

Medicinal properties of laccase from Basidiomycetes mushroom: a review

Ashwak Jasim^{1,2*}

¹Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, P R China

²Department of Analytical Diseases, College of Health and Medical Technology, Baghdad, Iraq

Abstract

Laccases (EC: 1.10.3.2) are a group of multi-copper proteins. These are found in many plants, fungi, and microorganisms, belonging to the group of oxidases. Laccases catalyze variety of phenolic compounds as well as diamines, and aromatic amines with concomitant reduction of molecular oxygen to water that is a mean act on phenols and similar molecules, performing a one-electron oxidations. It requires oxygen as a second substrate for the enzymatic action. Due to their ability to oxidize, both phenolic and non-phenolic lignin related compounds as well as highly recalcitrant environmental; these biocatalysts can be very useful for their application in several biotechnological processes. Therefore, laccases have been subject of intensive research in the last decades to use in the food as well as pharmaceutical industries. The mushrooms are rich sources of laccases. The main medicinal uses of laccase in Basidiomycetes reported so far, are as anti-oxidant, anti-diabetic, hypocholesterolemic, anti-tumor, anti-cancer, immunomodulatory, anti-allergic, nephroprotective, and anti-microbial agents. This review helps to understand the medicinal properties of this important enzyme for efficient use in the pharmaceutical industry. This paper reviews the occurrence and location of laccase, mechanism of actions, mediators involved in the metabolic pathways, substrate specificity, properties, and their application in medicine.

Keywords: Laccase, Basidiomycetes, Oxidases, Copper proteins, Bio-active metabolites, Medicinal application

1. Introduction

The use of fungal enzymes in the diverse fields of biotechnological based industries has been increased in recent decades. The search for efficient and green oxidation technologies have increased the interest in the use of enzymes to replace the conventional non-biological methods. Among the different existing oxidant enzymes, the fungal laccase has been are of great interest for researchers since they have low substrate specificity; do not require the addition or synthesis of a low molecular weight cofactor; more stable and utilize the enzyme in an immobilized form.

The bioactive compounds of mushrooms include polysaccharides, proteins, fats, ash, glycosides, alkaloids, volatile oils, tocopherols, phenolic, flavonoids, carotenoids, foliates, ascorbic acid enzymes, and organic acids. Laccase belongs to a small group of enzymes called oxidases multicopper blue, have the potential antioxidant capacity. It belongs to the enzymes that have the innate characteristics of the production of roots reaction, but was ignored used in many areas because of the lack in the commercial sphere. There are a variety of sources of producing organisms such as bacteria, fungi and plants. In fungi, it found in Ascomycetes, missing, basidiomycetes very plentiful in many of the white rot fungi that degrade lignin. Laccases can degrade both phenol and phenolic compounds. It also has the ability to detoxify a range of environmental pollutants. Because of their properties to detoxify a range of pollutants, it has been used for several purposes in many industries including paper industries, pulp, textile and petrochemical industries. Some other applications of kerosene in the food and medical industries, health care included. Applications in recent times, to laccase found in other fields such as the design of sensors and nanotechnology (Upadhyaya et al., 2016).

Laccase are belonging to the group of oxidases. Laccase (Benzenediol; oxygen oxidoreductase, EC: 1.10.3.2) are also called as a blue copper oxidases or blue copper proteins. They are sometimes referred to as polyphenol oxidases (PPOs). They are extracellular enzymes. The proteins of laccase are characterized by containing 4 catalytic copper atoms, present in their catalytic sites. Laccase catalyze the oxidation of a variety of phenolic compounds such as di- amines and aromatic amines pigment formation, lignin degradation and detoxification (Kiiskinen, et al., 2002). The production of laccase is affected by many distinctive fermentation factors. Laccase activity in fungal culture can be increased by the addition of different aromatic compounds (Telke, et al., 2004). The first laccase was discovered in the Japanese lacquer tree *Rhus vernicifera* in the

nineteenth century by (Yoshida, 1883). Although laccase are present in higher plants, fungi, bacteria, and insects, the most studied group of enzymes to date is from fungal origin, including the genera of Ascomycetes, Deuteromycetes, Basidiomycetes, and cellulolytic fungi (Hatakka, 1994; Schneider, et al., 1999; Pandey, et al., 2001; Baldrian, 2006; Sharma, et al., 2007). The fungal laccases are responsible for detoxification, fructification, sporulation, phytopathogenicity, and lignin degradation (Widsten and Kandelbauer, 2008). Basidiomycetes have a strong ability to degrade lignin due to the high laccase activity they produce, and to their well-developed hypha organization that can efficiently penetrate plant cell walls (Grove and Bracker, 1970). The use of laccase in pharmaceutical industry is under progress and is growing with a fast pace. Besides their use in industrial applications for biodegradation, laccase is used for organic synthesis of several novel compounds that exhibit beneficial antibiotic properties (Pliz, et al., 2003), enhancing antioxidant capability (Hosny, 2002), antiproliferative activity (Li, Miao et al., 2010), reducing the effect of poison ivy dermatitis, oxidizing iodide to iodine a disinfectant widely used in the medical field (Xu, et al., 1999). Over 60 fungal strains belonging to Ascomycetes, Deuteromycetes and especially Basidiomycetes show laccase activities.

Among the latter group, white-rot fungi are the highest producers of laccase but also litter decomposing and ectomycorrhizal fungi secrete laccase (Baldrian, 2006). Many popular mediators of laccase are recognized also among the natural products of lignin and humus degradation and syringaldehyde, acetosyringone, vanillic, p-coumaric and ferulic acids can be mentioned. These natural mediators represent an alternative to synthetic mediators which are more efficient, but unfortunately more toxic for environment and more expensive in exploitation. Potential applications of laccase include textile dye bleaching, pulp bleaching effluent, detoxification biosensors, and bioremediations. However, a grave problem often encountered with industrial exploitation of fungal laccase is the low production level by the native hosts. This problem may be overcome by heterologous production in fungal hosts capable of producing high amounts of extracellular enzymes, which generally include *Trichoderma Reesei* or *Aspergillus*.

The activity of fungal laccase are influenced by environmental factor such as temperature, pH, culture condition, and medium composition (Diaz Godinez et al 2016). Laccase isoenzyme expression can be constitutive (Tellez-Tellez et al 2012). There are various research on the production, characterization, and uses of phenoloxidases in white-rot fungal species corresponding (Diaz-Godines 2012).

In addition, applications of laccase are on the medicine Fig 1. Among physiological groups of fungi, laccase is typical for the wood-rotting basidiomycetes causing white-rot and a related group of litter-decomposing saprotrophic fungi, i.e. the species causing lignin degradation. Almost all species of white-rot fungi were reported to produce laccase to varying degrees (Hatakka, 2001), and the enzyme has been purified from many species. In the case of *Pycnoporus Cinnabarinus*, laccase was described as the only ligninolytic enzyme produced by this species that was capable of lignin degradation (Eggert, 1996). Although the group of brown-rot fungi is typical for its inability to decompose lignin, there have been several attempts to detect laccases in the members of this physiological group. A DNA sequence with a relatively high similarity to that of laccases of white-rot fungi was detected in *Gloeophyllum trabeum*. Oxidation of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) as an indirect indication of oxidative activity was also found in this fungus as well as in a few other brown-rot species (D'Souza, 2003). Although no laccase protein has been purified from any brown-rot species, the oxidation of syringaldazine – a reliable indication of laccase presence – has recently been detected in brown-rot fungus *Coniophora puteana* and oxidation of ABTS was reported in *Laetiporus Sulphureus* (Schlosser and Höfer, 2002). The occurrence and role of laccases in the brown-rot decay of wood is still unclear, but it seems to be rare. The main aim of this review work is to summarize the importance of laccase from basidiomycetes sources, which has provided more insights about properties, occurrence, metabolic pathways, substrate specificity, and medicinal application of laccase.

2. Occurrence and location

Laccase is generally found in higher plants and fungi, but recently, it was found in some bacteria, such as *Streptomyces lavendulae*, *Streptomyces cyaneus*, and *Marinomonas mediterranea* (Juarez, et al., 2005). In fungi, laccases appear more than the higher plants. Basidiomycetes such as *Phanerochaete chrysosporium*, *Theiophora terrestris*, and *Lenzites betulina* (Viswanat et al., 2008), and white-rot fungi (Kiiskinen et al., 2004) such as *Phlebia radiata* on *Pleurotus ostreatus* (Palmieri et al., 2000), and *Trametes versicolour* produce laccase reported by (Bourbonnais et al., 1995). Many *Trichoderma* species such as *T. atroviride*, *T. harzianum* and *T. longibrachiatum* are the sources of laccases (Holker et al., 2002). Several reports can be referred; the white-rot basidiomycetes are the most efficient degraders of lignin and also the most widely studied. The enzymes implicated in lignin degradation are lignin peroxidase, which catalyses the oxidation of both phenolic and non-phenolic units, manganese-dependent peroxidase, laccase, which oxidises phenolic compounds to give phenoxy radicals and quinones; glucose oxidase and glyoxal oxidase for H₂O₂ production, and cellobiose-quinone

oxidoreductase for quinine reduction (Thakker, 1992).

3. Mechanism of laccase

Basidiomycetes mushrooms have an extracellular laccase enzyme; the laccase catalysis occur due to the reduction of one oxygen molecule to water accompanied with the oxidation of one electron with a wide range of aromatic compounds which includes polygonal, methoxy-substituted monophenols, and aromatic amines (Bourbonnais, et al., 1995). Also laccase consists of 4 copper atoms termed Cu T1 where the reducing substrate, place, and trinuclear copper cluster T2/T3 where oxygen binds and is reduced to water. As a one-electron substrate oxidation is coupled to the four-electron reduction of oxygen, the reaction mechanism cannot be entirely straight forward. Laccase can be thought to operate as a battery, storing electrons from individual oxidation reactions in order to reduce molecular oxygen. Hence the oxidation of four reducing substrate molecules is necessary for the complete reduction of molecular oxygen to water. Substrate oxidation by laccase is a one-electron reaction generating a free radical. The initial product is typically unstable and may undergo a second enzyme catalyzed oxidation or otherwise a non-enzymatic reaction such as hydration, disproportionation or polymerization. The bonds of the natural substrate, lignin, that are cleaved by laccase include C α -oxidation, C α -C β cleavage and aryl-alkyl cleavage (Figure 1).

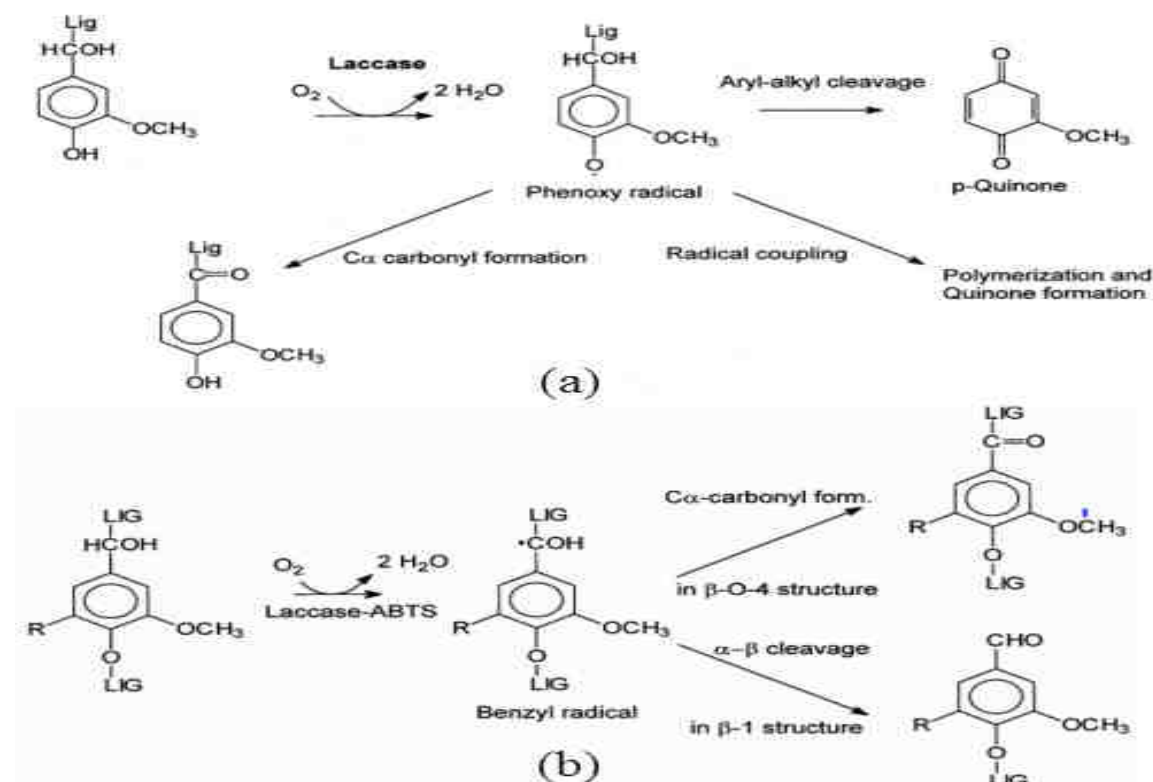


Figure 1: Laccase-catalyzed intermediary metabolism, (Archibald et al., 1997)

4. Mediator substances

Mediators are a group of low molecular-weight organic compounds that can be by oxidized laccase first and form highly active cation radicals to reach with carious chemicals, including non-phenolic compound that laccase alone cannot oxidase. The most common synthetic mediators are 1-hydroxybenzotriazole (HOBT), N-hydroxyphthalimide (NHPI) and 2,2'-azinobis(3-ethylthiazoline-6-sulfonat) (ABTS). The substrate rang of laccase can be extended to non-phenolic subunit by the inclusion of mediators. There are instances in which the substrates of interest cannot be oxidized directly by laccase, either because they are too large to penetrate into the enzyme active site or because they have a particularly high redox potential. By mimicking nature, it is possible to overcome this limitation with the addition of so-called chemical mediators, which are suitable compounds that act as intermediate substrates for the laccase, (Figure 2) whose oxidized radical forms are able to interact with the bulky or high redox-potential substrate targets. Approximately 100 different potential mediator compounds have been described for the LMS, but ABTS and syringaldehyde remain the most commonly used. Synthetic

mediating substrates are heterocyclic compounds belonging to the general classes of phenoxazinones, phenothiazines or phenoxy benzothiazoles (Eggert, et al., 1996). Natural mediators include phenol, aniline, 4-hydroxybenzoic acid and 4-hydroxybenzyl alcohol. The use of natural mediators proved to be as efficient as the commonly used ABTS and syringaldehyde. Activity of laccase-mediating substrate systems towards compounds depends on a combination of two main factors: the redox potential of the enzyme and the stability and reactivity of the radical generated by oxidation of the mediating substrate. Several hypotheses have been proposed for the mechanism of mediating substrate systems. The first hypothesis is that the mediating substrate can act as a redox mediating substrate, i.e.; reversible. It is thought that laccase oxidizes the mediating substrate and this oxidized form of the mediating substrate can oxidize the substrate, and is consequently, reduced back to its non-oxidized form: the species responsible for the oxidation of the substrate would be the oxidized mediating substrate. Another hypothesis is that active intermediates are generated during the oxidation of the mediating substrate by laccase. LMS has resulted highly efficient in many biotechnological and environmental applications as regards the numerous research articles and invention patents.

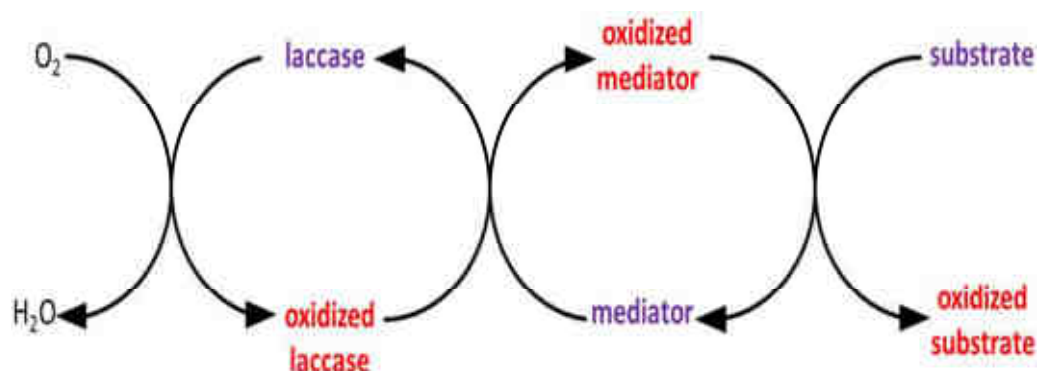


Figure 2:
Substrates for the laccase oxidized

produced by (Banci et al.,1999)

Many artificial published (Morozova et al., 2007, Kunamneni et al., 2008) mediators have been widely studied, from ABTS the first described laccase mediator (Bourbonnais et al., 1990), to the use of synthetic mediators of the type -NOH- such as HBT, violuric acid (VIO), N-hydroxyphthalimide (HPI) and N-hydroxyacetanilide (NHA); the stable 2,2,6,6 tetramethyl-1-piperidinyloxy free radical (TEMPO), or the use of phenothiazines and other heterocycles (e.g. promazine or 1-nitroso-naphthol-3,6-disulfonic acid), (Bourbonnais *et al.*, 1990). More recently, complexes of transition elements (polyoxometalates) have been also demonstrated to mediate lignin degradation catalyzed by laccase.

5. Substrate specificity

For the laccase, specificity is remarkably non-specific as to their reducing substrates, and the range of substrates oxidised varies from one laccase to another. These enzymes catalyse the one-electron oxidation of a wide variety of organic and inorganic substrates, including polyphenols, methoxy-substituted phenols, aromatic amines and ascorbate with the concomitant four electron reduction of oxygen to water (Thurston, 1994). Laccase has broad substrate specificity towards aromatic compounds containing hydroxyl and amine groups, and as such, the ability to react with the phenolic hydroxyl groups found in lignin was reported by (Youn, 1995). The kinetic data of laccase from different sources were reported (Yaropolov, 1994). K_m values are similar for the co substrate dissolved oxygen (about 5-10 M), but V_{max} varies with the source of laccase (50-300 M/s). The turnover is heterogeneous over a broad range depending on the source of enzyme and substrate/type of reaction. The kinetic constants differ in their dependence on pH-independent for both substrate and co substrate while K_{cat} , is pH-dependent.

6. Medicinal properties

i. Anti-Tumour Effect

The effect of laccase from Basidiomycetes mushroom are found in the few studies for the no 60 μM (Li, et al., 2008) isolated a homodimeric 32.4 kDa lectin from fresh fruiting bodies of the mushroom *Pleurotus citrinopileatus*. The lectin exerted potent anti-tumour activity in mice bearing sarcoma 180, and caused approximately 80% inhibition of tumour growth when administered intraperitoneally at 5 mg/kg daily for 20

days. (Sun, et al., 2011; Zou, et al., 2012). Among the laccase with antiproliferative activities towards tumor cells, *Agricus placomyces* laccase possesses lower IC₅₀ values. *A. biennis* laccase demonstrates antiproliferative activities against Hep G2 and MCF-7 cells with IC₅₀ values of 12.5 μ M and 6.7 μ M, respectively (Zhang, 2011). *Cucurbita maxima* laccase shows antiproliferative activity against Hep G2 and MCF-7 tumor cells with IC₅₀ values of 12.3 μ M and 3.0 μ M, respectively (Zhang, 2010). It indicates that the present laccase shows potential applications in cancer treatments. (Wang, et al., 1998). It is reported herein that a laccase can be purified from the fruiting bodies of the mushroom *Pleurotus nebrodensis*. The procedure employed in the present study was useful for isolating *Pleurotus nebrodensis* laccase. Proteins with little or no laccase activity were separated from the laccase enriched fraction in each of the chromatographic steps on DEAE-cellulose, CM-cellulose, Q-Sepharose and Superdex 75. In contrast to some of the previously reported laccase, e.g., those from *Coriolus hirsutus* (Shin and Lee, 2000) and *Rigidoporus lignose's* (Cambria, et al., 2000), which are adsorbed on cationic and anionic exchanger.

ii. Anticancer effects

Biologically active substances of medicinal mushrooms with anticancer action comprise polysaccharides, polysaccharide-protein complexes, dietary fiber, certain types of proteins, terpenoids, steroids, phenols. Laccases deserve special interest among mushroom enzymes because of their potent role in the biodegradation of lignin and phenolic pollutants. In addition, recently was found that some mushroom laccases possess direct cytotoxicity in vitro, particularly toward Hep G2 and MCF-7 cell lines. Laccases (benzenediol: oxygen oxidoreductase) form a class of ligninolytic enzymes which demonstrate a rather low degree of specificity with regard to the reducing substrate: they catalyze the oxidation of ortho- and para-diphenols and aromatic amines by removing an electron and a proton from a hydroxyl group to generate a free radical. It is likely that cytotoxicity of laccases can be associated with their capacity to oxidize wide range of substrates. The effect of anticancer by many studies from extracts the mushroom including species *Pleurotus* may modulate the response of host immune system, in particular, various mushroom polysaccharides are likely to affect promotion and progression stages towards cancer as reported by (Chatterjee, et al., 2011). Benefits of these medicinal mushrooms have been assessed by various clinical trials. (Lu et al., 2009) reported the potential of hispolon, a phenolic compound from to induce apoptosis of breast cancer cell. Similarly (Lavi, et al., 2006) reported the use of polysaccharide extract from on HT-29 colon cancer cells. Or Maitake enhances the efficacy of anti-cancer agent cisplatin, checking the decrease in the number of immunocompetent cells (Masuda, et al., 2009). The extract of *Phellinus linteus* was reported to contain antimutagenic activities and play a role in the prevention of cancer by inducing NAD (P) Quinone oxidoreductase and glutathione S-transferase activities. Hispolon, the phenolic compound extracted from this mushroom has been potential to induce apoptosis of breast- and bladder-cancer cell (Lu, et al. 2009).

iii. Immunomodulation

The immunomodulatory effects of the basidiomycetes are well described in the literature and are related to the increased function of monocytes in the production of Interleukin-1 (Takeshita, et al., 1996) and expression of cytokines. Molecules like glucans are relatively resistant to the stomach acid and are trapped by macrophage receptors present on the intestinal wall as the dectin-1, the toll-like receptor 2 (a class of proteins that play a role in immune system) and lactosylceramides. The β -glucans with its various structures have different affinities for these receptors to elicit different host response. In vivo, studies showed that the analysis of cytokine expression after administration of β -glucan isolated from *Lentinan* revealed a significant increase in mRNA levels of Interleukin-1 α , interleukin-1 β , tumor necrosis factor- α (TNF- α) and interferon- δ (IFN- δ). And another report on mushroom like *Agrocybe aegerita* (Chestnut mushroom, Velvet pioppino, *Agrocybe cylindracea*, Yanagimatsutake, Zhuzhuang-tiantougu) contains compounds with inhibitory properties against the enzyme cyclooxygenase (the same enzyme which is the target of Advil, Tylenol, and other NSAIDs) (Zhang, et al., 2003). An in vitro experiment, revealed the mushroom may offer immune-stimulating properties (Yoshida, et al., 1996).

iv. Antiviral Activities

The aim of the antiviral chemotherapy is the discovery of antiviral agents who are specific for the inhibition of viral multiplication without affecting normal cell division. It is necessary to identify and develop new antiviral agents without adverse side effect and viral resistance. Many studies have determined of extracts from LEP on the replication of poliovirus type 1 (PV-1), and bovine herpes virus type 1 (BoHV-1), and the results were anti-virus activity in promotin (Rincao, et al., 2012). The isolated from compound lentinan suppressed the activity of HIV-1 reverse transcriptase. In combination with antiretroviral 3'-azido-3'-deoxythymidine (AZT) lentinan suppressed the in vitro expression of surface antigens of HIV more efficiently compared to AZT monotherapy. It was also shown that it can increase the in vitro antiretroviral effect on HIV replication. In the other studies by (Tochikura, et al., 1989) tested many substances using non-sulfated polysaccharides (EP-LEM) and achieved inhibition for HIV-1, HIV-2 and HTLV-1. Various fractions of LEM caused inhibition of infectivity and

cytopathic effect of HIV. The mechanism of action is unclear, but it suggests that it may be related to activation of macrophages and stimulation of IL-1 (Wasser, 2002). *Pleurotus* mushroom contain substances that exert direct or indirect antiviral effects as a result of immune-stimulatory activity. Ubiquitin, an anti-viral protein was isolated and identified from fruiting body of oyster mushroom (Piraino and Brandt, 1999). Water-insoluble β -glucan, isolated from sclerotia of *Pleurotus tuber-regium* and their corresponding water-soluble sulphated derivatives were active against herpes simplex virus type-1 and type-2. The anti-viral activity was due to binding of sulphated β -glucan, to viral particles thereby preventing them from infecting the host cells. Not only intracellular proteins of *Pleurotus ostreatus* but its extracellular extract also contains polysaccharides that have immuno-modulating effects (Selegean and Rugea, 2009).

v. Antimicrobial Activities

It has been reported that extracts of shiitake possess antibacterial activity enhancing host immunity against infections (Rao, et al., 2009; Mantovani, et al., 2008; Hatvani, et al., 2001) used solvents like chloroform and ethyl acetate in dried mushroom and demonstrated bactericidal activity. Lenthionine, a cyclic organo sulfur compound partially responsible for the taste of shiitake showed inhibitory effects against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Several studies have shown the ability of the extract of *Lentinula edodes* to inhibit oral pathogens, mainly causing cavities and gingivitis (Lingstrom, et al., 2012; Zaura, et al., 2011). According to (Spratt, et al., (2012), the fraction of low molecular weight (LMM) isolated from the aqueous extract of also has potential activity against oral pathogens in vitro. And other Antimicrobial:OM has been explored to combat simple and multiple drug resistant isolates of *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus* (Akyuz, et al., 2010) and species of *Candida* (Wolff, et al., 2008), *Streptococcus*, *Enterococcus* (Kotra and Mobashery, 1998). Methanolic extracts of *Pleurotus* species demonstrated an inhibition in growth of *Bacillus megaterium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, *Candida glabrata*, species of *Trichophyton* and *Epidermophyton* to different degrees that were lower with respect to two antifungal agents: Streptomycin and Nystatin. Antimicrobial and antifungal activity of OM depended upon the nature of the solvent, ether extract were more active in Gram-negative bacteria as compared to acetone extract.

vi. Antineoplastic

Antineoplastic activity of polysaccharide extracted from the fruiting body of mushroom from species *Pleurotus ostreatus* these studied detected by (Watanabe, 1969), later polysaccharides extracted from mycelium of *Pleurotus sajor-caju* (Zhuang, et al., 1993) were also shown to have antineoplastic activities. These polysaccharides are components of the cell wall of polysaccharide extracted from *Pleurotus ostreatus* culture broth when injected intra-peritoneally (i.p.) in the female Swiss albino mice (Wolff, et al., 2008) caused 76% reduction in the number of neoplastic cells. Similar results were observed with extracts extracted from cell walls of *Pleurotus sajor-caju* in Ehrlich as acetic tumor in ascetic form. Of late (Li, et al., 2007) observed up-regulated gene expression of antioxidant enzymes and consequently, their activities were increased. However, a water extract of *P. sajor-caju* fruiting bodies had no such activity since it did not prevent HO induced oxidative damage to cellular DNA (Shi, et al., 200).

vii. Direct anti-hormone activity

The effect of mushroom on hormone by enzyme assay analysis due to able to influence the production of certain human hormones, and the other of mushrooms like *Agaricus bisporus* (Grube, et al., 2001; Chen, et al., 2006) may be able to partially inhibit the activity of aromatase, the enzyme responsible for producing estrogen. Mushrooms like Reishi (Liu, et al., 2006), may be able to partially inhibit the activity of 5- α reductase, the enzyme responsible for producing dihydrotestosterone.

viii. Anti-inflammatory activity

The anti-inflammatory activity of mushroom showed by (Choi, et al., 2004; Fu, et al., 2009) showed that methanol extracts of *Pleurotus Pulmonarius* and *Pleurotus Florida* fruiting bodies decreased induced paw oedema in mice and ameliorated acute and chronic inflammation, respectively. *Pleurotus* has also been shown to possess anti-inflammatory activity by exerting antioxidant and immunomodulatory effects on rats with induced colitis. Hyper sensitive immune responses, such as inflammation in delayed allergy, were suppressed by an ethanol extract of *Pleurotus eryngii*. It exhibited anti-allergic activity after oral or percutaneous administration to mice with oxazolone-induced type IV allergy (Selvi, et al., 2008).

ix. Hepatoprotective

The hepatoprotective and therapeutic activity determination of hot-water extract of *Pleurotus ostreatus* by the mechanism of inhibition through preventive regimen caused less leakage of alkaline phosphatase, less pronounced increase in hepatic malondialdehyde concentration, less notable reduction in hepatic total protein, RNA and DNA contents and in contrast increased hepatic superoxide dismutase glutathione peroxidase and

glutathione reductase, activities. Polysaccharopeptides extracted from fruiting body of *Pleurotus ostreatus* alleviated the thioacetamide-induced alterations, inflammation, steatosis, necrosis and fibrosis especially in the therapeutic regimen (a systemic plan for therapy) as reported by (Refaie et al., 2009). Liver damage by hepatotoxic agents is of vital consequence because chronic liver injury leads to fibrosis, end stage cirrhosis and hepato-carcinoma hence, there is an increasing need to search of an agent which could protect the liver from such damages. Many species of basidiomycetes contains some active compounds like β -glucans, phenol and vitamin C that increase the activity of antioxidant-enzymes viz. catalase, superoxide dismutase; these enzymes are responsible for reduction hepatic cell necrosis (Fu et al., 2009). Antioxidant is (Hepatoprotective activity of serum aminotransferase enzymes in However, Hepatoprotective activity was due to the lipid).

x. Antioxidant effect

Basidiomycete's species of *Pleurotus* possessed in plasma triglyceride, low density lipoprotein, total lipid higher concentration of antioxidants than other phospholipids etc. Whereas 5% mushroom powder of commercial mushrooms (Mau, et al., 2001; Yang, et al., 2002). This activity was *Pleurotus salmoneos tramineus* reduced total lipid, phospholipids mainly due to presence of polysaccharide pleuran and LDL/HDL ratio by 61 and 65.31 % (β -glucans) that has been isolated from *Pleurotus ostreatus* respectively Showing a positive effect on rat colon with pre-cancerous lesions (Bobek, et al., 2001). *Pleurotus ostreatus* increased the activities of Hyperglycemic a compound related to the important antioxidant enzymes (viz. superoxide dismutase, bi-guanide class of oral antidiabetic drugs was isolated catalase and peroxidase) thereby reducing oxidative from the *Pleurotus* species that exerted anti-hypoglycemic damage in humans (Keyhani, et al., 2007). Oyster mushrooms are now effect Endo-polymer from submerged mycelial widely used as ingredients in dietary supplements in the cultures of *Pleurotus ostreatus* possesses hypoglycemic effect shape of maintaining health and preventing diseases (Kim, et al., 1997). High fibre and proteins content and low fat content due to their higher free radical scavenging activities of edible mushrooms it ideal food for diabetic patient These free radical scavenging activities of oyster Aqueous extracts of *Pleurotus Pulmonarius* upon oral mushrooms depend upon the colour of fruiting bodies as administration decreased serum glucose level.

7- Conclusions

The up dated comprehensive information made available in this review shows that laccase from basidiomycetes have the potential for the synthesis of several useful drugs in pharmaceutical industry because of their high value of oxidation potential. They have many industrial applications because of their innate ability of oxidation of a broad range of phenolic and non-phenolic compounds. Laccase have also tremendous ability of oxidation of harmful and industrial products and belongs to those enzymes, many promising medicine properties that require more high-tech approaches for deeper exploration. Though in the most cases, biological activity is better understood but in many cases, there is a need to identify the active principle to understand the exact mechanism(s) for its exploration in right perspective. Availability of high-tech methods should allow the researchers to explore novel metabolites from mushroom. In the era of 'nomics' it would be much easier to study mechanism of action with biomarker-based approach for mushroom in medicine. Therefore, it is not surprising that this enzyme studies intensively and yet remains a topic of intense research today.

ACKNOWLEDGEMENTS

The authors would like to thank Ministry of Higher Education and Scientific Research, Middle- technical University /Baghdad for giving me the opportunity to complete a PhD graduate.

References

- Upadhyay, P., Shrivastava, R. & Agrawal, P.K. (2016). Bioprospecting and biotechnological applications of fungal laccase. *Biotechnology*, 6:15.
- Archibald, F.S., Bourbonnais, R., Jurasek, L., Paice, M. G., and Reid, I.D. (1997). "Kraft pulp bleaching and delignification by *Trametes versicolour*. *Journal of Biotechnology*, 53, 215-336.
- Akyuz, M., Onganer, A., Nerecevit, P., and Kirbag, S. Lv, H., Kong, Y., Yao, Q., Zhang, B., Leng, F.W. (2010). Antimicrobial activity of some edible mushroom *Pleurotus nebrodensis* with apoptosis, 49-62.
- Banci, L., Ciofi-Baffoni, S., and Tien, M. (1999). Lignin and Mn peroxidase-catalyzed oxidation of phenolic lignin oligomers. *Biochemistry*, 38, 3205.
- Baldrian P. (2006). Fungal laccases—occurrence and properties. *FEMS Microbiology Reviews*, 30(2): 215-242
- Diaz-Godines, G., Tellez-Tellez, M, et al (2016). Enzymatic, Antioxidant, Antimicrobial, and Insecticidal Activities of *Pleurotus Pulmonarius* and *Pycnoporus clinnabarinus* Grown Separately in an Airlift

- Reactor. *Bioresources* 11(2), 4186-4200.
- Tellez-Tellez, M., Diaz-Godinez, G., Aguilar, M.B., et al. (2012). Description of laccase gen from *Pleurotus* expressed under submerged fermentation conditions, *Bioresources* 7, 2038-2050. DOI :10.15376/biores.7.2.2038-2050.
- Diaz-Godinez, G. (2012). Production of laccase by *Pleurotus ostreatus* in solid-state and submerged fermentation, in , *Biotechnology of Microbial Enzymes*, Nova Science Publishers, Hauppauge.
- Bobek, P. and Galbavy, S. (2001). Effect of pleuran glucan from *Pleurotus ostreatus* on the antioxidant status of the organism and on dimethylhydrazine induced precancerous lesions in rat colon. *British Journal of Biomedical Sciences*, 58: 164-168.
- Bourbonnais, R, Paice, M.G. (1990). Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. *FEBS Letters* 267:99-102.
- Bourbonnais, R., Paice M.G, Reid I.D, Lanthier P, Yaguchi M. (1995). Lignin oxidation by laccase isozymes from *Trametes versicolor* and role of the mediator 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonate) in kraft lignin depolymerization. *Applied and Environmental Microbiology*. 61(5):1876-1880.
- Cambria, M.T. Cambria, A, Ragusa, S., Rizzarelli, E. (2000). Production, purification, and properties of an extracellular laccase from *Rigidoporus lignosus*. *Protein Express Purif* 18: 141-147.
- Choi, D.B. Cha, W.S. Kang S.H. and Lee, B.R. (2004). Effect of *Pleurotus ferulae* Extracts on Viability of Human Lung Cancer and Cervical Cancer Cell Lines. *Biotechnology Bioprocess Engineering*, 9: 356-361.
- Chatterjee, S.G. Biswas, S.K. and Acharya, K. (2011). Antineoplastic effect of mushrooms: a review. *A Journal Crop Sciences*. 5(7): 904-911.
- Chen, L. and Shao, H. (2006). C "Extract from *Agaricus blazei* Murill can enhance immune responses elicited by DNA vaccine against foot-and-mouth disease," *Veterinary Immunology and Immunopathology*, 109(1-2):177-182.,
- D' Souza C.G.M., Peralta R.M. (2003). Purification and characterization of the main laccase produced by the white-rot fungus *Pleurotus pulmonarius* on wheat bran solid state medium. *Journal of Basic Microbiology* 43: 278-286.
- Eggert, C., Temp, U., and Eriksson, K.L. (1996). The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus* purification and characterization of the laccase . *Applied and Environmental Microbiology*, 62(4):115-1158.
- Fu, H.Y. Shieh D.E. Ho, C.T. (2009). Antioxidant and free radical scavenging activities of edible mushrooms. *Journal of Food Lipids*, 9: 35-43.
- Grove, S.N. Bracker, C.E. and Moore, D.J. (1970). An ultrastructural basis for hyphal tip growth in *Pythium ultimum*. *American Journal of Botany*, 57:245-266.
- Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiology Review*, 13, 125- 135.
- Hatakka, A. (2001). "Biodegradation of lignin," in *Lignin, Humic Substances and Coal*, M. Hofrichter and A. Steinbuechel, Eds, Wiley-VCH, Weinheim Germany 12:79-91.
- Hatvani, N. (2001). Antibacterial Effect of the Culture Fluid of *Lentinus edodes* Mycelium Grown in Submerged Liquid Culture. *International Journal of Antimicrobial Agents*, 17, 71 -74.
- Jimenez-Juarez, N. Roman-Miranda, R. Baeza, A. Sánchez-Amat, A. Vazquez-Duhalt R., and Valderrama, B (2005). "Alkali and halide-resistant catalysis by the multipotent oxidase from *Marinomonas mediterranea*," *Journal of Biotechnology*, 117(1)73-82.
- Hosny, M., Rosazza, Hood., E., Bailey, M.R. Beifuss, K., MagallanesLundback, M., Horn, M. (2002). *JPN Journal Agriculture Food Chemistry* 50:5539-5545.
- Kiiskinen, L.L. Viikari, L. and Kruus, K. (2002). "Purification and characterisation of a novel laccase from the ascomycete *Melanocarpus albomyces*," *Applied Microbiology and Biotechnology*, 59 (2-3): 198-204.
- Kim, M.Y. M.H. Park, G.H. Kim, (1997). Effects of mushroom protein-bound polysaccharides on the blood glucose levels and energy metabolism in streptozotocin induced diabetic rats. *A Journal of Korean Neutral*. 30: 743-750.
- Kiiskinen, L.L. Viikari, L. and Kruus, K. (2002). "Purification and characterisation of a novel laccase from the ascomycete *Melanocarpus albomyces*," *Applied Microbiology and Biotechnology*, 59 (2-3): 198-204.
- Kiiskinen, K., Kruus, M., Bailey, E., Ylosmaki, M., Siikaaho, S., and Saloheimo, S. (2004). "Expression of *Melanocarpus albomyces* laccase in *Trichoderma reesei* and characterization of the purified enzyme," *Microbiology*, 150(9): 3065-3074.
- Kotra, L.P. and Mobashery, S. (1998). *lactamRinsho*, antibiotics, lactamases and bacterial resistance. 27: 1759.61-64.

- Kunamneni, A., Plou, F.J. Alcalde, M., Ballesteros A. (2008). Laccases and their applications: A patent review. *Recent Patent Biotechnolss* 2:10-24.
- Lavi, I., Friesem, D., Geresh, S., Hadar, Y., Schwartz, B. (2006). Aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro apoptotic effects on HT-29 colon cancer cells. *Cancer Letters*,70(2);44-61.
- Lu, T.L. Huang, G.J. Lu, T.J. Wu, J.B. Wu, C.H. Yang, T.C. Iizuka, A.,Chen, Y.F. (2009). Hispolon from *Phellinus linteus* has antiproliferative effects via MDM2-recruited ERK1/2 activity in breast and bladder cancer cells. *Food Chemistry Toxicology*, 47:2013–2021.
- Lingstrom, P., Zaura, E., Hassan, H., Buijs, M.J., Hedelin, P., Pratten, J., Spratt, D., Daglia, M., Karbowski, A., Signoretto, C., et al. (2012) .The Anticaries Effect of a Food Extract (Shiitake) in a Short-Term Clinical Study. *Journal of Biomedicine and Biotechnology*, 1110-7243.
- Liu, Z., Zhang, D., Hua, Z., Li, J., Du, G., and Chen, J.A. (2006). Newly *Paecilomyces* sp. WSHL07 for laccase production: Isolation, identification and production enhancement by complex inducement, *Journal of Industry Microbiology and Biotechnology*, 36: 1315-1321.
- Li, L., T.B. Ng, M. Song, F. Yuan, Z.K. Liu, C.L. Wang, Y. Jiang, M. Fu and F. Liu. (2007). A polysaccharide-peptide complex from abalone mushroom (*Pleurotus abalonus*) fruiting bodies increases activities and gene expression of antioxidant enzymes and reduces lipid peroxidation in senescence-accelerated mice, *Applied Microbiology and Biotechnology*., 75: 863-869.
- Li, M., Zhang, G, Wang, H., Ng, T. (2010). Purification and characterization of a laccase from the edible wild mushroom *Tricholoma mongolicum*. *Journal of Microbiology and Biotechnology*,20(7):1069–1076..
- Li, J., Li, .P, Liu, F. (2008). Production of theanine by *Xerocomus badius* (mushroom) using submerged fermentation. *LWT. Food Sciences and Technology*,41:883–889..
- Morozova, O.V., Shumakovich, G.P., Shleev, S.V., Iaropolov, Y.I. (2007) .Laccase mediator systems and their applications: A review. *Prikl Biochemistry Mikrobiology* 43(5):583-597.
- Mantovani, M.S., Bellini, M.F., Angeli, J.P., Oliveira, R.J., Silva, A.F. and Ribeiro, L.R. (2008). β -Glucans in Promoting Health: Prevention against Mutation and Cancer. *Mutation Research*, 658:154-161.
- Masuda, Y., Inoue, M., Miyata, A., Mizuno, S., Nanba, H. (2009). Maitake β -glucan enhances therapeutic effect and reduces myelosuppression and nephrotoxicity of cisplatin in mice. *International Immunopharmacol* 9:620–626.
- Pandey, A., Szakacs, G., Soccol, C. R., Rodriguez-Leon, J. A., and Soccol, V.T. (2001). Production, purification and properties of microbial phytases. *Bioresour. Technology*. 77: 203–214.
- Palmieri, G. Giardina, P. Bianco, C. Fontallella B., and Sannina, G. (2000). “Copper induction of laccase isoenzyme in the lignolytic fungus *Pleurotus ostreatus*,”*Applied Microbiology and Biotechnology*, (66): 920–924,
- Pilz, R., Hammer, E., Schauer, F., Kragl. (2003). Ecology and Management of Commercially Harvested Chanterelle Mushrooms .*Unites Apply Microbiology and Biotechnology*,60:708-712.
- Piraino, F. and Brandt, C.R. (1999). Isolation and partial characterization of an antiviral, RC-183, from the edible mushroom *Rozites caperata*. *Antiviral Research*, 43(2): 67-78.
- Rao, J.R., Smyth, T.J., Millar, B.C. and Moore, J.E. (2009). Antimicrobial Properties of Shiitake Mushrooms (*Lentinula edodes*). *International Journal of Antimicrobial Agents*, 33:591 -592. F. M.
- Refaie, F.M. Smat, A.Y. Daba, A.S. and Taha S.M. (2009). Characterization of Polysaccharopeptides from *Pleurotus ostreatus* mycelium: assessment of toxicity and immunomodulation in vivo. *Micologia Aplicada internacional*, 21(2):67-75.
- Rincao, V.P., Yamamoto, K.A., Ricardo, N.M., Soares, S.A., Meirelles, L.D., Nozawa,C.andLinhares,R.E. (2012).Polysaccharide and Extracts from *Lentinula edodes*: Structural Features and Antiviral Activity. *Virology Journal*, 9, 37.
- Selegean, M., Putz, M.V. and Rugea, T., (2009). Effect of polysaccharide extract from the edible mushroom *Pleurotus ostreatus* against infectious bursaldisease virus. *International Molecular Science s.*, 10: 3616-3634.
- Selvi, B. R., Jagadeesan, D., Suma, B. S., Nagashankar, G., Arif, M., Balasubramanyam, K., swaramoorthy, M. and Kundu, T. K. (2008). Intrinsically Fluorescent Carbon Nanospheres as a Nuclear Targeting Vector: Delivery of Membrane-Impermeable Molecule to Modulate Gene Expression In Vivo. *Nano letters*,8(10):3182-85.
- Schlosser, D. and Hofer, C. (2002). “Laccase-catalyzed oxidation of Mn^{2+} in the presence of natural Mn^{3+} chelators as a novel source of extracellular H_2O_2 production and its impact of manganese peroxidase. *Applied and Environmental Microbiology*, 68(7):3514–3521.

- Souza, C. Zilly, A. and Peralta, R. M. (2002). "Production of laccase as the sole phenoloxidase by a Brazilian strain of *Pleurotus pulmonarius* in solid state fermentation," *Journal of Basic Microbiology*, 42(2): 83–90.
- Spratt, D.A., Daglia, M., Papetti, A., Stauder, M., O'Donnell, D., Ciric, L., Tymon, A., Repetto, B., Signoretto, C., Hourri-Haddad, Y., et al. (2012). Evaluation of Plant and Fungal Extracts for Their Potential Antigingivitis and Anticaries Activity. *Journal of Biomedicine and Biotechnology*, 510198.
- Sun, J., Wang, H., and Ng, T.B. (2011). "Isolation of a laccase with HIV-1 reverse transcriptase inhibitory activity from fresh fruiting bodies of the *Lentinus edodes* (Shiitake mushroom), *Indian Journal of Biochemistry and Biophysics*, 48(2): 88–94.,
- Schneider, P., Caspersen, M.B., Mondorf, K., Halkier, T., Skov, L.K., Østergaard, P.R., Brown, K.M., Brown, S.H., Xu, F. (1999). Characterization of a *Coprinus cinereus* laccase. *Enzyme Microbiology and Technology*, 25:502–508.
- Sharma, P., Goel, R., and Capalash, N. (2007). Bacterial laccases. *World Journal of Microbiology and Biotechnology* 23, 823–832..
- Shin, K.S., Lee, Y.J. (2000). Purification and characterization of a new member of the laccase family from the white-rot basidiomycete *Co-riolus hirsutus*. *Archives of Biochemistry and Biophysics*, 384: 109–115..
- Takeshita, K., Hayashi, S., Tani, M., Kando, F., Saito, N. and Endo, M. (1996). Monocyte Function Associated with *Intermittent Lentinan* Therapy after Resection of Gastric Cancer Surgical Oncology, 5:23-28.
- Tochikura, T.S, Nakashima, H., Yamamoto, N. (1989). Antiviral agents with activity against human retroviruses. *Journal of Acquired Immune Deficiency Syndromes*, 2 (5): 441–7.
- Telke, A.A., Kalyani, D.C. Jadhav, U.U., Parshetti, G.K., and Govindwar, S.P. (2004). Purification and characterization of an extracellular laccase from a *Pseudomonas* sp. LBC1 and its application for the removal of bisphenol.. *A Journal of Molecular Catalysis B*, 61 (3-4): 252–260.
- Thakker, G.D., Evans, C.S., and Koteswara R.K., (1992). "Purification and characterization of laccase from *Monocillium indicum Saxena*," *Applied Microbial .Biotechnology*, 37:130-137..
- Thurston, C.F. (1994). "The structure and function of fungal laccases," *Microbiology and biotechnology*, 140 (1)19–26.
- Mau, J.L., Chao G.R. and Wu, K.T. (2001). Antioxidant properties of methanolic extracts from several ear mushrooms. *Journal of Agriculture. Food Chemistry*, 49: 5461-5467.
- Viswanath, B. Subhosh M. ChandraPallavi, , H. and Rajasekhar Reddy, B. (2008). "Screening and assessment of laccase producing fungi isolated from different environmental samples, *African Journal of Biotechnology*, 7(8): 1129–1133.
- Yang, J.H., Lin H.C. and Mau, J.L. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry*, 77: 229-235..
- Xu, F, M.C. and Drew S.W. (1999). Laccase In Flicking, *Encyclopaedia of Bioprocess technology fermentation, Biocatalyst ,Bio separation*, John Wiley and Sons Inc, New York 1545-1554.
- Wasser, S.P. (2002). Medicinal Mushrooms as a Source of Antitumor and Immunomodulating Polysaccharides. *Applied Microbiology and Biotechnology*, 60:258-274.
- Wang, HX., Ng, TB., Ooi, V.C. (1998) .Lectin activity in fruiting bodies of the edible mushroom *Tricholoma mongolicum*. *Biochemistry Molecular Biology International*, 44: 135–141.
- Watanabe, T., (1969). Antineoplastic activity of ostreatus. *Applied Biochemistry and. Biotechnology*, S 151: 402-412.
- Widsten, P. and Kandelbauer, A. (2008). Laccase applications in the forest products industry: a review. *Enzyme and Microbial Technology*, 42(4):293-307.
- Yaropolov, A.I. Skorobogatk, O.V. Vartanov, S.S. and Varfolomeyev, S.D. (1994). Laccase properties, catalytic mechanism. *Applied Biochemistry and Biotechnology*, 49:257-280.
- Wolff, E.R.S.E. Wisbeck, M.L.L. Silveira, R.M. Gern, M.S.L. Pinho and Furlan, S.A. (2008). Inducing and anti-HIV-1 effects. Phytomedicine, Antimicrobial and antineoplastic activity of *Pleurotus*, 16:198205.
- Yoshida, H. (1883). LXIII- Chemistry of lacquer (Urushi). Part I. Communication from the Chemical Society of Tokio. *Journal of the Chemical Society, Transactions*, 43: 472-486..
- Yoshida, I., Kiho, T., Usui, S., Sakushima, M., Ukai S. (1996). "Polysaccharides in fungi. XXXVII. Immunomodulating activities of carboxymethylated derivatives of linear (1-->3)-alpha-D-glucans extracted from the fruiting bodies of *Agrocybe cylindracea* and *Amanita muscaria*. *Biological and Pharmaceutical Bulletin* 19(1): 114–21.
- Zaura, E., Buijs, M.J. Hoogenkamp, M.A. Ciric, L., Papetti, A., Signoretto, C., Stauder, M. (2011). The Effects of Fractions from Shiitake Mushroom on Composition and Cariogenicity of Dental Plaque Microcosms

- in an in Vitro Caries Model. *Journal of Biomedicine and Biotechnology*,135034.
- Zou, Y.J. Wang, H.X. Ng, T.B. Huang, C.Y. and Zhang, J.X. (2012). "Purification and characterization of a novel laccase from the edible mushroom *Herichium coralloides*. *The Journal of Microbiology*, 50(1): 72–78.
- Zhuang, C.T. Mizuno, A., Shimada, H., Ito, C., Suzukiand, Y.. Mayuzumi, M.(1993). Antitumor protein-containing polysaccharides from a Chinese mushroom *Fengweiguor Houbitake, Pleurotus sajor-caju* (Fr.) Sings. *Bioscinsers. Biotechnology. Biochemistry*,57: 901-906.
- Zhang, Y., Mills, G.L. Nair, M.G. (2003). "Cyclooxygenase inhibitory and antioxidant compounds from the fruiting body of an edible mushroom, *Agrocybe aegerita*". *Phytomedicine* 10 (5): 386–90.
- Zhang, G. Q. Wang, Y. F. Zhang, X. Q. Ng, T. B. and Wang, H. X. (2010). "Purification and characterization of a novel laccase from the edible mushroom *Clitocybe maxima*," *Process Biochemistry*, 45(5):627–633.
- Zhang, G. Q. Tian, T. Liu, Y. P. Wang, H. X. and Chen, Q. J. (2011). "A laccase with anti-proliferative activity against tumor cells from a white root fungus *Abortiporus biennis*,". *Process Biochemistry*, 46(12):2336–2340.
- Zhu, X., and Williamson, P.R. (2004). Role of laccase in the biology and virulence of *Cryptococcus neoformans*. *FEMS Yeast Resarch*. 5, 1–10.