A Review: Partial Purification ,Characterization And Applications of Manganese Peroxidase From White Rot Fungi

Dheevar Poonam
Dept of Microbiology
Veer Narmad South Gujarat University
K.B.S and Nataraj professional science college vapi

Abstract- *Microbial ligninolytic enzymes like laccase, manganese peroxidase, and lignin peroxidase have gained much more attention in various industrial applications. It is mainly oxidizes Mn (II) ions that remain present in various wood and soils, they are strength more reactive Mn3+ form, stabilizing by fungal chelators like oxalic acids. It has a various and great application potential and ample opportunities in very different from each other area, such as alcohol industry, pulp and paper industry, biofuel, agriculture, cosmetic, textile, and food industries. This review article is focused on the sources, catalytic reaction mechanisms and different biotechnological applications and partial purifications of this enzyme .Thus, this review article is mainly focused and emphasize nowadays outline and bring up to date information on MnP enzyme.*

Keywords- Microbial MnP, Phenolic and non-phenolic compounds, Biotechnological applications, Partial purifications.

I. INTRODUCTION

Enzymes are habitual biocatalysts which have important biological, catalytic and physiochemical properties. Increased rate of reaction and specificity renders microbiological enzymes highly perspective for various industrial applications. In todays, enzymes are being systematically developed as eco-friendly catalysts for wide ranges of commercial and industrial applications (Zhang and Kim,2010). Enzymes have major application in the fields of industrial, food, medical and, environmental applications (Kumar A. G. *et al.,* 2014).

Manganese peroxidase, lignin peroxidase and laccase are the key ligninolytic enzymes yielded by white -rot fungi that are being asses for their applications in industrial and environmental technology (Buchanan *et al.,* 2004). The microorganisms keep up the important source for lignin-degrading enzymes. The producing of ligninolytic enzymes from very microbial sources has been well known and recorded. Fungi are the best puissant basis of lignin-degrading enzymes. The saprophytic fungi are these enzymes to breaking down the lignin polymer (Pandya N. *et al*., 2020). White-rot fungi (WRF) so named because of the whitish color fungi of the delignified wood, are the only known organisms able to mineralize by the recalcitrant and bulky heteropolymer lignin degraded.

OXIDOREDUCTASES (EC 1)

Oxidoreductases are enzymes catalyzing the redoxreaction between the transfer of an electron from donor to an electron acceptor. Normally, Oxidation-reduction reactions are countless in the living cells and included in many essential biological processes such as tricarboxylic acid cycle, glycolysis, amino acid metabolism, and oxidative phosphorylation reactions; hence, oxidoreductases are between the housekeeping enzymes essential for cell`s life and activity.

TRANSFERASES (EC 2)

Transferases catalyze the move or interchange of certain groups (amino group) among many compounds. This process has a essential role in creating vital amino acids for protein synthesis in all cells. Some transferases such as glutathione transferase may assist in environmental and oxidative stress tolerance in pathogenic fungi.

HYDROLASES (EC 3)

Hydrolases are the most widely studied groups of enzymes; they catalyze the hydrolysis of their substrate between the adding of water. To date, hydrolases represent the most commercially marketed enzymes dur to their wide applications in different industrial sectors. Fungal proteases, amylases, lipases, and cellulases represents the most commercially demanded enzymes.

LYASES (EC 4)

Lyases are a group of enzymes that catalyzed the addition or elimination reaction. The result of this type of adding or abolish is a new compound with both cyclic structure or new double bonds.

ISOMERASES (EC 5)

Isomerases are a group of enzymes that catalyzed the rearrangement of substrate structure through the interchange of a specific group within the same compound.

LIGASES (EC 6)

Ligases, in general, are those classes of enzymes that catalyze the joining of two compounds through the formation of new bonds (Ahmed K. Saleh *et.al.,* 2021) .

Oxidoreductase

Oxidoreductases is the first class of enzyme in the classification of IUPAC. Oxidoreductases are enzymes catalyzing the redoxreactionbetween the move of an electron from donor to an electron acceptor. Fungal oxidoreductases were characterized to play a major role in pathogenicity and protection against host defense mechanism. Yu *et.al.* ,announce the importance of oxidoreductase-like protein Olp1 in Cryptococcus neoformans for sexual reproduction between increase the meiotic division and its vital role in the pathogenicity by defend the fungal cells from lithium-ion toxicity. Benzoquinone oxidoreductases another reported fungal virulence factor produced by Beauveria bassiana (Entomopathogenic fungus) to overcome the exposure to benzoquinone from the host cell. To date, more than twenty classes of oxidoreductase have been recognized; however, the most studied and reported oxidoreductases include:

- Dehydrogenases - transfer of hydrogen to an electron acceptor.
- Oxygenase – the final electron acceptor is oxygen.
- Peroxidases – the final electron acceptor is peroxide.

Peroxidase are oxidoreductase where peroxide compound (H2O2) act as the final electron acceptor. The major part of peroxidases concede to date are metal-dependent enzymes, mainly iron; however, metal-independent peroxidases were also reported. Two of the most extensively report lignin peroxidases (LiP) and manganese peroxidases (MnP) (Ahmed K. Saleh *et al.,* 2021).

1.1 Lignin-degrading enzymes

Ligninolytic enzyme play a key role degradation and detoxification of lignocellulosic waste in environment. The majority of ligninolytic enzyme are laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase. The pursuit of these enzymes are enhanced by various cocilator as well as some other enzymes (feruloyl esterase, aryl-alcohol oxidase, quinone reductase, lipases, catechol 2, 3-dioxygenase) to make possible the process for degradation and detoxification of lignocellulosic waste in environment.

1.2 Manganese peroxidase enzyme

The ligninolytic enzyme manganese peroxidase (E.C.1,11.1.13. Mn2+: H2O2 oxidoreductase) is ever present in nature. This enzyme has more demands in the current years due to its various applications in a great many biotechnological areas. MnP has the most potential, well recognized, and studied enzymatic activities, which is highly compliant in nature with ample industrial applications. The phenolic and non- phenolic compounds are oxidized during the oxidation of Mn(II) to Mn(III) by MnP enzyme. MnPs are the extensively distribute extracellular potential peroxidases formed/produced by fungi (white-rot fungi). It is also with laccases and LiPs, which are considered to play a vigorous role in the lignin depolymerisation process. Around about 20 years ago, these hemecontaining glycoproteins are appear to be produced by the fungus *Phanerochaetechrysosporium*(Chowdhary P. *et al.,* 2018)

1.3 Purification of enzyme

Enzyme play an important role in overcast the energy of awakening and accelerating numerous biological reactions that are critical in nourishing the life without causing any enduring modifications. Enzymes are extensively give out in all forms of living organisms, including animal, plant, and microbial sources. Among the microbial sources, fungi representing an interesting source for industrial enzymes, sharing with more than one has a enzymes in the market. Fungal enzymes are characterizing by high production strength , easier purification and separation requirements, especially filamentous fungi, and efficient catalysis with desirable stability against harsh conditions. Enzyme purification is of great importance into acquired by the knowledge about structural and functional properties and its applications. The eventual degree of purity of a particular enzyme depends upon its end use. The objective behind deciding the strategies for purification is to obtained the greatest possible yield of the desired enzyme with the most highest catalytic activity and the greatest possible purity.Many of the purification method used in laboratory research and can be easily scaled to industrial processes (Bajpai P., 2014)

These method are:

- Filtration
- Centrifugation
- Ltrafiltration
- Diafiltration
- Precipitation
- Chromatography (ion exchange and gel filtration chromatography

1.4 Entrapment

The immobilization of enzymes by solid support of their continual or repeated use in industrial applications, since their steadiness are improved and also provide more rapid separation from the reaction media leading to an economical operations (Fernandez *et al.,* 2012). Enzymes have been immobilized on a great many supports, such as amorphous silica, organic gels, alginate, chitosan, acrylamide etc. Enzyme immobilization is a method to restrict enzyme that can be recycled and reused in a certain region without changing its characteristic catalyzing function. Enzyme immobilization is a method particularly designed to limited the enzyme movement. Immobilization providing are support for enzymes. For immobilization you are have to decide the support material first, and then the immobilization method, taking into account the intended use and applications . Many methods have been utilized previously for immobilization of enzymes, for example: adsorption, covalent binding, entrapment, encapsulation, and cross linking (Hassan Mohamed *et al.*,2016).

1.5 Mechanism of MnP

MnP catalysis occurs in a sequence of irreversible oxidationreduction (redox) reactions which follow a ping-pong mechanism with second order kinetics. In the first steps of the catalytic cycle, H2O2, or an organic peroxide, enters the active site of MnP. There the O2 in H2O2 hold together an Fe(III) ion in the heme cofactor to formed an iron peroxide complex. Two electrons are transferred from Fe3+ to peroxide, breaking the oxygen-peroxide bond to form H2O and a Fe(IV) oxoporphyrin radical complex. This oxidized intermediate is known as MnP compound I. MnP compound I then binds to a mono chelated Mn (II) ion, which donates an electron to quench the radical and form Mn(III) and MnP compound II, a Fe(IV) oxo porphyrin complex. MnP compound II oxidizes another Mn(II) ion to Mn(III) and is reduced by the reaction of two H+ ions and iron bound oxygen. This reforms the Fe (III) ion in the heme and releases a second water molecules. There are more difference from this conventional catalytic cycle. MnP compound I can be nearly new to oxidize free Mn (II), ferrocyanide, as well as phenolics, and further aromatic compounds.

1.6 Chelators

Mn (III) is unstable in aqueous media, therefore MnP releases it as a Mn (III)-carboxylic acid chelate. There are a variation of carboxylic acid chelators together with oxalate, malonate, tartrate, and lactate, however oxalate is the most common.

The peroxidase shape favors Mn(III)-chelates over unbound Mn(III) ions. The Mn(III) chelates interacts with the active site to facilitate product release from the enzyme. The chelator can have an effect on the kinetic and even the catalyzed reaction. If the substrate Mn(II) is chelated with lactate, MnP instead catalyzes the evolution of O2. However, this site reaction has little impact on enzymatic activity because it follows slower third order kinetics.

1.7 Applications

In paper and pulp industry, peroxidase use results in reduce , in energy expending in mechanical pulping and it is using for degradation of pollutants and xenobiotic compounds like a degradation of polyaromatic hydrocarbons, dioxins, chlorinated phenols, nitroaromatic compounds, metalloorganic compounds containing As, Sn, nylon, coal, humus (Annele H *et al.,* 1998). Ligninolytic enzymes was contemplate to be a key enzyme in lignin biodegradation due to its ability to catalyse directly the oxidation of compounds with a high oxidstion-reduction potential. In current 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 imperious role in bioremediation of industrial wastes because it oxidizes number of xenobiotics compounds as well as nontoxic substrate and also used in various industries like food industry, biosensor designing, paper and pulp, textile and distillery industry, diagnostic kits development in environmental protection. MnP is documented to catalyse the oxidation of various types of phenolic and non-phenolic compounds, with the aid of small molecules referred to as mediators (Nidhi P. *et al.,*2020 Ligninolytic enzymes acquire more attention in various type of biotechnological application such as in alcohol, pulp and paper, textile, food, medicals, and in cosmetic industry and also for biodegradation of various toxic compounds (Chowdhary P. *et al.,* 2018).

1.7.1 Degradation of phenolic and non- phenolic compound

Phenolic and non-phenolics compounds are found in many different industrial wastewaters, including coal conversion, petroleum refining, resins, plastics, wood preservation, metal coal, dyes and other chemicals, textiles, mining and dressing, and pulp and paper. Almost all applications have focused on the treatment of phenolic contaminants in the presence of H2O2. MnP-generated Mn (III) chelator can prompt the oxidation of phenolic compounds, including phenols, amines, dyes, and also dimers and lignin-containing phenolic structure. Various researchers build that under physiological conditions Mn(III), chelator acting as mild oxidant and limitation to the oxidation of lignin-containing phenolic structures. The Mn (III) formed is separated from the enzyme and stabilized by forming complexes with α-hydroxy acids at a high oxidation-reduction potential of 0.8–0.9 V. There are two optimal chelators, i.e., malonate and oxalate that are producing in significant amount by fungal species. The oxidation by Mn (III) captures the development of reactive radicals for non-phenolic substrate in presence of second mediator. In contrasting , LiPcatalyzedreaction, which involves e- reduction from the aromatic ring forming a radical cation. In the presence of thiols (R-S–H) such as glutathione, Mn (III) mediates the oxidation of substituted benzyl alcohols and di aryl propane structures into their own aldehydes (Zhang *et al*.,) found that MnP-Tra-48424, which obtaining from *Trametes sp.,* has the ability to decolorize various group of dyes such as indigo, anthraquinone, triphenylmethane and azo dyes.

1.7.2 Pulp and paper industry

Pulp and paper industry is a key sponsors in world's economy, but regrettably it causes diverse environmental toxicity and health problems. During the paper manufacturing process pulp and paper industry uses enormous amount of lignocellulosic materials. Paper manufacturing process involves three main steps like;

(1) pulping
(2) bleaching
(3) paper production.

Further pulping divided into three type

- mechanical,
- chemical
- chemi-mechanical or combination pulping.

These processes generate high strengthness of wastewater, which causes inauspicious impact on environment. There are several chemicalbased treatment technologies obtainable , but are risky to living beings, costly and insecure of green environment, while biological approaches are reported by the cost-effective, commercially and environmentally safe approaches for wastewater treatment. For the sufficient treatment of dischargable wastewater and pollutants, various group of microorganisms are playing an necessary role. Among these, white-rot fungi are found more and more effective for the degradation of many lignin and other similarity wood materials to CO2 and H2O. Some of them are influential degrader of lignin preferably than cellulose and hemicellulose.

1.7.3. Food industries

Food quality is not a function of nutritional values, but it can be also of the presence of bioactive compounds, which apply various positive effects on human and animal health. Microbial ligninolytic enzyme displays great outlook for variety of industrial application like pulp delignifcation, wastewater treatment, biofuel production, dye decolorization, biosensors, and juice extraction clarification. (Bilal*e tal* .,)immobilized MnP gives commendable result in which reduction in apple juice color and turbidity was 42.7% and 36.3, respectively. In synchronous study, it was observed that reduction in color and turbidity 51.5% and 43.6%, respectively. Further,(Bilal *et al*.,)founded that treatment by immobilized ligninolytic enzyme decreasing turbidities up to 84.02%, 57.84, 86.14% and 82.13% which was observed for apple, grape, orange and pomegranate juice, respectively. In recent years, MnP have also gained more demands in various applications in food industry. It has great possibility to generate natural aromatic favors such as vanillin production, ferulic, p-coumaric, syringic and p-hydroxybenzoic.

1.7.4 Alcohol industries

Ligninolytic enzymes play a very important role in distillery waste treatment process. Enzymatic degradation/detoxification has been put in an application in the mitigation of toxicity after dissimilar pre-treatments such as steam explosion, strong acids, organosolv, and hot liquid water. In inclusion , ligninolytic enzyme enhances detoxification, fermentation rates and ethanol production processes in alcohol industry. The enzymatic degradation or decolorization give rise to ethanol yields five times more in differentiation with ion exchange degradation in sugarcane bagasse. The enzyme has capacity to catalyze non-activated alcohols, which is an imperious factor for future degradation or detoxification procedures. For Manganese peroxidase , highest production was in *Aspergillus niger* TERI DB20 grown on corncob with wastewater.

1.7.5 Use of lignocellulosic materials and MnP in bio-ethanol production

In current years, concerning renewable biofuel production and bioethanol from lignocellulosic feedstocks is contemplate the most practicable choice for fossil fuels replacing, since these raw materials do not take part with food or feed crops . Treatment by ligninolytic enzyme of waste biomass could be of accurate interest, because it seems to be an environmental friendly method for making bio-ethanol. In future, biorefineries will combine biomass conversion process to produce fuels, power, heat and value-added chemicals. Due to its cheap and wide distribution, lignocellulosic biomass is anticipate to play an important role toward this aim .

2.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis of the samples before and after degradation were performed. The FTIR analysis was carried out in the mid IR region of 400-4000 cm-1 (Sekar. S. *et al;* 2012). Fourier transform infrared spectroscopy (FTIR) can supply basic information on the molecular structure of organic and inorganic components, and is one of the most versatile systematic techniques for the non-destructive, chemical representation of geological samples, such as coal, shale, fluid and melt inclusions, silicate glass, minerals, and microfossils.FTIR spectroscopy is used to quickly and definitively identify compounds such as compounded plastics, blends , fillers , paints , rubbers, coatings, resins and adhesives. It can be put in to the other side of all phases of the product lifecycle together with design, manufacture , and failure analysis.

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