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Purification and Characterization of Novel Peroxidase Enzyme from wild white Rot Fungi- A Review

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ABSTRACT

Wood-decomposing fungi colonizing dead or dying tree trunks and stumps utilize cellulose while modifying the hemicellulose and lignin constituents cause either brown-rot or more commonly, white-rot via the utilization of hemicellulose and cellulose during the degradation of lignin. White Rot fungi (WRF) produce different types of enzymes that are characterized by different or specialized group of Peroxidases. Versatile Peroxidase (syn. Hybrid peroxidase, manganese- lignin peroxidase) is a new ligninolytic enzyme, combining catalytic properties of manganese peroxidase, oxidation of Mn (II), lignin peroxidase (Mn-independent oxidation of non-phenol aromatic compounds) and plant peroxidase (oxidation of hydroquinone and substituted phenols). The ligninolytic enzymes of white-rot fungi have broad substrate specificity and have been implicated in the transformation and mineralization of organopollutants with structural similarities to lignin.

Keywords: Rot Fungi, Ligninolytic enzyme

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INTRODUCTION

Mushrooms are earliest known fleshy fungi that are widely distributed in tropical and temperate regions of the whole world. Mushrooms are very much attractive in colour, design and well shape that are intimately associated with human civilizations. They are ubiquitous saprophytes i.e symbiotic in nature. Many species that are edible and have their medicinal properties, some are deadly poisonous and are called toadstools. By the taxonomic point of view all mushrooms represent ascomycotina and basidiomycotina. Some mushrooms produce hallucinogens that are consumed on some of festive occasions. Mushrooms have received greater attention as food for healthy life. They being fibrous in nature, low lipid and sugar content mushrooms are a recommended food for diabetes and heart patients. In spite of all these good attributes, very few mushrooms have been studied and still many more await study. In spite of these facts, only about 20 mushrooms are being cultivated. Therefore, there is not only an urgent need for survey of these fungi in unexplored and non-accusable regions but also a need to develop methods for their cultivation in an economical way. Further efforts to increase shelf life and processing different edible mushrooms need to be taken up. The fungi that produce lignin are called ligninolytic fungi. Ligninolytic fungus can be used for remediation of pollutants in water as well as in soil. Extracellular peroxidases and laccase have been shown to oxidize recalcitrant compounds in vitro but the likely significance of individual enzyme levels in vivo remains unclear. It has reported that fungi have more than one laccase encoding gene. During the last decade, research on the lignin-degradation ability of fungi has focused mainly on basidiomycetes commonly known as white-rot fungi. The complexity of the lignin attack mechanisms depends upon a number of different enzymes. The relative importance of which depends on the fungus considered that the importance of the search for novel fungal isolates as a potential source of new enzymes with improved performances considering kinetics and substrate specificity.

Lignin:

Lignin is a naturally synthesized aromatic polymer of cell wall that provides strength to the wood. Combination of cellulose and hemicellolose, lignin forms a complex lignin-carbohydrate network known as lignocellulose. Based on its main constituents of high value, with an annually many million tons, Lignocellulose is the most abundant renewal raw material on earth.

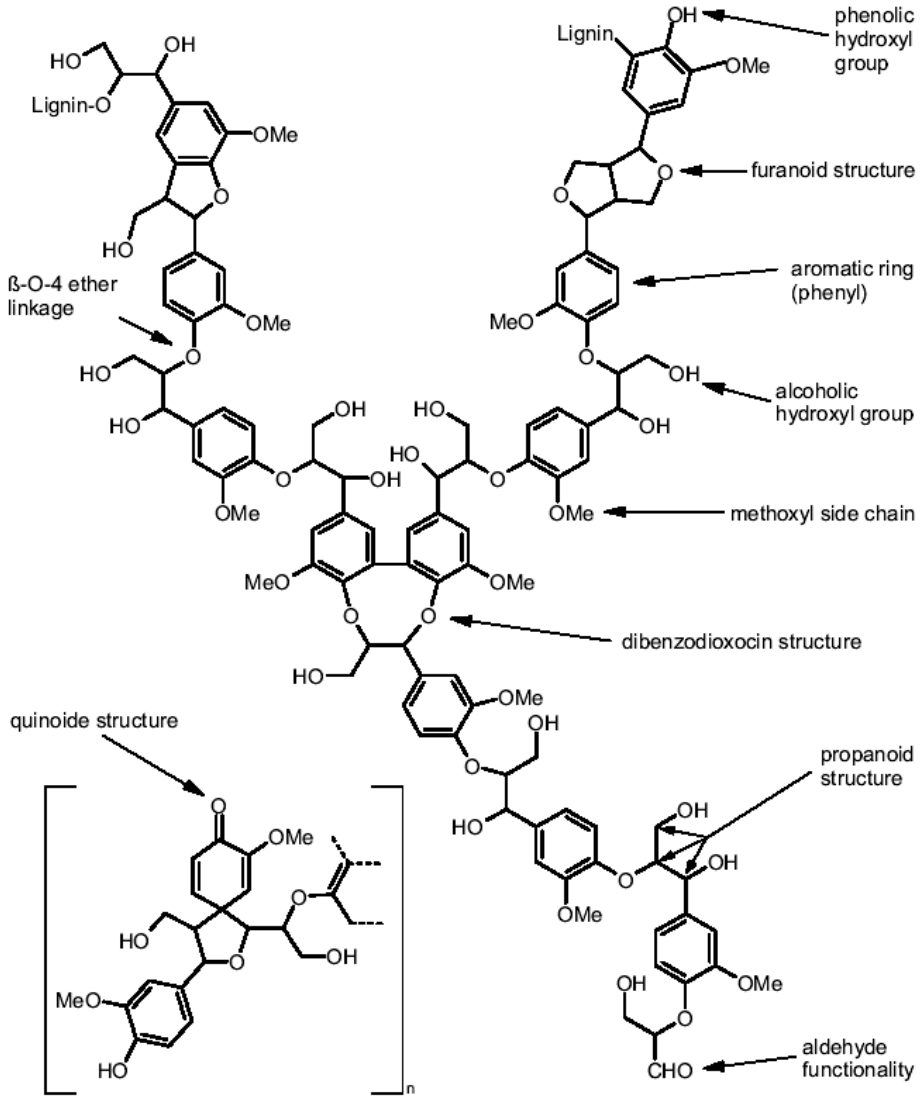

Figure 1: Lignin model after Brunow and coworkers (Brunow, 2001) including a structure called dibenzodioxocin (Karhunen et al., 1995a, b).

Macro-fungi:

Most of the Macrofungi are composed of elongated chains of cells called hyphae. They forming a cobwebby structure that is called mycelium, which grows in soil, wood or other substrata. In some species sporocarps are short-lived; in others they are persistent and may be perennial. Fungi constitute an essential component in forest and grassland ecosystems because of their roles as parasites of plants and animals, decomposers of organic matter, and mutualistic symbionts such as in lichens and mycorrhizae. Data on distribution patterns of macrofungi are important for understanding the evolution of fungi and the relationship between fungi and their associated plants. Macrofungi (fungi forming conspicuous sporocarps) are mostly either ectomycorrhizal. Ectomycorrhizal fungi form a mutually beneficial, often times obligatory, relationship with vascular plants and provide the plants with access to the key elements that are essential for plant growth (Read, 1991). Saprophytic

fungi are actively involved in nutrient recycling and vegetational succession in forest ecosystem. Therefore, knowledge of the diversity and ecology of macrofungi is crucial for forest management plans and conservation efforts, and they can also be used as a bioassay of ecosystem health.

White-rot fungi:

White-rot fungi have received too much attention in the recent years for their valuable enzyme systems that effectively degrade lignocellulosic compounds. These fungi have powerful extracellular oxidative and hydrolytic enzymes that degrade lignin and cellulose. These enzymes include ligninolytic enzymes (laccase, manganese peroxidase, lignin peroxidase, and versatile peroxidase) and cellulolytic enzymes (endo-glucanase, cello-biohydrolase, and beta-glucosidase). The use of these fungi for low-cost lignocellulolytic enzyme production might be attractive for bio-fuel production. Lignocellulose biomass is a complex biopolymer consisting of cellulose, hemicellulose, pectin, and lignin. Lignin is the one of the main constituents of wood that contains cellulose and hemicelluloses. It is the most recalcitrant compound of wood, due to its complex structure derived from the coupling of monolignols and three alcohols (*p*-coumaryl, coniferyl and sinapyl; Kaneda et al., 2008). White-rot fungi (WRF) belong to the class of basidiomycetes and certain ascomycetes. They constitute the most important wood rotting fungi since they are the only microorganisms able to mineralize lignin producing carbon dioxide and water. The term white-rot has been traditionally used to describe forms of wood decay where lignin-as well as cellulose and hemicellulose is broken down, leaving a light, white, rather fibrous residue completely different from the brown powder left by brown rot fungi (Schwarze et al., 2000). Generally, WRF are unable to use lignin as a sole carbon source but they degrade it in order to gain access to cellulose and hemicellulose. Within this group, *Phanerochaete chrysosporium* is the most extensively studied species, although other fungi such as *Bjerkandera adusta, Trametes versicolor, Pleurotus ostreatus* are also well-known (Schwarze et al., 2000). Delignification is based on the WRF capacity to produce one or more extracellular lignin-modifying enzymes (LMEs) which lack the of substrate specificity, are also capable of degrading a wide range of xenobiotics also at relatively low concentrations since they are not induced by either lignin or other related compounds (Mester and Tien, 2000). The use of fungal cultures has been considered as an environmental tool to remove organic pollutants such as polycyclic aromatic hydrocarbons, chlorinated and phenolic compounds, dyes, pharmaceutical compounds, among others.

Brown Rot Fungi:

Brown rot fungi decays the cellulose and hemicellulose in wood, leaving a brown residue of lignin, the substance which hold cell together. Cellulose is break down by hydrogen peroxide that is produced during breakdown of hemicellulose because hydrogen peroxide is a small molecule it can diffuse rapidly the wood leading to decay. Wood affected by brown rot fungi is usually dry as well as fragile that readily crumbles into cubes because of longitudinal and transverse cracks. Brown rot fungi are the major group of organisms which is associated with degradation of in-service wood, and they also play an important role in the forest ecosystem as well as biogeochemical cycling of nutrients (Gilbertson, 1981). These basidiomycetes are unusual in that they rapidly depolymerize the cellulose in wood without removing the surrounding lignin and normally prevents microbial attack. Examples of this group of fungi include *Laetiporus portentosus, Formitopsis lilacinogiva, Gleophyllum trabeum* and *Serpula lacrymans.*

Mycorrhizal Fungi:

A mycorrhiza is a mutualistic symbiosis association between a fungus and plant in which the fungal partner is physically attached to the roots of the vascular plant. Literally, the word mycorrhiza derive from Greek mykos "fungus" and riza, "roots" that means “fungus-root”. There are two types of mycorrhiza that are known: ecto- and endo mycorrhizas. The ectomycorrhizas are characterized by an extracellular fungal growth in the root cortex. They are more common in temperate and boreal forest trees and number over 5000 species mainly within the Basidiomycetes. About 80% of all terrestrial plant species form this type of symbiosis and 95% of the world’s present species of vascular plants belong to families that are characteristically mycorrhizal (Quilambo, 2000).

Litter Decomposing Fungi:

Wood and litter decomposing fungi employ a range of strategies to decompose organic matter. Many litter colonizing basidiomycetes are efficient degraders of needle litter (Boberg, 2009). Boreal litter decomposing fungi must have a well-developed enzymatic system to be able to obtain C and nutrients from the chemically complex litter, which is relatively rich in lignin and tannins (Berg and McClaugherty, 2003). As litter decomposers, free-living saprotrophs dominate the litter layer in boreal forest ecosystems, presumably by suppressing in growth of root- associated biotrophs (Lindahl et al., 2007). The basidiomycetous LDFs mostly belong to the families Agaricaceae, Bolbitiaceae, Coprinaceae, Strophariaceae and Tricholomataceae (Steffen, 2003). Many basidiomycetes form rhizomorphic mycelia. With the help of these chords, many litter decomposers can extend their organismal size up to a decimeter scale, which allows them to transport resources over a larger distance between heterogeneous substrates. Thereby, N can be re-allocated to freshly colonized litter, which

minimizes N losses in decomposition to the soil solution (Boberg, 2009). This fungal trait is an adaptation for persistence in an N limited ecosystem like the boreal forest

Lignin modifying enzymes:

MEs are the oxidoreductases which catalyze the electron transfer from one substrate to another. LMEs act by generating free radicals that randomly attack the lignin molecule, breaking covalent bonds and releasing a range of phenolic compounds. There are two main types of LMEs: peroxidases and laccases (phenol oxidases). The main LMEs are lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP) and laccase. In addition, these fungi secrete mediators of high molecular weight increasing the range of potentially biodegradable compounds. White-rot fungi start LMEs production during their secondary metabolism, since lignin oxidation provides no net energy to fungi (Mester and Tein, 2000).

Extracellular ligninolytic enzymes:

White rot fungi produce large number of extracellular oxidative enzymes with low specificity that are involved in the degradation of lignin content in a plant cell wall. Due to the low specificity of enzymes white rot fungi also have an ability to degrade many environmental pollutants. Main extracellular enzymes participating in lignin degradation are heme containing lignin peroxidase, manganese peroxidase and copper containing laccase (Hattaka, 2001). A new group of ligninolytic heme containing peroxidase, combining its structural and functional property are called versatile peroxidase. The versatile peroxidase is capable of oxidation of Mn^{2+} and phenolic compounds, as well as non phenolic aromatic compounds such as veratryl alcohol. LiP and MnP belong to the family of oxidoreductases. LiP oxidizes a variety of substrates that have high redox potentials. MnP oxidizes Mn^{+2} to Mn^{+3}, which in turn attacks phenolic structures in lignin .All extracellular peroxidases and laccases have the ability to catalyze one-electron oxidation resulting in the formation of radicals, which undergo several spontaneous reactions. These, in turn lead to various bond cleavages including aromatic ring fission (Hattaka, 2001).

Table 1: Extracellular ligninolytic enzymes involved in the degradation of lignin (Hatakka, 2001)

Enzymes and abbreviation	Cofactor	Donor, substrate and mediator	Main effect or reaction
Laccase	O_2	O_2,phenols, mediators, e.g HBT, ABTS	Phenols are oxidized to phenoxy radicals : other reactions in the presence of mediators
Aryl alcohol oxidase , AAO		Aromatic alcohols (anisyl, Veratryl alcohol)	Aromatic alcohols oxidized to Aldehydes :H_2O_2 production
Lignin	H_2O_2	H_2O_2,Veratryl Alcohol	Aromatic ring oxidized to

Peroxidase, LiP			cation radicals
Mangenese Peroxidases, MnP	H_2O_2	H_2O_2, Mn(II), organic acids as chelators, thiols ,unsaturated lipids	Mn(II), oxidized Mn(III), further oxidation of phenolic compounds of phenoxy radicals
Versatile Peroxidase, VP	H_2O_2	Same or similar Compounds as LiP and MnP	Same effect on aromatic and Phenolic compounds as LiP and MnP

Laccase:

The enzyme laccase (E.C.1.10.3.2) is a multi-copper oxidase that catalyzes the one-electron oxidations by transferring one electron from four substrate molecules to one molecule of molecular oxygen which is reduced to water (Wesenberg et al., 2003). Laccase shows low substrate specificity and can react with diphenols, aryl diamines, aminophenoles. Laccase are the glycosylated phenol oxidases that exist as monomers, homodimers or homotetramers (Solomon et al., 1996) and it belongs to the multicopper oxidase family. It was originally discovered in 1883 in the Japanese Lacquer tree *Rhus vernicifera.* LiP and MnP belong to the family of oxidoreductases (Nitta et al., 2002). Laccase is capable of catalyzing the oxidation of phenolic and non phenolic compounds and it is able to degrade wide range of synthetic dyes. Laccase are first isolated from plants but it is also present in fungi and some bacteria. Fungal laccase contribute to several processes such as lignin degradation, sporulation, pigment production, fruiting body formation and plant pathogenesis, (Mayer and staples, 2002).

Aryl alcohol oxidase:

Aryl-alcohol aoxidase (EC.1.1.3.7) is a FAD-containing enzyme in the GMC (glucose-methanol choline oxidase).family of oxidoreductases. Aryl oxidase activity was described for first time in 1960, in the fungus *Polystictus versicolor*. AAO participates in fungal degradation of lignin, a process of high ecological and biotechnological relevane, by providing the hydrogen peroxide required by ligninolytic peroxidases.

Lignin peroxidase:

Lignin peroxidase (LiP) (LiP, E.C.1.11.1.14) is also referred to the diaryl propane oxygenase and it is a heme-containing enzyme that catalyzes the hydrogen peroxide-dependent oxidative degradation of lignin. Ligninase I similarly serve same function as diaryl propane peroxidase. These enzymes are inclusive of the peroxidase-catalase superfamily (Zamocky and Obinger, 2010). Structurally, LiP is a monomeric hemoprotein. The nonplanarity of the heme cofactor of LiP and those in the other class-II peroxidases has been well documented (Piontek, Glumoff and Winterhatter, 1993), and observable in the structures of the different ligninolytic peroxidases deposited in the Protein Data Bank (PDB).

Manganese peroxidase:

Manganese peroxidase (MnP, E.C.1.11.1.13) is an extracellular enzyme discovered in *Phanerochaete chrysosporium* by (Kuwahara *et al.*,1984) and it is considered the most widespread ligninolytic peroxidase produced by almost all white-rot basidiomycetes and by various litter-decomposing fungi (Mester and tein ,2000) MnP is a glycoprotein with molecular weights between 32 and 62.5 kDa. This enzyme has a similar catalytic properties to other peroxidases involving a two-electron oxidation; however, MnP is able to oxidize Mn^{2+}, resulting in the formation of diffusible oxidants ($Mn3^{+}$) capable of penetrating the cell wall matrix and oxidizing mainly phenolic substrates (Wong, 2009) .

Versatile peroxidase:

Versatile Peroxidase (E.C.1.11.1.16) oxidizes $Mn2^{+}$, as MnP (EC 1.11.1.13) does, and also high redox-potential aromatic compounds, as LiP (EC1.11.1.14) does. Due to their Mn-oxidizing activity, the *Pleurotus* VP isoenzymes were first described as MnP isoenzymes (Martinez et al., 1996; Giardina et al., 2000), but they were later recognized as representing a new peroxidase type (EC 1.11.1.16). VP is also able to efficiently oxidize phenolic compounds and dyes that are the substrates of generic peroxidases (EC 1.11.1.7), such as the *C.inerea* peroxidase (CIP) (Baunsgaard et al.,1993) and related peroxidases, or the well-known horseradish peroxidase (HRP) (Veitch , 2004). By contrast, LiP is not able to oxidize phenolic compounds efficiently because of inactivation in the absence of veratryl alcohol (VA) or related substrates, and MnP only oxidize phenols in the presence of $Mn^{2+,}$ although a *P. radiata* short MnP seems to be an exception (Hilden *et al*., 2005). Moreover, VP directly oxidizes high redox-potential compounds, for example, the dye Reactive Black 5 (RB5), that LiP can oxidize only in the presence of redox mediators such as VA (Heinfling *et al*., 1998b) The enzyme VP is a peroxidase which combines the substrate specificity characteristics of the three other fungal peroxidases (MnP, LiP and *Coprinopsis cinerea* peroxidase). In this way, it is able to oxidize a variety of high and low redox potential substrates including Mn^{2+}, phenolic and non-phenolic lignin dimers, α-keto-γ-thiomethyl-butyric acid (KTBA), veratryl alcohol dimethoxybenzenes, different types of dyes (Reactive Black 5), substituted phenols and hydroquinones (caramel et al.,1999 ,Martizez, 2002). VP is only produced by fungi from the genera *Pleurotus*, *Bjerkandera* and *Lepista* (Heinflinger et al., 1998). It is interesting to underline that VP enzyme shows different optimal pH for the oxidation of Mn^{2+} (pH 5) or aromatic compounds (pH 3), similar to those of optimal LiP and MnP activity (Martizez, 2002). The VP catalytic cycle includes two-electron oxidation of the resting peroxidase (VP, containing, Fe^{3+}) by hydrogen peroxide to yield compound I (C-IA, containing Fe4+-oxo and porphyrin cation radical), whose reduction in two one-electron reactions, producing Mn^{3+},

results in the intermediate compound II (C-IIA, containing Fe^{4+}-oxo after porphyrin reduction) and then the resting form of the enzyme. Compounds C-IB and C-IIB, which are in equilibrium with C-IA and C-IIA respectively, are involved in the oxidation of veratryl alcohol and other high redox potential aromatic compounds (M. Perez-Boada,2005) . The presence of Mn^{2+} at moderate concentrations was demonstrated to strongly inhibit the oxidation of LiP substrates, such as VA (T. Mester, J.A. Field, 1988).

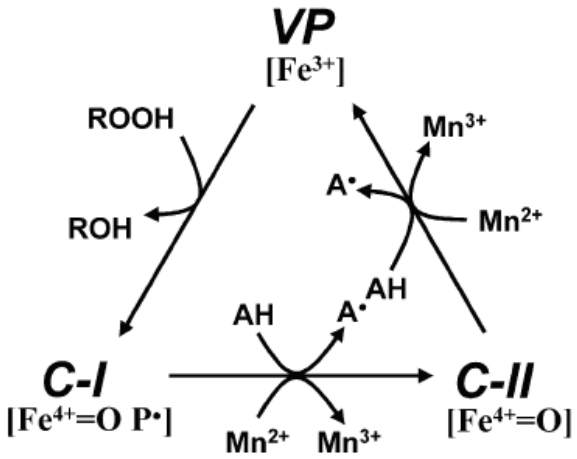

Figure 2: The catalytic cycle of VP (Ruiz-Duenas et al., 1999)

Versatile peroxidase (VP) has been recently described as a new family of ligninolytic peroxidases, together with lignin-peroxidase (LiP) and manganese peroxidase (MnP) both reported for *Phanerochaete chrysosporium* for the first time.(Martinez,A.T.,2002). Versatile peroxidase (VP) is a novel heme peroxidase type that is described in fungi from the genera *Pleurotus* and *Bjerkandera*, whose biochemical, molecular and structural aspects are being thoroughly investigated (Martinez et al., 1996 Banci et al., 2003). The most intriguing characteristics of this new enzyme is its ability to use a variety of electron donor substrates that were previously considered as characteristic of other peroxidase types, such as manganese peroxidase (MnP) lignin peroxidase (LiP) and horseradish peroxidase (HRP) (Martinez, 2002 Heinfling et al., 1998) .The complete genome of this model fungus has been recently sequenced revealing two families of LiP and MnP genes together with a "hybrid peroxidase" gene .However, the sequence of the latter gene is more related to non-ligninolytic CIP (Baunsgaard et al., 1993) than to VP.. This fourth fungal peroxidase family should be included in peroxidase class II together with the MnP, LiP and CIP families. VP genes (and cDNA) were first cloned and sequenced from *Pleurotus eryngii* in 1999–2000. (Ruiz-Duen et al .,1999 Camarero et al., 2000), Versatile peroxidase (syn. hybrid peroxidase, manga-nese-lignin peroxidase) is a new ligninolytic enzyme that has combining catalytic properties of manganese peroxidase (oxidation of Mn(II)), lignin peroxidase (Mn-independent oxidation of non-phenolic aromatic com-pounds) and plant peroxidase

(oxidation of hydro-quinones and substituted phenols). The manganese peroxidase component catalyzes the oxidation of Mn(II) to Mn(III) by H_2O_2. The highly reactive Mn(III) is stabilized via chelation in the presence of dicarboxylic acid. It was suggested that the catalytic properties of the new peroxidases were due to a hybrid molecular architecture combining different substrate binding and oxidation sites (Camarero, 1999). Recently, VP from *P.eryngii* was investigated using several techniques in order to understand the structural and functional peculiarities of this new peroxidase family. The most important aspects of ligninolytic peroxidase activity, i.e. the identification of the aromatic substrate-binding site as well as how 386 Electron Transfer Pathways in Versatile Peroxidase oxidation of high redox-potential aromatic compounds occurs. The crystal structure of wild-type and native recombinant (non-glycosylated) *P. eryngii* VP has been recently determined at high resolution. The ligninolytic system of white-rot fungi is composed of a variety of oxidative enzymes, i.e. lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Pelaaez et al., 1995, Keyser et al 1978). Moreover, the existence of a versatile peroxidase (VP) sharing LiP and MnP catalytic properties has been recently reported (Martoanez et al., 1996 Ruiz-Duenaas, 1999). The H_2O_2 required by ligninolytic peroxidases is generated by several direct and indirect enzymatic mechanisms. Direct reduction of O_2 to H_2O_2 is catalyzed by the extracellular enzymes glyoxal and aryl-alcohol oxidases (Kersten and Kirk, 1987 , Guillean et al., 1992]. Very recently, the crystal structure of recombinant *P. eryngii*. VP expressed in *Escherichia coli* and activated *in vitro* (Perez-Boada et al., 2002) has been determined at 1.33-Å resolution (Protein Data Bank code 2BOQ). VPs are characterized for their extraordinary wide substrate specificity and retain features of the other two fungal peroxidase families, manganese peroxidases (MnPs) and lignin peroxidases (LiPs). Therefore, a highly efficient VP over production system is desired for biotechnological applications in industrial processes and bioremediation of recalcitrant pollutants, and also detailed analysis of the structure–function relationship of the enzyme.

Purification of enzymes:

Purification of laccase enzyme was carried out by the method of (Cheftz et al., 1998). The culture filtrate was first filtered and centrifuged at 5000 rpm, supernatant and subjected to precipitation. The precipitate obtained was dialyzed and lyophilized and then loaded onto a DEAE-Cellulose anion exchange and equilibrated with 10mM sodium acetate buffer (pH 4.5), with a linearly increasing NaCl concentration gradient 0.5M) in the same buffer. The six fractions containing laccase activity were pooled, concentrated, and dialyzed overnight against same buffer. Gel filtration chromatography was performed using sephadex G-100 column 2.0 × 40 cm. The DEAE-purified sample was loaded on to the column and 3mL

fraction was collected. The eluted active fractions were dialyzed and protein content was determined by Bradford's method (Alberts et al., 2009) with crystalline bovine serum albumin as the standard. The extracellular proteins were purified with different folds and yields using different purification steps including ammonium sulphate precipitation , DEAE cellulose column chromatography and gel permutation using Sephadex G-100 column chromatography. The purified enzymes was homogenous that shows single band on SDS-PAGE with a molecular mass of 40 to 45 kda when compared to authentic standards (Cheftz et al., 1998).

Effect of pH and Temperature on Purified enzyme:

The purified enzyme was active in broad range of pH 3-9 with optimum activities. The purified laccase has a broad temperature sensitive to different optimum temperature ranges 35-70°C. Temperature kinetics of the enzyme suggests that the enzyme activity increases sharply from 60 to 65ºC followed by a decline after 70ºC.

Effect of activators/ Inhibitors:

Several activators/inhibitors such as $CuSO_4$, $MnSO_4$, $FeSO_4$, EDTA, Cysteine and ethanol with a concentration of up to 1mM were evaluated for the effect on ligninolytic enzyme activity. Activators including $CuSO_4$ and $MnSO_4$ had an enhancing effect on the production of each of the ligninolytic enzymes whereas inhibitors EDTA and Cysteine caused inhibition in $CuSO_4$ among $MnSO_4$ and $FeSO_4$ was an effective activator for laccase while MnSO4 enhanced MnP production. Zhu et al. (2003) reported an increase in laccase production due to the addition of $CuSO_4$ and found that Cu^{2+} (1mM) also had a positive effect on laccase production, thus enhancing activity to 360 U/ml. It has also been reported that, the activation of the laccase by Cu^{2+} may be due to the filling of type 2 copper binding sites with copper ions. It was also determined that the activation or inhibition of proteolytic enzymes by trace metals can influence extracellular enzymes production by changing their turnover rate (Sadhasivam *et al.*, 2008).

REFERENCES:

1. Alberts JF, Gelderblom WC, Botha A, Vanzyl WH. Degradation of aflatoxin B1 by fungal laccase enzymes. International Journal of Food Microbiology, 2009; 135(1): 47-52.
2. Banci L, Camarero S, Martinez AT, Martinez MJ, Perez- Boada M, Pierattelli R, Ruiz-Duenas FJ, J. Biol. Inorg. Chem. 8, 2003; 751-760.
3. Baunsgaard L, Dalboge H, Houen G, Rasmussen EM, Welinder KG. Amino acid sequence of Coprinus macrorhizus peroxidase and cDNA sequence encoding

Coprinus cinereus peroxidase: a new family of fungal peroxidases. European Journal of Biochemistry, 1993; 213: 605-611.

4. Berg B, McClaugherty C. Plant litter decomposition, humus formation, carbon sequestration. *Berlin, Germany: Springer-Verlag*, 2003.
5. Boberg J. Litter Decomposing Fungi in Boreal Forests. Their Function in Carbon and Nitrogen. Doctoral Thesis Acta Universitatis Agriculturae Sueciae, 2009; 75.
6. Brunow G. Methods to reveal the structure of lignin. In M. Hofrichter and A. Steinbüchel (eds.) Biopolymers, Vol. 1, Wiley-VCH, Weinheim, Germany. 2001; pp. 89-116.
7. Chefetz B, Chen.Y, and Hadar Y. Purification and characterization of laccase from *Chaetomium thermophilium*and its role in humification. Applied and Environmental Microbiology, 1998; 64(9): 3175-3179.
8. Camarero S, Ruiz-Duenas FJ, Sarkar S, Martinez MJ, Martinez AT. The cloning of a new peroxidase found in lignocellulose cultures of Pleurotus eryngii and sequence comparison with other fungal peroxidases. FEMS Microbiol. Letters, 2000; 191: 37-43.
9. Camarero S, Sarkar S, Ruiz-Duenas FJ, Martinez MJ, Martinez AT. Description of a versatile peroxidase involved in natural degradation of lignin that has both Mn-peroxidase and ligninperoxidase substrate binding sites. J. Biol. Chem, 1999; 274: 10324-10330.
10. Caramelo L, Martinez MJ, Martinez AT. A search for ligninolytic peroxidases in the fungus *Pleurotuseryngii* involving alpha-keto-gammathiomethylbutyric acid and lignin model dimers, Applied and Environmental Microbiology,1999; 65: 916-922.
11. Giardina P, Palmieri G, Fontanella B, Rivieccio V, Sannia G. Manganese peroxidase isoenzymes produced by Pleurotus ostreatus grown on wood sawdust. Archives of Biochemistry and Biophysics, 2000; 376: 171-179.
12. Guillean, F, Martoanez, AT, Martoanez, MJ. Substrate specifcity and properties of the aryl-alcohol oxidase from the ligninolytic fungus Pleurotus eryngii. Eur. J. Biochem, 1992; 209: 603-611.
13. Gilbertson RL. North American wood-rotting fungi that cause brown rots. *Mycotaxon*, 1981; 12: 372-416.
14. Hatakka AI. Biodegradation of lignin. Inbiopolymers lignin, humic substances and coal,(Hofrichter,M.andteinbuchel,A.editors) Wiley-VCH, Weinheim,Germany, 2001; 1:129-131.

15. Heinfling A, Ruiz-Duenas FJ, Martinez MJ, Bergbauer M, Szewzyk U, Martonez AT. A study on reducing substrates of manganese-oxidizing peroxidases from Pleurotus eryngii and Bjerkandera adusta. FEBS Letters. 1998; 428: 141–146.
16. Hilden K, Martinez AT, Hatakka A, Lundell T. The two manganese peroxidases Pr-MnP2 and Pr-MnP3 of Phlebia radiata, a lignin-degrading basidiomycete, are phylogenetically and structurally divergent. Fungal Genetics and Biology, 2005; 42: 403-419.
17. Kaneda M, Rensing KH, Wong JC, Banno TB, Mansfield SD, Samuels AL. Tracking monolignols during wood development in lodgepole pine, Plant Physiology, 2008; 147- 175.
18. KarhunenP, Rummakko P, Sipila J, Brunow G, Kilpelainen I. The formation of dibenzodioxocin structures by oxidative coupling - a model reaction for lignin biosynthesis. Tetrahedron Lett. 1995; 36: 4501-4504.
19. Kersten PJ, Kirk TK. Involvement of a new enzyme, glyoxal oxidase, in extracellular H2O2 production by *Phanerochaetechrysosporium.* J. Bacteriol, 1987; 69: 2195-2201.
20. Keyser P, Kirk TK, Zeikus JG. Ligninolytic enzyme system of *Phanerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. J. Bacteriol.1978; 135: 790-797.
21. Kuwahara M, Glenn JK, Morgan MA, Gold MH. Separation and characterization of 2 extracellular H O 2 2-dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium.* Federation of European Biochemical Societies Letters.1984; 169: 247-250.
22. Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Hogberg P, Stenlid J, Finlay RD. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist, 2007; 173: 611-20.
23. Martinez MJ, Martinez AT. in K.M .a.E. Srebotnik Ed., Biotechnology in the Pulp and Paper Industry: Recent Advances in Applied and Fundamental Research, Facultas-Universita tsverlag, Vienna, 1996; 417-420.
24. Martinez AT. Molecular biology and structure–function of lignin-degrading heme peroxidases. Enzyme Microb. Technol, 2002; 30: 425-444.
25. Martoanez MJ, Ruiz-Duenaas FJ, Guillean F, Martoanez AT. Purification and catalytic properties of two manganese peroxidase isoenzymes from Pleurotuseryngii. Eur. J. Biochem, 1996; 237: 424-432.

26. Mayer AM, Staples RC. Laccase: new functions for an old enzyme. Phytochemistry, 2002; 60: 551-565.
27. Mester T, Field JA. Characterization of a novel manganese peroxidaselignin peroxidase hybrid isozyme produced by Bjerkandera species strain BOS55 in the absence of manganese, Journal of Biological Chemistry, 1998; 273: 15412-15417.
28. Mester T, Tien M, Oxidation mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants, International Biodeterioration & Biodegradation, 2000; 46: 51-59
29. Nitta K, K. Katoka and T. Sakurai Primary structure of a Japanese lacquer tree Laccase as a prototype enzyme of multicopper oxidases. Journal of Inorganic Biochemistry, 2002; 91: 125-131.
30. PelaaezF, Martoanez MJ, Martoanez AT. Screening of 68 species of basidiomycetes for enzymes involved in lignin degradation. Mycol. Res, 1995; 99: 37-42.
31. Perez-Boada M, Doyle WA, Ruiz-Duenas FJ, Martinez MJ, Martıinez, AT, Smith AT. Enzyme Microb. Technol. 2002; 30: 518-524.
32. Perez-Boada M, Ruiz-Duenas FJ, Pogni R, Basosi R, Choinowski T, Martinez MJ, Piontek K, Martinez AT. Versatile peroxidase oxidation of high redox potential aromatic compounds: Site-directed mutagenesis, spectroscopic and crystallographic investigation of three long-range electron transfer pathways, Journal of Molecular Biology, 2005; 354: 385- 402.
33. Piontek K, Glumoff T, Winterhatter K. Low pH crystal structure of glycosylated lignin peroxidase from *Phanerochaetechrysosporium*. FEBS Letters, 1993; 315: 119-124.
34. Quilambo OA. Functioning of peanut (Arachishypogaea L.) under nutrient deficiency and drought stress in relation to symbiotic associations. PhD thesis. University of Groningen, the Netherlands. Van Denderen BV, Groningen. ISBN 903671284X (2000).
35. Read, DJ. Mycorrhizas in ecosystems. *Experientia*.1991; 47: 376-391.
36. Ruiz-Duenas FJ, Martinez MJ, and Martinez AT. Molecular characterization of a novel peroxidase isolated from the ligninolytic fungus Pleurotus eryngii. Mol. Microbiol, 1999; 31: 223–236.
37. Sadhasivam S, Savitha S, Swaminathan K, Lin FH. Production, purification and characterization of mid-redox potential laccase from a newly isolated *Trichoderma harzianum*s doi:10. 1016/j.biotechadv,2008;11-001

38. Schwarze F, Baum S, Fink S. Dual modes of degradation by Fistulina hepatica in xylem cell walls of Quercus robur, Mycological Research, 2000;104: 846-852.
39. Solomon EI, Sundaram UM, Machonkin TE. Multicopper oxidases and oxygenases. *Chemical Reviews*, 1996; 96: 2563-2606.
40. Steffen K. Degradation of recalcitrant biopolymers and polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. *DissertationesBiocentri Viikki UniversitatisHelsingiensis*, 23/2003. Ph.D. Thesis. Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, 2003;68.
41. Veitch NC. Horseradish peroxidase: a modern view of a classic enzyme. Phytochemistry. 2004; 65: 249-259.
42. Wesenberg D, Kyriakides I, Agathos SN. White-rot fungi and their enzymes for the treatment of industrial dye effluents, Biotechnology Advances.2003; 22:161-187.
43. Wong DWS. Structure and Action Mechanism of Ligninolytic Enzymes, Applied Biochemistry and Biotechnology, 2009; 157:174-209.
44. Zamocky M, Obinger C. Molecular phylogeny of heme peroxidases. In E. Torres, & M. Ayala (Eds.), *Biocatalysis based on heme peroxidases*,Heidelberg,Springer.2010; pp. 7-35.
45. Zhu X, Gibbons J, Zhang S, Peter RW. Copper-mediated reversal of defective laccase in a *_vph1* avirulent mutant of *Cryptococcus neoformans*. Molecular Microbiology 2003; 47: 1007-1014.