IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-14, Issue1, 2023, pp24-38
http://www.bipublication.com

Review Article

Fungi Laccases: Structure, Functions, and Potential Application in the Biodegradation of Pharmaceutical Micropollutants

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[Received 09 Feb-2023, Accepted 01 March-2023, Published 04 March-2023]

DOI: 10.5281/zenodo.7697775

ABSTRACT

Laccases are a family of oxidoreductases with copper centres found in bacteria, insects, fungi, and plants, and they catalyse the oxidation of a wide range of substrates with the conversion of molecular oxygen to water. They possess immense potential in the degradation of dyes, crude oil, pollutants, phenolic compounds and pharmaceuticals. Fungi laccases have unusual enzyme machinery which enables them to catalyze several complex chemical reactions. Fungi as well as their enzymes have been found to be of immense value in pollution management and control such as peroxidases, tyrosinases, laccases and cytochrome monooxidases. Pharmaceuticals are a broad class of emerging recalcitrant contaminants that have found their way into water bodies through different means and have become a major source of pollution and concerns to pollution management agencies.

In this review, we looked at the structure and function of fungi laccases and their potential application in the biodegradation of emerging pollutants like pharmaceuticals used as analgesics, antibiotics, antiepileptic, antihypertensive, antidepressants. antidiabetic and anti-inflammatory drugs. We concluded that laccases hold a lot of promise in their application in degrading pharmaceuticals but there is the need for its application outside the laboratory and translation into large industrial use. Engineering laccases for improved yield and efficiency is another area that can be explored in the degradation of pharmaceuticals micropollutants.

Keywords: Fungi Laccases, Biodegradation, Pharmaceutical Micropollutants, antidiabetic and anti-inflammatory drugs

1.0 INTRODUCTION

Globally, environmental pollution remains a lingering issue which has negatively affected the environment and the world at large. The different consequences of environmental pollution such as flooding, climate change and diseases are some of the challenges the world is currently facing

today. A lot of industries and households are known to empty their wastes on land and into lakes, rivers, ponds and streams to evade detection by environmental agencies and to reduce costs (1). Lots of studies have been on the use of microbial enzymes to transform and detoxify some of this environmental contaminants and pollutants (2). Fungi are known to be quite robust than bacteria for these purposes, as they can tolerate high amount of pollutants and consequently degrade or transform them (3). Fungi express significant levels of metabolic enzymes that of immense potential on a large scale and are eco-friendly and affordable (3).

Fungi possess unusual enzyme machinery capable of catalyzing complex chemical reactions. This ability of fungi makes them thrive in toxic living conditions (4). Laccases have attracted a lot of interest lately in the scientific community because of their potential application in the degradation of dyes, crude oil, pollutants, phenolic compounds and pharmaceuticals (5).

Laccases (*p*-diphenol:dioxygen oxidoreductase, EC 1.10.3.2) are a family of copper-containing polyphenol oxidases found in bacteria, insects, fungi, and plants and belongs to the multicopper oxidases (MCOs)(6). Laccase were initially identified in the exudates of *Rhus vernicifera*, a Japanese lacquer tree, by Yoshida in 1883. (7).

They are known to catalyse the oxidation of broad spectrum of substrates such as aromatic compounds, organic pollutants and inorganic compounds like aryl diamines, hydroxyindiols, anilines, methoxy-substituted phenols, aromatic diamines, benzenethiols, inorganic/organic metal compounds and other close compounds with the conversion of molecular oxygen to water via reduction (8,9).

1.1 LACCASE-PRODUCING FUNGI

Since laccase was first discovered from the plant *R. vernicifera* exudates, other organism like bacteria, insects and fungi have been shown to possess the enzyme. Fungi laccases have been intensively studied by researchers and delignification, decolorization, pigmentation, and pathogenesis and several other physiological processes have been associated with fungal laccases (6). Classes of fungi like, ascomycetes, deuteromycetes and basidiomycetes are well known producers of laccases. The white-rot fungi of the basidiomycetes class are the most efficient laccase producers and lignin biodegraders (10). The white rot fungi secretes laccases alongside other ligninolytic enzymes including lignin peroxidase, manganese peroxidase, tyrosinses, lyases and monoxidases, and the type of enzymes secreted differs with the strain or specie of the fungus (10,11).

Two model fungi that have been used in laccase research are *Pleurotus ostreatus and Trametes versicolor.* Other known *basidiomycetes* that produces laccase include *Cerrena unicolor, Ganoderma lucidum, Panus rudis, Phlebia radiata, Rigidoporus microporus, Polyporus brumalis, Coprinopsis cinerea, Cyathus bulleri, Coriolopsis gallica, Pycnoporus cinnabarinus, Cryptococcus neoformans, Agaricus bisporus, Pycnoporus sanguineus, Schizophyllum commune, Fomes fomentarius,* as well as various *Trametes (e.g. T. pubescens, T. hirsuta, and T. villosa*) and Pleurotus (e.g., *P. florida, P. eryngii* and *P. sajor-caju)* species (6,11). Efforts are geared towards screening for naturally occurring fungi laccases from other habitats like fresh water and marine habitat with desired yield and properties that can withstand certain extreme conditions as found in industries and with better biodegradation application (12, 13). The yield of laccase by fungi differs and it is dependent on the fungi species and strain, although poor laccase production from naturally-occurring species occurs but these organisms can be improved on for better enzyme production (11).

1.2 LACCASE STRUCTURE

The active site of fungi laccase are composed of two disulfide bonds from the amino acid cysteine alongside four copper atoms which are situated

in three copper centers namely: type 1 (T1), type 2 (T2), and type 3 (T3). Type 1 (T1) is a mononuclear centre with an absorption band at around 610 nm, which accounts for the characteristic blue color of the enzyme. T2/T3 are a trinuclear cluster, with type 2 (T2) having normal copper invisible in the UV-Vis absorption spectrum and type 3 (T3) with a coupled binuclear copper center with an absorption at 330 nm (14, 15). During catalysis, the oxidation of the substrates is carried out at the T1 centre of the enzyme, through a tripeptide sequence of His-Cys-His. Electrons are extracted during the oxidation of the substrate at the T1 centre and these electrons are subsequently transferred to the T2/T3 site where molecular oxygen is finally reduced to water molecule (9, 16). This explains why the enzyme laccase is generally considered as a "green enzyme," due to its ability to catalyse reactions by utilizing the only co-substrate, molecular oxygen rather than hydrogen peroxide like other oxidoreductases like peroxidases and the formation of a harmless product "water". (17). Laccase posses the ability to react better with the help of some small compounds called "mediators" and oxidize non-phenolic compounds, and as a result, its catalytic activity is not restricted to only phenolic compounds (18). The mediators are oxidized thereby releasing one or more electrons to the enzyme, thereafter, oxidized form of the mediator diffuses away from the catalytic active site, where it oxidizes the substrate. Mediators have the ability to oxidize substrates that are usually inaccessible for the laccase enzymes due to its small size (19).

Till date, over 100 laccases have been whollly characterized and studied. Most of the well studied fungi have been those isolated from decaying or decomposing woods especially white-rot fungi as: *Trametes versicolor, Pleurotus pulmonarius, Agaricus bisporus, Pleurotus ostreatus*, etc. (9, 20). Laccases are secreted, with typical molecular weight around of 60 kDa and are usually produced during the secondary metabolic processes of the fungi (21). Most fungal laccases have an isoelectric point around 4.0, although some exhibit basic isoelectric points (22). Fungi laccase function optimally at a temperature range of 40–70^{o}C (23). Laccases are generally glycosylated, and this contributes to the high stability of the enzyme (24). A great number of laccase have found industrial application in the decolorization of dyes in textile industries, bioremediation of soils, delignification and brightening of pulp paper, biosensor technology, food processing and organic synthesis of medications. These abilities of laccase in industries are due to its high stability, broad range of substrates and its versatility (25).

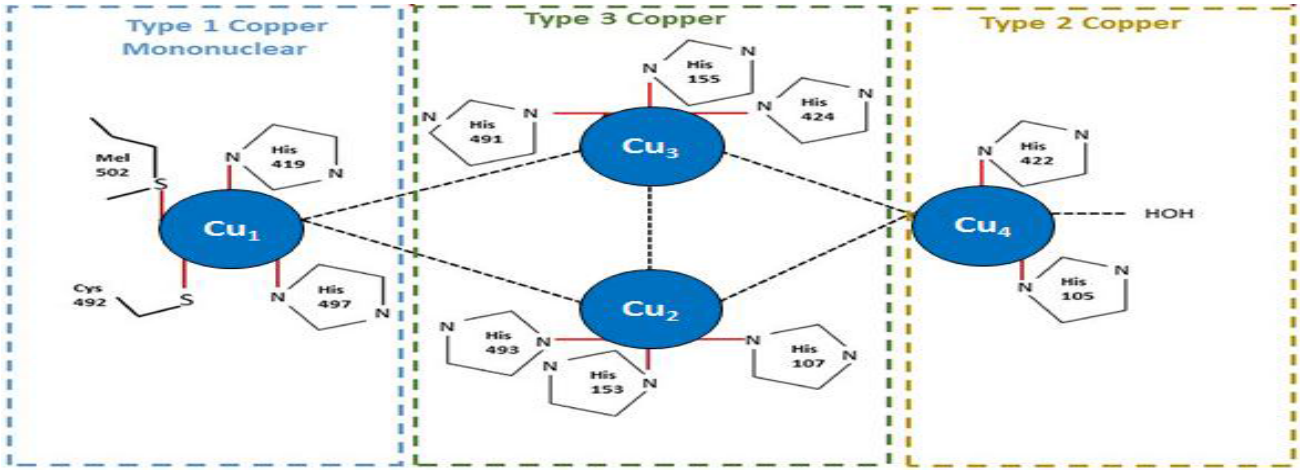

Figure 1: Schematic representation of the active copper center in the laccase (26)

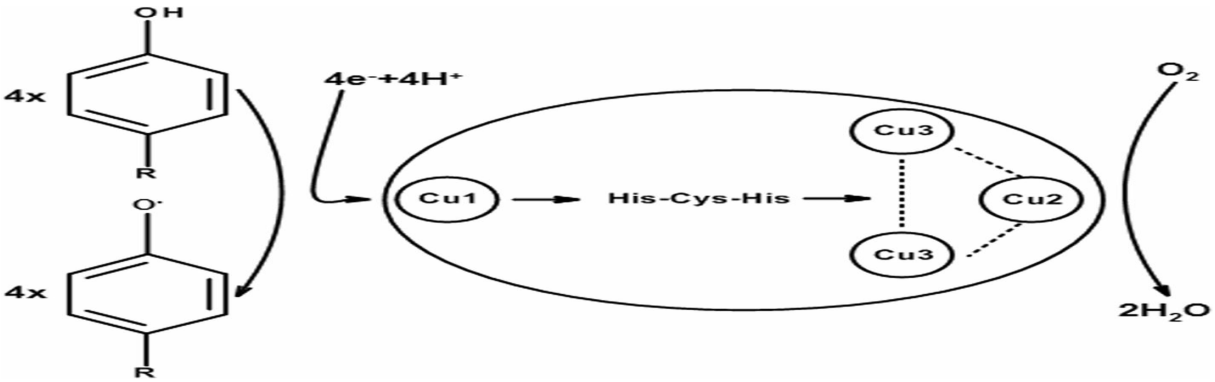

Figure 2: Diagram showing laccase catalytic cycle (27).

1.3 LACCASE MEDIATORS

The difference in the redox potential of the enzyme substrate and the T1 Cu centre of the enzyme affects the efficiency of laccase to oxidize its substrate. Laccase possess a lower redox potentials of about 0.8 V when compared to ligninolytic peroxidases of 1 V (28, 29), as a result of this reduced redox potential of laccase, oxidation of higher redox potential non-phenolic substrates by the enzyme is difficult. To overcome this limitation, redox mediators are employed in laccase catalysis to broaden the range of substrate the enzyme can act on or speed up the catalytic rate, especially for substrates that are too large to fit into the active site of the enzyme. Laccase mediators can be grouped into synthetic and natural mediators. Synthetic mediators include 1-hydroxybenzotriazole (HBT) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) while natural mediators are syringaldehyde (SA) and acetosyringone (AS) (11). Oxidation of the substrates by laccase may proceed with or without in a mediator in different directions depending on the type of substrate involved. Substrate oxidation by laccase mediated by ABTS occurs through electron transfer. Firstly, $ABTS^{+}$ radical is formed by the oxidation of ABTS and then converted to the di-cation ($ABTS^{2+}$) with redox potentials of 472 and 885 mV, respectively. Laccase mediators such as HBT and violuric acid an (N-OH type mediator), when oxidized by laccase form the N-oxy radical and upon deprotonation; the radical accepts the benzylic hydrogen atom from the substrate (11). Phenolic laccase mediators also involve the removal of hydrogen by the radical, but with a phenoxy radical intermediate formed (30, 31). The efficacy of laccase oxidation by the mediator varies with the enzyme and substrate and depends on the enzyme stability in the presence of the mediator, the radicals formed and on how fast the mediator is recycled (32,33). The use of mediators in laccase catalysis incurs more costs and leads to toxicity (34, 35) and sometimes laccase inactivation (36, 37).

1.4 PHARMACEUTICAL COMPOUNDS AS EMERGING CONTAMINANTS

In recent years, the quality assessment level of water resources have drawn a lot of attention based on findings from studies carried out on the problem of contamination borne out of the increased emergence of a spectrum of newly identified compounds with, bio-accumulative and bioactive properties (38, 39). This array of compounds with increasing occurrence known today as emerging or evolving contaminants (ECs) are defined by UNESCO (2014) as any synthetic or naturally-occurring chemical or any microorganism that is not routinely monitored or regulated in the environment with a known or suspected potential adverse effect on the ecosystem and human health (40). Therefore, pollution management due to the presence of ECs creates a new challenge in water quality in the globe.

Among the ECs, one of the most relevant compounds of interest are the pharmaceuticals, and this is due to their physicochemical properties, universal usage, and their capacity to be partially removed by normal wastewater treatment processes (41). They have been found to be present in several environmental compartments such as ground water, surface water, wastewater and drinking water (42). Pharmaceuticals constitute more than 3000 different molecules with several thousand tons of yearly total consumption and are used for the treatment of diseases in human and animal alongside aquaculture and livestock (43).

Pharmaceuticals find their way into the ecosystem at various stages of the lifecycle of the product starting from production (pharmaceutical industries) to consumption (humans and animals) down to excretion and disposal. These compounds are constantly funneled into water bodies via various ways, such as direct discharges of treated and untreated wastewaters from hospitals, industries, households, animal husbandry and wastewater treatment plants (WWTPs) (11, 44). After the various route of administration in humans, these compounds are excreted in their parent forms (unchanged) or as metabolites (changed) and enter the sewage system to reach WWTPs (45).

Unfortunately, most wastewater treatment processes do not properly or effectively eliminate these pharmaceuticals from treated water and as a result they remain latent while some adsorb on sewage sludge (46), and if the sewage sludge is used as manure or fertilizer or if the treated water is used for irrigation purposes, they find their way into the soil (45, 47). Similarly, veterinary drugs administered to animals can be excreted on agricultural fields and when these wastes are used as fertilizers, they find their way into the soil and crops (47). Also, pharmaceuticals in soils can be washed off from the soil into ground and surface waters (48) and can enter the food chain by crops uptake (45).

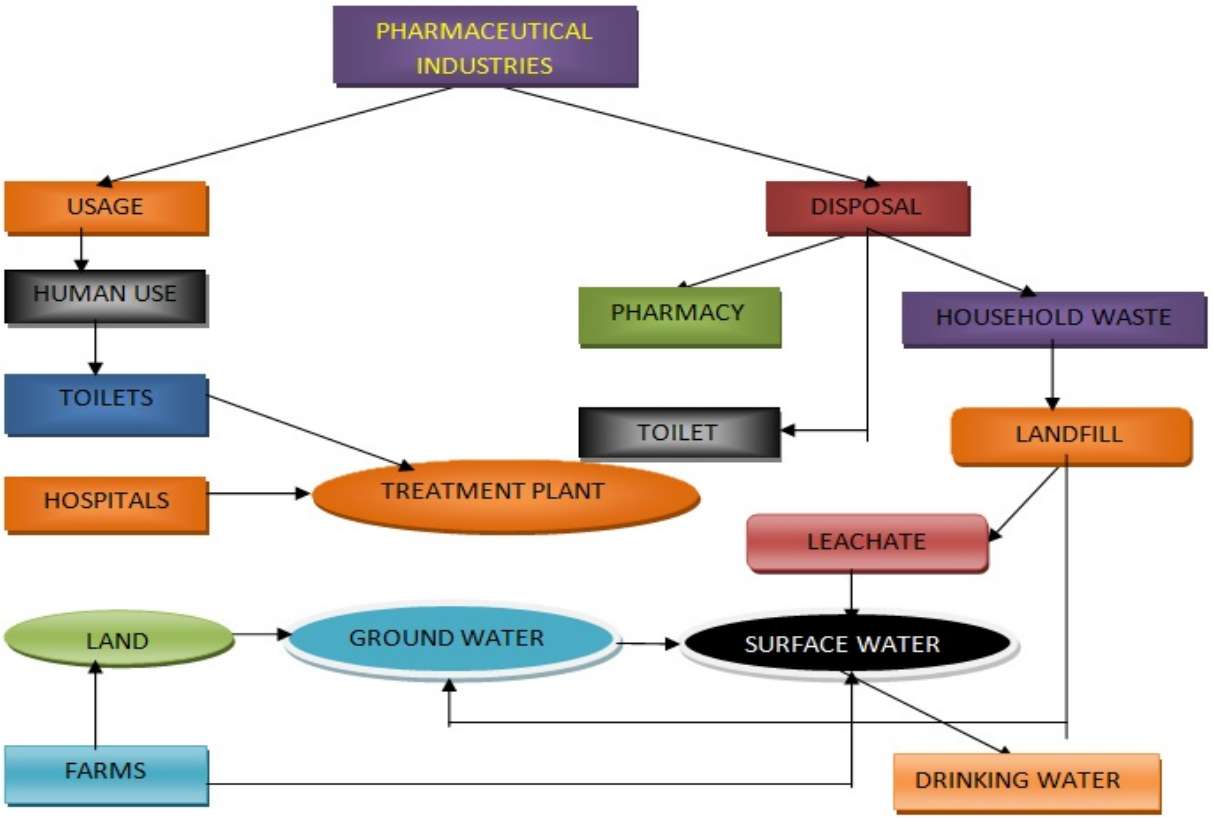

Figure 3: A flowchart of the sources and pathways of pharmaceutical pollution.

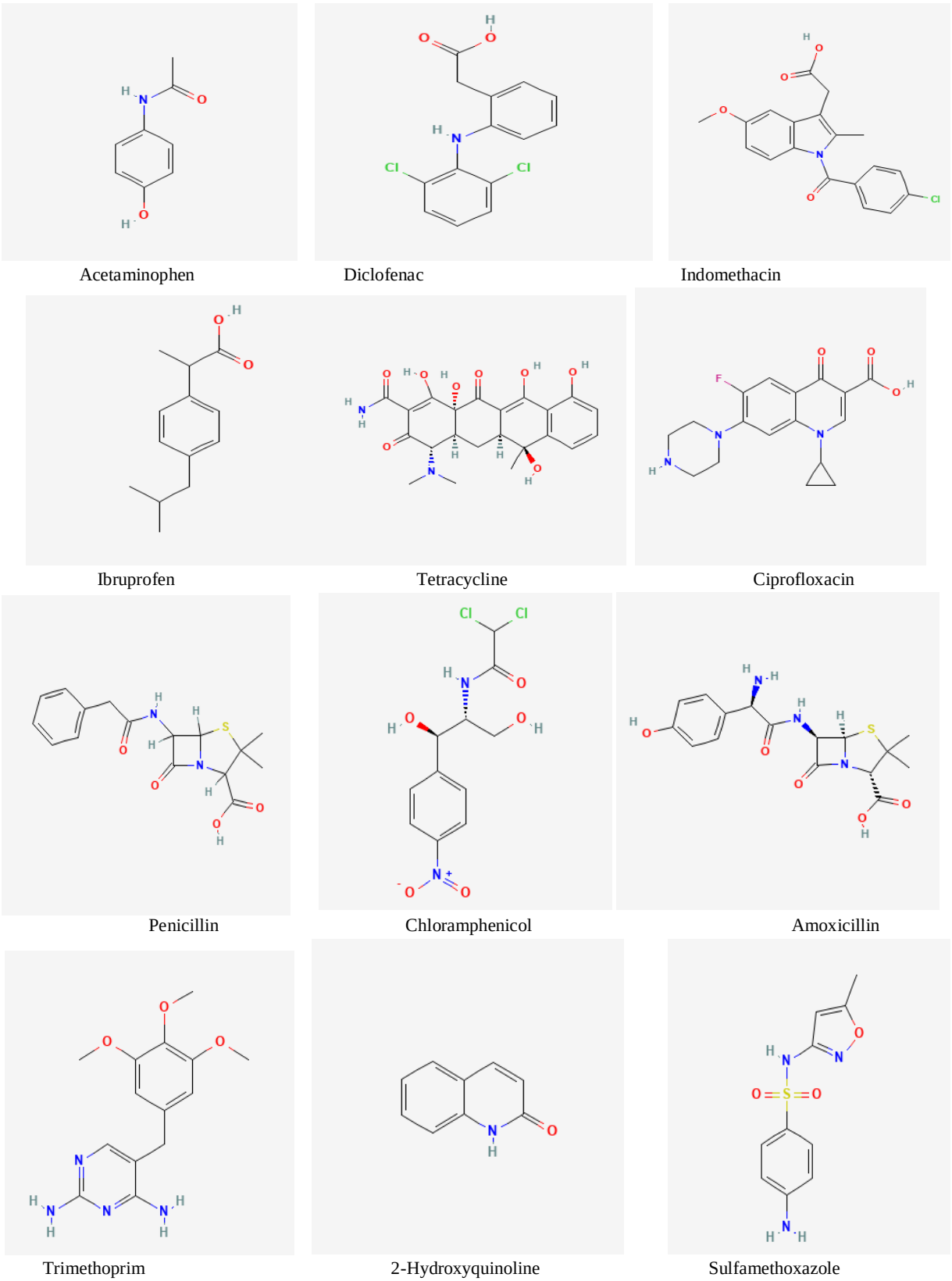

Figure 4: Structures of some pharmaceutical pollutants degraded by fungi laccases (49)

2.0 APPLICATION OF LACCASES IN BIODEGRADATION OF PHARMACEUTICALS MICROPOLLUTANTS

Fungi as well as their enzymes have been found to be of immense value in pollution management and control. Several enzymes such as peroxidases, tyrosinases, laccases and cytochrome monooxidases have been used extensively in pollution control and environmental management (11, 50). Laccases have been used to effectively biodegrade and detoxify a wide range of recalcitrant organic pollutants and have been employed in bioremediation (51, 52), and also as enzymatic biosensors in monitoring for environmental pollution (53). They have catalyzed reaction with substrates like pesticides (54), polycyclic aromatic hydrocarbons (PAHs) (11), dyestuffs (55) and endocrine disrupters (8).

Pharmaceuticals micropollutants are emerging contaminants which are becoming widespread in the environment. These pollutants are increasingly finding their way into water bodies. Due to the low absorption rate of most pharmaceuticals, they are excreted from the body through the renal system either unaffected or as metabolites, thereby ending in water bodies (44, 56). Laccases can be employed for the removal of these pharmaceutical micropollutants (8) and also to synthesize novel antibiotics (57, 58). Analgesics, antimalarial antibiotics, antiepileptic, antihypertensive, antidepressants. antidiabetic and anti-inflammatory drugs as are amongst the most prescribed pharmaceuticals (56). The use of techniques like ozonation and UV-treatment to improve the degradation processes has generated concerns on the byproducts of the processes having even greater negative implication on the ecosystem (59, 60). To remove these undesirable effects of pharmaceutically active compounds on the ecosystem, the binding of the pharmaceuticals to their specific receptors must be inhibited.

This review focuses on pharmaceuticals as emerging contaminants and the application of laccases in their biodegradation and detoxification.

2.1 DEGRADATION OF ANTIBIOTICS

The discussion on the resistance of antibiotics in organisms is an area that has been well researched. One factor responsible for this resistance is because antibiotics are one of the most used class of drugs in the world; their usage cuts across human, veterinary and livestock. Antibiotics that are not metabolized find their way into environment and persists (61) as water treatment processes are unable to effectively remove them (62), while more efficient sophisticated treatment methods have some drawbacks such as high costs and secondary pollution (63). The health risk of antibiotics is compounded by the evolution of some bacteria that are resistant to antibiotics (antibiotic-resistance bacteria, ARB). ARB, Antibiotics and the associated genes have been detected in soils, sediments and water bodies such as marine water, fresh water and drinking water (64). There has been an increased findings in the literature on the utilization of laccase in antibiotic removal, within the past 6 years, but this topics have not been exhaustively reviewed (61). Some antibiotics that have been studied are penicillins, quinolones, sulfonamides, trimethoprim and tetracyclines, while tetracycline and sulfamethoxazole are the most studied (11). The biodegradation time of some of these antibiotics ranges from minutes to hours based on the laccase, antibiotics and the treatment parameter used (11). Laccase mediators such as ABTS, SA and HBT are often used to hasten the bioconversion of antibiotics by laccases. (65, 66). In the biodegradation of tetracycline by laccase, the addition of HBT increased laccase

catalysis at a rate higher than that of some peroxidases like manganese peroxidase (67, 68) although it was still slower than that of lignin peroxidase with a degradation efficiency of 95% in 5 minutes (69). In the degradation of sulfamethoxazole the mediators used, i.e., AS, ABTS, SA and were consumed without obvious catalytic activity (70). Becker *et al.* (35) showed that sulfonamides and tetracyclines were more prone to attack by laccase than with quinolones. This could be as a result of the absence of electron donating aromatic amine group in quinolones (66). In the transformation of tetracycline, based on the intermediates identified, it suggests that the primary target of laccase oxidation is not the phenol group and that the steps involves oxidation, demethylation and the removal of water (11, 71). For sulfonamides, desulphonation (sulfapyridine and sulfathiazole) and cross coupling by laccase and mediators (SA or AS) occurs (65, 70), but not with ABTS (70). Laccase from *T. versicolor*, has been then most frequently used in biodegradation studies of antibiotics as well as other micropollutants. Other laccases include laccases from basidiomycetes *Perenniporia strain TFRI 707*, *Echinodontium taxodii, P. sanguineus and Cerrena sp. HYB07*, from ascomycetes *Phoma sp*. and *Myceliophthora thermophila* (recombinantly expressed in *Aspergillus oryzae*) and from actinobacteria *Streptomyces ipomoeae*. Immobilized laccase have also been used in degrading several antibiotics using different methods. Methods such as ultrasound (72) and soil adsorption (66) have also been employed with laccases in the treatment of antibiotics. These processes have facilitated the degradation of recalcitrant antibiotics like quinolones. The efficiency and stability of the removal of antibiotics by other organisms can also be enhanced by laccases (73).

The bulk of the studies on antibiotic degradation by laccases are in aqueous environments, few studies exists on remediation of soil (74), and sludge (75), river sediment (76).

2.2 DEGRADATION OF OTHER PHARMACEUTICALS

Several other pharmaceuticals aside from antibiotics, have been used as substrate for laccase such as anticonvulsants (e.g., benzodiazepines), anti-inflammatory drugs (e.g.,ibruprofen) (77), fungicides (e.g., ketoconazole) (78), (79), lipid regulators (e.g., lovastatin) (80), antidepressants (e.g., imipramine) (81), biocides (triclosan and chlorophene) (82), insect repellents (e.g., N,N-diethyl-m-toluamide) (83), and sunscreen agents (e.g., oxybenzone) (84). Diclofenac, carbamazepine, and triclosan are some of the most studied drugs. Diclofenac, carbamazepine are non-phenolic drugs while triclosan is phenolic. Carbamazepine which contains a strong electrophilic amide group is the most recalcitrant to oxidation by laccase (85) or peroxidases (86). Triclosan is susceptible to laccase oxidation because of the presence of a strong electron donating hydroxyl group even though it possess an electron withdrawing chlorinated groups, (87). Diclofenac is more prone to laccase oxidation than carbamazepine, which could be due to the presence of chlorine atoms along with aromatic amine (88). Laccase has also been used in synergy with other enzymes, such as glucose oxidase (89) and peroxidase (90) in the removal of pharmaceuticals. Based on studies, phenolic compounds have been shown to be more easily degraded than non-phenolics (91), since they are considered to be the natural laccase substrates.

3.0 CONCLUSIONS

A lot of studies have been carried out on the application of laccase in the biodegradation of different compounds and pollutants. In this review, we looked at the structure and function of laccase and its potential applications in the

degradation of pharmaceutical. Laccase holds a lot of promises in its usage in degrading recalcitrant pollutants but there is the need for its application outside the laboratory and its translation into large industrial application. There is also the need to improve the yield and efficiency of the enzyme and to make the process cheaper and ecofriendly. The metabolites produced from the degradation should be analyzed alongside their toxicity as this will help in understanding the biodegradation process.

CONFLICT OF INTEREST

No potential conflict of interest declared.

ACKNOWLEDGEMENT

This work was funded by the TETFUND IBR, Nigerian Defence Academy, (TETF/DR&D/CE/NDA/KADUNA/IBR/2022/VOL. 1).

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