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Review

Enzymatic Degradation of Lignin in Soil: A Review

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Abstract: Lignin is a major component of soil organic matter and also a rich source of carbon dioxide in soils. However, because of its complex structure and recalcitrant nature, lignin degradation is a major challenge. Efforts have been made from time to time to understand the lignin polymeric structure better and develop simpler, economical, and bio-friendly methods of degradation. Certain enzymes from specialized bacteria and fungi have been identified by researchers that can metabolize lignin and enable utilization of lignin-derived carbon sources. In this review, we attempt to provide an overview of the complexity of lignin's polymeric structure, its distribution in forest soils, and its chemical nature. Herein, we focus on lignin biodegradation by various microorganism, fungi and bacteria present in plant biomass and soils that are capable of producing ligninolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP), and dye-decolorizing peroxidase (DyP). The relevant and recent reports have been included in this review.

Keywords: ligninolytic enzymes; lignin peroxidase; manganese peroxidase; versatile peroxidase; dye-decolorizing peroxidase; degradation

1. Introduction

Lignin is the most common aromatic organic compound found in the lignocellulose component of the plant cell wall. Its characteristic ability to absorb UV (ultraviolet) radiation makes it susceptible to degradation [1] on being exposed to sunlight. The source of lignin in soil can be of plant origin or lignocellulosic waste from the food processing industry [2,3]. It represents a significant part of plant litter input (approximately 20%) into the soil [4,5]. Lignin is an amorphic three-dimensional polymer composed of phenylpropanoid subunits. It acts as a binding material and is involved in cross-linking of cellulose that provides extra strength, rigidity, and stiffness to the cell wall [6]. Lignin protects plant cells from enzymatic hydrolysis [7] and various other environmental stress conditions [8]. The complex structure of lignin makes it recalcitrant to most degradation methods and continues to pose a critical challenge.

Lignin is the most abundant source of carbon in the soil after cellulose [9]. Lignin degradation can thus play a major role in improving earth's biofuel resources and also serve as an alternative to harsh technologies used in the paper and pulp industry. Degradation studies are mainly biotic, aerobic, and co-metabolic. Studies have shown that certain bacteria [10] and fungi [11] are able to break down various biopolymers in soil. Lignocellulosic biomass degradation has been widely studied in wood-rotting Baciomycetes fungi due to their potential to degrade lignin.

In this review, we discuss the structural diversity of the lignin molecule, its distribution in soil, and also biodegradation of lignin by various groups of fungi and bacteria by reviewing the pertinent literature.

2. Lignin Structure and Its Biosynthesis

In the plant cell, lignin is biosynthesized by the combination of three basic hydroxycinnamoyl alcohol monomers or monolignols:

1. *p*-Coumaryl alcohol;
2. Coniferyl alcohol;
3. Sinapyl alcohol.

These monolignols are often referred to as phenylpropanoids, which differ in the substitutions at the 3-C and 5-C positions in the aromatic ring [12,13] (Figure 1).

Lignin synthesis starts with the random self-replicating radical coupling of phenoxy radical to form an oligomeric product. After polymerization, these polymers are referred as *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (from *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, respectively). Monolignols are linked either by C–C bond or C–*O*–C bond, and more than two third of monolignols are joined by ether linkages [14].

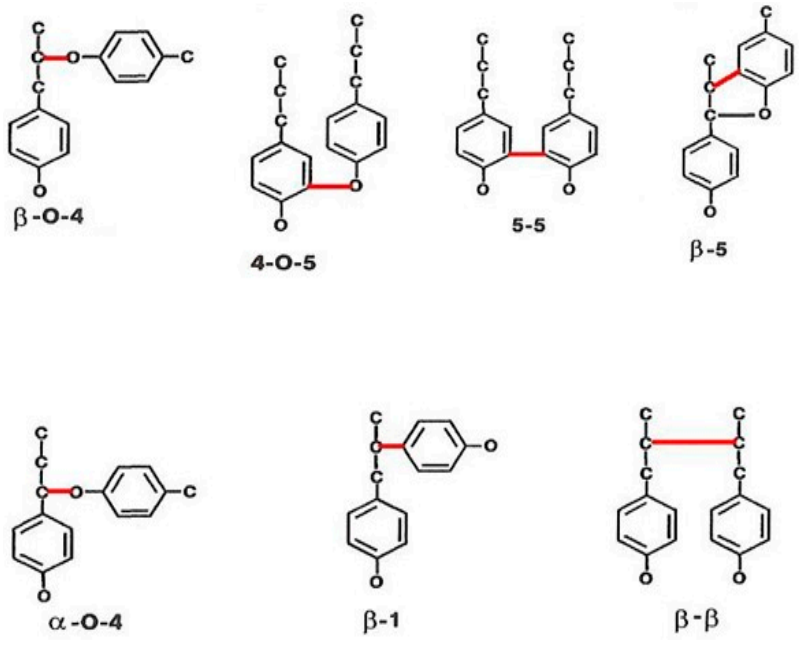

Figure 1. Different type of linkages in the lignin molecule [15].

3. Lignin in Soil

Because of the high inflow of organic aromatic matter into the soil, lignin is considered a major component of soil organic matter. The High stability and low degradability of lignin soil contribute to increasing humus formation [16]. The copper oxidation (CuO) method is commonly used for the characterization and quantification of lignin in soil. Oxidation by CuO yields phenolic compounds

such as vanillyl, syringyl, and cinnamyl type compounds. These compounds reflect the origin and extent of lignin decomposition in the soil. The sum of the above three monomeric phenolic components gives an estimate of total lignin in the soil, whereas the carboxylic acid to aldehyde ratio conveys the extent of lignin decomposition [17]. Studies have shown that biotic, aerobic, and co-metabolic degradation are the main processes involved in lignin degradation [18].

4. Lignin Distribution in Different Soil Horizons

Distribution of lignin in different soil limits has been discussed by many groups [19–22]. The lignin content in the soil decreases from the upper soil horizon to the lower soil horizon. However, in a few cases, an increase in lignin content of soil organic matter (SOM) with depth has been observed [23–25]. Lignin distribution in soil could also vary with location.

A relatively higher rate of lignin degradation is found in lower horizon of soil as compared to the upper horizon because the acid-to-aldehyde ratios of the vanillyl and syringyl units are greater in lower horizon. An increase in gradient is found from organic to mineral horizon. This is in accordance with the decreasing vanillyl, syringyl, and cinnamyl phenolic-lignin contents with the depth and limited supply of fresh organic materials in deep soil horizons.

The particle size of soil components also influences the lignin content and acid-to-aldehyde ratio. The lignin content of SOM decreases from the coarse to the finest particle-size fractions while the acid-to-aldehyde ratio increases with decreasing particle size and is the highest in the clay fraction [26–29].

5. Lignin Degradation in Soil

The Lignocellulosic complex in the plant cell wall contains approximately 40 to 60% cellulose, 20 to 40% hemicellulose, and 10 to 25% lignin [30], which provides rigidity to the cell wall structure. Certain enzymes from specialized bacteria and fungi have been identified by researchers that can catalyze a number of oxidative and hydroxylation reactions, depolymerize the phenolic and non-phenolic lignin polymer, and also mineralize the insoluble lignin. The orientation, adsorption, and diffusion of the ligninolytic enzymes in the soil solid phase affect the lignin degradation in soil [31]. In laboratory studies, the impact of soil particle size on soil respiration was observed by Datta et al., which can, in turn, affect lignin degradability in soil [32].

The biodegradation of lignocellulosic biomass has been widely studied in wood rotting Baciomyecetes microorganisms. These baciomyecetes are categorized as white-rot and brown-rot fungi. White-rot fungi are the most effective bio-degraders of lignocellulosic biomass (e.g., *Phanerochaete chrysosporium*) and can degrade lignin faster than other microorganisms. Su et al. reported that the degradation rate of lignin by *Phanerochaete chrysosporium* in tobacco stalk was 53.75% in 15 days [33].

White-rot fungi produce a number of extracellular enzymes that directly attack lignin, cellulose, and hemicellulose of the plant cell wall to decompose it. These enzymes include laccases and peroxidases, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP) (Table 1). Laccases and peroxidase enzymes can also cause lignin degradation through low molecular weight free radicals such a OH, depolymerize the phenolic and non-phenolic lignin polymer, and mineralize the insoluble lignin (Figure 2).

On the other hand, the brown-rot fungi are less efficient in degrading lignin compared to white-rot fungi. Lignin degradation by brown- rot fungi mainly involves non-enzymatic oxidation reactions producing hydroxyl radicals via Fenton chemistry [35–37]. Brown-rot fungi partially oxidize lignin via aromatic ring demethylation. During this process, the phenolic hydroxyl content of the reaction mixture increases due to partial oxidation and partially due to the addition of new carboxyl and carbonyl groups [38].

Table 1. Comparison of the properties of MnP, LiP, Laccase (Lac), and Dye-decolorizing Peroxidase (DyP) from white-rot fungi.

E.C.	LiP 1.11.1.14	MnP 1.11.1.13	Lac 1.10.3.2	DyP 1.11.1.19
Structure	Monomer, glycoprotein up to 15	Monomer, glycoprotein	Mono-, di-, or tetramer, glycoprotein,	Dimer
Prosthetic group	Heme	Heme	Four Cu atoms per active protein unit	Heme
Glycosylation	N–	N–	N–	N–Acetyl-glucosamine and mannose
Isoforms	Monomers; up to 15	Monomers; up to 11	Mono-, di-, tetramers; several	Dimeric α + β barrel structure
pH Range	2.0–5.0	2–6	2.0–8.5	3.2 (Optimum)
C–C Cleavage	Often	Often	No	Yes
H_2O_2 Regulated	Yes	Yes	No	Yes
Stability	Low	Immense	Immense	Highly
Natural mediators	Unknown mediators	Mn^{2+}; Mn^{3+}	3-Hydroxy-anthranilic acid	Mn^{2+}
Specificity	Broad, aromatics, incl. nonphenolics	Mn^{2+}	Broad, phenolics	Non-phenolics, phenolics, veratryl alcohol.
Catalytic center	Fe-protoporphyrin	Fe-protoporphyrin	Four copper atoms	Fe-protoporphyrin
Mediators	NO	Thiols, unsaturated fatty acids, organic acids as chelators, Mn^{3+}.	Hydroxyben-zotriazole or ABTS, HBTo, syringaldazine, 3-HAA, RBB.	Chelated Mn^{3+}
Cofactor	H_2O_2	H_2O_2	O^{2-}	H_2O_2
Substrate	Halogenated phenolic compounds, polycyclic aromatic compounds	Lignin and other phenolic compounds	Ortho- and para-diphenols, aminophenols, polyphenols, polyamines, lignins, and aryl diamines	Phenols, hydroquinones, dyes, amines, aromatic alcohols and xenobiotics.

Modified from [34].

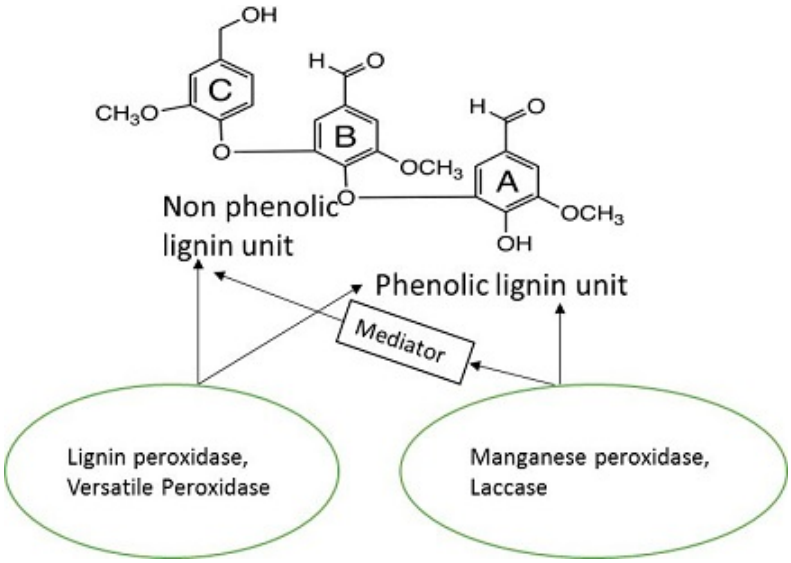

Figure 2. Illustration of ligninolytic enzymes and their selective action on lignin components.

Research in terms of lignin degradation by bacteria has been limited. Although fungi are reported to be more potent in lignin degradation, many soil bacteria such as Actinomycetes are also capable of mineralizing and solubilizing polymeric lignin and lignin-related compounds. Spiker [39] showed that bacteria (e.g., *Streptomyces. viridosporus*) could oxidize phenolics but not the non-phenolic compounds [40]. Various types of cleavages in lignin molecules, e.g., aromatic ring, demethylation, and oxidation, are catalyzed by bacterial enzymes [41]

Lignin-degrading fungi and bacteria can produce primarily four major extracellular heme peroxidases, including lignin peroxidase (LiP, EC 1.11.1.14), manganese-dependent peroxidase (MnP, EC 1.11.1.13), versatile peroxidase (VP, EC 1.11.1.16), and laccase (Lac, EC 1.10.3.2) [42] (Figure 3). These enzymes can directly attack lignin, cellulose, and hemicellulose of the plant cell wall to decompose it. Litter decomposing fungi of families such as *Strophariaceae*, *Tricholomataceae*, and *Bolbitiaceae* have been found to have an evident expression of MnP (Table 2). Recently, a new group of peroxidases has been identified in fungi and bacteria that is capable of degrading lignin, known as dye-decolorizing peroxidases (DyPs, EC 1.11.1.19) [43].

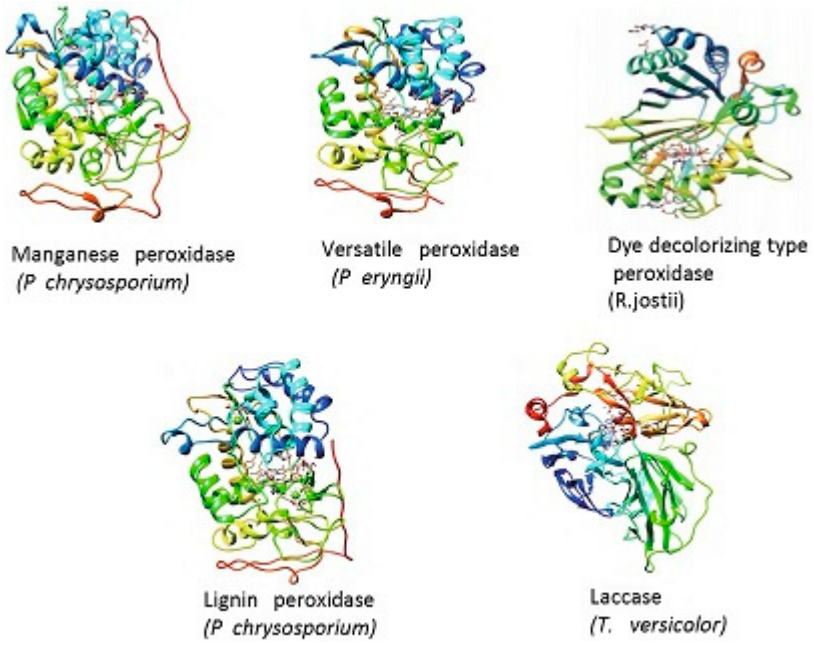

Figure 3. 3D Structure of lignin degrading enzymes [44].

Table 2. Soil litter decomposing fungi secreting MnP.

Fungal Species	Phylum	Family	Mol. Wt (kDa)	Culture Medium	Reference
Agaricus bisporus	Basidiomycota	Agaricaceae	40	Solid	[45,46]
Agrocybe praecox	Basidiomycota	Bolbitiaceae	41–42	Liquid	[47]
Collybia dryophila	Basidiomycota	Tricholomataceae	43	Liquid, solid	[47]
Marasmius quercophilus	Basidiomycota	Tricholomataceae	ND	Liquid	[48]
Phallus impudicus	Basidiomycota	Phlallaceae	ND	Liquid	[49]
Pleurotus sp. *Bhutan*	Basidiomycota	Lentinaceae	ND	Solid	[50]
Panaeolus sphinctrinus	Basidiomycota	Strophariaceae	42	Liquid	[51]
Stropharia aeruginosa	Basidiomycota	Strophariaceae	ND	Liquid	[47]
Stropharia coronilla	Basidiomycota	Strophariaceae	40–43	Liquid	[47]
Stropharia cubensis	Basidiomycota	Strophariaceae	ND	Liquid	[47]
Stropharia rugosoannulata	Basidiomycota	Strophariaceae	41–43	Liquid, solid	[47,52]
Nematoloma frowardii	Basidiomycota	Strophariaceae	42–44	liquid	[47]

6. Steps in Lignin Degradation

Lignin biodegradation involves both depolymerization and aromatic ring cleavage. Extracellular enzyme brought about oxidation of lignin in the following steps:

1. Oxidation of β–*O*–4 linkages to arylglycerol compounds;
2. Aromatic rings cleavage, mostly follows the β–ketoadipate pathway [53];
3. Cleaved aromatic rings coupled with β–*O*–4 oxidation leads to the formation of cyclic carbonate structures.

The lignin–protein and lignin theories [16] hypothesize that during the formation of humic acid from lignin, lignin first breaks down into smaller constituents, and later, these small constituents recombine to form more complex organic molecules.

7. Humification

Humification is a process of conversion of dead organic matter (leaves, twigs, etc.) into humus by the action of decomposers such as bacteria and fungi. Humification affects soil property and nature. Due to its complex nature, chemical composition, and structure, humus is still not well understood. Different theories have been proposed on humification but doubt still exists, and none of them are universally accepted. The heterogeneous nature of soil makes it difficult to generalize different experimental results. A study was done in 2009 by Alianiello et al. [54] to investigate the different steps of humification and the changes that occur during humification. At the beginning of the experiment, a high degree of humification is seen in lignin treated soil. The overall result shows that all the natural substances tend to be almost completely mineralized or converted to substances similar to those of the treated soil, excluding lignin, which after a year incubation is still analytically recognizable in the soil.

8. Lignin Peroxidase (LiP, EC 1.11.1.14)

Lignin peroxidase (LiP, EC 1.11.1.14) is a glycosylated enzyme containing heme protein with an iron protoporphyrin prosthetic group that requires hydrogen peroxide (H_2O_2) to catalyze the oxidation of non-phenolic lignin units and mineralize the recalcitrant aromatic compounds.

Lignin oxidation takes place via electron transfer, non-catalytic cleavages of various bonds, and aromatic ring opening [55] (Figure 4). The catalytic cycle of LiP consists of one oxidation and two reduction steps as follows:

Step 1 Two-electron oxidation of the resting (native) ferric enzyme ([LiP]-Fe(III)) by H_2O_2 to form the Compound I oxo-ferryl intermediate [Fe(IV)];

Step 2 Reduction of Compound I by the non-phenolic aromatic reducing substrate (A) to form Compound II by gaining one electron;

Step 3 Finally, the oxidation cycle ends when Compound II is returned to the resting ferric state with a gain of one more electron from the reducing substrate A.

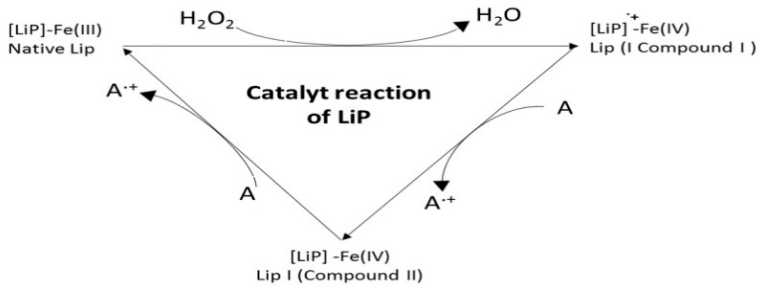

Figure 4. Illustration of the catalytic cycle of lignin peroxidase (LiP).

LiPs have a high redox potential (1.2 V at pH 3.0) [56] as compared with other peroxidases and can oxidize phenolic and nonphenolic structures of lignin directly without a mediator. The bacterial and fungal sources of LiP are listed in Table 3.

Table 3. Fungi and their ligninolytic enzymes involved in lignin degradation.

Enzyme	Fungi	Reference
DyP	*Auricularia auricular-judae*	[57]
LiP	*Phanerochaete chrysosporium*	[58]
Lip	*Phlebia radiata*	[59]
LiP	*P. tremellosa*	[60]
MnP	*Phanerochaete sordida*	[61]
MnP	*P.chrysosporium*	[62]
MnP	*Trametes versicolor*	[63]
MnP	*Ceriporiopsis subvermispora*	[64]
Lac	*P. radiata*	[65]
Lac	*C.subvermispora*	[65]
Lac	*Pleurotus eryngii*	[65]
Lac	*T. versicolor*	[65]
Lac	*T.hirsuta*	[65]
Lac	*T. ochracea*	[65]
VP	*P. eryngii*	[66]
VP	*Pleurotus ostreatus*	[67]
VP	*Bjerkandera fumosa*	[68]

9. Manganese Peroxidase (MnP, EC 1.11.1.13)

Manganese (Mn) is essential for the formation of MnP. The enzyme MnP plays an important role during the initial stages of lignin degradation [69]. Compared to laccase, MnP causes greater degradation of phenolic lignin due to its higher redox potential [70] with the eventual release of carbon dioxide [71]. MnP is mainly produced by a broad species of white-rot basidiomycetes such as *Phanerochaete chrysosporium* [71].

The catalytic cycle of MnP is similar to that of LiP (Figure 5). Like LiPs, MnPs are also heme-containing glycoproteins which require H_2O_2 as an oxidant. Manganese acts as a mediator during MnP enzymatic activity. To begin with, MnP oxidizes Mn^{2+} to Mn^{3+}. The enzymatically generated Mn^{3+} oxidant is freely diffusible and participates in the oxidation reaction as a redox couple [72].

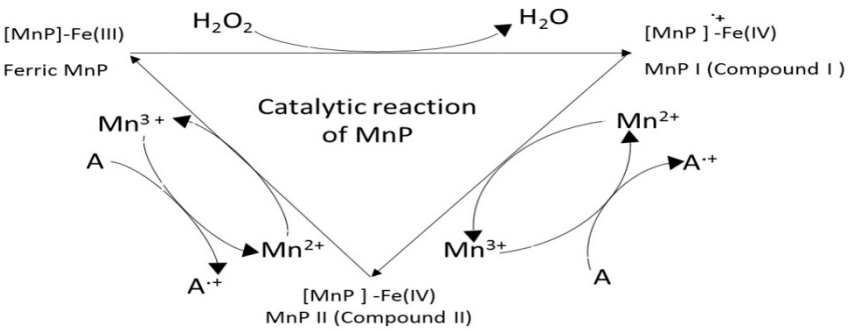

Figure 5. Illustration of the catalytic reaction of manganese peroxidase (MnP).

In addition, organic acids such as lactate and malonate can chelate Mn^{3+} ion [72]. The chelated Mn^{3+}—organic acid complex oxidizes the phenolic compounds in lignin to phenoxy radicals [73]. High levels of Mn can stimulate MnP enzymatic activity and enhance the degradation process of lignin in soils [74]. The bacterial and fungal sources of MnP are listed in Table 3.

10. Versatile Peroxidase (VP, EC 1.11.1.16)

Versatile peroxidase, as the name suggests, has catalytic properties of both LiP and MnP. VP was first purified from the genera of fungi *Bjerkandera* [75] and was found to transform lignin even without an external mediator.

The VP enzyme possesses a hybrid molecular architecture with several binding sites including Mn^{2+} and is able to oxidize Mn^{2+} like MnP and LiP. However, unlike MnP, VP has the dual ability to oxidize Mn^{2+} in the independent oxidation of simple amines and phenolic monomers [76]. VP can also oxidize a variety of substrates (with high and low redox potentials) including Mn^{2+}, phenolic and non-phenolic lignin dimers, and aromatic alcohols [77]. The bacterial and fungal sources of VP are also listed in Table 3.

11. Laccase (Lac, E.C. 1.10.3.2)

Lac (EC 1.10.3.2, *p*-diphenol oxidase) is a copper-containing enzyme belonging to the oxidoreductase group which oxidizes a wide variety of organic and inorganic substances (Figure 6).

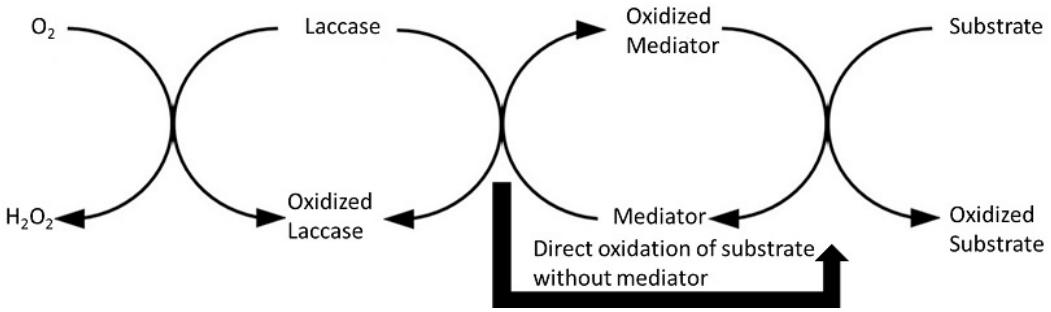

Figure 6. Illustration of the catalytic cycle of laccase [78].

Lac was extracted by Yoshida in 1883 for the first time from the *Rhus vernicifera* tree [79,80]. In 1896, it was first showed to be present in fungi by Bertrand and Laborde [80]. Thereafter, many Lacs have been identified from fungi (Table 3). The white-rot fungus produces high levels of Lac and is very efficient at decolorizing dyes. Some bacterial Lac have also been reported (Table 4).

Table 4. Bacteria and their ligninolytic enzymes involved in lignin degradation.

Ligninolytic Enzyme	Bacteria	Reference
DyP A	*Amycolatopsis* sp.	[81]
DyP A	*Escherichia coli*	[81]
DyP A	*Rhodococcus jostii*	[82]
DyP A	*Steptomyces viridosporus*	[81]
DyP A	*S. coelicolor*	[81]
DyP A	*S. viridosporus*	[81]
DyP A	*Thermobifida fusca*	[83]
DyP A	*T. fusca YX*	[81]
DyP B	*Escherichia coli*	[81]
DyP B	*Pseudomonas* sp.	[81]
Dyp B	*Rhodococcus jostii*	[82]
DyP B	*R. jostii*	[81]
DyP B	*S. coelicolor*	[81]
Laccase	*Bacillus atrophaeus*	[81]
Laccase	*B. licheniformis*	[84]
Laccase	*B. pumilus*	[81]
Laccase	*B. subtilis*	[85]
Laccase	*S. coelicolor*	[86]
Laccase	*S. griseus*	[87]
Laccase	*S. ipomoea*	[88]
Laccase	*S. lavendulae*	[89]
Laccase	*Streptomyces cyaneus*	[90]
Laccase	*Thermus thermophilus*	[91]

It was initially assumed that Lac could only oxidize phenolic compound [92], due to its lower redox potential (450–800 mV) as compare to Lips (>1 V). However, with the involvement of a mediator, a wide variety of substance can be oxidized using Lac.

Mediators are low molecular weight compounds that are easily oxidized by Lacs and subsequently reduced by the substrate. Due to its large size, the substrate cannot reach the active site of the enzyme. A mediator, due to its small size, acts as a conveyer of an electron from the enzyme to the substrate [93]. The mediator reaches the enzyme active site easily and gets oxidized to a more stable intermediate with a high redox potential. The oxidized mediator diffuses away from the enzyme and oxidizes more complex substrates before returning to its original state [94]. The electrons taken by Lacs are finally transferred back to oxygen to form hydrogen peroxide [95,96].

Most of the enzymes are substrate specific, in contrast to Lac activity which oxidizes a variety of substrates like polyphenols, diphenols, benzenethiol, and aromatic amines.

12. Dye-Decolorizing Peroxidase (DyP, EC 1.11.1.19)

The DyP enzyme is also a heme-based peroxidase that can cause lignin breakdown through a radical-mediated oxidation process. The DyPs are phylogenetically distinct [97] from other peroxidases as they possess an α + β ferredoxin-like fold [98]. However, their oxidation mechanism is similar to VP and MnP [99]. They are widely found in microorganisms [100] and classified into four types: A, B, C, and D. Bacterial enzymes are predominantly found in type A to C, while type D is mostly clustered to fungal DyPs. All kinds of DyPs have peroxidase activities; however, they differ in substrate specificity values [40]. In addition to lignin, DyPs can also oxidize synthetic dyes [101], non-phenolic methoxylated aromatics [99], Mn^2 [102], and high redox synthetic dyes such as anthraquinone and azo dyes [101]. Tables 3 and 4 lists various fungi and bacteria, and their ligninolytic enzymes involved in lignin degradation.

13. Fenton Chemistry in Lignin Degradation

Brown-rot fungi hydrolyze and partially oxidize the lignocellulose component of the plant cell wall, in contrast to white-rot fungi which produce an array of extracellular lignin-degrading enzymes. During the oxidation process, a hydroxyl ion is generated via Fenton oxidation chemistry [35–37]. The Haber–Weiss reaction is a specific example of the Fenton reaction. In Haber–Weiss reaction, ferrous salt and hydrogen peroxide react to produce a highly reactive hydroxyl free radical capable of oxidizing a wide verity of substrates. In a nutshell, Fenton reaction is a cyclic redox reaction. In the Fenton reaction, there is neither a breaking of old bonds nor the formation of new bonds. As a result, a highly reactive hydroxyl radical is produced (Figure 7).

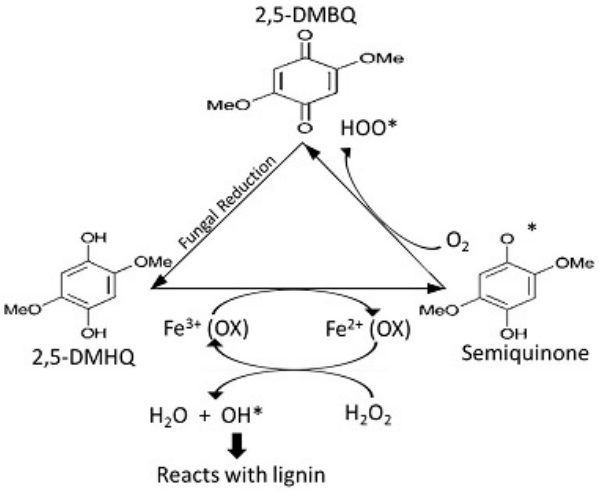

Figure 7. Fenton redox cycle found in brown-rot fungi.

A detailed mechanism of the Fenton reaction has been explained using two extracellularly produced quinones, 2,5-dimethoxy-1,4-benzoquinone (2,5-DMBQ) and 4,5-dimethoxy-1,2-benzoquinone (4,5-DMBQ), in *Gloeophyllum trabeum* [80]. The two hydroquinones have the ability to reduce ferric oxalate salt to ferrous oxalate. In the second step, ferrous oxalate reacts with hydrogen peroxide to generate highly reactive hydroxyl free radicals. This reactive hydroxyl free radical can react with many organic compounds including lignin. Quinone is then converted back to hydroquinone by fungi [103].

14. Low Molecular Weight Compounds Involved in Lignin Degradation

Electron microscopy studies have shown that enzymes such as peroxidase and laccase, due to their larger size than that of the pores of the cell wall, cannot reach to the native lignin molecule. Therefore, mediators are important parts of the ligninolytic enzyme system. Mediators help enzymes such as MnP and LiP [104–106] to reach native lignin present in the wood.

Many low molecular weight compounds have been identified which could play a fundamental role in the enzymatic ligninolytic systems of white-rot fungi [104,107–111]. Lip and MnP can oxidise various lignin compounds and synthetic lignin only in the presence of a cofactor [112–114].

Lignin degradation conditions support both depolymerization and repolymerization, so it is important to optimize several factors, such as H_2O_2 and lignin concentration, O^2, and the presence of a suitable mediator [112].

14.1. Manganese

A high amount of manganese (10–100 mg/kg of dry wood) is present in the wood [115]. Manganese deposits were found in wood decay resulting from white-rot fungi activity. Scanning electron microscopy revealed a 100-fold increase in manganese concentration as compared to delignified wood. Mn^{2+} precipitates in the form of MnO^2 [116].

During lignin degradation, Mn^{3+} assists oxidation of various phenolic compounds whereas Mn^{2+} acts as a substrate and triggers MnP production [112,114,117–119]. Mn^{2+} is a scavenger of the peroxide radical [74,120] and it decreases the oxygen stress of cell, resulting in decreased LiP production [121], whereas it increases under an oxidizing atmosphere [74,122]. Furthermore, Mn^{2+} induces MnsoD which further minimizes oxidative stress as well [74].

14.2. Veratryl Alcohol

Veratryl alcohol (VA) is synthesized from glucose [109,110]. Its production starts in parallel with LiP production during the early phase of secondary metabolism. Mester et al. [123] showed that manganese inhibits the production of VA in fungi Bjerkandera sp. strain BOS55 and *P. chrysosporium*.

In addition to de novo synthesis from glucose, several alternative pathways are present for VA production in *Phlebia radiata*. De novo synthesis of VA is repressed when products of lignin degradation are used as precursors.

VA is most likely the physiological substrate of LiP. Production of Lip and VA is triggered by the presence of secondary lignin metabolites. The introduction of VA to culture medium has been found to increase LiP [122,124,125]. VA plays a key role in LiP-catalyzed oxidation reactions and LiP-mediated electron transfer reactions [126].

14.3. Oxalate

White-rot fungi secrete oxalate as a major aliphatic organic acid [127,128]. White-rot fungi decompose oxalate to carbon dioxide and formate as they cannot accumulate acid [129]. This is further oxidized to a super-oxidized form under aerobic conditions. This superoxide directly participates in the oxidation of lignin [130].

The enzymes oxaloacetate and glyoxylate oxidase are responsible for the biosynthesis of oxalate. LiP and MnP can decompose oxalate in the presence of VA or Mn^{2+} [130–132].

Oxidation of oxalate is necessary; it can slow down the lignin mineralization by reducing VA^{+*} and Mn^{2+} [131].

14.4. 2-Chloro-1,4-dimethoxybenzene

Chlorinated anisyl metabolites (CAMs) are physiologically involved in lignin degradation. CAMs act as a substrate for the aryl alcohol oxidase enzyme involved in extracellular peroxide production. White-rot fungi produce a wide variety of halogen metabolites [133]. CAMs and chlorinated hydroquinone metabolites (CHMs) [133] are the most common.

The CHM biosynthesis pathway involves the formation of 9 metabolites found in basidiomycetes [57,59]. Among these, 9,2-chloro-1,4-dimethoxybenzene, 2,6-dichloro-1,4-dimethoxybenzene, tetrachloro-1,4-dimethoxybenzene, and tetrachloro-4-methoxy phenol were identified [134,135].

2-Chloro-1,4-dimethoxybenzene (2-Cl-1,4-DMB) acts as a substrate for LiP and is actively involved in lignin degradation [136] and acts as a redox mediator [136]. DMB free radicals are formed during LiP-catalyzed oxidation [137]. These free radicals easily diffuse away from enzyme active site and are regarded as diffusible redox mediators.

2-Cl-1,4-DMB involvement in lignin degradation is still not clear as the formation of LiP and its biosynthesis do not coincide [138].

15. Discovery of New Lignin-Degrading Bacteria

Greater knowledge of microbial biodiversity helps us to understand lignin degradation to a greater extent. In recent years, many new bacterial strains have been identified which are more actively involved in lignin degradation than previous ones [112]. Some of these new microbes have developed a new mechanism of lignin degradation, particularly if they originate from unusual environments such as wood-feeding insects [113,114,139], the rumen of cows [116], or active soils [140]. For example, an anaerobic lignin degradation environment is interesting regarding the degrading enzymes that depend on peroxide or oxygen [141]. There is a lack of knowledge about the genes involved in lignin degradation, but physiological [118] and structural studies [119] could help to identify new chemical transformations as a result of lignin degradation. However, microbial gene isolation from anaerobic environments, rainforest soil, or just forest area improves our understanding.

16. Conclusions

A surveying of the literature tells us that very little is known about the degradation of lignin in soil. Lignin is the most important and common aromatic organic compound found in the plant cell wall, and becomes a major source of humic acid in soil. Degraded lignin fragments are building blocks of the humic compounds in soil. Hence, lignin degradation has received vast attention from various researchers. The most efficient organisms for lignin mineralizing are white-rot fungi. Enzymes from specialized bacteria and fungi have been surveyed in this study that can metabolize lignin and enable utilization of lignin-derived carbon as a resource. The study summarizes lignin biodegradation by various fungi and bacteria present in plant biomass and soils that are capable of producing ligninolytic enzymes such as LiP, MnP, VP, and DyP. Most of these enzymes are substrate specific, in contrast to Lac activity which oxidizes a variety of substrates like polyphenols, diphenols, benzenethiol and aromatic amines. Recent efforts to identify new lignin-degrading microbes [142,143] and thorough study of their genomics, biochemistry, and proteomics will uncover the role of ligninolytic enzymes in the coming years.

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