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Purification, biochemical characterization and dye decolorization capacity of an alkali-resistant and metal-tolerant laccase from *Trametes pubescens*

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HIGHLIGHTS

- A novel laccase (Tplac) from white rot fungus *Trametes pubescens* was purified and characterized.
- Tplac performed better catalytic efficiency toward ABTS with k_{cat}/K_m at 8.34 s^{-1} μM^{-1}.
- Tplac was highly stable and resistant under alkaline conditions.
- Tplac was intrinsically highly metal-tolerant by enhancing the affinity toward substrate.
- Tplac could degrade and detoxify dyes used in textile industries.

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ABSTRACT

Extracellular laccase (Tplac) from *Trametes pubescens* was purified to homogeneity by a three-step method, which resulted in a high specific activity of 18.543 U mg^{-1}, 16.016-fold greater than that of crude enzyme at the same level. Tplac is a monomeric protein that has a molecular mass of 68 kDa. The enzyme demonstrated high activity toward 1.0 mM ABTS at an optimum pH of 5.0 and temperature of 50 °C, and under these conditions, the catalytic efficiency (k_{cat}/K_m) is 8.34 s^{-1} μM^{-1}. Tplac is highly stable and resistant under alkaline conditions, with pH values ranging from 7.0 to 10.0. Interestingly, above 88% of initial enzyme activity was maintained in the presence of metal ions at 25.0 mM, leading to an increase in substrate affinity, which indicated that the laccase is highly metal-tolerant. These unusual properties demonstrated that the new fungal laccase Tplac has potentials for the specific industrial or environmental applications.

1. Introduction

Laccase (benezenediol: oxygen oxidoreductase, EC 1.10.3.2), the most abundant member of the multicopper protein family, is widely distributed in plants, fungi, insects, and bacteria (Claus, 2004). This protein contains four histidine-rich copper binding domains, which coordinate copper atoms types I–III that differ in their environment and spectral properties (Thurston, 1994). The enzyme can catalyze the oxidation of an array of substrates, such as mono-, di-, and polyphenols, aromatic amines, methoxyphenols, and ascorbate through a one-electron transfer. The oxidation is coupled to the reduction of oxygen to H_2O (Thurston, 1994). Furthermore, laccase is of particular interest with regards to various commercial applications because of its ability to oxidize a wide range of reaction capabilities and relevant substrate specificities. Thus, research concerning laccase is being carried out in various fields of interest: textile, pulp and paper, food, and cosmetics industries, as well as in bioremediation, biosensor, biofuel, and organic synthesis applications (Arora and Sharma, 2010). To date, more than 100 laccases have been isolated from different microorganisms. However, most of these laccases are 'common' with a lower yield of enzymatic activity and tolerance to extreme conditions (Kim et al., 2012). This reduced performance hampers their large-scale commercial and industrial use for most applications. Therefore, it is necessary to search for novel laccases with higher yields of activity and versatile properties.

Global industrialization has resulted in the release of large amounts of potentially toxic compounds into the biosphere (Gomi et al., 2011). Among these compounds, dye-containing effluents represent highly problematic wastewaters due to their higher chemical (COD) and biochemical oxygen demand (BOD), suspended solids, and the content of toxic compounds, as well as their color, which makes them easily recognized and poses esthetic

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