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COMMENTARY

Progress and obstacles in the production and application of recombinant lignin-degrading peroxidases

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ABSTRACT

Lignin is 1 of the 3 major components of lignocellulose. Its polymeric structure includes aromatic subunits that can be converted into high-value-added products, but this potential cannot yet been fully exploited because lignin is highly recalcitrant to degradation. Different approaches for the depolymerization of lignin have been tested, including pyrolysis, chemical oxidation, and hydrolysis under supercritical conditions. An additional strategy is the use of lignin-degrading enzymes, which imitates the natural degradation process. A versatile set of enzymes for lignin degradation has been identified, and research has focused on the production of recombinant enzymes in sufficient amounts to characterize their structure and reaction mechanisms. Enzymes have been analyzed individually and in combinations using artificial substrates, lignin model compounds, lignin and lignocellulose. Here we consider progress in the production of recombinant lignin-degrading peroxidases, the advantages and disadvantages of different expression hosts, and obstacles that must be overcome before such enzymes can be characterized and used for the industrial processing of lignin.

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Introduction

Lignocellulose is the most abundant form of sustainable biomass on earth and is therefore highly promising for the production of renewable fuels and chemicals.[1] Two of the components of lignocellulose (cellulose and hemicellulose) are readily accessible for industrial processes because they are easily degraded by known enzymes and by chemical hydrolysis.[2] However, the third component (lignin) is much more resistant to degradation. Unlike the other components it is a non-carbohydrate polymer with aromatic subunits. Depending on the lignin source, up to 3 phenylpropanoid monomeric precursors are coupled to form the lignin polymer.[3] It is not currently possible to fully exploit the potential of this polymer due to the lack of knowledge and technologies for lignin degradation.[4] The lignin obtained from lignocellulosic biomass during industrial processes such as papermaking and biomass fractionation is therefore either incinerated to provide electricity for plants that produce cellulosic ethanol[5] or is chemically modified and used to manufacture products such as phenolic resins, polyurethane foams, epoxy resins and biodispersants.[6] However, lignin has the potential for conversion into high-value-added products such as vanillin, adipic acid, ferulic acid, vinyl guaiacol, optically-active lignans, the dimers of monolignols and *p*-coumaric acid.[7,8] Lignin could therefore replace the fossil-fuel-based feedstock used in many current industrial processes, increasing their sustainability and benefiting the environment.

Lignin-degrading enzymes

Studies focusing on the biological degradation of lignin date back to the 1980s. Enzymes from white-rot and brown-rot fungi have been studied more comprehensively than those from bacteria because they were initially considered to be more efficient, but recent reports have shown that bacteria also degrade lignin and produce potentially important peroxidases and laccases.[9] The first lignin-degrading enzymes to be identified were peroxidases from the white-rot fungus *Phanerochaete chrysosporium*.[10,11] Many further enzymes were subsequently identified and characterized, and these can be assigned to 2 major classes: heme peroxidases and laccases (Fig. 1).

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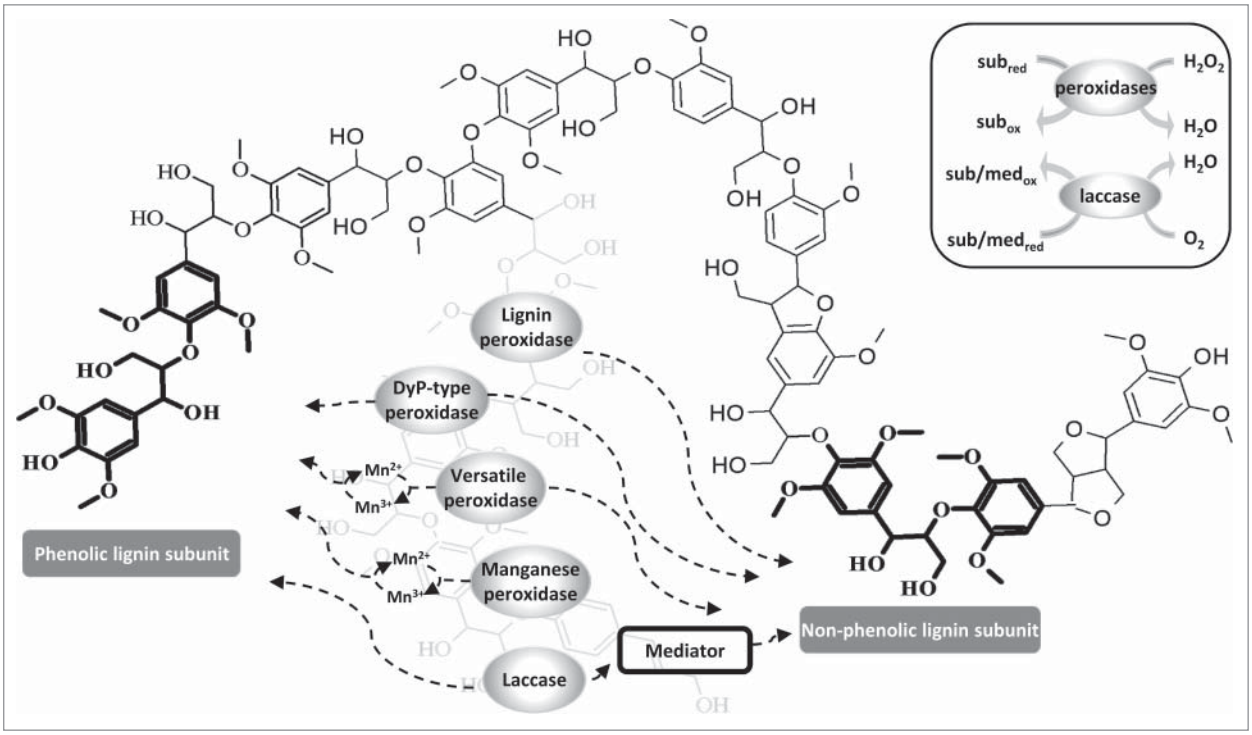

Figure 1. Lignin subunits attacked by enzymes and their most common reaction mechanisms. The bulky lignin polymer structure represents part of an organosolv lignin substrate.[65] Both superfamilies of lignin-degrading enzymes (heme peroxidases and laccases) can oxidize phenolic lignin subunits. In order to oxidize non-phenolic lignin subunits, laccase requires the presence of a mediator. Lignin peroxidase, versatile peroxidase and DyP-type peroxidase do not require a mediator to attack non-phenolic structures. Manganese peroxidase and versatile peroxidase oxidize phenolic lignin subunits via the oxidation of manganese ($Mn^{2+} \rightarrow Mn^{3+}$). The inset box shows heme peroxidases reducing H_2O_2 to water to catalyze the oxidation reactions, whereas laccases reduce molecular oxygen to water, which is accompanied by the oxidation of the substrates or mediators. The atoms and bonds of the phenolic and non-phenolic lignin subunits are highlighted in bold within the bulky structure. Abbreviations: med = mediator, sub = substrate.

The first heme peroxidase superfamily comprises lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP), which are typically produced by fungi.[12] Non-phenolic structures are thought to be degraded by LiP due to its high redox potential. MnP can oxidize phenolic model compounds via the oxidation of Mn^{2+} to Mn^{3+}. VP is so called because it combines the catalytic properties of LiP and MnP.[3]

The second heme peroxidase superfamily comprises the dye-decolorizing (DyP-type) peroxidases, which also oxidize β-O-4 non-phenolic and phenolic lignin model compounds. Although the first DyP-type peroxidase was isolated from fungi, subsequent genome sequence analysis has revealed that this superfamily of enzymes is also prominent in bacteria.[13] DyP-type peroxidases typically require hydrogen peroxide to form the oxo-ferryl intermediates of the enzymes, which subsequently oxidize the mediator or the substrate.

Unlike peroxidases, laccases reduce molecular oxygen to water using the copper atoms located within the active center in order to oxidize the mediator or substrate. Phenolic lignin units are oxidized directly whereas non-phenolic subunits are oxidized via a redox mediator system to overcome the low redox potential of laccase. The potential applications of laccases in lignocellulose degradation have been comprehensively reviewed.[14,15] The remaining enzymes that support lignin degradation are known as accessory enzymes.[3] These include enzymes that produce hydrogen peroxide and enzymes that reduce methoxy radicals generated by the peroxidases and laccases.

Recombinant lignin-degrading peroxidases

Although several groups have attempted to optimize the cultivation of *P. chrysosporium* to increase the yield of native LiP and MnP, a successful large-scale production system has yet to be reported. This could potentially be addressed by using heterologous expression systems to produce large amounts of unmodified

or modified recombinant enzymes for targeted industrial applications.

The mechanism of cellulose degradation is well understood and recombinant cellulases have been produced successfully in various heterologous systems.[16,17] In contrast, the mechanisms and interactions of lignin-degrading enzymes (especially peroxidases) are poorly characterized. Even so, many recombinant lignin-degrading enzymes from diverse fungal (Table 1) and bacterial (Table 2) sources have been expressed in different host organisms, although it is challenging to achieve high yields and satisfactory catalytic activity due to the complex secondary and tertiary structures of such enzymes. Specific posttranslational modifications may be necessary during maturation, such as heme assembly, glycosylation and the formation of disulfide bridges, which may differ between the native source and heterologous production host.

This challenge is clearly illustrated by recent progress toward the production of high enzyme titers with activity against complex lignin model compounds, as well as lignin and wood (Tables 1 and 2). The first report describing the production of recombinant fungal MnP and LiP was published in 1991, but although the authors used insect cells and the reliable baculovirus expression system, only low yields were achieved and the cost of the process was high (Table 1).[18-20] *P. chrysosporium* was used for the homologous production of MnP and LiP with yields of up to 2 mg of purified LiP per liter of culture.[20,21] Later, *Pleurotus ostreatus* was used to express the versatile peroxidase MnP2 homologously, with a yield of up to 21 mg L^{-1}.[22] However, the extensive screening required to identify mutant production strains, as well as contamination with the native enzyme, led to disappointing results. The model bacterium *Escherichia coli* has been extensively developed as an expression platform for fungal enzymes, and many recombinant enzymes have been isolated successfully from inclusion bodies and refolded with 1–28% efficiency[23-30] and maximum yields of 1.5–14 mg L^{-1} after *in vitro* activation and purification.[29] A soluble form of a VP was produced using a thioredoxin tag[31,32] or following intensive screening.[31] Few publications have described the activity of such recombinant enzymes against complex lignin model compounds or synthetic lignin.[29,30,33] Fungal and yeast expression systems have been used to ensure that posttranslational modifications such as glycosylation are carried out, because these are thought to increase the stability of many fungal enzymes. MnP, LiP and VP have been expressed in various *Pichia* and *Saccharomyces* strains, the recombinant enzymes were secreted into the medium (e.g. 100 mg L^{-1} and 21.6 mg L^{-1} for *Pichia methanolica* and *Saccharomyces cerevisiae*, respectively)[34,35] and catalytic activities were determined using common substrates such as 2,2'azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and veratryl alcohol (VA).[34-38] MnP produced in this manner was suitable for the treatment of Kraft lignin as determined by measuring the kappa number and the increase in Klason lignin residue.[39] MnP from *P. chrysosporium* has also been expressed in the filamentous fungi *Aspergillus oryzae* and *A. niger*,[40,41] yielding up to 100 mg L^{-1} in *A. niger*. A VP from *Pleurotus eryngii* was expressed successfully in another *Aspergillus* strain (*A. nidulans*) with a yield of 0.4 mg L^{-1} after protein purification.[42] Recently, native LiP and MnP were expressed in *P. chrysosporium* in concert with an exogenous VP, generating a recombinant strain with higher LiP and MnP activity and detectable VP activity.[43] Further strain engineering included the introduction of an additional laccase.[44] In addition to LiP, MnP and VP, fungal DyP-type peroxidases have also been expressed in various heterologous production platforms,[45-48] with yields of 0.1 mg L^{-1} purified enzyme achieved using *E. coli*[47] and up to 62 mg L^{-1} recovered from the supernatant of *A. oryzae* expression cultures.[45]

The production of several recombinant bacterial lignin-degrading enzymes has also been achieved over the last few years (Table 2). The first recombinant LiP was produced successfully in 1998 by cloning a 4.1-kb fragment of *Streptomyces viridosporus* genomic DNA and expressing it in the yeast *Pichia pastoris*.[49] Several DyP-type peroxidases from bacteria have been expressed in *E. coli*, including enzymes from *Thermobifida fusca*,[50,51] *Amycolatopsis* sp.,[52] *Pseudomonas aeruginosa*,[53] *Anabaena sp.*,[54] *Pseudomonas putida*,[55] *Bacillus subtilis*,[55,56] *Saccharomonospora viridis*,[57] *Pseudomonas fluorescens*,[58] and 2 enzymes (DypA and DypB) from *Rhodococcus jostii* RHA1.[59,60] The yields were generally in the range 3–25 mg L^{-1}, but exceptional yields of 106.5 mg L^{-1} and 2.1 g L^{-1} have been achieved in *E. coli* for *S. viridis*[57] and *B. subtilis*[56] peroxidases, respectively. The recombinant enzymes have been extensively characterized and optimized using a range of dyes as well as

Table 1. Recombinant fungal lignin-degrading peroxidases. Examples of successfully produced fungal lignin-degrading peroxidases and the substrates that have been used. The activity of the enzymes (if stated) is shown in parentheses. ABTS: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate), DCP: 2,4-dichlorophenol, DFAD: 4-[(3,5-difluoro-4-hydroxyphenyl)azo]benzenesulfonic acid, DMP: 2,6-dimethoxyphenol, icb: inclusion body; MHG: methoxyhydroquinone, VA: veratryl alcohol.

enzyme	origin	host organism	localization	substrate (activity)	ref
LiP H8	*Phanerochaete chrysosporium*	baculovirus	extracellular	VA (20 U mg^{-1})	18
LiP H8	*P. chrysosporium*	*P. chrysosporium*	extracellular	VA (K_m 89.4 μM, k_{cat} 23.2 s^{-1})	21
LiP H8	*P. chrysosporium*	*E. coli*	cytoplasm (ib)	VA (29.7 s^{-1}) ABTS (27.0 s^{-1}) DFAD (31.1 s^{-1})	24
LiP H2	*P. chrysosporium*	*E. coli*	cytoplasm (ib)	Mn^{2+} (14 s^{-1}) VA (39 s^{-1})	25
LiP H8	*P. chrysosporium*	*E. coli*	cytoplasm (ib)	β-0-4 tetrameric lignin model compound	33
LiP	*Trametes cervina*	*E. coli*	cytoplasm (ib)	ABTS VA (K_m 3240 μM, K_{cat} 17.7 s^{-1}) 1,4-dimethoxybenzene ferrocytochrome c	28
LiP H8	*P. chrysosporium*	*Pichia methanolica*	extracellular	VA (α-mating factor: 1933 U L^{-1}) VA (native leader peptide: 932 U L^{-1})	34
LiP H2 variants	*P. chrysosporium*	*Saccharomyces cerevisiae*	extracellular	DCP (78.1 – 81.8 U mg^{-1}) DCP (k_{cat} 3520 – 3960 min^{-1}, K_m 163 – 190 μM)	37
MnP H4	*P. chrysosporium*	baculovirus	extracellular	phenol red (83 mU L^{-1}) vanillylacetone (201 mU L^{-1}) guaiacol (94 mU L^{-1})	19
MnP isozyme 1 (*mnp1*)	*P. chrysosporium*	*P. chrysosporium*	extracellular	Mn^{2+} (341.3 U mg^{-1}) DMP (158.3 U mg^{-1})	20
MnP H4	*P. chrysosporium*	*E. coli*	cytoplasm (ib)	Mn^{2+}(140 U mg^{-1})	23
MnP H4 S168W	*P. chrysosporium*	*E. coli*	cytoplasm (ib)	Mn^{2+} (k_{cat} 260 s^{-1}; K_m 0.11 mM) VA (k_{cat} 11 s^{-1}; K_m 0.49 mM)	26
MnP	*P. chrysosporium*	*Aspergillus oryzae*	extracellular	Mn^{2+} (0.33 U mL^{-1}; k_{cat} 132 $\pm$ 15 s^{-1}) DMP	40
MnP H4 (*mnp1*)	*P. chrysosporium*	*Aspergillus niger*	extracellular	ABTS ($\sim$66.2 $\pm$ 12.1 ΔAbs min^{-1} mL^{-1})	41
MnP (*mnp1*)	*P. chrysosporium*	*Pichia pastoris*	extracellular	DMP (160 U mg^{-1}) Kraft lignin (Kappa number: 49.2 to 42.0, Klason lignin: 7.41% to 6.75%)	39
MnP6 S168W*	*Ceriporiopsis subvermispora*	*E. coli*	cytoplasm (ib)	Mn^{2+} (k_{cat} 60.5 $\pm$ 5.0 s^{-1}; K_m 8.5 $\pm$ 1.2 μM) ABTS (k_{cat} 1.4 $\pm$ 0.1 s^{-1}; K_m 60.2 $\pm$ 13.4 μM) VA (k_{cat} 0.54 $\pm$ 0.04 s^{-1}; K_m 2740 $\pm$ 560 μM) Reactive Black 5 (k_{cat} 7.8 $\pm$ 1.1 s^{-1}; K_m 812.6 $\pm$ 3 μM) DMP 4-O-methylsyringylglycerol-ß-guaiacyl ether (k_{cat} 0.47 $\pm$ 0.0 s^{-1}; K_m 1.1 $\pm$ 0.2 μM)	29
MnP1 – MnP6	*Pleurotus ostreatus*	*E. coli*	cytoplasm (ib)	Mn^{2+} ** ABTS ** VA ** Reactive Black 5 ** DMP **	30
VP (MnPL2 -*mnpl2*)	*Pleurotus eryngii*	*Aspergillus nidulans*	extracellular	Mn^{2+} (K_m 20 μM; k_{cat} 99 s^{-1}) VA (K_m 1780 μM; k_{cat} 12 s^{-1}) MHQ (K_m 23 μM; k_{cat} 10 s^{-1}) DMP	42
versatile MnP2 (*mnp2*)	*P. ostreatus*	*P. ostreatus*	extracellular	Mn^{2+} (K_m 22.3 μM) guaiacol (7300 U L^{-1}) VA (K_m 5080 μM)	22
VP	*Bjerkandera adusta*	*E. coli*	cytoplasm	Mn^{2+} (27.5 mU mg^{-1}) DMP (0.13 mU mg^{-1}) VA (0.25 mU mg^{-1}) 1-napthol (70 mU mg^{-1})	31
VP (*vpl2*)	*P. eryngii*	*E. coli*	cytoplasm	Mn^{2+} (194 $\pm$ 3.1 U mg^{-1}; k_{cat} 138 $\pm$ 5.2 s^{-1}; K_m 78 $\pm$ 7.9 μM) ABTS (8.8 $\pm$ 0.03 U mg^{-1}; k_{cat} 5.4 $\pm$ 0.1 s^{-1}; K_m 0.7 $\pm$ 0.04 μM) VA (7.2 $\pm$ 0.6 U mg^{-1}; k_{cat} 6.4 $\pm$ 0.3 s^{-1}; K_m 4090 $\pm$ 523 μM) Reactive Black 5 (2.2 $\pm$ 0.2 U mg^{-1}; k_{cat} 2.7 $\pm$ 0.08 s^{-1}; K_m 4.8 $\pm$ 0.3 μM)	32
VP1 VP2 VP3	*P. ostreatus*	*E. coli*	cytoplasm (ib)	Mn^{2+} ** ABTS ** VA ** Reactive Black 5 ** DMP ** 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether synthetic lignin	30

(*Continued on next page*)

Table 1. (*Continued*).

enzyme	origin	host organism	localization	substrate (activity)	ref
VP (*vpl2*) variant	*P. eryngii*	*S. cerevisiae*	extracellular	ABTS (717 U mg^{-1})	35
VP (*vpl2*)	*P. eryngii*	*P. chrysosporium*	extracellular	Mn^{2+}(9.084 U mL^{-1} culture)	43
MnP (*mnp1*)	*P. chrysosporium*			DMP (20 U mL^{-1} culture)	
LiP (*lipH8*)	*P. chrysosporium*			VA (~3.8 U mL^{-1})	
DyP	*Thanatephorus cucumeris (Geotrichum candidum Dec 1)*	*A. oryzae*	extracellular	(relative substrate specificity) Reactive Blue 5 (100 %) Reactive Blue 19 (100 %) Reactive Black 5 (0.9 %) Reactive Red 33 (1.8 %) VA (0 %) guaiacol (0.17 %) DMP (0.54 %)	46
DyP	*T. cucumeris*	*A. oryzae*	extracellular	1-amino-2-sulfonyl-4-aminomethyl-9,10-anthraquinone sodium salt (600 U mg^{-1})	45
DyP	*T. cucumeris*	*E. coli*	periplasm	(relative substrate specificity) Reactive Blue 5 (100 %) Reactive Blue 19 (119 %) Reactive Blue 21 (26 %) Reactive Blue 114 (4.4 %) Reactive Black 5 (1.0 %) Reactive Red 33 (2.3 %) Reactive Red 120 (0.96 %) Reactive Orange 13 (0.22 %) VA (0 %) guaiacol (9.2 %) DMP (9.3 %)	47

*see Ref. [29] for kinetic constants for recombinant peroxidases from *C. subvermispora* (MnP6, MnP6-S168W-environment variants), *P. ostreatus* (VP1), and *P. chrysosporium* (LiP-H8).
**see Ref. [30] for kinetic constants (K_m, k_{cat}, k_{cat}/K_m) for the recombinant peroxidases from the *P. ostreatus* genome.

simple and also more complex lignin model compounds (Table 2). These studies revealed that DyPs are active against both non-phenolic and phenolic substrates and can oxidize polymeric lignin. Another DyP from *R. jostii*, which was expressed in its native host, was shown to be active against wheat straw lignocellulose and produced potentially valuable products, namely vanillin and smaller amounts of ferulic acid and 4-hydroxybenzaldehyde.[61]

Future challenges

Detailed studies describing the activity of recombinant enzymes on complex lignin model compounds, lignin and lignocellulose substrates have been published only in the last few years. Activity assays using lignin as a substrate are time-consuming because both the measurements and data analysis take a long time. The oligomeric nature of lignin model compounds affects the catalytic activity of lignin-degrading enzymes, e.g., the activity of LiP on the model compound β-O-4 lignin was 25-fold lower than on the simple artificial substrate VA.[62] To increase the convenience of protein characterization and engineering approaches, artificial substrates such as ABTS, VA and DMP are used for initial testing and simple phenolic or non-phenolic model compounds are used in subsequent assays. However, the degradation of these simple aromatic compounds may require different catalytic mechanisms, which makes it difficult to extrapolate the results to typical lignin and lignocellulose samples. For example, LiP has two substrate interaction sites: a heme-edge site which is typical for peroxidases and a second site for the oxidation of VA.[24] The optimization of enzyme activity by screening will only select for increases in activity against the test substrate under the specific conditions of the test and will not necessarily have a positive impact on the degradation of typical lignin substrates found in plant biomass or organosolv lignin. It is also clear that the successful degradation of lignin and lignocellulose requires multiple peroxidases and other lignin-degrading enzymes and accessory proteins.[63] Each enzyme individually may not show promising results *in vitro* because optimal activity requires the presence of other enzymes, or conversely an individual enzyme may show promising *in vitro* activity alone but this may not be fulfilled under natural conditions when other enzymes are present. Lignin degradation is not an isolated process in nature, but is instead embedded in the more

Table 2. Recombinant bacterial lignin-degrading peroxidases. Examples of successfully produced recombinant bacterial lignin-degrading peroxidases and the substrates that have been used. The activity of the enzymes (if stated) is shown in parentheses. ABTS: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate), DCP: 2,4-dichlorophenol, DMP: 2,6-dimethoxyphenol, DOPA: L-3,4-dihydroxyphenylalanine, VA: veratryl alcohol.

enzyme	origin	host organism	localization	substrate (activity)	Ref
extracellular lignin peroxidase	*Streptomyces viridosporus*	*Pichia pastoris*	extracellular	DCP (~1.1 (AU) min^{-1}) DOPA	[49]
DyP-type (*Tfu*_3078)	*Thermobifida fusca*	*Escherichia coli*	periplasm	Reactive Blue 19 (K_m 29 μM, k_{cat} 10 s^{-1})	[50]
*Tfu*DyP	*T. fusca*	*E. coli*	intracellular	ABTS (K_m 0.86 ± 0.07 mM, k_{cat} 28.1 ± 1 s^{-1}) DCP (K_m 5.51 ± 0.5 mM, k_{cat} 2.86 ± 0.1 s^{-1}) guaiacol phenol (K_m 0.126 ± 0.01 mM, k_{cat} 0.136 ± 0.03 s^{-1}) pyrogallol (K_m 11.29 ± 1 mM, k_{cat} 6.63 ± 0.25 s^{-1}) Reactive Blue 4 (K_m 0.179 ± 0.01 mM, k_{cat} 1.88 ± 0.07 s^{-1}) guaiacylglycerol-β-guaiacyl ether Kraft lignin	[51]
DyP (DyP2)	*Amycolatopsis sp. 75iv2*	*E. coli*	extracellular	k_{cat}/ K_m (M^{-1} s^{-1}) ABTS ((6.6 ± 0.9) × 10^6) Reactive Blue 5 ((7.1 ± 0.9) × 10^5) Reactive Black 5 ((1.6 ± 0.1) × 10^5) Mn^{2+} ((1.2 ± 0.2) × 10^5) guaiacylglycerol-β-guaiacol ether, veratrylglycerol-β-guaiacol ether	[52]
DyP-type (*dyp* Pa)	*Pseudomonas aeruginosa*	*E. coli*	intracellular	Reactive Blue 5 (4395 μM mg^{-1} min^{-1}) Reactive Blue 19 (107 μM mg^{-1} min^{-1}) Reactive Black 5 (704 μM mg^{-1} min^{-1}) DMP (786 μM mg^{-1} min^{-1})	[53]
DyP	*Anabaena sp strain PCC7120*	*E. coli*	intracellular	Reactive Blue 5 (K_m 3.6 M, k_{cat}/K_m 1.2 × 10^7 M^{-1} s^{-1})	[54]
PpDyP BsDyP	*Pseudomonas putida* *Bacillus subtilis*	*E. coli*	intracellular	(PpDyP // BsDyP) ABTS (40 ± 0.9 U mg^{-1} // 15 ± 1 U mg^{-1}) Reactive Blue 5 (15 ± 0.2 U mg^{-1} // 11 ± 0.6 U mg^{-1}) guaiacol (0.2 ± 0.004 U mg^{-1} // 0.2 ± 0.006 U mg^{-1}) syringaldehyde (0.11 ± 0.005 U mg^{-1} // 0.004 ± 0.0002 U mg^{-1}) acetosyringone (0.16 ± 0.008 U mg^{-1} // 0.012 U mg^{-1})	[55]
*Bs*DyP	*B. subtilis*	*E. coli*	intracellular	ABTS (66.80 U mg^{-1}) VA (0.13 U mg^{-1}) Reactive Blue 19 (11.55 U mg^{-1}) Reactive Black 5 (17.65 U mg^{-1}) veratrylglycerol-β-guaiacolether (0.086 U mg^{-1})	[56]
*Svi*DyP	*Saccharomonospora viridis*	*E. coli*	intracellular	Reactive Blue 19 (1.29 U mg^{-1}) Reactive Green 19 (1.32 U mg^{-1}) Reactive Yellow 2 (4.86 U mg^{-1}) Reactive Black 5 (0.96 U mg^{-1}) Reactive Red 120 (0.69 U mg^{-1}) Brilliant Green (12.24 U mg^{-1}) Malachite Green (8.4 U mg^{-1}) Crystal Violet (4.11 U mg^{-1}) Azure B (1.62 U mg^{-1}) DMP (0.06 U mg^{-1}) VA (0.03 U mg^{-1}) eucalyptus kraft pulp (Kappa decrease 21.8 %)	[57]
DyP1B	*Pseudomonas fluorescens*	*E. coli*	intracellular	ABTS (K_m 1.13 ± 0.1 mM, k_{cat} 13.5 ± 0.4 s^{-1}) DCP (K_m 1.25 ± 0.1 mM, k_{cat} 0.66 ± 0.02 s^{-1}) guaiacol (K_m 0.056 ± 0.006 mM, k_{cat} 0.058 ± 0.001 s^{-1}) pyrogallol (K_m 4.0 ± 0.6 mM, k_{cat} 2.5 ± 0.1 s^{-1}) Reactive Blue 4 (K_m 0.12 ± 0.01 mM, k_{cat} 1.04 ± 0.03 s^{-1}) Kraft lignin (K_m 0.006 ± 0.001 mM, k_{cat} 0.9 ± 0.1 s^{-1})	[58]
DypB	*Rhodococcus jostii*	*E. coli*	intracellular	ABTS (0.0549 ΔAbs min^{-1}) β-aryl ether lignin model Kraft lignin (0.24 ΔAbs 10 min^{-1}) nitrated lignin (0.0081 ΔAbs 20 min^{-1}) wheat straw lignocellulose	[59]
DyPA	*R. jostii*	*E. coli*	intracellular	ABTS (K_m 8.2 mM, k_{cat} 16.83 s^{-1})	[60]
peroxidase	*R. jostii*	*R. jostii*	extracellular	wheat straw lignocellulose (96 mg L^{-1} vanillin 53 mg L^{-1} p-hydroxybenzaldehyde 3-120 mg L^{-1} vanillic acid 23-86 mg L^{-1} ferulic acid)	[61]

complex process of lignocellulose degradation. For example, the oxidative activity of lytic polysaccharide monooxygenases against cellulose was recently linked to lignin via long-range electron transfer.[64]

In conclusion, the production and characterization of recombinant single enzymes is necessary to understand the different reaction mechanisms required for successful and tailored lignin degradation. Optimized expression, intensive characterization and the analysis of enzyme structures and reaction mechanisms will help to determine the optimal reaction conditions of these enzymes. Nevertheless, successful lignin degradation currently appears to require a combination of enzymes. In the future, the combination of certain lignin-degrading enzymes will enable the effective and targeted degradation of lignin feedstock sources to obtain specific value-added products.

Abbreviations

ABTS	2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)
DMP	2,6-dimethoxyphenol
DyP-type peroxidase	dye-decolorizing peroxidase
LiP	lignin peroxidase
MnP	manganese peroxidase
VA	veratryl alcohol
VP	versatile peroxidase

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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