–243.
55. Tavares, F., Bernardo, L., & Sellstedt, A. (2003). *Plant and Soil, 254*, 75–81.
56. Alskog, G., & Huss-Danell, K. (1997). *Physiologia Plantarum, 99*, 286–292.
57. Steele, D. B., & Stowers, M. D. (1986). *Canadian Journal of Microbiology, 32*(5), 409–413.
58. Vera-Cabrera, L., Johnson, W. M., Welsh, O., Resendiz-Uresti, F., & Salinas-Carmona, M. C. (1999). *Journal of Clinical Microbiology, 37*(6), 1971–1976.
59. Wu, G., Nie, L., & Zhang, W. (2005). *Antonie van Leeuwenhoek, 89*(1), 135–136.
60. Passardi, F., Zamocky, M., Favet, J., Jakopitsch, C., Penel, C., Obinger, C., et al. (2007). *Gene, 397*, 101–113.
61. Youn, H.-D., Yim, Y.-I., Kim, K., Hah, Y. C., & Kang, S.-O. (1995). *The Journal of Biological Chemistry, 270*, 13740–13747.
62. Hahn, J.-S., Oh, S.-Y., & Roe, J.-H. (2000). *Journal of Bacteriology, 182*(13), 3767–3774.
63. Mliki, A., & Zimmerman, W. (1992). *Applied and Environmental Microbiology, 58*(3), 916–919.
64. Menéndez, M. C., Ainsa, J. A., Martín, C., & García, M. J. (1997). *Journal of Bacteriology, 179*(22), 6880–6886.
65. Rafii, F., Lunsford, P., Hehman, G., & Cerniglia, C. E. (1999). *FEMS Microbiology Letters, 173*, 285–290.
66. Ro, Y. T., Lee, H. I., Kim, E. J., Koo, J. H., Kim, E., & Kim, Y. M. (2003). *FEMS Microbiology Letters, 226*(2), 397–403.
67. Ghiladi, R. A., Knudsen, G. M., Medzihradszky, K. F., & Ortiz de Montellano, P. R. (2005). *The Journal of Biological Chemistry, 280*(24), 22651–22663.
68. Kapetanaki, S. M., Choucane, S., Yu, S., Zhao, X., Magliozzo, R. S., & Schelvis, J. P. M. (2005). *Biochemistry, 44*(1), 243–252.
69. Wayne, L. G., & Diaz, G. A. (1982). *International Journal of Systematic Bacteriology, 32*(3), 296–304.
70. Martilla, H. J., Soini, H., Huovinen, P., & Viljanen, M. K. (1996). *Antimicrobial Agents and Chemotherapy, 40*(9), 2187–2189.
71. Rouse, D. A., DeVito, J. A., Li, Z., Byer, H., & Morris, S. L. (1996). *Molecular Microbiology, 22*(3), 583–592.
72. Böttger, E. C., & Springer, B. (2008). *European Journal of Pediatrics, 167*, 141–148.
73. Kim, S. J., & Shoda, M. (1999). *Applied and Environmental Microbiology, 65*(3), 1029–1035.
74. Sugano, Y. (2009). *Cellular and Molecular Life Sciences, 66*, 1387–1403.
75. Sugano, Y., Muramatsu, R., Ichiyanagi, A., Sato, T., & Shoda, M. (2007). *The Journal of Biological Chemistry, 282*(50), 36652–36658.
76. Shimokawa, T., Shoda, M., & Sugano, Y. (2009). *Journal of Bioscience and Bioengineering, 107*(2), 113–115.
77. Van Bloois, E., Pazmiño, D. E. T., Winter, R. T., & Fraaije, M. W. (2010). *Applied Microbiology and Biotechnology, 86*(5), 1419–1430.
78. Pettigrew, G. W., Echalier, A., & Pauleta, S. R. (2006). *Journal of Inorganic Biochemistry, 100*, 551–567.
79. Niebisch, A., & Bott, M. (2001). *Archives of Microbiology, 175*(4), 282–294.
80. Zámocký, M., Furtmüller, P. G., & Obinger, C. (2010). *Archives of Biochemistry and Biophysics, 500*, 45–57.
81. Ilyina, A., Huerta-Guel, P. J., Martinez-Hernandez, J. L., Rodriguez-Martinez, J., & Gorokhovsky, A. (2008). *Journal of Molecular Catalysis. B, Enzymatic, 51*, 1–9.
82. LaFleur, G. J., Jr., Horiuchi, Y., & Wessel, G. M. (1998). *Mechanisms of Development, 70*(1–2), 77–89.
83. Dayer, R., Fischer, B. B., Eggen, R. I. L., & Lemaire, S. D. (2008). *Genetics, 179*, 41–57.
84. Immenschuh, S., & Baumgart-Vogt, E. (2005). *Antioxidants and Redox Signalling, 7*(5–6), 768–777.
85. Barranco-Medina, S., Lázaro, J.-J., & Dietz, K.-J. (2009). *FEBS Letters, 583*, 1809–1816.
86. Rhee, S. G., Chae, H. Z., & Kim, K. (2005). *Free Radical Biology & Medicine, 38*(12), 1543–1552.
87. Schröder, E., & Ponting, C. P. (1998). *Protein Science, 7*, 2465–2468.
88. Trujillo, M., Mauri, P., Benazzi, L., Comini, M., De Palma, A., Flohé, L., et al. (2006). *The Journal of Biological Chemistry, 281*(29), 20555–20566.
89. Sherman, D. R., Mdluli, K., Hickey, M. J., Barry, C. E., III, & Stover, C. K. (1999). *Biofactors, 10*(2–3), 211–217.
90. Pelletier, I., Altenbuchner, J., & Mattes, R. (1995). *Biochimica et Biophysica Acta, 1250*(2), 149–157.

91. De Mot, R., De Schrijver, A., Schoofs, G., & Parret, A. H. A. (2003). *FEMS Microbiology Letters, 224*, 197–203.
92. De Mot, R., Schoofs, G., & Nagy, I. (2007). *Archives of Microbiology, 188*, 257–271.
93. Butler, A. (1998). *Current Opinion in Chemical Biology, 2*(2), 279–285.
94. Littlechild, J., Rodriguez, E. G., & Isupov, M. (2009). *Journal of Inorganic Biochemistry, 103*(4), 617–621.
95. Neumann, C. S., Fujimori, D. G., & Walsh, C. T. (2008). *Chemistry & Biology, 15*, 99–109.
96. Herbette, S., Roeckel-Drevet, P., & Drevet, J. R. (2007). *The FEBS Journal, 274*(9), 2163–2180.
97. Koshkin, A., Knudsen, G. M., & Ortiz de Montellano, P. R. (2004). *Archives of Biochemistry and Biophysics, 427*(1), 41–47.
98. Ito, K., Arai, R., Fusatomi, E., Kamo-Uchikubo, T., Kawaguchi, S.-I., Akasaka, R., et al. (2006). *Protein Science, 15*(5), 1187–1192.
99. Adam, W., Lazarus, M., Saha-Möller, C. R., Weichold, O., Hoch, U., Häring, D., et al. (2000). Biotransformations with peroxidases. In K. Faber (Ed.), *Biotransformations*. Berlin: Springer.
100. Kennedy, K., Alemany, K., & Warith, M. (2002). *Water SA, 28*(2), 149–158.
101. Duarte-Vazquez, M. A., Ortega-Tovar, M. A., Garcia-Almendarez, B. E., & Regalado, C. (2003). *Journal of Chemical Technology and Biotechnology, 78*(1), 42–47.
102. Wright, H., & Nicell, J. A. (1999). *Bioresource Technology, 70*(1), 69–79.
103. Caza, N., Bewtra, J. K., Biswas, N., & Taylor, K. E. (1999). *Water Research, 33*(13), 3012–3018.
104. Al-Kassim, L., Taylor, K. E., Nicell, J. A., Bewtra, J. K., & Biswas, N. (1994). *Journal of Chemical Technology and Biotechnology, 61*(1), 179–182.
105. Antonopoulos, V. T., Rob, A., Ball, A. S., & Wilson, M. T. (2001). *Enzyme and Microbial Technology, 29*, 62–69.
106. Winter, B., Fiechter, A., & Zimmermann, W. (1991). *Applied and Environmental Microbiology, 57*(10), 2858–2863.
107. Hernandez-Pérez, G., Goma, G., & Rols, J. L. (1997). *Biotechnology Letters, 19*(3), 285–289.
108. Pizzul, L., del Pilar Castillo, M., & Stenström, J. (2006). *World Journal of Microbiology and Biotechnology, 22*, 745–752.
109. Pizzul, L., Castillo, M., & Stenstrom, J. (2007). *International Biodeterioration and Biodegradation, 59*(2), 111–118.
110. Bogan, B. W., & Lamar, R. T. (1995). *Applied and Environmental Microbiology, 61*(7), 2631–2635.
111. Lee, B., Pometto, A. L., III, Fratzke, A., & Bailey, T. B., Jr. (1991). *Applied and Environmental Microbiology, 57*(3), 678–685.
112. Sundaramoorthy, M., Gold, M. H., & Poulos, T. L. (2010). *Journal of Inorganic Biochemistry, 104*, 683–690.
113. Sarkanen, K. V., & Ludwig, C. H. (1971). *Lignins: Occurrence, formation, structure and reactions* (pp. 1–18). New York: Wiley-Interscience.
114. Conesa, A., Punt, P. J., & Hondel, C. A. (2002). *Journal of Biotechnology, 93*, 143–158.
115. Aitken, M. D., Venkatadri, R., & Irvine, R. L. (1989). *Water Research, 23*(4), 443–450.
116. Ferrer, I., Dezotti, M., & Duran, N. (1991). *Biotechnology Letters, 13*(8), 577–582.
117. Field, J. A., de Jong, E., Feijoo-Costa, G., & de Bont, J. A. M. (1993). *Trends in Biotechnology, 11*(2), 44–49.
118. Tuncer, M., Ball, A. S., Rob, A., & Wilson, M. T. (1999). *Enzyme and Microbial Technology, 25*(1–2), 38–47.
119. Boerjan, W., Ralph, J., & Baucher, M. (2003). *Annual Review of Plant Biology, 54*, 519–546.
120. Ralph, J., Peng, J. P., Lu, F. C., Hatfield, R. D., & Helm, R. F. (1999). *Journal of Agriculture and Food Chemistry, 47*(8), 2991–2996.
121. Hamid, M., & ur-Rehman, K. (2009). *Food Chemistry, 115*, 1177–1186.
122. Spadaro, J. T., Gold, M. H., & Renganathan, V. (1992). *Applied and Environmental Microbiology, 58*(8), 2397–2401.
123. Ulson de Souza, S. M., Forgiarini, E., & Ulson De Souza, A. A. (2007). *Journal of Hazardous Materials, 147*(3), 1073–1078.
124. Aitken, M. D., & Irvine, R. L. (1989). *Biotechnology and Bioengineering, 34*(10), 1251–1260.
125. Bhunia, A., Durani, S., & Wangikar, P. P. (2001). *Biotechnology and Bioengineering, 72*, 562–567.
126. Pasti, M. B., & Crawford, D. L. (1991). *Canadian Journal of Microbiology, 37*(12), 902–907.
127. Ball, A., & Colton, J. (1996). *Journal of Basic Microbiology, 36*(1), 13–18.
128. Goszczynski, S., Paszczynski, A., Pasti-Grigsby, M. B., Crawford, R. L., & Crawford, D. L. (1994). *Journal of Bacteriology, 176*(5), 1339–1347.
129. van de Velde, F., Könemann, L., van Rantwijk, F., & Sheldon, R. (2000). *Biotechnology and Bioengineering, 67*(1), 87–96.

130. Zhao, S. S., & Luong, J. H. T. (1996). *Analytica Chimica Acta, 327*, 235–242.
131. Jia, J. B., Wang, B. Q., Wu, A. G., Cheng, G. J., Li, Z., & Dong, S. J. (2002). *Analytical Chemistry, 74* (9), 2217–2223.
132. Ruzgas, T., Csöregi, E., Emnéus, J., Gorton, L., & Marko-Varga, G. (1996). *Analytica Chimica Acta, 330*(2–3), 123–138.
133. Krell, H. W. (1991). Peroxidase: An important enzyme for diagnostic test kits. In J. Loburzewski, H. Greppin, C. Penel, & T. Gaspar (Eds.), *Biochemical, molecular and physiological aspects of plant peroxidases* (pp. 469–478). Switzerland: University of Maria Curie-Sklodowska and University of Geneva.
134. Agostini, E., Hernandez-Ruiz, J., Arnao, M. B., Milrand, S. R., Tigier, H. A., & Acosta, M. (2002). *Biotechnology and Applied Biochemistry, 35*(1), 1–7.
135. Ragland, B. D., Kourad, R. J., Chaffin, C., Robinson, C. A., & Hardy, R. W. (2000). *Clinical Chemistry, 46*(11), 1848–1851.
136. Ayala, M., Roman, R., & Vazquez-Duhalt, R. (2007). *Biochemical and Biophysical Research Communications, 357*(3), 804–808.
137. Burton, S. G. (2003). *Trends in Biotechnology, 21*(12), 543–549.
138. Demain, A. L., & Adrio, J. L. (2008). *Molecular Biotechnology, 38*(1), 41–55.