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Applications of Ligninolytic Enzymes from a White-Rot Fungus *Trametes versicolor*

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Abstract:

The growing concern over the pollution issues by the rapid industrialization has posed a serious problem forcing researchers around the world to seek alternative eco-friendly technologies. Textile, pulp and paper industries discharge a huge quantity of waste in the environment, and the disposal of this waste is an immense problem. To solve this problem, work has done to discover such a biological process, which can detoxify these wastes and is not damaging the environment. Lignocellulosic agro-wastes comprising over 60% of the existing plant biomass are the potential renewable resources for bio fuels, bio fertilizers, animal feed, bioremediation and chemical feed stock. *Trametes versicolor* is one of the extra cellular, non-specific and efficient ligninolytic enzymes producers with high activities as secondary metabolites under optimum growth conditions, especially lignin peroxidases (LiPs), manganese peroxidases (MnPs) and laccases. Laccases use oxygen and produce water as by product. They can degrade a range of compounds including phenolic and non-phenolic compounds. They also have capability to detoxify a range of environmental pollutants. The present review therefore aims to bring out a comprehensive analysis of available biological agent which can replace the present highly polluting chemical processes for effluent treatment from pulp and paper industries, textile industries, food industries, bio remediation and bioethanol production.

Keywords: Pollution, Ligninolytic enzymes, Secondary metabolites, Bio-remediation, Laccases

1.0 Introduction:

The rapid improvement in industrialization and globalization has led to the high standard of living and betterment of mankind (Srinivasan and Meenakshi, 1999). This has positive effect on lifestyle but negative effect on the living beings and environment like the depletion of fossil fuels, global warning, soil, air and water pollutions. The growing concern over the pollution has posed a serious problem forcing researches around the world to seek alternative eco-friendly technologies. The agricultural and industrial waste comprise of the worlds 75% of waste and steps are taken to treat these wastes worldwide (Thomson, 2001).

Agriculture and some industrial wastes, which mainly consists of cellulose, hemicelluloses and lignin is the most abundant renewable lignocellulosic biomass. Most of these materials are disposed by burning, which results in environmental pollution (Sanchez, 2009). Lignocellulosic agro-wastes comprising over 60% of the existing organic biomass in the world. These are potential renewable resources for bio fuels, bio fertilizers, animal feed, bio remediation. Lignin is the second most available renewable bio polymers in nature after cellulose, but it is degraded by only a small number of microorganisms (Patil, 2014).

To solve above problems, research has being carried out in finding, such an enzymes which can detoxify, degrade the lignocellulosic wastes and are not harmful to the environment. Myco remediation is a process of using fungi to degrade the environmental contaminantes to a less contaminated state (Mayuri and shoba, 2014). White-rot fungi are efficient degraders of the lignocellulosic materials such as lignin, cellulose and hemicellulose by synthesizing ligninolytic enzymes (Ruhl *et al*, 2008).

Trametes versicolor is one of the extra cellular, nonspecific and efficient ligninolytic enzymes producer, especially lignin peroxidases (LiPs), manganese peroxidases (MnPs) and laccase with high activities as secondary metabolites under optimum growth conditions (Pandey *et al*, 2000). LiPs are heme proteins with high oxidation potential and are capable of oxidizing phenolic and non phenolic substrates (Shrivastava, 2005). MnPs are glyco proteins with an iron protoporphyrin IX prosthetic group (Baborova *et al*, 2006) that are capable of oxidizing phenolic substrates and unable to oxidize non-phenolic substrates, although they have the capability of depolymerizing synthetic or natural lignin invitro (Elisashvili *et al*, 2001). Laccases are N-glycosylated blue multi-copper oxidases (Daphne *et al*,2013) that are versatile mineralizers of lignin as well as a variety of recalcitrant aromatic compound including synthetic dyes (Murugesan *et al*; 2006).

This paper clearly explains the applications of *Trametes versicolor* as a potential fungal species for bioethanol production, bioremediations, treating soil, air and water pollutions.

2.0 Applications

2.1 Food Industry:

2.1.1 Wine Stabilization

In food industry, wine stabilization is the main application of the ligninolytic enzymes like laccases. Polyphenols have undesirable effects on wine production and on its organoleptic characteristics, so their removal from the wine is very necessary. Many innovative treatments, such as enzyme inhibitors, complexing agents, and sulfate compounds, have been proposed for the removal of phenolics responsible for discoloration, haze, and flavor changes but the possibility of using enzymatic laccase treatments as a specific and mild technology for stabilizing beverages against discoloration and clouding represents an attractive alternative (Morozova *et al*., 2007).

The work carried out by Muhammad Imran *et.al* (2012) stated that laccase enzyme is not yet allowed as a food additive, the use of immobilized laccase might be a suitable method to overcome such legal barriers as in this form it may be classified as technological aid. So laccase could find application in preparation of must, wine and in fruit juice stabilization.

2.1.2. Bakery Products:

In the bread-making process laccases affix dough-enhancement additives to the break dough, these results in improved freshness of the bread texture, flavor and the improved machinability (Minussi, *et al*, 2002). Selinheimo, et al. (2006) reported that when laccase was added to the dough, the strength of the gluten structures was improved. Prolonged incubation of flour dough with laccase results in softening of the dough and the extent of softening increases with increase in concentration of laccase.

2.2. Pulp and Paper Industry:

Pulp and paper industries discharge a huge quantity of waste in the environment, and the disposal of this waste is a big problem. The extent of pollution caused by the paper and pulp industry can be adjusted by the fact that overgrowing demand of the paper makes paper and pulp industry among the world's largest contributors of air and water pollutants, hazardous waste products and the toxic gases that cause the considerable change in the climate. The pressure of demand exceeding the supply of required wood is leading to the forest being logged for timber and cleared for growing plants that have reduced ecological value, and use of toxic chemical fertilizers and herbicides that leads to secondary consequences (Tavares *et al*, 2005).

The situation becomes worse considering the fact that industrialized and developing nations with just 20 percent of the world's total population, consume 87 percent of the world's paper. According to the reports of OECD Environmental outlook, 2012 the global scenario of paper and pulp production is expected to be increased by 77% from 1995 to 2020. Indian paper mills use a wide variety of cellulosic and non cellulosic based raw materials for paper production accounting for about 43% from forest wood, 28% from agro based product and 29% from recycling of waste paper. Industrial pollution is global concern. In India, more than 55% of industries do not have any proper treatment and around 20% of them have partial treatment facilities (Pokhrel and Viraraghavan, 2004).

2.2.1 Delignification of Lignocelluloses:

In the manufacture of paper, separation of lignin from cellulose fibres is required. Chlorine, sulphite and oxygen based oxidants are conventionally used in the degradation of lignin for the production of paper. A mild strategy of delignification using ligninolytic enzyme laccase has replaced conventional and polluting chlorine-based methods without affecting the integrity of cellulose. Fermentation of lignocelluloses to generate fuels such as ethanol requires delignification (Kristensen J.B *et al,* 2008).

2.2.2. Biopulping and Biobleaching:

Enzymatic pretreatment of wood chips before chemical methods is known as bio pulping. Ligninolytic enzymes produced by the *Trametes versicolor* have many applications in the pulp and paper industrial processes. The important one is the pulp bleaching (i.e.) demethylation and delignification of the pulp (on Kraft pulp). The chemical bleaching is a very fast process but it also affects the cellulose in the pulp and the environment is also polluted by the various effluents from the paper plants. The white-rot fungus *Trametes versicolor* delignifies and bleaches pulp. This process is rather slow compared with chemical bleaching, but it is an eco-friendly method. One of the important ligninolytic enzymes found to bleach, depolymerize synthetic lignin and to degrade high molecular weight chloro lignin (Ana Maria. *et. al,* 2007).

The work carried out by M.G. PAICE et.al. (1993) show that Manganese Peroxidase is produced by bleaching cultures of *Trametes versicolour* that the peak production of the enzyme occurs at the same time as the maximum rate of fungal culture bleaching, and that manganese and peroxide, depending demethylation and delignification of kraft pulp occur in vitro. This pulp bleaching will not affect the fibre strength.

2.3 Textile Industry:

2.3.1 Dye Decolourization:

Textile industry effluents are of great threat to the environment. Textile waste waters contain various dyes which are hardly decolorized by conventional treatment system. Generally, these effluents are highly coloured with high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Nagraj, C.M.2006). Dye decolourization through biological means has gained momentum as this method is cost effective and can be applied to wide range of dyes. Decolourization of dye waste waters using the Ligninolytic enzymes of white-rot fungus *Trametes versicolor* has found to have an enormous potential for waste water treatment (Rose Jebapriya, G. 2013). Vijaykumar et al, 2006 isolated a new fungus from coal sample which decolourized five different azo and tri phenyl methane dyes like acid blue 193, acid black 210, and crystal violet, reactive black and reactive black BL/LPR.

2.3.2. Denim Finishing:

Application of laccase enzyme in denim finishing is a new trend. Several steps are involved in the manufacture of denim garments. The traditional technology of producing a stone-washed look in denim fabric involves washing the fabrics with pumice to generate the desired erosion of the fabrics. Then the fabric is treated with sodium hypochlorite to partially pre-bleach it, followed by neutralization and a rinsing step. All these steps cause substantial environmental pollution. In 1996 Novozyme (Novo Nordisk, Denmark) launched a DeniLite, the first industrial laccase and the first bleaching enzyme which acts with the help of a mediator molecule. Present research in this field had led to discovering novel microorganism to produce high quality of laccase at normal environmental Conditions. So, *Trametes versicolor* was found to be the best microorganism for enzymatic bleaching of denim fabric (Sharma, H.S.S., 2005).

2.3.3. Cotton Bleaching:

Cotton bleaching is done to remove natural pigments caused by the presence of flavonoids and to give a white appearance to the fibres. The most common bleaching agent used is hydrogen peroxide which is applied at alkaline p Hand high temperatures which causes a decrease in the degree of polymerization thus damaging the fibres. Bleaching using an enzymatic system will lead to less fibre damage and less use of water and gives rise to a better quality product. Ligninolytic enzymes have bleaching properties at low concentrations and works without reducing the quality of the fabric (Tzanov. T., 2003).

2.4 Bioremediation:

2.4.1 Cyanide Degradation:

Large quantities of cyanide are produced during the extraction of metal ores, photographic processing, synthetic fibre manufacturing, and production of organic chemicals and steel making. Cyanide is often removed from industrial effluents by alkaline chlorination or by using H_2O_2 or ozone. However, biotechnological treatment of cyanide is considered as most cost-effective and environmentally acceptable than chemical methods (Akcil and Mudder, 2003). The biotechnological processes still being used in this field are mainly based on the results of the studies of the hydrolytic, oxidative, reductive and substitution/transfer pathways for biodegradation of cyanides. Aerobic treatments have received much greater attention than anaerobic processes. The existence of the cyanide-resistant, alternative respiratory pathway in fungi has allowed the development of biotechnological process for degradation of cyanide compounds in polluted effluents (Ebbs 2004). The cyanide degradation abilities of three white rot fungi, *Trametes versicolor* ATCC 200801, *Phanerochaete chrysosporium ME 496 and Pleurotus sajor-caju*, were examined. *Trametes versicolor* was the most effective with 0.35 g dry cell/100 ml degrading 2 nM KCN (130 mg/1 over 42 h, at 30^0 c, pH 10.5 with stirring at 150 rpm. The above work carried out by Ahmet Cabuk *et. al* (2006) showed that, *Trametes versicolor* had the highest yield of cyanide degradation capacity as compared with the others examined, it was used to determine the necessary incubation conditions for improving yield of KCN degradation.

2.4.2 Degradation of Pollutants in Soil:

White-rot fungi *Trametes versicolor* are used extensively in biodegradation processes. The oxidative ability of these fungi is related to their extracellular and intracellular enzymatic system. Their non-specific enzymatic system allows them to degrade and/or mineralize a wide range of pollutants resistant to other microorganisms, such as dyes, PAHs, PCBs, pesticides, penta chloro phenols and endocrine disruptors (Tahseen Sayara *et al,* 2011). Two major strategies have been developed for use of white-rot fungi for degradation of pollutants in bioreactors. One is treatment with enzymes, whether purified or using broths from fungi cultures, such as laccases, manganese peroxidases and lignin peroxidases. The other is direct degradation of pollutants using active cultures of fungi. The main advantage of using fungi lies in the broad range of enzymes produced and the further transformation of the intermediate biodegradation products. On the other hand, use of enzymes allows easier operation and, in some cases, faster biodegradation (Eduard Borras *et al,* 2008).

2.5 Bio-deterioration:

Wood is degraded by microorganisms, when moisture, oxygen and other environmental factors favour microbial growth. Bio deterioration represents a complex of natural physical and chemical spoilage processes in various materials, caused by the growth of very different organisms. These are generically called biodeteriogens, but they are all characterized by the saprotrophic ability of using substrates to sustain their growth and reproduction. The growth characteristics of the microorganisms in wood and the type of degrading system produce different decay patterns. Depending on the type of decay, different physical, chemical and morphological changes occur in wood (Sanchez, 2011).There are reports that white rot fungi produce extracellular -OH, but they also secrete peroxidases, laccases and celluloses, which undoubtedly participate in lignocelluloses degradation. Paulownia is a fast growing tree. If appropriate conditions are provided, the tree can reach about 15–25 m high in a 5-year period(Jonsson, I. J *et al* ,1998).Paulownia wood is used for a variety of applications such as furniture, construction, musical instrument, shipbuilding, aircraft, packing boxes, coffins, paper, ply wood, cabinet making, and molding. In this study we investigated the biological resistance and microscopy properties of Paulownia wood treated with nano silver, nano copper, and nano zinc oxide after exposure to a white-rot fungus (*Trametes versicolor*). Knowing the morphological and structural changes of the wood decayed by this fungus and its deterioration potential is essential both for understanding the ageing of wooden cultural heritage objects and for conducting the biodegradation of fallen and old trees wood in the forests (Maliheh Akhtari *et. al,* 2013).

2.6 Bio-Ethanol Production:

Fermentation of wood hydrolysates to desirable products, such as fuel ethanol, is made difficult by the hydrolysate. White-rot fungi (Eriksson *et al*. 1990) are known for their ability to degrade lignin. Hardwood and softwood in general contain 20%-25% and 26%-32% lignin respectively. As white-rot fungi degrade large amounts of wood aromatics the enzymes they secrete for this purpose should be of interest for investigations concerning the detoxification of aromatics derived from wood. One of the best-studied white-rot fungi is the basidiomycete *Trametes versicolor,* which secretes enzymes such as the phenol oxidase laccase and perioxidase, which take part in the transformation of aromatic compounds. (Lipin *et al ,* 2013).

A novel method to increase the fermentability of lignocellulosic hydrolysates; enzymatic detoxification, laccase a phenol oxidase and lignin peroxidase purified from the ligninolytic basidiomycete fungus *Trametes versicolor* were studied using a ligno cellulosi hydrolysate. The results show more rapid *Saccharomyces cerevisiae* was employed for ethanolic fermentation of the hydrolysates. The results show more rapid consumption of glucose and increased ethanol productivity for samples treated with laccase. Treatment of the hydrolysate with lignin peroxidase also resulted in improved fermentability (Olsson, L., *et al*, 1996).

3.0 Conclusion:

Present review on applications of Ligninolytic enzymes from *Trametes versicolor* provides detailed information on the applications of this fungus in the various fields like pulp and paper industries, textile industries, bio remediation, food industries and bio ethanol production. However, despite of large technological advancements and stringent government regulations, little has been done to promote safe environmental friendly treatment process. Literature shows several significant studies done on the lignin degradation using approaches in biotechnology but the fungi used in various methods suffers drawbacks owing to the environmental conditions they are exposed to. Most of the fungi studied so far are acidophilic and thus they are inefficient to grow on medium containing effluents from various industries that are highly alkaline. High concentrations of ligninolytic enzymes can be produced with proper optimisation of various process conditions and usage of en expensive sources like agro wastes should be explored. Hence, ligninolytic enzymes from the white-rot fungi *Trametes versicolor* are receiving much attention of researches around the globe.

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