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(REVIEW ARTICLE)



## Biotechnological applications of white rot fungi: A review

Tirsit Tibebu Bogale <sup>1,\*</sup>*Microbial Biotechnology Research Program, National Agricultural Biotechnology Research Center, Ethiopian Institute of Agricultural Research, Holeta, Ethiopia*

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Article DOI: <https://doi.org/10.30574/gscarr.2020.5.2.0043>**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 biotechnological applications such a biological process, which can detoxify wastes and is not damaging the environment. Examples of white-rot fungi that possess selective decay at least under certain condition are *C. subvermispota*, *Dichomitus squalens*, *P. chrysosporium*, and *Phlebia radiata*. Examples of white-rot fungi that possess non-selective decay are *Trametes versicolor* and *Fomes fomentarius*. These enzymatic complexes mainly consist of lignin peroxidases (LiPs), manganese peroxidases (MnPs) and laccases. They also have capability to detoxify a range of environmental pollutants. The present work explores the potential of WRF in more recent areas of their applications such as, textile industries, food industries, bio remediation, pulp and paper industries and animal feed digestibility.

**Keywords:** White-Rot Fungi; Lignin Peroxidases (Lips); Manganese Peroxidases (Mnps); Laccases; Textile Industries; Food Industries; Bioremediation; Pulp And Paper Industries; Animal Feed

**1. Introduction**

Fungi that are active in the biodegradation of wood can be classified into three main groups according to their methods of degrading biomass, specifically white-rot, brown-rot, and soft-rot fungi. White-rot and brown-rot fungi belong to Basidiomycetes, whereas soft-rot fungi belong to Ascomycetes (Hatakka, 2001). White-rot fungi are able to decompose all wood fractions, including lignin, and leave the wood with a white, fibrous appearance. Mostly, white-rot fungi grow on hardwoods e.g. birch and aspen. However, certain species such as *Heterobasidion annosum*, *Phellinus pini*, and *Phlebia radiata* grow on softwoods such as spruce and pine (Blanchette 1995).

White-rot fungi degrade lignin with two modes of action, namely selective and non-selective decays (Blanchette 1995). Selectivity of white-rot fungi regarding lignin degradation depends on the lignocellulose species, cultivation time, and other factors (Hatakka and Hammel, 2010). Examples of white-rot fungi that possess selective decay at least under certain condition are *C. subvermispota*, *Dichomitus squalens*, *P. chrysosporium*, and *Phlebia radiata*. Examples of white-rot fungi that possess non-selective decay are *Trametes versicolor* and *Fomes fomentarius*. In selective decay, lignin and hemicellulose fractions are selectively degraded while the cellulose fraction is essentially unaffected. In non-selective degradation, approximately equal amounts of all fractions of lignocellulose are degraded (Hatakka, 2001).

\* Corresponding author: Tirsit Tibebu Bogale

Microbial Biotechnology Research Program, National Agricultural Biotechnology Research Center, Ethiopian Institute Of Agricultural Research, P.O. Box 249, Holeta, Ethiopia.

Related to lignin degradation, white-rot fungi face three major challenges associated with lignin structure, i.e. (1) the lignin polymer is large; therefore ligninolytic systems must be extracellular, (2) lignin structure is comprised of inter-unit carbon-carbon and ether bonds, therefore the degradation mechanism must be oxidative rather than hydrolytic, and (3) lignin polymer is stereo-irregular, therefore the ligninolytic agents must be much less specific than degradative enzymes (Kirk and Cullen, 1998).

WRF are ubiquitous in nature, particularly in hardwood forests as hardwood (e.g. birch and aspen) is more susceptible to the attack of WRF than softwood (e.g. spruce and pine). WRF can degrade all wood components (i.e. cellulose, hemicellulose and lignin) or preferentially lignin. The former are named simultaneous or non-selective WRF and the latter selective WRF. The selective WRF are of special bio industrial interest, since they remove lignin leaving the valuable cellulose intact. There are also WRF that cause both types of white-rot attack within one substrate (Blanchette, 1984)

White-rot fungi (WRF), so-named because of the whitish colour of the delignified wood, are the only known organisms able to mineralize the recalcitrant and bulky heteropolymer lignin. This ability is due to the secretion of extracellular non-specific enzymatic complexes during their secondary metabolism (Wesenberg *et al.* 2003), usually under limited nutrient availability (C: N ratio) with nitrogen being the limiting nutrient for fungal growth in most wood and soils. These enzymatic complexes mainly consist of lignin peroxidases (LiPs), manganese-dependent peroxidases (MnPs), and laccases together with accessory enzymes (Ruiz-Dueñas & Martínez 2009). The non-specificity of these enzymes enables them to transform a great variety of persistent chemicals with a structure similar to lignin. Furthermore, their extracellular nature allows the fungi to access non-polar and insoluble compounds (Levin *et al.* 2003).

This paper will review the research thus far on the potential use of microorganisms (especially white-rot fungi) in degrading some of the top pollutants throughout the world and the mechanisms involved in this process such as textile industries, food industries, bio remediation, pulp and paper industries and animal feed digestibility.

## 2. Potential applications of white-rot fungi

### 2.1. The role of white rot fungi in Bioremediation

Bioremediation is defined as the application of biological processes to the treatment of pollution. Most research within the field of bioremediation has focused on bacteria, with fungal bioremediation (mycoremediation) attracting interest just within the past two decades. The toxicity of many of the above-named pollutants limits natural attenuation by bacteria, but white rot fungi can withstand toxic levels of most organopollutants (Aust *et al.*, 2004). White rot fungi is a physiological grouping of fungi that can degrade lignin (and lignin-like substances). Four main genera of white rot fungi have shown potential for bioremediation: *Phanerochaete*, *Trametes*, *Bjerkandera*, and *Pleurotus* (Hestbjerg *et al.*, 2003). These fungi cannot use lignin as a source of energy, however, and instead require substrates such as cellulose or other carbon sources. Thus, carbon sources such as corncobs, straw, and sawdust can be easily used to enhance degradation rates by these organisms at polluted sites. Also, the branching, filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil. The main mechanism of biodegradation employed by this group of fungi, however, is the lignin degradation system of enzymes. These extracellular ligninmodifying enzymes (LMEs) have very low substrate specificity so they are able to mineralize a wide range of highly recalcitrant organopollutants that are structurally similar to lignin (Cajthaml *et al.*, 2002).

The three main LMEs are lignin peroxidase, Mn-dependent peroxidase, and laccase. All three of these enzyme groups are stimulated by nutrient limitation (Aust *et al.*, 2004). They are most effective at degrading lignin and lignin-like substances when certain nutrient levels, primarily nitrogen, are low. Conversely, activities of these enzymes are completely suppressed in media containing high levels of nitrogen (Reddy and Mathew, 2001). This characteristic is advantageous for the fungi inhabiting highly contaminated sites with very low productivity due to toxic levels of organopollutants. Lignin peroxidase is a glycosylated heme protein that catalyzes hydrogen peroxide-dependent oxidation of lignin-related aromatic compounds. They have a higher redox potential than most peroxidases and so are able to oxidize a wide range of chemicals, including some non-phenolic aromatic compounds (Reddy and Mathew, 2001). Mn-dependent peroxidase also requires hydrogen peroxide to oxidize Mn<sup>2+</sup> to Mn<sup>3+</sup>. The Mn<sup>3+</sup> state of the enzyme then mediates the oxidation of phenolic substrates (Mester and Tien, 2000).

Laccase, a multicopper oxidase enzyme, is the primary enzyme involved in the degradation process. It was first described in 1883, making it one of the oldest enzymes ever described (Mayer and Staples, 2002). It uses dioxygen as an oxidant, reducing it to water and it has the ability to catalyze the oxidation of a wide range of dihydroxy and diamino aromatic compounds.

### 2.1.1. Cyanide Degradation:

Large quantities of cyanide are produced during the extraction of metal ores, photographic processing, synthetic fiber manufacturing, and production of organic chemicals and steel making. Cyanide is often removed from industrial effluents by alkaline chlorination or by using H<sub>2</sub>O<sub>2</sub> 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. The cyanide degradation abilities of three white rot fungi, *Trametes versicolor*, *Phanerochaete chrysosporium* 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/l over 42 h, at 300 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.

## 2.2. Applications of white rot fungi in textile industry

### 2.2.1. Dye decolourization by white rot fungi

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 and Kumar, 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. (Vijaykumar *et al.*, 2006) isolated a new fungus from coal sample which decolorized 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

### 2.2.2. Cotton Bleaching

Cotton bleaching is done to remove natural pigments caused by the presence of flavonoids and to give a white appearance to the fibers. The most common bleaching agent used is hydrogen peroxide which is applied at alkaline Ph and high temperatures which causes a decrease in the degree of polymerization thus damaging the fibers. Bleaching using an enzymatic system will lead to less fiber 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 *et al.*, 2003).

## 2.3. Pulp and paper industry

During pulp and paper production, it is necessary to separate the cellulose fibers from lignin. This is performed by using mechanical or chemical methods. In chemical pulping, lignin is solubilized by chemicals resulting in a brown residual material that must be removed to produce white paper. For this, elemental chlorine has been used for a long time but currently delignification with oxygen and hydrogen peroxide is being used. However, they are less efficient in achieving a high degree of brightness than the chlorine reagents. The treatment of wood chips with ligninolytic fungi prior to conventional pulping methods (mechanical, chemical or a combination of both) is named bio-pulping.

WRF have been considered as potentially useful agents for bio-pulping because they reduce not only energy consumption but also chemicals, thus, being environmentally-friendly in contrast with the conventional pulping. In addition, bio-pulping not only removes lignin but also some of the wood extractives, thereby reducing the pitch content and effluent toxicity (Ali & Sreekrishnan 2001). The biological delignification of wood by WRF was first considered by Lawson & Still (1957) at the West Virginia Pulp and Paper Company Research Laboratory (now Westvaco Corp). Since then, many researchers have studied the potential use of WRF in pulping processes and pilot mill trials have been started in the last decades. The efficiency of fungal pre-treatment utilizing different lignocellulosic materials has been described and several patents have been published, the one based on the use of *Ceriporiopsis subvermispora* being the most optimized one (Gutiérrez *et al.* 2001). This species has also proven to be very competitive on both softwoods and hardwoods. The pre-treatment of wood chips by WRF has shown to improve the effectiveness of kraft pulping and paper brightness (Fonseca *et al.* 2014). Therefore it can be considered as a possible alternative to chemical pulping since, in addition to this, it requires simpler equipment and produces an effluent with reduced biological oxygen demand (BOD).

## 2.4. Food Industry

### 2.4.1. Wine Stabilization

In food industry, wine stabilization is the main application of the

lignolytic 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.

### 2.4.2. 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. (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.

Laccase application in the food industry is based on its ability to polymerize molecules. Laccases can be applied to certain processes that enhance or modify the colour appearance of food or beverage for the elimination of undesirable phenolics, responsible for the browning, haze formation and turbidity in clear fruit juice, beer and wine.

Laccase is also employed to ascorbic acid determination, sugar beet pectin gelation, baking and in the treatment of olive mill wastewater. Researcher showed that a laccase from the white-rot fungus *Trametes hirsuta* increased the maximum resistance of dough and decreased the dough extensibility in both flour and gluten dough. Also laccase in a bi-enzyme system with novel cellobiose dehydrogenase-3-ethylbenzothiazoline-6-sulphonic acid (CDHABTS-laccase) has been used for fast oxidation of lactose to lactobionic acid (LBA) which is a valuable organic acid, with numerous applications in pharmaceutical, food, and cosmetics industries (Hildebrand, 2015).

## 2.5. Pharmaceuticals and Cosmetics

Laccases are biomolecules which act specifically; this make pharma-tech companies to use this enzyme for synthesis of complex medicinal compounds such as anesthetics, anti-inflammatory, antibiotics, sedatives, etc. More recently cosmetics for skin lightening have been developed which are laccase-based hair dyes could be less irritant and safer than current hair dyes. Protein engineered laccase may be used as deodorants, toothpaste, mouthwash, detergent, soap, and diapers with reduced allergenicity (Pannu and Kapoor, 2014).

## 2.6. Animal feed

Aerobic environment such as the rumen most of lignocellulosic agricultural residues are enriched in microbial protein, enzyme and bio-factors that can be used as animal feed stuff directly or after partial treatments. Microbial degradation of lignocellulosic materials brings a variety of changes in their bio- physicochemical properties. Lower digestibility of various agricultural residues can be enhanced by microbial treatment. White rot fungi are the potential candidates, which can improve the nutritional quality of lignocellulosic residues by degrading lignin and converting complex polysaccharides into simple sugars. (Chen and Dixon, 2007).

Changes in physical qualities of lignocellulosics that is texture, colour and aroma have been an interesting area of study along with chemical properties. Degradation of lignocellulose not only upgrades the quality of degraded biomass, but helps simultaneous production of different commercial enzymes and other by-products of interest. In-vitro digestibility, lignocellulose degradation, solid state fermentation, white rot fungi the availability of green fodder is a worldwide problem, resulting in their scarcity for animal consumption. To overcome the crisis of feed, several cereal crop residues like wheat straw (WS), paddy straw (PS), corn stover and so on are generally used as substitutive fodder and feed supplement.(Chen *et al.*, 2010)

A variety of food crops are produced around the globe, which generate enormous agricultural residues. This lignocellulosic biomass can serve as low cost feed stocks for production of fuel, ethanol and other value-added commodity chemicals. As compared to forage, cereal straws are of poor nutritive value and low digestibility because of their higher lignin content. Lignin is a phenyl-propanoid biopolymer, resistant to most of the microbial enzymatic systems and non-digestible by ruminants as well. The presence of lignin and its hemicellulose binding matrix affects the accessibility of energy rich polysaccharides. Degradation of lignin has got the potential to improve the digestibility of such residues. Natural degradation of plant biomass is mainly caused by different fungal and bacterial species. Holocellulose and lignin are the biopolymers that are converted into low molecular weight compounds by the enzymatic action of these microorganisms (Chen *et al*, 2010).

Fungi are usually better degraders of plant cell wall constituents because of their extracellular enzymatic system and hyphal penetration power. Many fungal species have been screened for their potential to degrade various agricultural residues (Li *et al.*, 2001,). White rot fungi degrade lignin efficiently and selectively by using their ligninolytic enzyme system which mainly comprises of laccase, lignin peroxidase and manganese peroxidase, along with many other enzymes. Fungal ligninolysis and break down of cellulose- hemicellulose matrix liberate simple degradable components that can be easily utilized by rumen microflora, thus improving the ruminant digestibility. Bioconversion of these residues into more nutritive animal feed has fascinated many workers to work on the problem and make it practically possible at lab-level fermentation (Li *et al.*, 2001).

## 2.7. The Role of White-Rot Fungi and Their Enzymes on Second-Generation Bioethanol

There is a growing need to find sustainable alternatives to avoid further increases in global warming due to the greenhouse effect. The utilization of renewable resources for ethanol production has been given much attention in a drive to find a substitute for petrochemical fuels. Bioethanol is produced by the alcoholic fermentation of sugar or starch-based crops such as maize, wheat, sugarcane, or sugar beet, among others. In recent years, increasing interest has been shown in second-generation bioethanol produced from lignocellulosic materials in agricultural and forestry waste. The main advantages in favor of using second-generation bioethanol is that the raw materials are sufficiently abundant and do not compete with food chains.

The process to obtain ethanol from lignocellulose involves the enzymatic hydrolysis of cellulose, and, in some cases, hemicellulose, into fermentable monomers. However, the hydrolysis of these polysaccharides is hampered by the presence of lignin and the compact architecture of the cell wall, which makes their degradation to fermentable sugars with hydrolytic enzymes much more difficult than that for starch. Therefore, a pretreatment of this material is necessary to remove or alter the lignin in order to improve the rate of enzymatic hydrolysis of the sugar fraction. White-rot fungi have been studied as an alternative biological pretreatment for degrading the lignin barrier. Moreover, ligninolytic enzymes from white-rot basidiomycetes have been employed for reducing the free phenol content released after physicochemical pretreatment, which might have a negative impact on both enzymatic hydrolysis of sugars and fermentation by microorganisms (Hahn-Hägerdal, 2007).

### 2.7.1. Biological Pretreatment of Lignocellulose for Ethanol Production

The biological pretreatment of lignocellulose is an environmentally friendly alternative to other chemical and physical methods, which are expensive, pollute the environment, and generate a number of inhibitors, including acetic acid, furfural or hydroxymethyl-furfurals, and free phenols, which remain embedded in the biomass and can affect downstream processes. The biological pretreatment basically consists of a solid state fermentation process in which microbes grow on the lignocellulosic biomass, preferentially degrading lignin and increasing the accessibility of cellulose and hemicellulose. White-rot basidiomycetes are the most effective organisms for the biological pretreatment of lignocellulosic materials, as previously commented (Rodríguez, 2004).

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## 3. Conclusion

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. White rot fungi are ubiquitous in nature, particularly in hardwood forests as hardwood and can degrade all wood components (i.e. cellulose, hemicellulose and lignin) or preferentially lignin. The former are named simultaneous or non-selective WRF and the latter selective WRF. The selective WRF are of special bio industrial interest, since they remove lignin leaving the valuable cellulose intact. The biotechnological significance of these white rot fungi (enzymes that are produced) are promising to replace the conventional chemical processes of several industries. Thus, there is a broad field of investigation that is almost entirely open to new findings and it is quite reasonable to propose that many new applications will be found in the near future.

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## Compliance with ethical standards

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