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A Review: MANGANESE PEROXIDASE: Screening, Production and Applications

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Abstract: *Microbial ligninolytic enzymes like laccase, manganese peroxidase, and lignin peroxidase have gained much attention in many industrial applications. Among these, manganese peroxidases are key contributors in the microbial ligninolytic system. It mainly oxidizes Mn (II) ions that remain present in wood and soils, into more reactive Mn^{3+} form, stabilized by fungal chelators like oxalic acids. However, Mn^{3+} acts as a diffusible redox intermediate, a low molecular weight compound, which breaks phenolic lignin and produces free radicals that have a tendency to disintegrate involuntarily. It has a great application potential and ample opportunities in diverse area, such as alcohol, pulp and paper, biofuel, agriculture, cosmetic, textile, and food industries. This review article is focused on the sources, catalytic reaction mechanisms and different biotechnological applications. Thus, this review article is mainly focused and highlighted the current scenario and updated information on manganese peroxidase enzyme.*

Keywords: Microbial MnP, Phenolic and non-phenolic compounds, Biotechnological applications

1. INTRODUCTION:

Manganese peroxidase, lignin peroxidase and laccase are the key ligninolytic enzymes yielded by white-rot fungi that are being evaluated for their applications in industrial and environmental biotechnology (Buchanan *et al*., 2004). The microorganisms persist the important source for lignin-degrading enzymes. The production of ligninolytic enzymes from various microbial sources has been well known and documented. Fungi are the best puissant basis of lignin-degrading enzymes. The saprophytic fungi make these enzymes to break down the lignin polymer.

1.1 Manganese peroxidases:

Manganese peroxidases belong to the family of oxidoreductases (Martinez *et al.*, 2009; Hammel and Cullen, 2008). Activity of the enzyme was stimulated by simple organic acids, which stabilise the Mn^{3+}, thus producing diffusible oxidizing chelates (Glenn and Gold *et al.*, 1985). The catalytic cycle of MnP starts with the binding of H_2O_2 to the reactive ferric enzyme. H_2O_2 is produced by the fungus using other enzymes (GLOX, AAO) or by MnP in the oxidation of glutathione (GSH), NADPH and dihydroxy malic acid (Paszczynski *et al*., 1985). The cleavage of the oxygen-oxygen bond requires the transfer of two electrons from the heme, forming the MnP compound I. This activated state of the heme centre is able to form a radical complex and to remove an electron from the Mn^{2+} donor resulting in the formation of a highly reactive Mn^{3+} ion. MnP-compound II in this process is also able to oxidize a Mn^{2+} ion (Kishi *et al.*, 1994). This step closes the cycle and the input of one H_2O_2 results in the formation of two H_2O and two Mn^{3+} (Wariishi *et al.*, 1992). The Mn^{3+} formed is highly reactive and complexes with chelating organic acids such as oxalate or malate (Cui and Dolphin *et al.*,1990; Kishi *et al.*, 1994), which are produced by the fungus (Galkin *et al.*, 1998, Hofrichter *et al.*, 1999; Makela *et al.*, 2002). With the help of these chelators, Mn^{3+} ions get stabilized and can diffuse into materials such as wood. The redox potential of the MnP-Mn system is lower than that of LiP and preferably oxidizes phenolic substrates (Vares *et al.*, 1996). Mn^{3+} or chelated Mn^{3+} is, in turn, able to oxidize various monomeric and dimeric phenols, as well as carboxylic acids, thiols and unsaturated fatty acids forming radicals (Hofrichter *et al.*, 2002). Recently, physiological levels of oxalate in *P. chrysosporium* cultures have been shown to stimulate manganese peroxidase activity (Kuan and Tien *et al.*, 1993). The basic version of reaction mechanism of MnP is mentioned below and entails the oxidation of Mn (II) by compound I (MnP-I) and compound II (MnP-II) to profit Mn (III). In view of nonspecific landscape of its action, focus on biochemical properties of MnP has been made.

$MnP + H_2O_2 = MnP\text{-}I + H2O$

$MnP\text{-}I + Mn^{2+} = MnP\text{-}II + Mn^{3+}$

$MnP\text{-}II + Mn^{2+} = MnP + Mn^{3+} + H_2O$

Mn (III) in turn facilitates the oxidation of organic substrates.

These enzymes are also directly involved in the degradation of various xenobiotic compounds. Due to the fact that ligninolytic enzymes are basically nonspecific, they are able to attack a series of molecules chemically similar to lignin including pesticides, polycyclic aromatic hydrocarbons, synthetic polymers, and synthetic dyes (Maciel *et al*., 2012). The activity of MnP was approximately 950 U/L, with the maximum activity observed at the bottom layer of the bed at 84 h (Zhao *et al*., 2015).

The ligninolytic enzyme manganese peroxidase is ubiquitous in nature (Qin, X. *et al.,* 2017). This enzyme has more demands in the recent years due to its diverse applications in numerous biotechnological areas (Asgher M *et al*., 2014). MnP has the most potential, well recognized, and studied enzymatic activities, which is highly adaptable in nature with ample industrial applications (Nayanashree G *et al*., Manavalan T *et al*.,2015). Manganese peroxidases (MnPs) (EC 1.11.1.13) belong to a unique enzyme groups that hydrolyse lignin substrates in nature; other members are lignin peroxidases,laccases, and versatile peroxidases. MnPs are extracellular glycoproteins with an iron protoporphyrin IX (heme) prosthetic group. MnPs are assumed to be the first in the line of proteins expressed during fungal catabolism of lignin (Janusz, G *et al*.,2013, Pant, D *et al*.,2007.

2. Screening and Production of Manganese peroxidase:

Lignin is an amorphic three-dimensional substance whose molecular weight is difficult to determine because lignins are highly poly disperse materials (Kosa and Ragauskas *et al.*, 2012; Vanholme *et al.*, 2010). The chemical structure of lignin has also been difficult to determine, and even very recently, new bonding patterns have been described in softwood lignin, e.g., dibenzodioxodn structures. Also, the isolation of native lignin is complicated, if possible, at all (Buswell *et al*., 1987). Therefore, lignin model compounds, e.g., dimeric /J-O-4 model compounds and synthetic lignin [dehydrogenation polymerizate, (DHP)], are commonly used in microbiological studies. The results obtained using these substrates are not easy to extrapolate to natural conditions, and thus little is known what happens when microorganisms, such as white-rot fungi, degrade lignin in wood. Wood-rotting basidiomycetous fungi that cause white rot in wood are the most efficient lignin degraders in nature (Kirk and Farrell et al., 1987; Hong *et al.*, 2012), and they are perhaps nature's major agents for recycling the carbon of lignified tissues. No other microorganisms as pure culture has been described to mineralize lignified tissues as efficiently (Kirk and Cullen *et al.*, 1998). They are a group of taxonomically heterogeneous higher fungi, characterized by their unique ability to depolymerize and mineralize lignin using a set of extracellular ligninolytic enzymes. The enzymology and molecular biology of lignin degradation has been mainly studied in *P. chrysosporium* (Kirk and Cullen *et al.*, 1998; Selvam *et al*., 2012). However, many other species of white-rot fungi degrade lignin as efficiently as *P. chrysosporium* (Hatakka *et al.*, 2011), *Pycnoporus* species (Lomascolo *et al.*, 2011). Moreover, several fungi show better selectivity for lignin removal (Messner and Srebotnik *et al.*, 1994). Physiological conditions for lignin degradation, as well as secretion patterns of the ligninolytic enzymes, vary between different fungal species (Hatakka *et al.*,2011). Various authors have tried to establish correlations between ligninolytic enzymes and lignin degradation (Hatakka *et al.*,2011). However, many of the enzymes necessary for lignin degradation were not characterized before the beginning of the 1980s when virtually only laccase had been known. Since the discovery of two important peroxidases in the beginning of the 1980s, namely lignin peroxidases (LiPs) in 1983 and manganese peroxidases (MnPs) in 1984 (Kirk and Farrell *et al.*,1987), an array of enzymes has been isolated from fungi and characterized in detail. Lignin degradation is in a central position in the earth's carbon cycle, because most renewable carbon is either in lignin or in compounds protected by lignin from enzymatic degradation (cellulose and hemicellulose) (Kirk *et al.*, 1983). On the other hand, potential applications utilizing lignin degrading organisms and their enzymes have become attractive, because they may provide environment friendly technologies for the pulp and paper industry and for the treatment of many xenobiotic compounds, stains, and dyes. Despite its significance, lignin biodegradation has slowly been defined chemically and biochemically. One of the main reasons for that was the poor knowledge of the chemical structure of lignin until the late 1960s when it became better known (Eriksson *et al.*, 1990). The scheme for structural features of conifer lignin presented by Adler (Adler *et al.*,1977) has been commonly used in the literature, and only recently new structures have been discovered (Argyropoulos and Menachem *et al.*, 1997). Successful studies on the biodegradation of lignin require a good cooperation between microbiologists, biochemists, and chemists, and relatively expensive equipment and materials, e.g., 14C-labeled lignins or lignin model compounds that have not been commercially available.

Biodegradation assays based on 14C-lignins were developed in the 1970s (Haider and Trojanowski *et al.*, 1975), and using these techniques, called radio respirometry, it was revealed how lignin was optimally degraded under

laboratory conditions by white-rot fungi. The white-rot fungus *P. chrysosporium* was used as the main experimental organism in USA, while in some other laboratories, the anamorph of the same fungus, *Sporotrichum pulverulentum*, had been chosen for lignin biodegradation studies (Ander and Eriksson *et al.*, 1976). Before that, *Trametes versicolor* was a popular experimental fungus (Cowling *et al.*, 1961). In the late 1970s and in the beginning of the 1980s many important findings in the physiology of lignin degradation by *P. chrysosporium* were made. The experiments were carried out almost only in synthetic liquid media and using 14C-labeled synthetic lignin (DHP) (Kirk *et al.*, 1978).

Some other fungi were also found to readily degrade lignin and lignin model compounds in a similar way (Hatakka and Uusi-Rauva *et al.*, 1983). A breakthrough in the enzymology of lignin biodegradation occurred in 1983 when the first extracellular enzymes involved in the degradation of lignin were discovered (Kuwahara *et al.*, 1984). Large amounts of studies on the biochemistry and molecular biology of these enzymes have been published, but they were again strongly concentrated on the enzymes of one fungus, *P. chrysosporium*. The catalytic mechanism of lignin peroxidase, based on initial one-electron oxidation of the lignin model compounds followed by subsequent breakdown reactions via radical cation intermediates was proposed and experimentally verified (Kirk *et al.*, 1986). Numerous publications describing the effect of ligninolytic enzymes on lignin model compounds also appeared and were frequently reviewed (Kirk and Farrell *et al.*, 1987). In the 1990s, in addition to detailed studies on catalytic and enzymatic properties of the lignin-modifying peroxidases as well as their molecular biology, major lines of research have dealt with the potential applications of white-rot fungi and their enzymes in biopulping (biomechanical pulping) and pulp bleaching (Maciel *et al.*, 2010). However, the optimal conditions for lignin degradation and expression of LiP did not seem to be appropriate for many other fungi, and especially, the most efficient selective lignin degraders apparently did not degrade lignin under these artificial conditions. Moreover, some of these fungi did not even produce LiP at all, e.g., the most promising fungus for biopulping, *Ceriporiopsis subvermispora*, only produces MnP and laccase. When many different fungi had been studied in detail, it became clear that MnP is the most commonly occurring peroxidase while it was difficult to demonstrate the expression of LiP (Hatakka *et al.*, 1994).

Molecular biological studies as reviewed by Gold and Alic *et al.*,(1993) began with cloning and sequencing of LiP gene (Tien and Tu *et al.*, 1987), cloning and sequencing of MnP gene (Pribnow *et al.*, 1989), heterologous expression of laccase (Saloheimo and Niku-Paavola *et al.*, 1991), 3-D structure of LiP (Poulos *et al.*, 1993) and MnP (Sundaramoorthy *et al.*, 1994), heterologous (Stewart *et al.*, 1996) expression of peroxidases, 3-D structure of laccase (Ducros *et al.*, 1998), homologous expression of peroxidases (Gelpke *et al.*, 1999). Production of MnP, LiP and laccase in various species of ligninolytic fungi grown in liquid medium and their effect on mortification of polycyclic aromatic hydrocarbons, polychlorinated biphenyl mixture and a number of synthetic dyes has been reported (Cenek *et al.*, 2011). High levels of MnP were correlated with efficient decolorization of reactive dyes. Production of the extracellular ligninolytic enzyme like MnP influenced the enzymatic activity in shaken flasks and aerated cultures by *Nematoloma frowardii* (Rogalski *et al.*, 2006). A low nitrogen medium, 1.36 mM nitrogen added as ammonium tartrate, containing 16 g/L glucose inoculated with immobilized polyurethane foam mycelium, made it possible to obtain a MnP yield of 2304 nkat/L in 8 days. Under these operational conditions, the enzyme productivity in the immobilized cells of *N. frowardii* was 1.4 times higher than that obtained with the free fungus. Lignocellulosic enzymes were produced in solid-state fermentation using soy and wheat bran as substrates by *Fomessclero dermeus* (Papinutti and Forchiassin *et al.*, 2007). High enzyme production along with the very low cost of the substrate showed the suitability of the system of *F. sclerodermeus* for industrial purposes. The major isoenzyme, MnP2 of manganese peroxidase produced by *Lentinulae dodesin* corncob SSF medium was purified by using ultrafiltration, acetone precipitation and gel filtration (Boer *et al.*, 2006). The optimum pH and temperature of purified MnP-2 was 4.5 and 40°C, respectively. The enzyme was stable in acidic pH range 4.5-6.0 and 45°C temperatures. The Km values of MnP for H_2O_2 and Mn^{+2} at pH 4.5 was 20.8 and 22.2 mM, respectively. When compared with other MnP enzymes, MnP2 was more stable in the Merve and Urek (2012) carried out a research using a substrate from the fruit juice industry for the production of ligninolytic enzymes such as LiP, MnP and Lac in solid-state fermentation using *Pleurotus eryngii.* The highest MnP activity was attained as 2198 U/L on day 15 in the presence of 500 µM Mn^{+2}(Ramzan, M *et al.*, 2013).6789)

3. Applications of manganese peroxidase:

In paper and pulp industry, peroxidase usage results in decrease in energy consumption in mechanical pulping and it is used for degradation of pollutants and xenobiotic compounds like degradation of polyaromatic hydrocarbons, dioxins, chlorinated phenols, nitroaromatic compounds, metallo-organic compounds containing As, Sn, nylon, coal, humus (Annele H *et al.*, 1998). Lignolytic enzymes was considered to be a key enzyme in lignin biodegradation due to its ability to catalyse directly the oxidation of compounds

with a high redox potential. In recent years, the application of ligninase to the degradation of xenobiotics such as polycyclic aromatic hydrocarbons (PAHs), dyes and a wide range of pesticides has been intensively studied (Leandro p.*et al.*, 2006). MnP plays an imperative role in bioremediation of industrial wastes because it oxidizes number of xenobiotics compounds as well as nontoxic substrate and also used in industries like food industry, biosensor designing, paper and pulp, textile and distillery industry, diagnostic kits formation and in environmental protection. MnP is reported to catalyse the oxidation of several types of phenolic and non-phenolic compounds, with the aid of small molecules referred to as mediators. However, in recent years a new field of application for MnP is emerging in the cosmetic industry too, because these enzymes are used especially for the synthesis of flavonoids, pigments, cosmetic dyes as well as aromatic aldehydes and heterocyclic compounds, which are active ingredients in cosmetic products. As Manganese peroxidase is biodegradable, it is eco-friendly. Since it is protein, it is nontoxic to human body.

One of the most important industrial uses of WRF and their oxidative enzymes is in bioleaching and biopulping in the pulp and paper industry to replace eco-unfriendly toxic chlorinated chemicals to save on mechanical pulping energy costs (Hakala *et al*., 2005). Additional benefits are obtained by removal of wood extractives with less pitch problems and less effluent toxicity. The potential benefits of biochemical pulping by WRF include decreased lignin content of pulp, reduction of pulping time, reduced consumption of bleaching chemicals and improved tensile or bursting strength properties of pulps and paper strength properties of pulp (Bajpai *et al*., 2001; Selvam *et al*., 2006; Franco *et al*., 2006; Mardones *et al*., 2006).

4. References:

- Boer, C. G., Obici, L., de Souza, C. G. M., & Peralta, R. M. (2004). Decolorization of synthetic dyes by solid state cultures of Lentinula (Lentinus) edodes producing manganese peroxidase as the main ligninolytic enzyme. Bioresource Technology, 94(2), 107-112.
- Glenn, J. K., & Gold, M. H. (1985). Purification and characterization of an extracellular Mn (II)-dependent peroxidase from the lignin-degrading basidiomycete, Phanerochaete chrysosporium. Archives of biochemistry and biophysics, 242(2), 329-341.
- Karigar, C. S., & Rao, S. S. (2011). Role of microbial enzymes in the bioremediation of pollutants: a review. Enzyme research, 2011.
- Kirk, T. K., & Farrell, R. L. (1987). Enzymatic" combustion": the microbial degradation of lignin. Annual Reviews in Microbiology, 41(1), 465-501.
- Kosa, M. and Ragauskas, A.J., 2012. Bioconversion of lignin model compounds with oleaginous Rhodococci. Applied microbiology and biotechnology, 93(2), pp.891-900.
- Leandro, J., Nascimento, C., de Almeida, I.T. and Leandro, P., 2006. Co-expression of different subunits of human phenylalanine hydroxylase: evidence of negative interallelic complementation. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1762(5), pp.544-550.
- Mäkelä, M., Galkin, S., Hatakka, A., & Lundell, T. (2002). Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. Enzyme and Microbial technology, 30(4), 542-549.
- Paszczyński, A., Huynh, V. B., & Crawford, R. (1985). Enzymatic activities of an extracellular, manganese-dependent peroxidase from Phanerochaete chrysosporium. FEMS Microbiology Letters, 29(1-2), 37-41.
- Praveen, K., Usha, K. Y., Viswanath, B., & Reddy, B. R. (2012). Kinetic properties of manganese peroxidase from the mushroom Stereum ostrea and its ability to decolorize dyes. J Microbiol Biotechnol, 22(11), 1540-1548.
- Ramzan, M., 2013. Production and Characterization of Manganese Peroxidase (Mnp) From Mutant And Native Strains Of Coriolus Versicolor (Doctoral dissertation, University of Agriculture Faisalabad).
- Rogalski, J., Szczodrak, J., & Janusz, G. (2006). Manganese peroxidase production in submerged cultures by free and immobilized mycelia of Nematoloma frowardii. Bioresource technology, 97(3), 469-476.