Insights into the mechanism of lignocellulose degradation by versatile peroxidases

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Lignocelluloses are imperative structural components of plant cell wall and are profusely found in agricultural crop residues. The structural heterogeneity and recalcitrance of lignin limit the accessibility of cell wall carbohydrates for constructive exploitation. During the past decades, diverse lignin degrading enzymes were characterized to facilitate the utilization of lignocellulosic biomass for technological applications. Versatile peroxidases are unique among ligninolytic enzymes for their remarkably high redox potential and ability to oxidize lignin without the requisite of redox mediators. The hybrid structural architecture of this enzyme bearing functional features of lignin peroxidase and manganese peroxidase demonstrates its versatility in aromatics oxidation. This review summarizes the distinctive structural aspects of fungal versatile peroxidase in correlation to its oxidation of aromatic substrates besides emphasizing on the catalytic environment conducive for substrate oxidation. This review also focuses on the general strategies employed for production of this enzyme, its molecular framework, potential biotechnological applications of versatile peroxidase and prospects on enhancing the production of enzyme. Finally, the significance of this enzyme in improving the nutritive value of crop residues to promote ruminal productivity is highlighted.

Keywords: Lignolytic enzyme, lignin degradation, ruminant nutrition, white rot fungi.

LIGNOCELLULOSES are major components of plant cell wall that impart mechanical strength and rigidity to plants. Lignin cements the cellulose and hemicellulose filaments together and acts as a barrier to prevent degradation of sugar polymers. Lignin is the second abundant organic polymer on earth next only to cellulose. Its metabolism is of great importance for the maintenance of carbon cycle. Interestingly, agricultural crop residues are one of the renewable sources of lignocellulosic biomass which in addition to energy-rich carbohydrates, preserve a significant proportion of nitrogen, phosphorus, sulphur and potassium of the total nutrient intake of the plant. Despite the energy-rich potential of these fibres, a large fraction of these crop residues are burnt on field pointing to the ease of clearance and lack of economically viable alternative for disposal of these crop residues post-harvesting. Ironically, this lignocellulosic biomass has attracted immense research in recent decades owing to its vast potential for utilization in diverse industrial applications vitally in paper pulp industries, for bioremediation, for second generation biofuel production and as animal feed. Delignification of lignocellulosic residues exposes the nutritionally-affluent and highly valuable polysaccharides as feedstock for these applications. The complex process of mineralization of lignin has been perceived with fungi of basidiomycetes and bacteria of the actinomycetes and proteobacteria group[1,2]. The gut microflora of wood boring termites, beetles and certain soil bacteria possess the capability to decompose aromatic lignin structures. *Streptomyces viridosporus*, *Rhodococcus jostii*, *Nocardia autotrophica* of actinomycetes and *Pseudomonas putida*, *Sphingobium*, *Burkolderia* of proteobacteria could depolymerize lignin through their extracellular enzymes. Albeit, the fungal system of biodegradation of lignin overarches most efficient bacterial lignin degraders in terms of redox potential and in oxidation of highly recalcitrant compounds[3]. The fungal system possesses integrative complete machinery requisite for selective degradation of lignin. To this end, selective degradation of lignin for access to energy-rich cellulose and hemicellulose is performed naturally by white rot fungi, in particular phylum basidiomycetes[1]. These fungi have evolved with the ability to efficiently degrade whole wood components by selective or simultaneous delignification with selective delignification more pronounced for their action focused on lignin with least attack on polysaccharides. Lignolytic fungi secrete a repertoire of extracellular enzymes during their secondary metabolism in response to nutrient limitation for non-specific oxidative degradation of lignin[4]. This enzyme array comprises phenol oxidases like laccase, heme peroxidases like lignin peroxidase, manganese peroxidase, versatile peroxidase and extracellular H_2O_2 generating oxidases[5]. Chemically, lignin macromolecule is a highly heterogenous aromatic polymer of phenyl propanoid units whose stereo irregular structure costs high redox potential for oxidation. Redox potential of laccases falls in the range of 780–800 mV, while manganese peroxidases and lignin peroxidases has potential of 1100–1500 mV. Recently explored versatile peroxidases operate with a redox potential of about

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1700 mV (ref. 6). The oxidizing ability of peroxidases is a summation of active site morphology, redox potential and substrate accessibility. Thus, peroxidases are more prominent of ligninolytic enzymes for their relatively high redox potential and capability to oxidize the heavily recalcitrant non-phenolic phenyl propanoid units of lignin in contrast to phenol oxidases which preferably oxidize the phenolic units[7]. Versatile peroxidases have been the focus of intense research in the past decade because of its unique feature of oxidizing phenolic and non-phenolic units of lignin, independent of redox mediators, contrary to other ligninolytic enzymes which require the presence of mediators for destruction of non-phenolic units. This trait of versatile peroxidase makes them potential biocatalysts for diverse biotechnological applications. This review focuses on the properties of versatile peroxidases and the reactions catalysed by them featuring their relevance in biotechnological applications.

Characteristics of versatile peroxidase

From a structural point of view, versatile peroxidase (EC 1.11.1.16), a reactive black 5 hydrogen peroxide oxidoreductase is a heme containing glycoprotein catalysing oxidative degradation of aromatic heterogenous compounds using hydrogen peroxide as electron acceptor. The most typical feature of this enzyme is the hybrid molecular architecture with Mn^{2+} to Mn^{3+} oxidizing property of manganese peroxidase and high redox potential

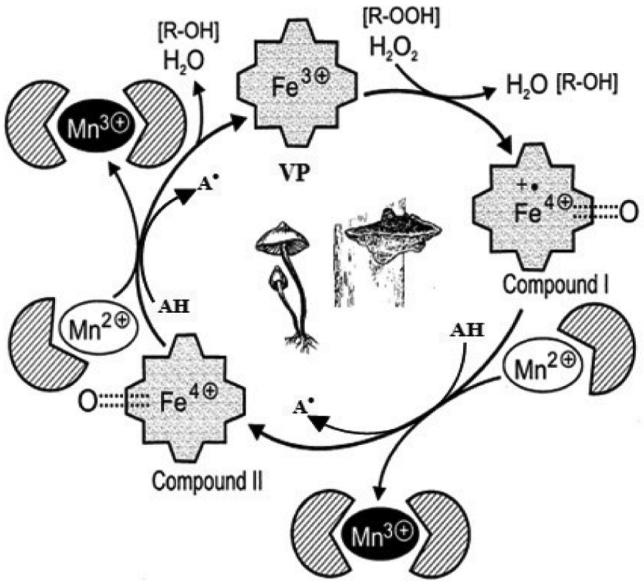

Figure 1. Substrate oxidation mechanism of versatile peroxidase. Resting enzyme is oxidized by hydroperoxides to a two electron deficient compound I followed by subsequent one electron reductions of compound I to compound II and the native enzyme. Enzyme abstracts electrons from either aromatic compound (AH) or Mn^{2+}. $Fe^{4+} = O^{+\bullet}$ represents high valent porphyrin cation radical (adapted from Ruiz-Dueñas *et al.*[41] and Hofrichter[43]).

compounds oxidizing behaviour of lignin peroxidase. This Mn^{2+} oxidizing property has initially led to designation of versatile peroxidase as manganese peroxidase isoenzyme. Nevertheless this enzyme was later identified as a novel peroxidase with added manganese independent activity[8]. This enzyme also exhibits higher stability among basidiomycetes peroxidases in oxidation of lignin units, whereas lignin peroxidase is inactivated by high concentration of phenolic compounds[9]. Additionally, manganese is an obligatory co-factor for manganese peroxidase while laccase and lignin peroxidase oxidize non-phenolic lignin structure through aromatic mediators like ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) and veratryl alcohol respectively. Although these mediators are secreted by fungi as part of their secondary metabolism, industrial production of these enzymes necessitates explicit supplementation of these redox compounds. Amazingly, versatile peroxidase leads the ligninolytic peroxidases with its ability to oxidize the dimeric lignin macromolecule free from the presence of redox mediators. From the taxonomical standpoint, versatile peroxidase belongs to class II 'plant, fungal and bacterial peroxidases', the major role of which appears to be the degradation of lignin in wood.

Catalytic mechanism of versatile peroxidase

Versatile peroxidase is a glycosylated heme protein with polyvalent catalytic sites for oxidation of manganese and low and high redox potential compounds. Structural elements that hold this property reveal a heme porphyrin ring in a central pocket surrounded by two channels for access by hydrogen peroxide, the activator of enzyme complex and for manganese ion. The heme pocket is stabilized by two helical domains with Ca^{2+} and proximal and distal basic histidine residues, a feature representative of the class II plant, fungal and bacterial peroxidases[10]. The catalytic cycle of versatile peroxidase for oxidation of low redox potential substrates follows a similar mechanism of oxidation as other constituent members of heme peroxidase family, but stands out in possessing an added ability to utilize manganese as substrate. Catalytic cycle is initiated by binding of hydrogen peroxide in the central heme pocket to form iron peroxide complex (Figure 1). The resting enzyme in ferric state (Fe^{3+}) reacts with H_2O_2 or any organic peroxide in distal side of heme to generate a Fe^{4+} oxo-porphyrin radical complex with activated heme known as compound I, a two-electron deficient complex. Compound I is then oxidized in two-consecutive single-electron reactions with reducing substrates yielding highly reactive radical products and water. The first reduction step results in the formation of another enzyme intermediate compound II, which is finally reduced back to ferric peroxidase, the native enzyme[7]. The radical products are highly unstable and go through several

non-enzymatic reactions resulting in breakdown of ether linkages, demethoxylation, aromatic ring cleavage, deprotonation and hydroxylation internally to yield simple breakdown products[11,12]. Hydrogen peroxide, the electron acceptor requisite for lignin degradation by these peroxidases is generated by white rot fungi through cyclic redox reactions involving aryl alcohol oxidases and dehydrogenases[13]. Aryl alcohol oxidases catalyse the oxidation of primary aromatic unsaturated alcohols whereas dehydrogenases reduce aromatic aldehydes to produce H_2O_2. The high affinity substrate of versatile peroxidase, manganese divalent cation is oxidized at a surface close to heme propionate where the cation is bound by tricarboxylates of glutamate and aspartate residues in closed gate conformation, the orientation of residues slightly different from manganese peroxidase, demonstrating higher stability of versatile peroxidase over the former. Site-directed mutagenesis studies have revealed the role of three acidic residues in coordination of manganese and established that versatile peroxidase exhibited paramount manganese oxidizing activity substantiated by experimental evidence that substituting one of the three carboxylate residues or reducing the length of acidic side chains did not have a significant effect on its catalytic activity. In contrast, manganese peroxidase lost its catalytic activity when one of the amino acid carboxylate was replaced, whereas in versatile peroxidase only triple deletion variant decreased its oxidizing action[14]. The natural lignocellulosic substrates, viz. wood and the soil, host good quantity of manganese which is oxidized by versatile peroxidase; this oxidized manganese chelates with organic acid secreted by fungi such as glyoxalate, oxalate, lactate to act as a diffusible oxidant in oxidation of phenolic lignin structures and also non-phenolic moieties through formation of lipid peroxyl radicals[15]. Organic acids are secreted by fungi through *de novo* synthesis and also during degradation of lignin[16]. However, high redox potential substrate oxidation by versatile peroxidases is inhibited by Mn^{2+} with decrease in rate of the former reaction, a reaction supposed to be non-competitive inhibition[17].

Another feature of versatile peroxidase which merits special mention is the different strategy employed in oxidation of high redox potential substrates in the absence of manganese. Reports had documented biphasic kinetics for certain large molecular weight bulky substrates[14] suggesting the presence of multiple oxidation sites wherein initially the large molecular weight substrates are likely to be oxidized at the enzyme surface and the degradation intermediates gain access to the heme propionate where they are further oxidized to end products through classical catalytic cycle of heme peroxidases. This oxidation at enzyme surface is possible by long range electron transfer (LRET) from the enzyme surface to redox cofactor heme, initiated at an exposed tryptophan neutral radical on the protein front[18]. Putative electron transfer pathways initiating at histidine and tryptophan residues were proposed for lignin peroxidase which operates through a similar electron transfer mechanism wherein site-directed mutagenesis studies established the only operative stable neutral tryptophan radical in LRET to heme. Proteins functioning in catalysis through LRET generally employ tryptophan and tyrosine residues in tandem for electron transfer to activated cofactor (Figure 2). In contrast, in ligninolytic enzymes especially versatile peroxidase, no tyrosine residues are observed demonstrating their distinguished property in oxidation of aromatic compounds. Tyrosine residues in degradation of aromatic compounds cross link with phenoxy radicals formed leading to enzyme inactivation. Absence of tyrosine residues in ligninolytic enzymes prevents enzyme inactivation by dimerization proving its competence in demineralization of complex aromatic and recalcitrant lignin in highly oxidative environment. Additionally, tryptophanyl radicals involved in electron transfer can exist in neutral and cationic form. The cationic form typically occurs on inner plane of protein and the tail tryptophan residue is usually deprotonated and is neutral as confirmed by spectroscopic and density functional theory studies[19]. Further, the tryptophan radical in lignin peroxidase exists in β-hydroxylated form in conditions where peroxide is in larger proportion than the reducing substrate. Regarding the surface chemistry of tryptophan in versatile peroxidase, the aromatic amino acid is present in a positively charged environment contributed by basic amino residues exposed to solvent, favourable for binding of anionic substrates, while in lignin peroxidase, tryptophan is surrounded by acidic residues creating a negatively charged environment with protruding side chain of phenylalanine residue around it. These structural features of lignin peroxidase

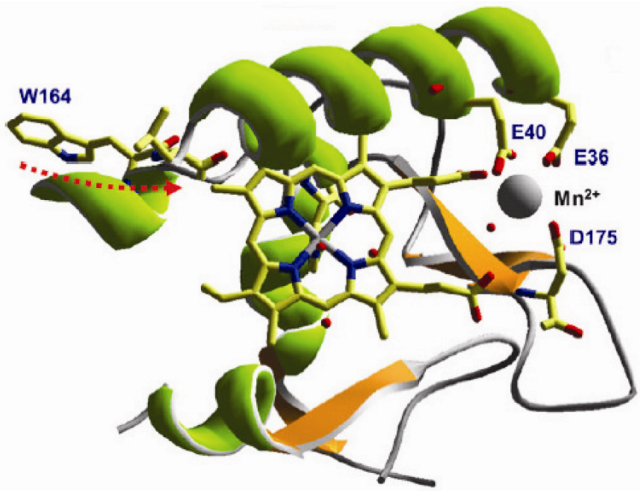

Figure 2. Structure of heme pocket of versatile peroxidase showing tryptophan residue (W164) involved in LRET (indicated by red arrow) and carboxylates of glutamate (E40, E36) and aspartate (D175) forming the manganese divalent cation binding site. Heme porphyrin centre is indicated in blue (adapted from Ruiz-Dueñas *et al.*[41]).

necessitate the use of redox mediators for degradation of high molecular weight substrates. Versatile peroxidase tryptophanyl environment, a strong oxidizing centre acts on large molecular substrates effectively, which are otherwise not oxidized at the activated heme due to steric hindrance[20]. Electron transfer reactions occur both in the enzyme and macromolecular lignin, where labile intramolecular bonds of the compound are subjected to non-enzymatic cleavage. Fungal hyphae then sequester these lignin degradation products for internal catabolism. The enzyme is also highly active in the presence of organic solvents thereby effectively binding the inorganic cation manganese due to favourable decrease in medium polarity[21]. These aspects deliver versatile peroxidases as a novel biocatalyst for direct oxidation of a broad spectrum of aromatic heterogenic substrates, a feature significant for biotechnological applications of this enzyme.

Biochemical aspects

The remarkable biotechnological applications of this enzyme demand availability of a large quantity of enzyme with enhanced stability. Conceptually, enzyme stability, kinetic parameters of catalytic reaction and substrate ionization are highly reliant on environmental parameters such as temperature and pH. Accordingly, versatile peroxidase of *Bjerkandera fumosa* was investigated[22] for its optimal activity on 2,6 dimethoxyphenol, an aromatic substrate at different pH values. The catalytic efficiency peaked at pH 4.0 in the absence of Mn^{2+} and remained constant in the pH range 4.0–6.0. Similarly, in the presence of Mn^{2+}, oxidation efficiency remained constant in the pH range 4.5–6.5 with maximum activity at pH 5.0. The results agree with the optimal activity values of versatile peroxidases from diverse basidiomycetes and oxidation on a range of different substrates. Interestingly, pH optima for versatile peroxidase for oxidation of manganese (pH 5) and aromatic compounds (pH 3) are comparable to the activity maximum of manganese peroxidase and lignin peroxidase enzymes respectively evidential of its hybrid architecture[23]. Redox potential of heme peroxidases increases at acidic pH; hence pH optimum in acidic range provides an added advantage of efficient aromatic degradation. Although natural wood degradation takes place at acidic pH, acidic and alkaline stability of enzymes is a property desirable for industrial application of an enzyme. To this fact, a versatile peroxidase isoenzyme from the *Pleurotus ostreatus* reportedly exhibits a remarkably high acidic and alkaline stability and also considerably high thermal stability[24]. Along these lines, *Pleurotus eryngii* versatile peroxidase has been subjected to protein engineering based on the stabilizing structural information that yields high stability to *Pleurotus ostreatus* versatile peroxidase. Similarly, production of ligninolytic enzymes and efficiency of lignin degradation strongly depends on fungal growth conditions and growth medium composition. Solid state fermentation on wheat straw, cotton stalks and wood chips has provided promising results with ligninolytic fungi owing to the fact that solid state fermentation mimics the natural habitat conditions of the fungi[8]. Solid state fermentation and liquid cultures stimulate the secretion of different isoenzymes such as differential expression of enzymes under different media composition. For instance, ligninolytic enzymes are strongly expressed when peptone is used as the nitrogen source while no activity was found in synthetic medium[8]. Recent studies reveal that *Pleurotus ostreatus*, *Pleurotus eryngii* and *Pleurotus pulmonarius* of white rot fungi secrete diverse peroxidases in solid and liquid cultures with minimal difference in their physiochemical properties[17]. The ability of different species to demineralize lignin is generally assessed by the oxidation of KTBA (α-keto-γ-thiomethylbutyric acid) as representative compound. Given the complexity of lignin degradation further insight is needed on the activity of ligninolytic enzymes on natural substrate lignin with characterization of novel ligninolytic peroxidases for extensive physiochemical stability.

Molecular configuration of lignolytic enzymes

Laccases, the phenol oxidases, are pervasive among fungi of ascomycetes and basidiomycetes, higher plants, bovine rumen microflora, bacteria, arthopods, insects, yeast and mold[25]. Although diverse laccase genes have been identified from the above families, the frailty of laccase in acting independently on non-phenolic units of lignin prevents complete depolymerization of the polymer with exclusive application of this enzyme. Bacterial ligninolytic system encompasses dye-decolourizing peroxidases (DyPs) substantiated by the recognition of multitude of putative dye-decolourizing peroxidase encoding genes in the bacterial genome[3]. However, no homologs to fungal peroxidases have been perused till date. Dye-decolourizing peroxidases with diverse potential from varying bacterial species still lags behind the fungal counterpart in efficiency and entirety of oxidation of lignin[3]. The genes of ligninolytic peroxidases – lignin peroxidase, manganese peroxidase and versatile peroxidase are exclusive to white rot group of basidiomycetes, confirming their role in preferential degradation of lignin[26]. These peroxidases are differentially expressed under the influence of environmental conditions, as substantiated by the presence of elements such as heat shock response, metal response, xenobiotic response, oxidative stress response and cAMP response revealed in their upstream regulatory sequences[24]. In *Pleurotus ostreatus*, gene family comprising nine genes encode for manganese peroxidase and versatile peroxidase with five genes representing the former and four for the latter[14]. Here, expression of specific

enzyme is transcriptionally regulated by the presence of manganese in the growth substrate. Also the pH and temperature stabilities of these isoenzymes differ symptomatic of environmental regulation. On the sequence homology front, versatile peroxidase genes of *Pleurotus eryngii* are reported to have high sequence homology to *Phanerochaete chrysosporium* lignin peroxidase than to manganese peroxidase[23]. However, there also exists a significant sequence identity between versatile peroxidase and the other peroxidases revealing it to be a 'hybrid peroxidase'.

Industrial applications of versatile peroxidase

Ligninolytic enzymes, especially peroxidases, have gained interest in recent years for their diverse biotechnological and industrial applications, owing to their high redox potential advantageous in degradation of various aromatic heterogeneous compounds. Versatile peroxidases are currently widely preferred among lignolytic enzymes, attributed by their significant advantage of oxidation of compounds without any expensive redox mediators. This is more central from the application perspective as cost of synthetic mediators and risk of mediator release to environment limit the usage of other heme peroxidases.

Xenobiotic compounds like polycyclic aromatic hydrocarbons (PAHs) are efficiently oxidized by versatile peroxidase through LRET in the absence of manganese and through diffusible oxidation in the presence of manganese[27]. For instance, bioconversion of chrysene by *Pleurotus ostreatus* was studied. Degradation of chrysene, an aromatic hydrocarbon commonly found in coal tar and produced by combustion of plant and animal material, was analysed under exclusive laccase production conditions and laccase and versatile peroxidase production conditions. Degradation resulted in accumulation of quinine metabolite under exclusive laccase action whereas with both laccase and versatile peroxidase, chrysene was completely degraded through phthalic acid pathway. This reveals that although laccase was effective in the initial stages of transformation, versatile peroxidase was required for complete oxidation of hydrocarbon[28]. Likewise, several azo and anthraquinone dyes are efficiently oxidized by versatile peroxidase implying its application in treatment of industrial dye effluents[29].

Recently, versatile peroxidase from *Bjerkandera adusta* was shown to successfully degrade β-carotene and exhibit high activity even at alkaline pH. Hence this alkaline stable enzyme was reported to high applicability in de-staining of fabrics in textile industries, leading to green processes in textile cleaning[30].

Paper pulp industries presently employ these ligninolytic enzymes for their pulp bleaching applications with versatile peroxidase as the biocatalyst of choice for kraft pulp bleaching applications. Also the ecofriendly process of pulp bleaching using manganese substituted polyoxometalates through versatile peroxidase-assisted system has proved to be effective without significant loss of viscosity of the pulp and good reduction in kappa number, an attribute preferred for production of high quality paper[31].

The abundance of lignocellulosic biomass merits its application in production of second generation biofuels. Delignification and detoxification for removal of toxic metabolites from pre-treatment methods are effectively achieved by the heme peroxidases[32]. Versatile peroxidase stands out in improving the yield and economics of fuel production through its catalytic versatility.

In developing countries, the growing livestock population faces the challenge of scarcity of quality feed. Ruminants possess the capability to utilize cellulose/hemicellulose of crop residues through their gut microflora, conversely their access is highly hindered by the presence of recalcitrant polymer lignin. Delignification of the lignocellulosic residues converts this biomass to nutritionally enriched and highly digestible animal feed. Several researches have illustrated the fungal pre-treatment of lignocellulosic biomass for application as good quality ruminant feed. Diverse fungal species have been studied in the past decade to enrich the utilization of lignocellulosic biomass like rice, wheat, corn residues, etc. for ruminant feed (Table 1). *Pleurotus ostreatus*, *Pleurotus eryngii*, *Ceriporiopsis subvermispora*, *Phlebia brevispora* and *Lentinula edodes* have been reported to be effective in selective degradation of lignin of wheat crop residues. Augmentation of *in vitro* digestibity of wheat straw by 72% was accounted by pre-treatment with *Phlebia brevispora*[33]. Similarly, for sugarcane residues the above fungal species pre-treatment resulted in a maximum of 88% increase in crude protein by *Ceriporiopsis subvermispora* followed by *Lentinula edodes* with 74% enhancement in crude protein[34]. Significant lignin digestion of corn straw by 54.6% and wheat straw by 39.7% was achieved through pretreatment by *Trametes versicolor* and *Pleurotus sajor-caju* respectively[35,36]. However, it is imperative to note that fungal pre-treatment comes with the disadvantage of prolonged incubation period and utilization of carbohydrates by fungi during pre-treatment. Ideally, characterization of ligninolytic enzymes involved in fungal degradation of lignocelluloses is needed to increase the effectiveness and for large scale commercial exploitation of lignocellulosic biomass for animal feed. A significant increase of 8% and 9% respectively in the *in vitro* dry matter digestibility (IVDMD) of ragi straw upon treatment with enzyme-rich media at a ratio of 2 : 5 obtained from culture of *Coriolus versicolor* and *Pleurotus flabellatus* was observed[37]. Highly significant ($P < 0.05$) improvement in the *IVDMD* was obtained in five common crop residues treated with fungal laccases[38] while a 20% increase in IVDMD was reported

Table 1. Fungal species utilized and the lignocellulosic substrates treated for animal feed

Lignocellulosic substrate	Fungal species	References
Wheat	*Bjerkandera adusta*, *Ceriporiopsis subvermispora*, *Cyathus stercoreus*, *Daedalea guercina*, *Dichomitus squalens*, *Ganoderma lucidum, Hericium clathroides*, *Inonotus andersonii*, *Inonotus dryophylus*, *Inonotus obliquus*, *Laccaria amethystine*, *Lentinus tigrinus*, *Lentinula edodes*, *Lyophyllum ulmarium*, *Phanerochaete chrysosporium*, *Phellinus laevigatus*, *Phlebia brevispora*, *Phlebia fascicularia*, *Phlebia floridensis*, *Phlebia radiata*, *Pleurotus eryngii*, *Pleurotus cornucopiae*, *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus sapidus*, *Polyporus brumalis*, *Polyporus ciliates*, *Schizophyllum commune*, *Trametes gibbosa*, *Trametes versicolor*, *Volvariella volvacea*	33, 44–58
Rice	*Ceriporiopsis subvermispora*, *Corprinus fimetarius*, *Lentinula edodes*, *Phanerochaete chrysosporium*, *Phlebia brevispora*, *Phlebia fascicularia*, *Phlebia floridensis*, *Phlebia radiata*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Pleurotus sajor-caju*	57, 59–61
Finger millet	*Pleurotus sajor-caju*, *Pleurotus ostreatus*, *Voriallae volvoraceae* and *Phanerochaete Chrysosporium*, *Coriolus versicolor* and *Ganoderma lucidium*, *Pleurotus flabellatus*, *Poria placenta*	37, 62, 63
Oil Palm	*Bjerkandera adusta*, *Ceriporiopsis subvermispora*, *Ganoderma lucidum*, *Lentinula edodes*, *Phanerochaete chrysosporium*, *Phlebia brevispora*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Schizophyllum commune*, *Trametes versicolor*	34, 64, 65
Bamboo	*Ceriporiopsis subvermispora*	66
Mustard straw	*Ganoderma lucidum*	67
Cedar	*Ceriporiopsis subvermispora*, *Lentinula edodes*, *Pholiota nameko*, *Pleurotus ostreatus*	68
Corn	*Ceriporiopsis subvermispora*, *Lentinula edodes*, *Pleurotus eryngii*, *Pleurotus ostreatus*	34
Sugarcane	*Ceriporiopsis subvermispora*, *Lentinula edodes*, *Pleurotus eryngii*, *Pleurotus ostreatus*	34
Birch	*Phanerochaete chrysosporium*, *Pleurotus sajor-caju*, *Trametes versicolor*	69
Spruce	*Phanerochaete chrysosporium*, *Pleurotus sajor-caju*, *Trametes versicolor*	69
Water hyacinth	*Pleurotus citrinopileatus*, *Pleurotus florida*	70
Cocoa	*Pleurotus ostreatus*	71

upon treatment of barnyard millet, foxtail millet and prosomillet with purified lignin peroxidase[39].

Conclusion

The catalytic versatility with non-requirement of redox mediators projects versatile peroxidase as a potential industrial biocatalyst[40]. Until recently, this enzyme was characterized only from *Pleurotus* and *Bjerkandera* and reported in *Panus*, *Trametes*, *Lepista*, *Dichomitous* and *Spongipellis*[41]. Despite extensive literature on characterization studies of this enzyme on oxidation of representative lignin compounds, research is lacking on activity of this member of peroxidases on its natural substrate lignin. Exploration of novel versatile peroxidases may yield more effective enzymes with respect to substrate specificity and reaction kinetics. Further, low level of enzyme in native state limits its practical use in contrast to demand for large quantity of enzyme for biotechnological applications. Ironically, heterologous expression studies in *Escherichia coli* and *Aspergillus nidulans* faced the problems of unproductive protein post-translation modification and low yield respectively[41]. Stability and solubility properties of the peroxidases are dependent on efficient post-translational modification for proper coordination of heme, inclusion of calcium and formation of disulphide bridges. Emphatically, an efficient heterologous expression system for versatile peroxidase is crucial with acquisition of knowledge of activity on its indigenous substrate, lignin for better exploitation of unique properties of this class of peroxidases.

Conflict of interest: The authors state there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

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