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Genomic and molecular mechanisms for efficient biodegradation of aromatic dye

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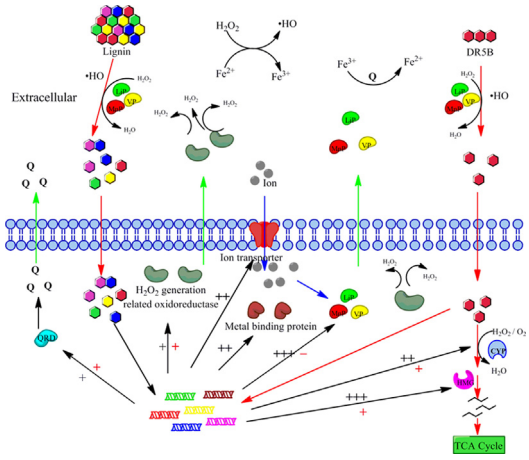
HIGHLIGHTS

- Azo dye bio-degradation was significantly improved in presence of lignin.
- Omics analysis revealed efficient dye degradation relied to synergistic enzyme network.
- Manganese peroxidase plaid significant roles on dye degradation by *I. lacteus* CD2.
- This study reported the first genomic characterization of the species *I. lacteus*.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the molecular mechanisms for aromatic compound degradation is crucial for the development of effective bioremediation strategies. We report the discovery of a novel phenomenon for improved degradation of Direct Red 5B azo dye by *Irpex lacteus* CD2 with lignin as a co-substrate. Transcriptomics analysis was performed to elucidate the molecular mechanisms of aromatic degradation in white rot fungus by comparing dye, lignin, and dye/lignin combined treatments. A full spectrum of lignin degradation peroxidases, oxidases, radical producing enzymes, and other relevant components were up-regulated under DR5B and lignin treatments. Lignin induced genes complemented the DR5B induced genes to provide essential enzymes and redox conditions for aromatic compound degradation. The transcriptomics analysis was further verified by manganese peroxidase (MnP) protein over-expression, as revealed by proteomics, dye decolorization assay by purified MnP and increased hydroxyl radical levels, as indicated by an iron reducing activity assay. Overall, the molecular and genomic mechanisms indicated that effective aromatic polymer degradation requires synergistic enzymes and radical-mediated oxidative reactions

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to form an effective network of chemical processes. This study will help to guide the development of effective bioremediation and biomass degradation strategies.

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1. Introduction

Synthetic aromatic dyes are widely used for paper printing and textile dyes with more than 7×10^5 t of annual global consumption. Azo dyes are the most widely used, yet they are also toxic to human, livestock and wildlife. Considering the strong toxicity, the bioremediation of azo dye with bacteria and fungi was extensively studied as an environmentally friendly, sustainable, and cost-effective approach for broad industrial applications [1]. Among the different microorganisms, white rot fungi belonging to basidiomycetes are distinct from other species for their outstanding capacity to degrade a large variety of recalcitrant pollutants [2,3]. More importantly, some white rot fungal strains are also highly tolerant to toxic compounds, making them uniquely fit for the remediation of azo dyes [4]. Despite the potential, one of the major challenges of aromatic compound bioremediation is the limited understanding of the molecular mechanisms and environmental factors related to dye degradation.

Advanced "omics" technologies have been widely applied to study the mechanisms for various biological processes [3,5–8]. Previous omics and chemical studies revealed a diverse group of enzymes and pathways involved in aromatic compound degradation. The degradation process normally involves both depolymerization and subsequent aromatic compound catabolism by microorganisms. Laccase, peroxidase, and many other enzymes are involved in the oxidation of the lignin macromolecule to depolymerize it into oligomers or monomers [5,8,9]. The non-enzymatic Fenton reaction complements enzymatic processes to further degrade the lignin macromolecule [5,8,9]. To understand the mechanisms of dye and lignin degradation, the patterns of these enzymes and their potential impacts on the chemical path of degradation need to be mapped out. In addition, the downstream aromatic compound catabolic pathways need to be characterized. Even with all the accumulated information, it is still unclear which pathways are important and how these pathways are regulated for dye degradation. Our discovery of enhanced azo dye Direct Red 5B (DR5B) (Fig. 1A) decolorization by lignin provides a unique and effective system to dissect the mechanisms for both aromatic compound degradation and lignin catabolism.

In our study, we first quantified the enhanced decolorization of azo dye DR5B by the white rot fungus *Irpex lacteus* CD2 when alkali lignin was added as a co-substrate. Based on the analysis, comparative transcriptomics analysis was then carried out to elucidate the molecular and genomic mechanisms for both dye degradation and aromatic compound catabolism. The molecular mechanisms were further elucidated by proteomics analysis, an iron reducing activity assay, and the dye decolorization by MnP. The molecular and genomic mechanisms added new perspectives to our current understanding of aromatic compound degradation and could lead to new strategies to improve both dye remediation and lignin degradation.

2. Materials and methods

2.1. Strain and chemicals

The white rot fungus *I. lacteus* CD2 was previously studied and characterized for its highly efficient lignin degradation system [10]. The strain was originally isolated from rotted wood samples in Shennongjia Nature Reserve (Shennongjia, China) and maintained on potato dextrose agar (PDA) plates. The dye Direct Red 5B (CAS: 2610-11-9) has been commonly used in the textile industry and is widely used as a model compound for azo dyes in biodegradation research [11,12]. The dye was purchased from Colorfran S.A. (Monterrey, Mexico). The alkali lignin (CAS: 8068-05-1) in this study was purchased from Sigma–Aldrich (St. Louis, Missouri, United States).

2.2. DR5B decolorization and lignin co-metabolism

Six 5 mm disks of *I. Lacteus* CD2 mycelium were cut from PDA plates and transferred to 100 mL modified Kirk medium [13] in 250-mL Erlenmeyer flasks. The fungus was incubated at 28 °C with a shaking speed of 120 rpm before inoculation for decolorization treatments. The dye decolorization medium was prepared by adding 150 mg L^{-1} DR5B to Kirk medium supplemented with 1% glucose as an additional carbon source in the presence or absence of 0.03 g L^{-1} sterilized alkali lignin. Alkali lignin was sterilized using ultraviolet light for 30 min. A 10.0% (v/v) inoculum of fungus was then added and cultivated in cotton-plugged 250-mL flasks containing 100 mL of the aforementioned decolorization medium for six days. Experiments were performed in triplicate biological experiments. Mycelia-free supernatant was taken periodically for measurement of dye decolorization.

The maximum absorption wavelength of DR5B was determined by absorption spectrum scanning (190–800 nm) using an UV–vis spectrophotometer. The decrease in absorbance was monitored using the spectrophotometer at the wavelength 510 nm.

2.3. Dye adsorption assay by fungal mycelia and lignin

The DR5B adsorption rate by alkali lignin was measured by tracking the changes of OD_{510} with the UV–vis Spectrophotometer in the same medium without fungus. The physical dye adsorption capacity of fungal mycelia during the DR5B decolorization process was detected by the following method: fungal mycelia from the dye decolorization medium were collected periodically by centrifugation at 7000 rpm for 15 min and then soaked in 70% (v/v) methanol for 24 h to recover the adsorbed dye. The recovered dye was measured by an UV–vis Spectrophotometer at 510 nm.

2.4. Total RNA extraction and sequencing

The fungal mycelium for RNA extraction was collected from *I. lacteus* CD grown in the following four different conditions after 72 h: Kirk medium only (CK), Kirk medium with 100 mg L^{-1} DR5B (CK + DR5B), Kirk medium with 0.03 g L^{-1} alkali lignin (CK + lignin), and Kirk medium with 100 mg L^{-1} DR5B and 0.03 g L^{-1} alkali lignin (CK + DR5B + lignin). Glucose was supplemented in all of the growth conditions to a final concentration of 1% as an additional carbon source. The fungal mycelia during the dye decolorization was harvested and immediately washed with RNase-free water, dried with a paper towel, and then ground in liquid nitrogen to a fine powder. RNA was extracted according to the manufacturer's manual for filamentous fungi in the Rneasy Plant Mini Kit (Qiagen Inc., TX, USA); the RNA concentration and quality were measured by bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) after RNA