

Research progress of ligninase system

Yingtian Xiao ^{1,2,3}, Yan Li ^{1,2,3}, Nan Lu ^{1,2,3}

¹Shaanxi Provincial Land Engineering Construction Group Co., Ltd. Xi'an 710075, China;

²Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd. Xi'an 710075, China;

³Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an 710075, China.

Abstract

Synthetic dyes are widely used in printing and dyeing, printing and other fields. However, due to its complex structure, difficult to degrade, and potentially toxic, it has caused serious pollution to the environment. At present, one of the more cost-effective methods for the treatment of dye wastewater is the biological method. Among them, the ligninase system secreted by the white rot fungus has a good effect on the decolorization of the dye. The ligninase system secreted by white rot fungi mainly includes Mnp, Lip, Lac. Studies have found that ligninase has different degrees of degradation of azo, anthraquinone and triphenylmethane dyes. Ligninases from different cultures also differ in their ability to degrade dyes.

Keywords

Synthetic Dyes; Laccase; Lignin peroxidase.

1. Laccase (Lac)

Laccase (Lac; EC 1.10.3.2) was first discovered by Bertrand in the sap of the Japanese sumac tree in 1985 and also verified that laccase is a metal oxidase ^[1]. Fungal laccase typically consists of three-domain single-molecule extracellular glycoproteins, each containing four copper ions ^[2]. Studies have shown that laccase is a copper-containing ionase that uses molecular oxygen as an electron acceptor and can oxidize various benzene and non-benzene compounds^[3]. According to the principle of crystal diffraction in spectroscopy and the related study of enzymatic kinetics, the method of catalyzing the substrate by laccase is inferred as shown in Figure 1-1. The substrate will first bind to the T1-type Cu ion site of the enzyme activity center to obtain electrons; T2 Cu ions and 2 His and T3 Cu ions and 6 His were retained. The electrons are then transferred to the trinuclear copper cluster through the Cys-His channel, which in turn is further transferred to the oxygen molecule bound to the active center, which is reduced to water ^[4]. At the same time, the free radicals formed by the substrate continue the enzymatic reaction, and they can bind themselves or conjugate to each other to form polymers or conjugate products. In the dye degradation reaction catalyzed by laccase, the decolorization rates of laccase to azo dyes (Orange 2, Acid Orange 6) from *Trametes versicolor* can reach 72.8% and 45.3%, respectively ^[5]; And laccase from *Oudemansiella canarii* can decolorize Congo red with a decolorization rate of 80% ^[6].

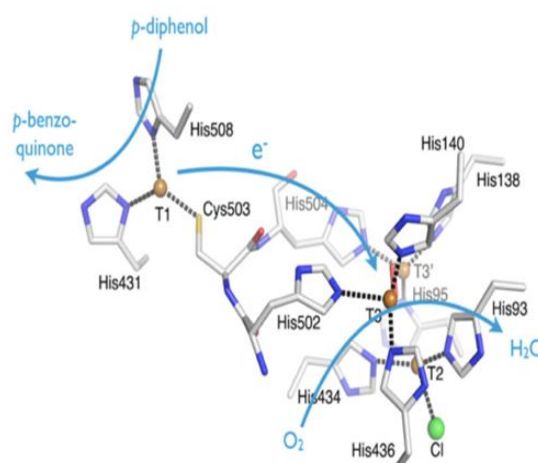


Figure 1 Lac catalytic mechanism

2. Lignin peroxidase (Lip)

Lignin peroxidase (Lip; EC 1.11.1.14) is a relatively nonspecific enzyme containing heme prosthetic groups, which can mineralize a variety of refractory aromatic and halogenated phenolic compounds, has a higher redox potential than peroxidase, and is a strong H_2O_2 -dependent oxidant [7]. The fungal lignin peroxidase is globular, mainly composed of spiral-shaped glycoproteins, which are about 38-40 kDa in size and include 343-344 amino acids [8], 370 water molecules, and heme, among others. Studies have shown that the isoelectric point of lignin peroxidase is about 3.5, the optimal pH is acidic, and the optimal temperature is 35-55 °C [9]. There are many microorganisms that secrete lignin peroxidase, such as *Phanerochete chrysosporium*, *Thametes versicolor*, and so on. Analysis of lignin peroxidase secreted by *P. xanthospora* found that there are 10 isoenzymes of lignin peroxidase encoded by different genes in their genomes, but these isoenzymes are not produced at the same time, and some can only be expressed in specific situations [10]. In recent years, many researchers have also reported the catalytic mechanism of lignin peroxidase, as shown in Figure 2. Lignin peroxidase can oxidize electron-rich phenolic or non-phenolic aromatic compounds, and electron-seize the substrate through the electron transporter, oxidize it to free radicals, and then produce many different free radicals in a chain reaction, resulting in the breaking of the main chemical bonds in the substrate molecule, and then a series of cleavage reactions [11].

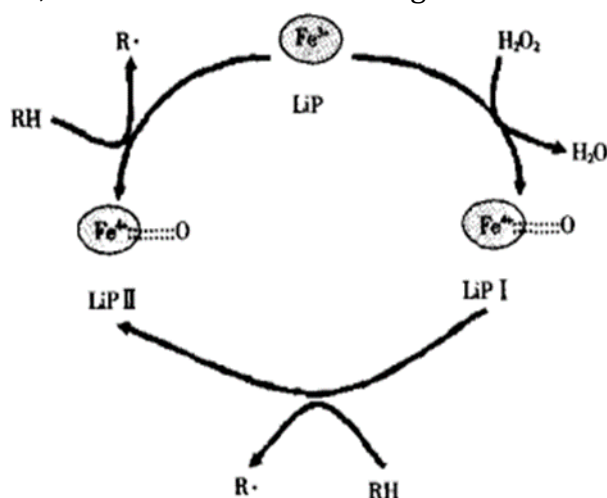


Figure 2 Lip catalytic mechanism

3. Manganese peroxidase (Mnp)

Manganese peroxidase (Mnp; EC 1.11.1.13) was first discovered in *Phanerochaete chrysosporium*, and later proved to be a heme-containing glycosylated peroxidase, which, in the presence of Mn^{2+} and H_2O_2 , oxidizes and decomposes aromatic cyclopolymers and is considered one of the key enzymes for lignin degradation. At present, the proposed catalytic mechanism of Mnp activity mainly involves manganese ions, as shown in Figure 3, Mn^{2+} oxidation to Mn^{3+} , and then further degradation of stubborn organic pollutants such as dyes and phenol compounds [12]. In general, MnPs always comes in the form of different types of isoenzymes, and the diversity of its amino acid sequences is mainly due to differences between the c-terminal and lysine residue numbers [13]. Studies have shown that *Ceriporiopsis subvermispora* secretes up to 11 isoenzymes of manganese peroxidase.

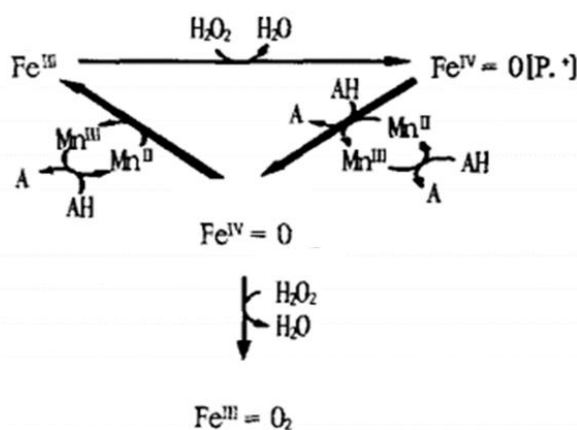


Figure 3 Mnp catalytic mechanism

Acknowledgements

The project was supported by the projects of Land Engineering Construction Group of Shaanxi Provincial (DJNY2022-18).

References

- [1] Giardina P, Faraco V, Pezzella C, et al. Laccases: a never-ending story [J]. Cellular & Molecular Life Sciences, 2010, 67(3):369-385.
- [2] Morozova O V, Shumakovich G P, Gorbacheva M A, Shleev S V, Yaropolov A I. "Blue" Laccases [J]. Biochemistry, 2007, 72(10):1136-1150.
- [3] Kumar M, Mishra A, Singh SS, et al. Expression and characterization of novel laccase gene from *Pandoraea* sp. ISTKB and its application [J]. International Journal of Biological Macromolecules, 2018.
- [4] B. Legerská, Chmelová D, Ondrejovic M. Decolourization and detoxification of monoazo dyes by laccase from the white-rot fungus *Trametes versicolor* [J]. Journal of biotechnology, 2018, 285: 84-90.
- [5] Iark D, Buzzo A J D R, Garcia J A A, Côrrea V G, Helm C V, Corrêa R C G, Peralta R A, Moreira R D F P M, Bracht A, Peralta R M. Enzymatic degradation and detoxification of azo dye Congo red by a new laccase from *Oudemansiella canarii* [J]. Bioresource Technology, 2019, 289: 121655.
- [6] Wong D W S. Structure and Action Mechanism of Ligninolytic Enzymes [J]. Applied Biochemistry & Biotechnology, 2009, 157(2):174-209.
- [7] Piontek K, Smith A T, Blodig W. Lignin peroxidase structure and function [J]. Biochemical Society Transactions, 2001, 29(2):111.

- [8] Xu H, Guo M Y, Gao Y H, Bai X H, Zhou X W. Expression and characteristics of manganese peroxidase from *Ganoderma lucidum* in *Pichia pastoris* and its application in the degradation of four dyes and phenol [J]. *BMC Biotechnology*, 17(1):19.
- [9] Fernández-Fueyo E, Ruiz-Dueñas F J, Martínez M J, Romero A, Hammel K E, Medrano F J, Martinez A T. Ligninolytic peroxidase genes in the oyster mushroom genome: heterologous expression, molecular structure, catalytic and stability properties, and lignin-degrading ability [J]. *Biotechnology for Biofuels*, 7(1):2.
- [10] Sun J, Peng R H, Xiong A S, Tian Y, Zhao W, Xu H, Liu D T, Chen J M, Yan Q H. Secretory expression and characterization of a soluble laccase from the *Ganoderma lucidum* strain 7071-9 in *Pichia pastoris* [J]. *Molecular biology reports*, 2012, 39(4):3807-3814.
- [11] Ma X, Liu L, Li Q, Liu Y, Yi L, Ma L, Zhai C. High-level expression of a bacterial laccase, CueO from *Escherichia coli* K12 in *Pichia pastoris* GS115 and its application on the decolorization of synthetic dyes [J]. *Enzyme Microbial Technology*, 2017, 103: 34–41.
- [12] Muneer M, Saeed M, Bhatti I A, Haq A, Khosa M K, Jamal M A, & Ali, S. Radiation induced degradation of Congo red dye: a mechanistic study [J]. *Nukleonika*, 2019, 64(2): 49–53.
- [13] Mester T, Field J A. Characterization of a Novel Manganese Peroxidase-Lignin Peroxidase Hybrid Isozyme Produced by *Bjerkandera* Species Strain BOS55 in the Absence of Manganese [J]. *Journal of Biological Chemistry*, 1998, 273(25):15412-15417.