

A Comprehensive Review on Function and Application of Plant Peroxidases

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Abstract

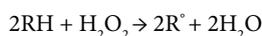
Peroxidases, one of the key antioxidant enzymes, are widely distributed in nature and catalyze oxidation of various electron donor substrates concomitant with the decomposition of H₂O₂. The non-animal plant peroxidases (class III peroxidase) are involved in various essential physiological processes of plant growth and development throughout their life cycle. In view of the capability of peroxidases to catalyze the redox reaction for a wide range of substrates, they are considered as one of the important enzyme from the point of view of their various medicinal, biochemical, immunological, biotechnological and industrial applications. They have been successfully used for biopulping and bio-bleaching in the paper and textile industries. Peroxidases have also been used in organic synthesis, bioremediation, as well as various analytical applications in diagnostic kits, ELISA. Peroxidase based biosensors find application in analytical systems for determination of hydrogen peroxide, glucose, alcohols, glutamate, and choline etc. Thus, in view of array of physiological functions as well as industrial applications, the peroxidases have conquered a dominant position in research groups and become one of the most extensively studied enzymes. In this direction, the present review embodies the classification, mechanism of action, major physiological functions as well as industrial applications of plant peroxidases.

Keywords: Heme peroxidase; Analytical applications; Biosensor development; Class III peroxidase; Dye decolorization

Introduction

Development of environmentally sustainable processes is a challengeable task for the current bioeconomy. In this direction, the use of biocatalysts, enzymes, in various processes is considered as an ecofriendly approach. The stability, activity and specificity of enzymes are the fundamental parameters that are required to develop enzymes for their optimal applications in various industrial processes. Therefore, identification of newer sources for such novel enzymes with desired properties is important. Furthermore, modern approaches such as *in-silico* molecular modeling concomitant with site directed mutagenesis to develop such novel enzymes with improved attributes are also in great demand. The present thesis is an effort in this direction.

Peroxidases (EC.1.11.1.x) are hydrogen peroxide (H₂O₂) decomposing enzymes concomitant with the oxidation of wide range of phenolic as well as non-phenolic substrates (RH)



They are ubiquitous in nature being found in bacteria, fungi, algae, plants and animals. The plant peroxidases, belonging to Class III peroxidase, are implicated in various vital processes of plant growth and development throughout the plant life cycle including cell wall metabolism, lignification, suberization, reactive oxygen species (ROS) metabolism, auxin metabolism, fruit growth and ripening, defense against pathogens etc. Due to versatility in reaction catalyzed by peroxidases, and their ubiquitous nature, they have immense potential to be an industrial enzyme with application in various medicinal, immunological, biotechnological and industrial sectors. The peroxidases find applications in bioremediation, textile synthetic dye decolorization, polymer synthesis, paper and pulp industry, in development of biosensor, diagnosis kits etc. In view of various applications, the identification of newer sources of novel peroxidase offering resistance towards temperature, pH, salts, heavy metals, organic solvents etc. is highly desirable. Thus, the present review is an attempt to summarize various physiological functions as well as the industrial applications of plant peroxidases.

Classification of peroxidases

On the basis of presence or absence of heme, the peroxidases have been classified into heme and non-heme peroxidases [1]. According to PeroxiBase database, >80% of known peroxidase genes are reported to code for heme-containing peroxidases. On the other hand, the non-heme peroxidases such as thiol peroxidase, alkylhydroperoxidase, NADH peroxidase constitute only a small proportion. Since majority of the peroxidase sequences are reported to be heme peroxidases, thus they are described in detail.

Heme peroxidases have further been assigned to two superfamilies, namely peroxidase-cyclooxygenase superfamily (PCOXS) and the peroxidase-catalase superfamily (PCATS) [2,3]. Description of these peroxidase superfamilies are presented as follows. A schematic classification of peroxidases is presented in Figure 1.

The Peroxidase-Cyclooxygenase Superfamily (PCOXS): The peroxidases of PCOXS superfamily exclusively contain animal peroxidases which have been suggested to be involved in the innate immunity, defense responses etc. [4,5]. The myeloperoxidase (MPO), eosinophil peroxidase (EPO), lactoperoxidase (LPO), thyroid peroxidase (TPO) are belonging to this family. In this superfamily, the prosthetic heme group is covalently linked with the apoprotein.

The Peroxidase-Catalase Superfamily (PCATS): The PCATS is the most intensively studied superfamily of non-animal heme peroxidases.

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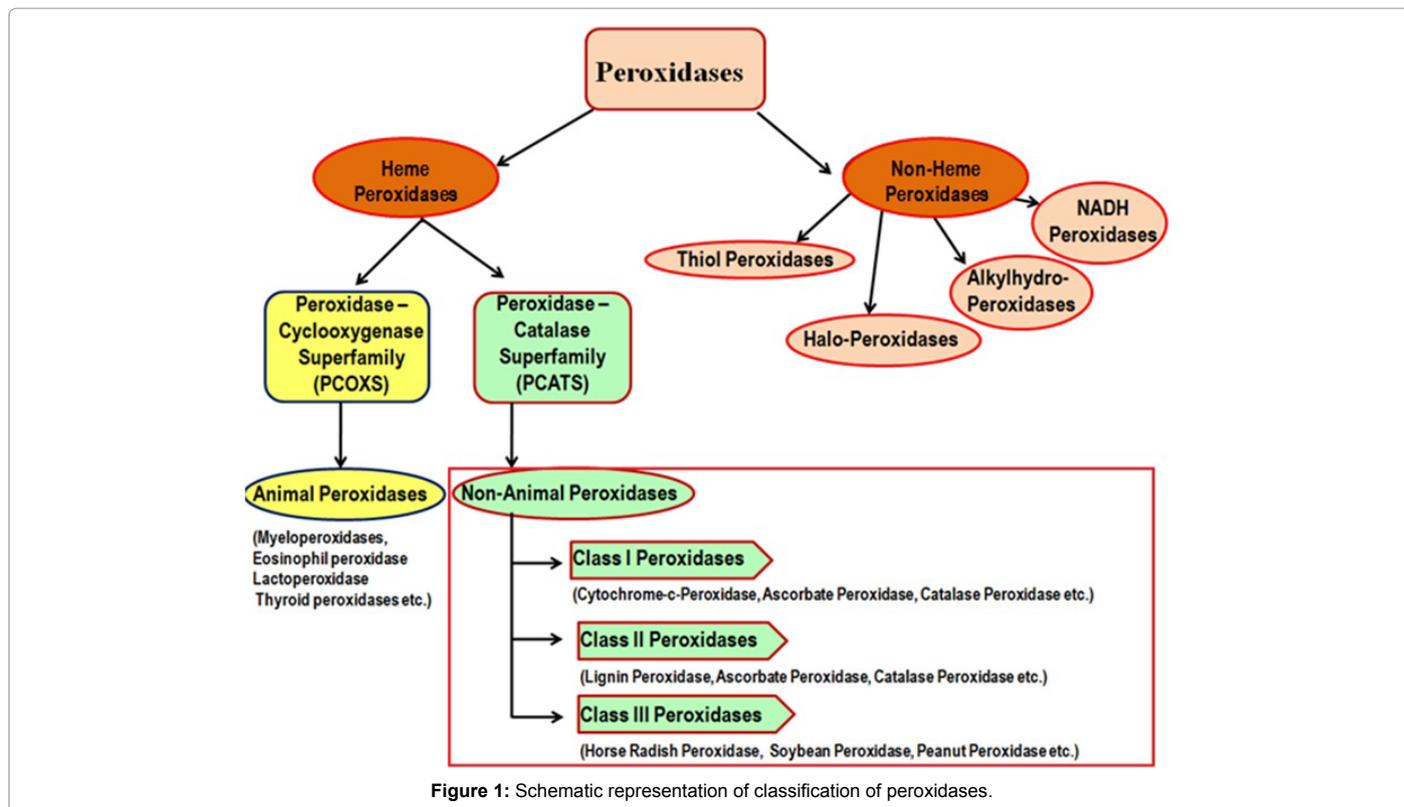


Figure 1: Schematic representation of classification of peroxidases.

Initially, the superfamily was named as the plant, fungal and bacterial heme peroxidase superfamily depending upon the sources of the peroxidases. Later on, due to emergence of new cnidarians peroxidase, the name of this superfamily was changed as peroxidase-catalase superfamily.

The non-animal peroxidases have further been sub-divided into three classes namely, class I, II and III peroxidases as described in following sections.

(a) Class I Peroxidases: The class I peroxidases include both prokaryotic and eukaryotic peroxidases belonging to non-animal sources. Currently, 1839 sequences of this class are reported in PeroxiBase database. They exhibited major role in oxidative stress i.e., detoxification of ROS (H_2O_2) [6-8]. They include cytochrome c peroxidase (CCP; EC 1.11.1.5), ascorbate peroxidase (APX; EC 1.11.1.11) and catalase peroxidase (CP; EC 1.11.1.6). The cytochrome c peroxidase (CCP), uses reducing equivalents from cytochrome c and reduces hydrogen peroxide to water. Ascorbate peroxidases (APx) are involved in hydrogen peroxide detoxification using ascorbate as reducing equivalents as well as in photo-protection of the chloroplasts and cytosol in higher plants [9,10]. The catalase-peroxidases (CPs), predominantly reported in bacteria, are bi-functional antioxidant enzymes that exhibit both catalase and peroxidase enzyme activity. Due to their unique catalytic capacity to dismutate hydrogen peroxide and ability to evolve molecular oxygen (O_2) by oxidation of H_2O_2 , they prevent bacteria from oxidative stress [11]. Evolutionary they are closely related to ascorbate peroxidases and cytochrome c-peroxidases [12,13]. At the structural level, the class I peroxidases lack disulphide bridges, calcium and an endoplasmic reticulum signal sequence.

(b) Class II Peroxidases: The class II peroxidases, exclusively containing fungal peroxidases, have major role in lignin biodegradation

[14]. In the PeroxiBase database, 609 sequences of class II peroxidases are reported till date. White-rot fungal lignin peroxidases (LiPs; EC 1.11.1.14) are secretory in nature and catalyze depolymerization of lignin as well as possess immense potentials for waste disposal of a number of phenolic as well as non-phenolic compounds. Manganese peroxidases (MnP; EC 1.11.1.13), are also secreted by lignin-degrading white-rot fungi. They catalyze the peroxide-dependent oxidation of Mn (II) to Mn (III) and the Mn (III) is released from the enzyme as oxalate-Mn (III) complex that serve as diffusible redox mediator having ability to oxidize lignin. Versatile peroxidases (VP; EC 1.11.1.16) exhibited a hybrid molecular architecture between LiPs and MnPs [15]. They are not only specific for Mn (II) as in 3MnPs, but also catalyze the oxidation of phenolic and non-phenolic substrates like LiPs, in the absence of manganese [16]. In contrary to class I peroxidases, the class II peroxidases have N-terminal signal peptides, four conserved disulphide bridges (differently located to those of class III) and calcium in their structure.

(c) Class III Peroxidases: The class III peroxidases are widely distributed in plant kingdom [17,18]. In the PeroxiBase database [1], 5692 sequences of class III peroxidases (~ 70% of total non-animal heme peroxidases) are reported till date. They include horseradish peroxidases (HRP), peanut peroxidase (PNP), soybean peroxidase (SBP), etc. and they are reported to play crucial roles in the plant life cycle [19]. Thus, they are involved in wide range of physiological processes such as cell wall metabolism [20], lignification [21], suberization [22], auxins metabolism [23], wound healing [24], reactive oxygen species (ROS) and reactive nitrogen species (RNS) metabolism [25,26], fruit growth and ripening [27] defense against pathogens [28] etc. These peroxidases exist as multigene family as evident by 73 and 138 peroxidase genes in the genomic sequences of Arabidopsis (Arabidopsis Genome Initiative, 2000) and rice (International Rice Genome Sequencing Project, 2005),

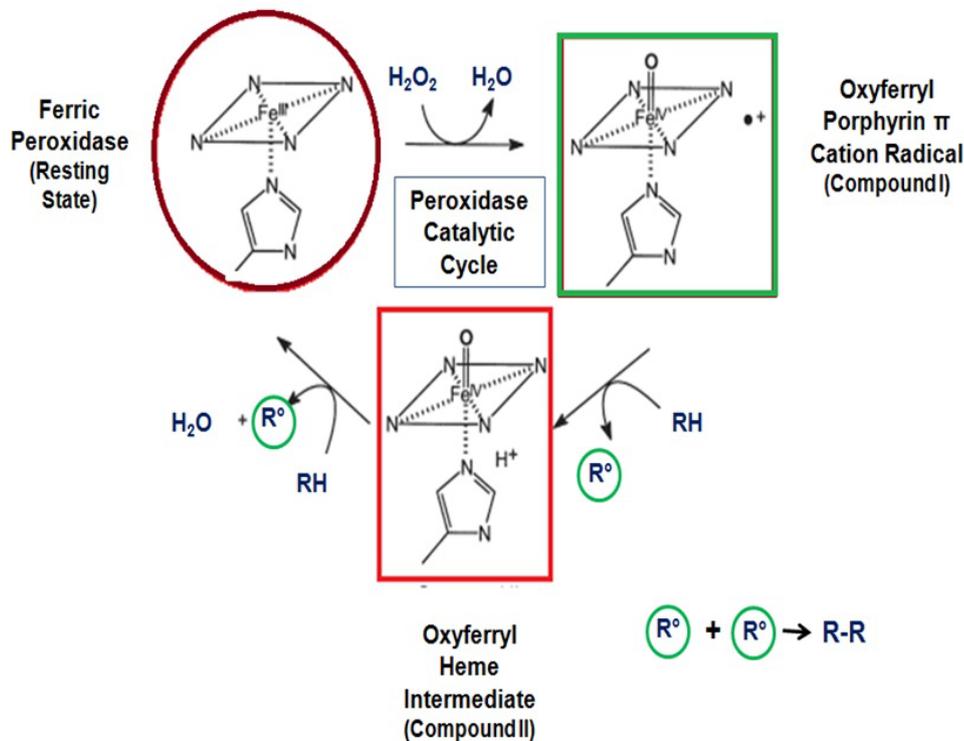


Figure 2: Catalytic cycle of heme peroxidases showing catalytic events (oxidation-reduction) occur at heme (Fe⁺ porphyrin IX) center of the enzyme. At the end of the reaction, H₂O₂ is degraded to H₂O and reducing equivalent (RH) is polymerized.

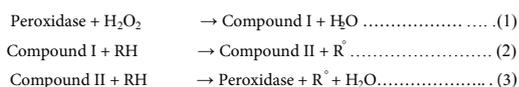
respectively [17,29,30]. Similar to class II peroxidases, in the structural fold, the class III peroxidases also contain N-terminal signal peptides, four conserved disulphide bridges and calcium. In the present review, the class III plant peroxidases are detailed below.

Evolutionary relationship between heme peroxidases

The heme peroxidases of non-animal origin show low amino acid sequence identity (less than 20%) but, share similar helical folds independent to the presence (in plant and fungal peroxidases) and absence (in bacterial peroxidases) of disulfide bridges and structural calcium ions. Recently [31] have also studied the comparative account of non-animal heme peroxidases and reported that the peroxidases are clustered into three major classes. In addition, [6] have suggested that the class I peroxidases are the origin point for the other two classes of peroxidases.

Mechanism of action

Peroxidases share a common catalytic mechanism for the degradation of hydrogen peroxide [32]. The peroxidase reaction is a two-electron oxidation-reduction with three distinct steps [33] :



Where, RH is a peroxidase substrate and R[•] is a free-radical product derived from it.

The catalytic cycle of heme peroxidases specific to plants begins with the coordination of peroxide to the ferric heme (Figure 2). The

coordinated peroxide undergoes rapid heterolytic cleavage, producing a molecule of water and the semi-stable intermediate referred to as compound I (green in color, and also referred as oxyferryl porphyrin π cation radical). The reaction involves transfer of a proton from peroxide O₁ to O₂, followed by breaking of O-O bond. Departure of O₂ as water molecule leaves O₁ that is already coordinated to the heme with only six electrons. It completes its octate by abstracting the two most readily available electrons from the enzyme. One electron is removed from the iron, creating an oxy-ferryl (Fe=O) center. Generally, in case of most of the peroxidases, the second electron is removed from the porphyrin ring, creating a porphyrin pi-cation radical.

This porphyrin radical accepts one electron from an electron donor substrate, yielding a substrate free radical and compound II (red in color and referred as oxyferryl heme intermediate). In the next one-electron reduction step from a second molecule of the substrate reduces compound II to the resting ferric peroxidase [34,35].

Another intermediate namely the compound III, in which the iron is in the ferrous state, is usually formed when there is a large excess of H₂O₂. It is likely that this intermediate is largely formed by combination of superoxide, generated by the oxidation of H₂O₂, with the ferric enzyme, although superoxide could also be generated by electron transfer from oxidized substrates to molecular oxygen. Compound III is not a catalytically active intermediate.

Physiological roles of plant peroxidases

Class III plant peroxidases have been reported to play diverse functions in the plant life cycle such as in cell wall metabolism, lignification, suberization, ROS metabolism, wound healing, fruit growth and ripening, seed germination etc. [36]. A schematic representation of diverse functions of class III peroxidases is presented

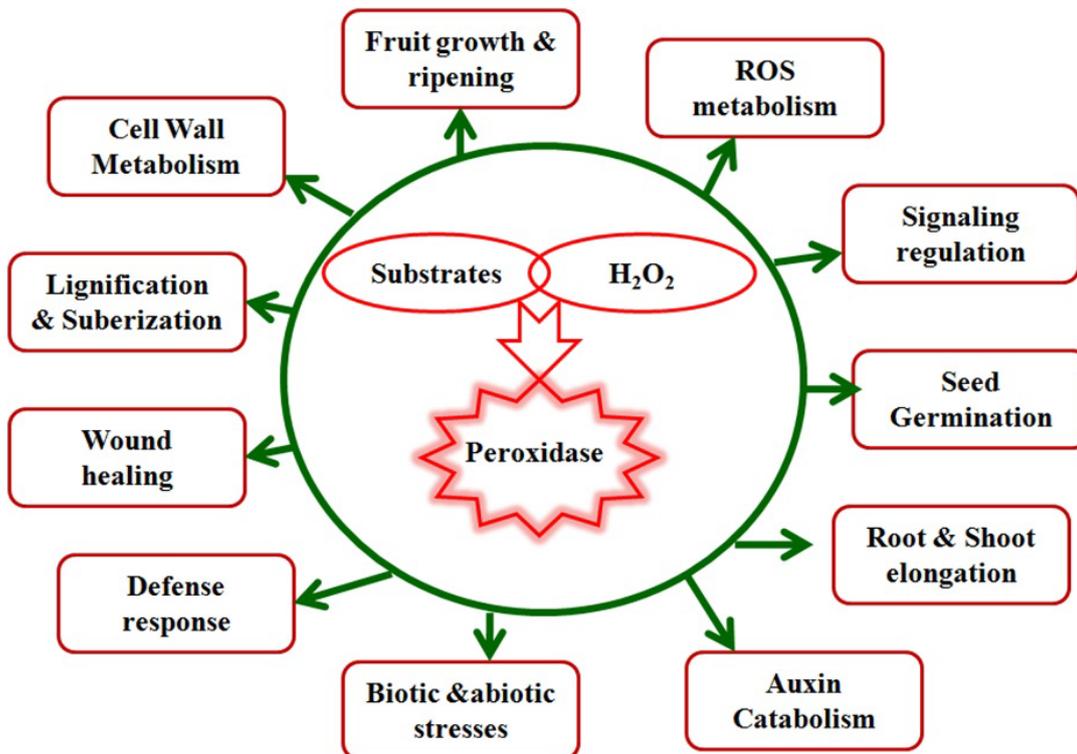


Figure 3: A schematic representation of various roles of plant peroxidases.

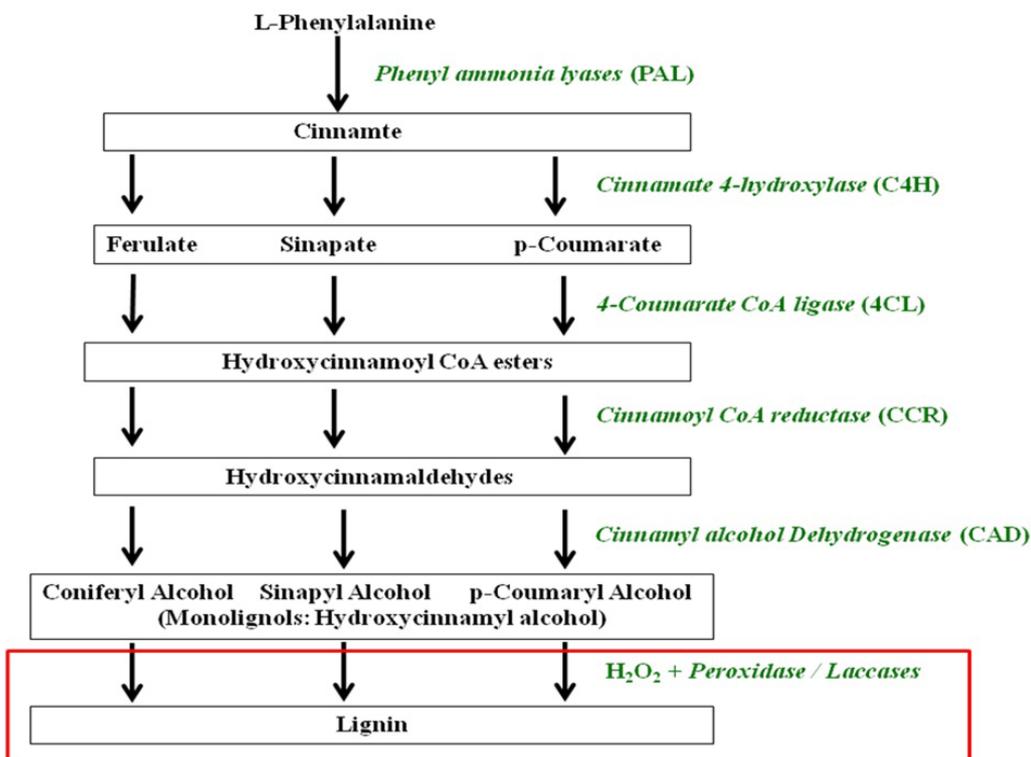


Figure 4: Generalized lignin biosynthesis pathway indicating the role of peroxidases.

in Figure 3. Some major roles of peroxidases are described in following sections:

Lignification and suberization

Lignification occurs during normal growth and defense responses in plants and is one of the classical functions attributed to Class III peroxidases [37]. Lignin, a phenolic heteropolymer, present in the cell wall of plants and provides rigidity, strength, and resistance to chemical, physical as well as biological attacks. Lignin which contributes about 25% of the plant biomass is considered as one of the greatest obstacle towards the optimal utilization of the plant biomass for various purposes such as paper manufacturing, production of highly palatable forage and bagasse utilization.

Chemically, lignin is made up of mainly three types of monolignols, namely p-coumaryl alcohol, sinapyl alcohol and coniferyl alcohol. These monolignols are synthesized via phenyl propanoid (PP) pathway which starts with deamination of phenylalanine, followed by successive hydroxylations, methylations, thiol ester formation and two reduction reactions leading to the formation of the three major kinds of monolignols. These monolignols are transported to cell wall in the form of glycosides and there glucosidase enzyme release the monolignols.

The polymerization of the monolignols, into lignin involves an oxidative mechanism with the consequent formation of phenoxy radicals, through the action of oxidative enzymes, such as peroxidases. The schematic representation of lignin biosynthesis pathway along with the role of peroxidase is represented in Figure 4.

There are several reports suggesting higher expression of peroxidase genes in the tissues undergoing lignification [38]. Thus, Mader and Amberg- Fisher [39] have reported that the peroxidases have ability to polymerize cinnamylalcohols in the presence of hydrogen peroxide. Furthermore, based on experimental study, Andrews et al. [40] have demonstrated the link between peroxidase isoenzymes and the cross linking of cell wall components and the deposition of lignin-like phenolics in the epidermis of the tomato fruits. Christensen et al. [38] have purified and characterized five peroxidases in poplar xylem and also analyzed their correlation with lignification via oxidation of syringaldazine (a lignin monomer analogue). The recombinant papaya peroxidase was also reported to be involved in defense response and lignification via qRT-PCR and activity measurement with coniferyl alcohol [41]. In addition, Ostergaard et al. [42] have reported an extracellular peroxidase from lignifying *Arabidopsis thaliana* cell suspension cultures. The authors have also done mutational studies and proposed a possible correlation between the enzyme and the increased levels of lignin in a mutant *Arabidopsis*. The evidence for a role of peroxidases in lignification has also been supported by studies on transgenic plants with altered peroxidase activity. For example, Quiroga et al. [43] showed that expression of a tomato peroxidase gene, in transgenic tobacco resulted in an increase in lignin content. Lagrimini et al. [44] have also found higher levels of lignin in transgenic tobacco with over-expressed peroxidase than the wild type plants.

Suberization has been believed to play a role in the defensive responses against the entry of pathogenic micro-organisms through a wounded part by developing physical barrier [43,45]. This protects tissue from water loss and pathogen invasion. Suberized tissues are found in various underground organs like roots, stolon and tuber as well as in periderm layer. They are formed as a part of wound and pathogen induced defenses of specific organs and cell types, perhaps the most familiar example being the browning of sliced potato tubers. Lignification and suberization are terminal processes of determinate

and highly differentiated plant cells capable of forming secondary cell walls. The cell wall peroxidases polymerize the hydroxycinnamic acid and their derivatives by converting them into phenoxy radicals that are then deposited on the extracellular surface. The accumulation of these polymers strengthens the cell wall, thereby restricting cell expansion and pathogen invasion, and confers structural strength to the plant body, which is especially important for trees and construction of xylem vessels.

Plant defense against pathogen infection

Plants protect themselves, after pathogen attack, through the passive and active defense mechanisms. The passive defense mechanisms involve structural barriers or existing anti-microbial compounds which prevent colonization in the tissue, while, the active or induced defense responses include the hypersensitive response (HR) and systemic acquired resistance (SAR) i.e., production of phytoalexins and pathogenesis-related (PR) proteins, reactive oxygen species (ROS) and reactive nitrogen species (RNS) (oxidative bursts), ion fluxes across the plasma membrane, lignification and the reinforcement of the cell wall through both the cross-linking of cell wall structural proteins. The active defense responses are regulated through a complex and interconnected network of signaling pathways mediated by salicylic acid (SA), jasmonic acid (JA) and ethylene (ET).

Among the proteins induced during the plant defense, the class III plant peroxidases are well known and they play roles through (1) reinforcement of cell wall physical barriers comprising lignin, suberin, feruloylated polysaccharides and hydroxyproline-rich glycoproteins [46,47]; (2) enhanced production of reactive oxygen species as signal mediators and antimicrobial agents [48-50]; and (3) enhanced production of phytoalexin [51].

Peroxidases have been reported to be induced by fungal infection [52,53], bacterial infection [54,55], infection caused by viruses and viroids etc. [56-59]. In tobacco, a positive correlation between peroxidase activity and resistance to tobacco wildfire disease was reported [60,61]. Furthermore, [54] have reported a rapid induction of a cationic peroxidase in rice plants, infected with *Xanthomonas oryzae pv. oryzae*.

Wound healing

Plants respond to wounding by activating self-defense systems to restore damaged tissues or to defend against attacks by pathogens and herbivores. Among the large number of wound-inducible proteins, peroxidase have been shown to express upon mechanical wounding in various plants, including tobacco [57,58], tomato [62], potato [62], cucumber [63], azuki bean [64], rice [65], horseradish [66] and sweet potato [67].

Reactive Oxygen Species (ROS) metabolism

The Reactive Oxygen Species (ROS) are partially reduced forms of atmospheric oxygen (O_2) and produced by the excitation of oxygen to form a singlet oxygen (O_2^1) or from the transfer of electrons to O_2 , to form superoxide radical in the case of one electron; hydrogen peroxide (H_2O_2) if two electrons are transferred; or hydroxyl radical (OH^{\cdot}) when three electrons have been transferred to oxygen. These reduced species of oxygen are highly reactive and are capable of oxidizing various cellular components leading to the oxidative damage of the plant cell that is associated with the peroxidation of membrane lipids, protein oxidation, enzyme inhibition and DNA damage that ultimately leads to Programmed Cell Death (PCD) [68-70]. In plant cells, ROS are produced in cell walls, chloroplasts, mitochondria, plasma membrane,

endoplasmic reticulum, and apoplasmic space [68,71]. In plants, the cellular level of H_2O_2 is mostly regulated by enzymatic actions of catalases and peroxidases. In addition to scavenging H_2O_2 , the cell wall peroxidases have been considered to catalyze O_2^- and H_2O_2 formation through oxidation of substrates such as NADH and IAA, in the absence of exogenous H_2O_2 [71].

Auxin catabolism

Peroxidases play an important role in auxin catabolism through the oxidative carboxylation of indole-3-acetic acid (IAA) [72]. IAA, one of the most studied plant growth regulators, is found throughout the plant, but is at highest concentrations in the apical and other meristematic regions. Auxin affects the plant development through apical dominance, cell elongation, ethylene formation and adventitious root formation. Plant peroxidases are involved in the oxidation of auxin either through the conventional H_2O_2 -dependent pathway or through a H_2O_2 -independent and O_2^- -dependent pathway. As specific IAA oxygenases, the peroxidases have domains, similar to auxin-binding proteins, which are missing in non-plant peroxidases [23].

Gazaryan et al. [23] have also reported that over-expressing peroxidase in tobacco plants showed depressed IAA levels with decreased root branching and closed stomata. On the other hand, plants with suppressed peroxidase showed increased levels of IAA and rapid shoot growth, fused leaves and early flowering. Based on experimental study, Jansen et al. [73] have reported the transgenic tobacco with over-expressed peroxidase exhibited increased UV tolerance and decreased IAA levels, suggesting the contribution of the peroxidases in UV tolerance and auxin catabolism. Furthermore, Schopfer [74] have also reported the involvement of plant peroxidases in cell elongation via IAA catabolism.

Seed germination

The seed occupies a unique position and has fundamental importance in plant physiology because most of the known physiological processes are concentrated in the growth and development of the seed. The exact reaction between activation of essential enzymes, sequential release of hormones and the energy relations of the process during the germination of seed are still unknown. An increase in peroxidase activity during seed germination has been reported [75]. Fridovich [76] and Gasper et al. [77] have suggested that the peroxidase removes various toxic products from the seeds as a natural scavenger for seeds. After 4 days of germination in mung bean seedlings, Dendsay and Sachar [78] have observed a 30-fold activation of peroxidase activity. In cotton plants, increase in peroxidase activity is also correlated with auxin induced ethylene biosynthesis [79,80].

Fruit ripening

Ethylene has been demonstrated as an essential plant hormone involved in initiation of fruit ripening as well as promotion of maturation and abscission of fruits and in the regulation of senescence and fading of flowers [81]. Peroxidases also contribute to the synthesis of ethylene. L- methionine is the precursor for ethylene in tissues of higher plants. Three enzymes are involved in this methionine-methional-ethylene pathway with peroxidase being most limiting enzyme in ethylene biosynthesis. Zymograms of peroxidase show change in activity pattern during initial stages fruit ripening [82]. Both positive and negative correlations between peroxidase activity and fruit ripening have been reported in literature. Thus, peroxidase activities in mango, apples, banana fruits etc. have been reported to increase with ripening [83-85] while, those of tomato, strawberry, capsicum, papaya fruits etc. decrease with ripening [86-89].

Stress tolerance

Peroxidases are considered as one of the stress indicators of plants because their level considerably increases after stress stimulation. Lignification also occurs due to stress responses [73].

The role of peroxidases and other antioxidant enzymes on heat stress have been documented by various researchers. Thus, Larkindale and Huang [90] have demonstrated that the expression level of peroxidases and superoxide dismutase increased, while those of catalase decreased after heat treatment of creeping bentgrass plants. Similarly, Edreva et al. [91] have also investigated the role of peroxidases in bean plants and they found that peroxidase activity is increased after heat treatment.

Salinity stress is caused by accumulation of dissolved salts in soil water either through natural process or due to human induced activity and the high salt accumulation in the soil can affect the growth of the plants [92,93]. Amaya et al. [94] have investigated the role of a cell wall peroxidase in the response to salt stress. They have observed the increased rates of seed germination in transgenic tobacco with over-expressed peroxidase under both osmotic and salt stress. They have also suggested that enhancement of peroxidase activity stabilizes the cell wall structure and higher germination rate might be due to the better capacity to retain water that reduces the effect of water deficit caused by the salinity. Pujari and Chanda [95] have also studied the effect of high salinity on the levels of peroxidase expression in vigna seedlings and have reported higher activity of peroxidases in salt treated plants.

Metals such as manganese (Mn), zinc (Zn), copper (Cu) etc. are necessary for the plant development in trace amounts but in excess they become toxic for the plant [96]. In general, increase in peroxidase expression in response to various metals are reported and it is suggested that this increase could be a kind of defensive response for scavenging the H_2O_2 generated due to metal toxicity [97]. Assche and Clijsters [98] have reported the induction in peroxidase activity in leaves and roots treated with toxic doses of Zn, Cd, Ni and Pb. In addition, Fang and Kao [99] have also reported the increase in peroxidases activity as well as changes in the isozyme patterns after exposure to iron, copper and zinc in rice leaves and have suggested that the toxic levels of the metals could be responsible for both quantitative and qualitative changes in the peroxidase. Abercrombie et al. [100] have also reported that in response to metals such as aluminum, arsenic, etc. the class III peroxidase genes are over expressed.

Ultraviolet-B radiation is reported to influence the plant metabolism, growth and development leading to affecting photosynthesis, flowering, pollination and transpiration by altering gene expression [101]. Jansen et al. [102-120] have analyzed various tobacco lines with altered peroxidase expression and suggested a clear link between peroxidase activity and UV tolerance. UV tolerance has been reported to be linked with levels of peroxidases in plant. Thus, Jansen et al. [73] demonstrated that UV-tolerant plants exhibited more peroxidase expression than the sensitive type.

Isolation, purification, and characterization of some peroxidases

Plant peroxidases from various sources such as *Eruca vesicaria*, tea, *Ficus*, lettuce, citrus, broccoli, royal palm, soybean, *Leucaena leucocephala*, papaya, wheat grass, *Solanum melongena*, lemon etc. have been isolated, purified and characterized. A brief description of physicochemical properties of some of them is presented in Table 1.

S. No.	Source	Mol. Weight	pH optimum	Temperature optimum	Km/affinity for substrates	Thermostability	Effect of metal/organic solvent	References
1	<i>Citrus jambhiri</i>	56 kDa	5.5	40°C	Guaiacol> O-dianisidine> pyrogallol	Retained 20-30% activity at 50, 60, and 70°C after 15, 10, and 5 min of incubation	Inhibited by Li ⁺ , Zn ²⁺ and Hg ²⁺	Mohamed, et al. (2008)
2	<i>Prunus persica</i>	29 kDa	5	40°C	o-dianisidine: 9.35 mM H ₂ O ₂ : 15.38 mM	Lost 55% activity at 65°C within 1 min	Not affected by Zn ²⁺ , Cu ²⁺ , Mg ²⁺ , Mn ²⁺ , NH ₄ ⁺ (1.5-6.0 μM)	Neves (2002)
3	<i>Lactuca sativa</i> L.	35 kDa	5.0	45°C	Guaiacol: 4.74 mM pyrogallol: 1.96 mM	Retained 55% activity at 60°C after 1 hr	<ul style="list-style-type: none"> Inhibited by Fe³⁺, Zn²⁺, Ca²⁺, Cu²⁺, Mn²⁺ 40-70% loss of activity in presence of 25% propylene glycol, ethanol, acetone and methanol 	Hu et al., (2012)
4	<i>Carica papaya</i>	240 kDa	7.0	40°C	Guaiacol: 0.8 mM O-dianisidine: 0.125 mM ascorbic acid: 5.2 mM H ₂ O ₂ : 0.25 mM	Retained 80% activity at 60°C upto 1 Hr	Mg ²⁺ as a potent activator and Ca ²⁺ as a weak activator	Pandey et al., (2012)
5	<i>Roystonea regia</i>	51 kDa	4-11	-	o-dianisidine: 9.35 mM H ₂ O ₂ : 15.38 mM	Stable at 70°C after 1 h incubation	-	Sakharov et al. (2001)
6	<i>Ficus sycomorus</i>	43 kDa	5.5-7.0	5°C to 40°C	Guaiacol: 9.5 mM O-dianisidine: 16.6 mM pyrogallol: 26 mM, H ₂ O ₂ : 1.2 mM	-	<ul style="list-style-type: none"> Activated by Ca²⁺, Ni²⁺ and Mg²⁺ inhibited by Mn²⁺, Fe³⁺, Zn²⁺ and Hg²⁺ 	Mohamed et al. (2011)
7	<i>Leucaena leucocephala</i>	200 kDa	5.0	55°C	Guaiacol: 2.9 mM, H ₂ O ₂ : 5.6 mM	Fully active at 65°C for 20 min	<ul style="list-style-type: none"> Activated by Ca²⁺, Mn²⁺, Na⁺ Azide insensitive 	Pandey and Dwivedi, 2011
8	<i>Eruca vesicaria</i> sbsp. <i>Sativa</i>	34 kDa	6.0	40°C	Guaiacol: 375.74 mM pyrogallol: 510.14 mM	Retained 50% activity at 80°C after 30 min incubation	<ul style="list-style-type: none"> Activated by Ni²⁺, Co²⁺, Cu²⁺ Inhibited by Mn²⁺, Hg²⁺, Zn²⁺, K⁺, Ca²⁺, Fe²⁺ 	Nadaroglu, et al., (2013)
9	<i>Citrus medica</i>	32 kDa	6.0	50°C	Guaiacol: 8 mM o-dianisidine : 1.8 mM H ₂ O ₂ : 0.66 mM	Stable at 60°C and 65°C	<ul style="list-style-type: none"> Activated by Cu²⁺, Co²⁺, Mg²⁺, K⁺ and Ca²⁺ Inhibited by Hg²⁺, Fe²⁺, Mn²⁺ and Zn²⁺ 	Mall et al. (2013)
10	<i>Fragaria ananassa</i> Duch.	56 kDa	6.0	30°C	-	Complete loss of activity> 60°C within 5 min	-	Civello et al. (1995)
11	<i>Brassica oleracea</i> Var. <i>Italica</i>	Neutral and basic: 43 kDa Acidic: 48 kDa	Neutral and basic : 6.0 Acidic: 4.0	-	Guaiacol: 0.305 mM (acidic), 8.789 mM (basic), 0.711 mM (neutral)	Stable at 80°C activity after 1hr incubation	-	Thongsook and Barrett, (2005)
12	<i>Citrus limon</i>	200 kDa	5.0	40°C	Guaiacol: 0.7 mM H ₂ O ₂ : 1.09 mM	Retained 92% activity at 80°C for 1 h	<ul style="list-style-type: none"> Activated by Na⁺, Zn²⁺, Mg²⁺, Ca²⁺ and Mn²⁺ tolerance towards Cd²⁺, Cs²⁺ and Ni²⁺ Retained 30-50% activity in the presence of 50% ethanol, methanol and isopropanol 	Pandey, et al., (2016)
	<i>Actinidia deliciosa</i>	29 kDa	5.5	50°C	Guaiacol: 7.4 mM H ₂ O ₂ : 1.3 mM		-	Soda et al., (1991)
14	<i>Camellia sinensis</i>	34.5 kDa	4.5 to 5.0	-	Pyrogallol > ascorbate > guaiacol	-	-	Kvaratskhelia et al. (1997)
15	<i>Solanum tuberosum</i>	45.8 kDa	4.5	40°C -60°C	-	-	-	Bernards et al. (1999)
16	<i>Trachycarpus fortunei</i>	50 kDa	3.0	61–67°C	-	Moderate stable	Inhibited by Na ⁺ , Ca ⁺⁺ , and Mg ⁺⁺	Caramyshve et al., (2006)
17	<i>Solanum melongena</i>	-	5.5	84°C	Guaiacol: 6.5 mM H ₂ O ₂ : 0.33 mM	-	-	Vernwal et al. (2006)
18	<i>Fagopyrum esculentum</i>	46.1 kDa (POX I) and 58.1 kDa (POX II).	8.0 (POX I) 4.5 (POX II)	30°C (POX I) and 10°C (POX II)	POX I- Guaiacol: 0.288 mM, o-dianisidine: 0.229 mM, Ascorbate: 0.043 mM POX II- o-dianisidine: 0.137 mM, Ascorbate: 0.029 mM	-	-	Suzuki et al. (2006)

19	<i>Gossypium hirsutum</i>	39.1 and 64 kDa	5.0 and 6.0	25°C.	-	-	<ul style="list-style-type: none"> Activated by Al³⁺, Fe³⁺, Ca²⁺, and Ni²⁺ Inhibited by Mn²⁺, K⁺, Zn²⁺ and Hg²⁺ 	Kouakou et al., (2009)
20	<i>Vigna</i> species	-	-	-	o-dianisidine: 1.55-2.24 mM	Retained 33%, 66% and 3.4% activity at 70°C, 75°C and 80°C, for 1 h	-	Yves et al. (2011)
21	<i>Brassica oleracea</i>	-	4.0	-	-	Retained <10% activity after 5 min at 76.6°C	-	Fortea, et al., (2012)
22	<i>Luffa aegyptiaca</i>	-	6.5	60°C	Guaiacol: 2.0 mM H ₂ O ₂ : 0.2 mM	-	-	Yadav et al., (2011)

Table 1: Physicochemical properties of some purified plant peroxidases.

Applications of peroxidases

Peroxidases are important from the point of view of their industrial applications by virtue of their ability to catalyze the oxidation-reduction reaction of a wide range of phenolic as well as non-phenolic substrates in the presence of hydrogen peroxide. A number of industrial applications of peroxidases have been reported in the area of agriculture, analytical, environmental, medical sectors etc. Thus, peroxidases have been used in bioremediation of contaminating environmental pollutants such as phenols, delignification in paper and pulp industry, diagnosis kit development, immunoassay, organic and polymer synthesis as well as in and biosensor technology. They are also used for developing convenient and quick methods for the determination and quantification of hydrogen peroxide in both the biological and industrial samples [121,122]. Additional applications of peroxidases include determination of extent of lipid peroxidation in meat food products, in polymerization and precipitation of aqueous phenols as well as in decolorization of industrial effluents [123]. Some of the important applications of peroxidases are described in detail in following sections.

Application as biosensor

Biosensors have significance in medicine, quality control, food and environmental monitoring as well as in research. Enzyme based biosensors have advantage over other analytical techniques with regards to high selectivity and high sensitivity. The performance of these biosensors depends upon the amount and bioactivity of enzyme immobilized onto the electrodes. Peroxidases have immense potential and wide spread application as biosensors [123].

Horse radish peroxidase has widely been used in development of biosensors [124-126]. HRP biosensors are made by applying various detection methods including an amperometric immunosensor, mass balance, potentiometric methods, photovoltaic spectroscopy, optical and chemiluminescent methods etc. [127-132]. Potentiometric based biosensors are developed by combination of an enzyme (eg. peroxidase) and a transducer that can detect the variation in protons. Glucose, maltose or lactate are reported to be detected by peroxidase based biosensor using potentiometric methods [133]. HRP-based biosensors for antioxidant monitoring have been applied in the detection of superoxide radical [134], nitric oxide [135], glutathione [134,136], uric acid [137,138] and phenolic compounds [139-141]. Besides HRP, other plant peroxidases such as sweet potato, tobacco, peanut, soybean etc. have been also explored for their applications as biosensors. The sweet potato peroxidase, due to its easy availability, high specific activity and superior electrochemical characteristics, is considered as advantageous for application as biosensor [126]. A recombinant tobacco peroxidase immobilized to graphite electrodes were reported to be advantageous for detection of aromatic phenols and amines [142].

Soybean peroxidase (SBP) has been reported to be advantageous

over HRP for the biosensor manufacturing. The first SBP biosensor was reported by Vreeke et al. [143] as a thermostable-wired enzyme electrode. Spring cabbage peroxidase (SCP), has been reported to provide a good bioelectrocatalytic system due to its good affinity for various substrates, stability towards temperature and pH and ability to bind to polyanionic matrices and low costs of extraction and purification [144]. Due to efficient bioelectrocatalysis of lignin peroxidases (LiP), the LiP-graphite electrode biosensor systems have been developed for detection of recalcitrant aromatic compounds [145].

Application in analytical and diagnostic kits

Peroxidases are widely used in the development of analytical as well as diagnostic kits. Among peroxidases, horseradish peroxidases are most commonly used for the analytical purposes [146]. However, other plant peroxidases having wide pH and temperature stability are emerging as option for HRP. Since, the peroxidase has capability to produce stable chromogenic products, thus, they are suitable candidate enzyme for the manufacturing of various diagnostic kits based on enzyme conjugated antibody technology [147]. Uric acid detection kits have been developed using turnip root peroxidases [148].

In combination with cholesterol oxidase and cholesterol esterase, peroxidases have also been exploited for developing cholesterol detection kits that help in quantification and monitoring of human serum cholesterol [149,150]. Peroxidases have been used in developing kits for the diagnosis of bladder and prostate cancers through the detection of 8-hydroxydeoxyguanosine and its analogs in urine [151]. The monitoring of glucose for diabetes mellitus and of lactate in hypoxia and ischemia, are of great significance in patient management [152] and it would be highly desirable to develop such a sensitive biosensor for the detection of H₂O₂, which would be stable at 37°C and higher temperatures for sustained periods of time. Lactose content monitoring strips have been developed using the combination of immobilized β-galactosidase, galactose oxidase and HRP enzymes [153]. In biomedical sectors, for cancer treatment gene-directed enzyme/prodrug therapy, (GDEPT) have been extensively and successfully used. Greco et al. [154] proposed a prompt and efficient peroxidase-IAA based GDEPT system for cancer treatment. This enzyme-prodrug system has been found to be effective against hypoxic and anoxic tumor cells and also has potential to be used in other anti-cancer strategies. Besides GDEPT, in antibody-directed enzyme/prodrug therapy (ADEPT), specific HRP-conjugated antibodies are used [155,156]. Influenza virus was reported to detect using ultra-sensitive colorimetric immunoassay with peroxidase-mimic of gold nanoparticles [157].

Application in de-colorization of industrial dyes

Dyes are used extensively for paper printing, color photography and as additive in petroleum products. These are synthetic aromatic

compounds having complex structures, biologically un-degradable and causing environmental problems. For the degradation of these synthetic industrial dyes, the currently available methods such as chemical oxidation, reverse osmosis, and adsorption, are highly efficient, but they suffer with some disadvantages. Now-a-days the interest is towards the use of microbial degradation of dyes, because this process is less expensive and ecofriendly alternative [158]. Enzymes such as lignin peroxidases (LiPs) and manganese peroxidases (MnPs), are involved in the decolorization of synthetic azo dyes such as orange II, and others [159]. Horseradish peroxidase (HRP) is reported to degrade phenol and substituted phenols via a free radical polymerization mechanism and can be effective in degrading and precipitating industrially important azo dyes, such as Remazol [160,161]. The properties of white-rot fungi to oxidize various recalcitrant xenobiotics released to the environment are thought to result from the activities of LiP, MnP, and laccases [162]. A purified peroxidase produced by *Geotrichum candidum* dec 1, was involved in decolorization of dyes [163]. Pandey et al. [164,165] have also reported that the purified lemon peroxidase was found to be oxidized the industrial dyes in the order of aniline blue>methyl orange>indigo carmine >trypan blue>crystal violet.

The textile industry, being one of the traditional industrial segments, consume large quantities of water, chemical products and synthetic dyes and generate large volumes of wastewater that contain a high organic load that are responsible for acute or chronic toxicity on the ecosystems. Thus, the wastewater from textile industries is considered as one of the most polluting among all industrial wastes, thereby requiring appropriate treatment technologies. Peroxidases have been shown to have great potential in the decolorization process to decrease textile industry pollutant residues [166,167]. Uses of peroxidases, such as horseradish, *Brassica campestris* turnip, tomato, bitter melon, soybean, *Ipomea palmata* and *Saccharum spontaneum* peroxidases, for degrading and detoxifying polyaromatic hydrocarbons, polychlorinated biphenyls, and other synthetic industrial dyes have been reported [168-178].

Application in bioremediation of phenolic compounds

Aromatic compounds such as phenols and its derivatives are a major class of pollutants in wastewater from a number of food and chemical industries [179]. Phenols are known to be toxic and also hazardous carcinogens that can accumulate in the food chain. Being highly toxic their discharge into the environment should be highly regulated [180]. The polymerization using redox enzymes is one of the phenol removal methods. The enzyme treatment offers a high degree of specificity, operation under mild conditions and high reaction velocity [180] concomitant with an ecofriendly approach. The ability of peroxidases to catalyze the formation of free-radical from various aromatic pollutants and their polymerization can be potentially exploited in bioremediation and wastewater treatment. Thus, peroxidases have been reported for removal of phenolic compounds from synthetic model effluents and also from real industrial effluents [160,161,181-183]. There are a number of reports in literature on detoxification of wastewater contaminated with phenols, cresols, and chlorinated phenols using HRP. Using HRP, Bewtra et al. [184] have determined the optimum pH for removal of 2, 4-dichlorophenol as 6.5. The removal efficiency is also affected by the hydrogen peroxide concentration. Soybean and turnip peroxidases have also been shown to have good potential for removal of phenolics compounds [185,171]. Additives, such as PEG or gelatin, usually improve removal efficiency by protecting the enzyme [186]. The addition of PEG-3350 or PEG-8000 to soybean peroxidase, increased the removal efficiency of 2, 4-dichlorophenol by a factor of 10 or 50, respectively [187]. Thus, the

potential of peroxidase for soil and water detoxification constitute a possible basis for the development of bioremediation technologies.

Application in pulp and paper industry

Lignin, a phenolic heteropolymer, shows negative impact on the proper exploitation of plant biomass for the pulp and paper industry. Thus, the lignin should be removed prior to the production of good quality paper. The chemical delignification leads to production of various pollutants. Thus, the enzymatic degradation of lignin is suggested as a better alternative. Lignin peroxidase (LiP) and manganese peroxidase (MnP) are successfully used for biopulping, biobleaching as well as selective delignification in the paper industry and selective delignification helps in the production of cellulosic materials that can be used as efficient feed and biofuel [188,189].

Hair dyeing

Traditionally the hair coloring dyes are synthesized via oxidative polymerization of dye precursors (phenols or aminophenols, and couplers). For this process, the hydrogen peroxide (3%) is used that initiates the polymerization reaction but it bleaches the natural hair pigment melanin. These concentrations of H₂O₂, when applied repeatedly, can cause hair damage. To achieve a gentler dyeing with milder oxidation process, the enzymes such as oxidases, peroxidases etc. have been proposed as better options [190].

Analytical applications

Enzyme Linked Immunosorbent Assays (ELISA) has advantages over other immunoassays in simplicity, low cost, reliability, simple equipment requirement etc. ELISA is usually developed to detect antigens or antibodies by producing an enzyme such as peroxidase catalyzed color changes. In these assays an enzyme-linked antibody specific to the antigen is required along with a chromogenic substrate, which give colored product in the presence of the enzyme and the color of the product is proportional to the amount antigen/antibody of interest. For such type of assays HRP is the most commonly used enzymes as linked with antibody. They are used to screen the monoclonal antibodies against dangerous mycotoxins of various fungal species like *Aspergillus*, *Penicillium* (ochratoxins), *Fusarium* (T-2 toxin, trichothecenes) etc [191-193]. A large number of reports regarding the development of enzyme immunoassays using peroxidase as reporter enzyme to detect toxins, pathogens, and other analyses are available. For example, in detection of Goynautoxins, Chlamydia, *Fusarium* toxin, Dengue virus proteins, Hepatitis-E virus peroxidases are used [194-199]. HRP-based immunoassays for the detection of undeclared milk proteins (eg. bovine b-lactoglobulin) in foods has also been developed [200].

The DNA detection using complementary DNA probes is of significance in diagnostics and research. The non-radioactive DNA probes associated with peroxidases, such as HRP have become safer than radioactive probes [201]. Peroxidases have been reported to become suitable for microarray analyses because it catalyses a large number of electron-transfer reactions with natural and synthetic substrates. It can be used either directly immobilized on the microarray [202], or as a labeling agent for nucleic acids, antibodies and other proteins [203,204]. The HRP-based or associated microarrays (biochips) have been used in various applications such as expression analysis, recombination and gene mapping, mutation analysis etc [205,206].

Application in organic polymer synthesis and grafting

The importance of heme peroxidases in polymer chemistry is

based on their ability to oxidize a variety of phenolic molecules thereby generating reactive species (phenoxy radicals) which provide ideal sites for cross-linking (coupling) leading to polymerization reactions. The grafting application of peroxidase is one of the important industrial applications of heme peroxidases. The free radicals generated from the reactions catalyzed by peroxidases, can induce formation of other new functional polymers such as phenolic resins [207,208]. The broad spectrum of peroxidase applications in organic synthesis is due to their ability to catalyze different types of reactions as 1) oxidative dehydrogenations ($2RH + H_2O_2 \rightarrow 2R^{\circ} + 2 H_2O$), 2) oxygen transfer reactions ($R + H_2O_2 \rightarrow RO + H_2O$), 3) oxidative halogenations ($RH + H_2O_2 + HX \rightarrow RX + 2H_2O$) and 4) H_2O_2 dismutation ($2 H_2O_2 \rightarrow 2H_2O + O_2$) [209,210]. The peroxidases have been used in the development of hybrid resins from renewable sources to replace phenol-formaldehyde based resins that are widely used in surface coatings, adhesives, laminates, molding, friction materials, abrasives, flame retardants, carbon membranes, glass fiber laminates, fiberboards, and protein-based wood adhesives etc [211].

Blinkovsky and Dordick [212] have demonstrated the HRP mediated polymerization of phenolics and incorporation of phenols into lignin leading to the formation of polymers of great potential as phenolic resins [213]. For example, enzymatically synthesized poly (p-phenylphenol) and poly (p-cresol) were reported to have high melting points, whereas, the poly (p-phenyl-phenol) exhibited higher electrical conductivity than that of phenol-formaldehyde based resins. The incorporation of cresol into lignin by peroxidase provides a platform for using lignin as a raw material for grafting molecules to obtain new functional polymers [214]. Thus, the ability of peroxidases to modify lignin and develop new functional polymers with excellent properties leads to the progress in lignin applications such as in development of polymer adhesives, biodegradable plastics, polyurethane copolymers, paints, dispersants in dyes, in pesticides, and printed circuit boards [207,215-217]. Using *Soybean* peroxidase (SBP), Ikeda et al. [218] successfully developed polyphenol resins without involvement of formaldehyde, which exhibited better properties than that of conventionally polymerized resins.

Kim et al. [219] have used SBP to catalyze the oxidative polymerization of cardanol to polycardanol. Cardanol is an excellent raw material for the preparation of high grade insulating varnishes, paints, enamels, laminating resins, and rubber. Thus, the cardanol-based resins show resistance towards softening action of mineral oils, acids and alkaline conditions, termite, and insects and have coefficient of friction less sensitive to temperature changes than phenol-formaldehyde based resins.

The free radical polymerization of methyl methacrylate (MMA) catalyzed by peroxidase (such as HRP) was developed by Karla and Gross [220]. Poly (methyl methacrylate) (PMMA) is a colorless polymer used extensively for the production of scratch resistance optical products, plastics, and PVCs. In addition, peroxidase mediated polymerization of acrylamide in to poly-acrylamide with good thermal properties have been reported [221-223]. Peroxidases have also been used to catalyze the free-radical polymerization of vinyl monomers, such as acrylamide, acrylic acid and methacrylates, such as methyl, phenylethyl, 2-hydroxyethyl methacrylate [220,224]. MnP also reported to catalyze the polymerization of acrylamide into a thermoplastic resin namely polyacrylamide in the presence of 2, 4-pentanedione, has been reported [225]. With the versatile properties of peroxidases, styrene was also polymerized into polystyrene that is widely used as packaging material, injection molded parts, UV screening agents, in disposable cutlery, and CD and DVD cases [226].

The chemical synthesis of conducting polymers such as polyaniline, the most extensively studied conducting polymers having high environmental stability and promising electronic application including electronic equipment, photovoltaic cells, plastic batteries, polymer light-emitting diodes, and optical displays, is not eco-friendly, thus HRP have been tried as an alternative for the synthesis [227-234]. But due to low activity of HRP towards the synthesis of Polyaniline, the other anionic peroxidase purified from *soybean*, african oil palm tree is developed as better substitute of HRP [235].

Peroxidase catalyzed polymerization of substituted and unsubstituted phenols and anilines have also been reported as better and striking alternative to the conventional chemical (formaldehyde) polymerization method [236-238]. Peroxidases also catalyze the stereospecific oxygen transfer reactions such as heteroatom oxidations, oxidation of C-H bonds in allylic/benzylic compounds, alcohols and indoles that yield various synthetic compounds of significance [210]. The plant peroxidases have also been shown to have application in the synthesis of α -3', 4'-anhydrovinblastine, by the coupling reaction of catharanthine and vindoline. The α -3', 4'-anhydrovinblastine is a metabolic precursor of vinblastine and vincristine which are used in cancer chemotherapy [238,239,240].

Conclusion

Peroxidases catalyze oxidation of a wide range of phenolic as well as non-phenolic compounds. Plant peroxidases, belonging to class III peroxidases, have been implicated in various plant growth and developmental processes such as cell wall metabolism, fruit growth and ripening, metabolism of reactive oxygen species (ROS), defense against pathogens etc. In view of the wide applications of peroxidases in key areas such as clinical biochemistry, immunology, biotechnology, environment and industry, they are considered as one of the important industrial enzyme. Thus, peroxidases have been used in bioremediation of contaminating environmental pollutants such as phenolic compounds, delignification in paper and pulp industry, diagnosis kit development, immunoassay, organic and polymer synthesis as well as in ELISA and biosensor technology. Furthermore, with the potentials of nano-based biosensor applications, in recent years, peroxidases have gained more prominence. The use of immobilized enzymes, in various industrial processes, is one of the advancement in their application. For all such applications of peroxidases there is a need for search of novel peroxidases offering tolerance towards the factors / ingredients of the reaction environment such as temperature, pH, salts, metals and organic solvents etc.

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