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Review

Fungal laccases

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ABSTRACT

Laccases are enzymes widely distributed in plants, fungi, bacteria, and insects. They are multicopper oxidases that catalyze the transformation of aromatic and non-aromatic compounds with reduction of molecular oxygen to water. These enzymes participate in processes such as biosynthesis and lignin degradation, morphogenesis, and pigment biosynthesis, among others. In this review we discuss relevant aspects of fungal laccases regarding the existence of fungal laccases gene families, the growing interest in investigating mechanisms of their molecular regulation, and factors that influence the production of laccases, due to their potential biotechnological applications. In addition we comparatively analyzed some structural similarities and differences depicting general features of laccases' active site, demonstrating their frequency as monomeric proteins with highly conserved cupredoxine type domains. Although inter- and intra-specific differences have been determined, structural differences encountered between fungal laccases remain unclear based on Crystallography and X-ray diffraction.

1. Introduction

Yoshida (1883) was the first to describe laccase in latex obtained from the tree *Rhus vernicifera*. A decade later, the enzyme was isolated and purified by Bertrand, who reported its mechanism of action (Bertrand, 1894; Yoshida, 1883). This type of activity was attributed exclusively to higher plants and fungi. However, it is now recognized that laccases are almost

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ubiquitous enzymes, since they have been isolated from plants, fungi (*Ascomycetes*, *Basidiomycetes* and *Deuteromycetes*), prokaryotes, and arthropods (Giardina *et al.*, 2010).

Laccases have been isolated from insects, where their main function is during sclerotization processing for epidermal cuticle synthesis (Nakamura and Goa, 2005; Sakurai and Kataoka, 2007). In bacteria laccases have several functions such as morphogenesis processes, copper homeostasis, pigment biosynthesis (such as melanin and brown spore pigment), and spore protection against UV light and hydrogen peroxide (Santhanam *et al.*, 2011; Strong and Claus, 2011). Furthermore, fungal laccases are involved in sporulation, pigment production, fruit body formation, and plant pathogenesis (Alcalde, 2007). The white rot *Basidiomycetes* are known for its efficient lignin, cellulose, and hemicellulose decomposition and transformation into carbon dioxide (Baldrian, 2006). Consequently, *Basidiomycetes* are a widely studied fungus.

At present, more than 100 laccases from *Basidiomycetes* and *Ascomycetes* fungi have been purified and characterized. Laccase purification from plant crude extracts is complex, and for this reason it has not been studied extensively (Strong and Claus, 2011).

Laccases (EC 1.10.3.2), also named p-diphenol: dioxide oxidoreductases are blue multicopper oxidases (MCOs) that have the ability to catalyze the oxidation of a wide variety of organic aromatic compounds, concomitantly with the reduction of molecular oxygen to water (Ruiz-Dueñas and Martínez, 2009; Sakurai and Kataoka, 2007). Although most laccase substrates are phenolic compounds (ortho and para-diphenols, methoxy-substituted phenols, polyphenols, aromatic amines, benzenethiols, hydroxindols, 1-naphthol, syringaldazine) enzyme activity can be extended to non-phenolic compounds by use of mediators like ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and HBT (1-hydroxybenzotriazole) (Solís-Oba *et al.*, 2005).

Addition of mediators extend the use of laccases for industrial processes related to bioremediation; including delignification of lignocellulosics, color removal and detoxification of industrial dyes, bioremediation of xenobiotic compounds, pesticides, explosives, wastewater treatment, and treatment of other pollutants such as polycyclic aromatic hydrocarbons (PAHs), (Desai and Nityanand, 2011). Other substrates that can also be reduced by laccases include inorganic/organic metal compounds, such as Mn^{2+} and Fe $(EDTA)^{2-}$ (Thurston, 1994).

Oxidases and oxidoreductases are classified in more than 200 types, but only six classes (cytochrome-c oxidase, laccases, L-ascorbate oxidase, ceruloplasmin, bilirubin oxidase, and phenoxazinone synthase) have the ability to catalyze this type of oxygen reaction (Nakamura and Goa, 2005). It is difficult to categorize laccases based on the substrate reduced, due to the wide range of compounds that can be catabolized. Laccases have the property to change their redox potential (E^0), thus substrate type can change from one laccase to another (Giardina *et al.*, 2010; Strong and Claus, 2011).

General enzymology of laccases has been discussed in numerous reviews and several of them describe the broad range of biotechnological applications (Desai and Nityanand, 2011; Kunamneni *et al.*, 2008a, 2008b; Majeau *et al.*, 2010; Rodríguez Couto and Toca Herrera, 2006; Shraddha *et al.*, 2011; Singh Arora and Kumar Sharma, 2010; Widsten and Kandelbauer, 2008). In particular fungal laccases use has increased in recent years for certain biotechnological processes, since they do not use hydrogen peroxide in their catalytic process. In addition, they present high stability, and can be used in an immobilized form (Loera Corral *et al.*, 2006).

This review offers an overview of what is known about fungal laccase's molecular characteristics, as well as some details of the structural diversity of emzyme's crystallography, their heterologous expression and biotechnological applications.

2. Main reactions of laccases

During laccase's catalytic process, different free radical reactions result, depending on structure and reaction conditions. The most frequent reactions are coupling of free radicals that generate dimeric products or polymeric compounds and oxidative carboxylations. The oxidation of substrates is coupled to reduction of molecular oxygen; generating two water molecules. For each oxygen reduced, four molecules of substrate are oxidized without hydrogen peroxide production: $4H^+ + 4\ \text{substrate} + O_2 \rightarrow 2H_2O + 4\ \text{substrate}^+$ (Solomon *et al.*, 2008). Consequently, laccases are considered "ideal green" catalysts because they employ O_2 as a co-substrate and generate H_2O as a byproduct (Fig 1).

3. Characteristics of laccases

One of the fundamental characteristics of these enzymes is the direct relation of their redox potential (E^0) with the energy required to remove an electron from the reducer substrate. In fact, the catalytic behavior of laccases on most reducer substrates depends on the electron acceptor: E^0 Cu T1 (Wong, 2009; Xu *et al.*, 1998). Thus, laccases with greater E^0 T1 display a special interest for biotechnology, due to their greater potential to oxidize substrates with greater E^0. This is the case for

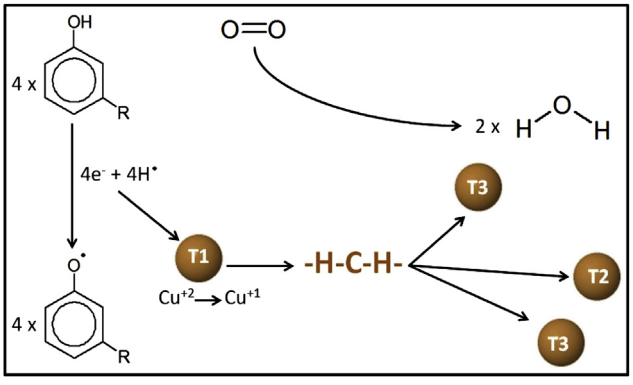

Fig 1 – Laccase catalytic cycle. Substrates are oxidized by the Cu-T1 center and electrons are transferred by a highly conserved motif: His-Cys-His (HCH) to the T2 and T3 copper centers. This is where reduction of molecular oxygen to water takes place. Figure modified from (Baldrian, 2006). In the scheme, copper atoms appear in brown, and were based on in the 1GYC structure (laccase-2 of *Trametes versicolor*).