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Topical Perspectives

Comparative modeling and molecular docking analysis of white, brown and soft rot fungal laccases using lignin model compounds for understanding the structural and functional properties of laccases

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ABSTRACT

Extrinsic catalytic properties of laccase enable it to oxidize a wide range of aromatic (phenolic and non-phenolic) compounds which makes it commercially an important enzyme. In this study, we have extensively compared and analyzed the physico-chemical, structural and functional properties of white, brown and soft rot fungal laccases using standard protein analysis software. We have computationally predicted the three-dimensional comparative models of these laccases and later performed the molecular docking studies using the lignin model compounds. We also report a customizable rapid and reliable protein modelling and docking pipeline for developing structurally and functionally stable protein structures. We have observed that soft rot fungal laccases exhibited comparatively higher structural variation (higher random coil) when compared to brown and white rot fungal laccases. White and brown rot fungal laccase sequences exhibited higher similarity for conserved domains of *Trametes versicolor* laccase, whereas soft rot fungal laccases shared higher similarity towards conserved domains of *Melanocarpus albomyces* laccase. Results obtained from molecular docking studies showed that aminoacids PRO, PHE, LEU, LYS and GLN were commonly found to interact with the ligands. We have also observed that white and brown rot fungal laccases showed similar docking patterns (topologically monomer, dimer and trimer bind at same pocket location and tetramer binds at another pocket location) when compared to soft rot fungal laccases. Finally, the binding efficiencies of white and brown rot fungal laccases with lignin model compounds were higher compared to the soft rot fungi. These findings can be further applied in developing genetically efficient laccases which can be applied in growing biofuel and bioremediation industries.

1. Introduction

Laccase (EC 1.10.3.2) is highly studied commercially important enzyme representing the major subgroup of multicopper oxidase (MCO) family, widely distributed among bacteria (prokaryotes), fungi and plants (eukaryotes) [1]. The function of laccases varies widely based on their host organisms, in plants it is involved in lignin biosynthesis, where as in fungi and bacteria it is involved in lignin degradation [1,2]. It was first discovered in the sap of plants (*Rhus vernicifera*) [3] and later it was demonstrated in fungi [4]. Other enzymes belonging to multicopper oxidases (copper containing enzymes) family are ferroxidase (EC 1.16.3.1), ascorbate oxidase (EC 1.10.3.3), ceruloplasmin monoxygenases, dioxygenases and various manganese oxidases [5]. Multicopper oxidase family enzymes usually found to contain one to six copper atoms per molecule, with the aminoacids ranging between 100–1000 per a single peptide chain [5,6]. Laccases are characterized by the presence of four catalytic copper atoms: the T1 copper site and the T2/T3 trinuclear copper cluster [7]. Substrate oxidation occurs at the T1 copper due to its high redox potential (up to +800 mV). The one electron substrate oxidation is coordinated with the four electron reduction of molecular oxygen at the T2/T3 cluster; oxidation of four substrates is necessary for complete reduction of molecular oxygen to water [7].

Laccases extensively uses the redox ability of copper ions for oxidation of various aromatic substrates concomitantly reducing the molecular oxygen to water [2,8]. Laccases directly oxidize ortho, *para*-diphenols, aminophenols, polyphenols, polyamines, aryl diamines and also some inorganic ions [2]. The use of the laccase mediator system allows for oxidation of non-phenolic compounds and substrates too large to bind to the active site [9–12]. A mediator is a low molecular weight compound (acting as elec-

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tron shuttle) with higher redox potential than the T1 copper (>900 mV) [13]. The most common laccase mediators used are 2,2′-azino-bis (3-ethybenzthizoline-6-sulfonic acid) (ABTS) and triazole 1-hydroxybenzotriazole (HBT). The mediator is initially oxidized at the T1 site, generating a strong oxidizing intermediate, which then diffuses out of the active site and oxidizes the substrate [13]. In this way, the laccase mediator acts as an electron transport shuttle. Laccases typically show low substrate-specificity, and the range of substrates oxidized can vary between laccases. Oxidizing ability of laccases also depends on the nature of substrate whether it is monomeric, dimeric, or tetrameric [13]. Possible substrates of laccases include polyphenols, methoxy-substituted phenols, aromatic amines, and ascorbate [14].

The comparative modeling of fungal and bacterial laccases was reported in the past, however studies on fungal laccases typically focused on white rot basidiomycetes due to the extrinsic lignolytic abilities. Rivera-Hoyos et al., reported the three dimensional (3D) homology models of white rot fungal (*Ganoderma lucidum* and *Pleurotus ostreatus*) laccase proteins, which revealed the laccase interactions with ABTS [15]. The 3D homology models of white rot fungi *Pycnoporus cinnabarinus* [16], *Lentinula edodes* [17], were reported earlier. Tamboli et al. has compared physio-chemical properties of bacterial and fungal (*Cryphonectria parasitica, Ganoderma lucidum, Phomopsis liquidambaris, Pycnoporus coccineus and Trametes sanguine*) laccases and generated the 3D comparative models of bacterial and fungal laccase proteins which can be used for molecular docking studies [18]. Molecular docking studies with fungal laccases were performed using various chemical substrates such as ABTS [15] and also with lignin model compounds such as sinapyl alcohol, dimer, trimer and tetramer [19] were reported earlier. However, studies comparing the structural and functional properties of white, brown and soft-rot fungal laccases were not been reported till today. As these fungi exhibit differential wood decaying properties white rot (can efficiently degrade lignin, cellulose and hemicellulose), brown rot fungi (efficient cellulose, hemicellulose degrading with lignin modifying) and soft rot fungi (exhibits partial decaying abilities). It would be interesting to understand the structural and functional differences among the laccases of these fungi.

In our present study, we have reported the three-dimensional homology models of the selected white, brown and soft fungal laccase protein sequences retrieved from public repositories and extensively discussed about their structural and functional properties using standard tools. Using a set of lignin model compounds (monomers, dimer, trimer and tetramer) we have performed the molecular docking experiments. Results obtained in our study demarcates the structural and functional properties of white, brown and soft rot fungi and highlights the significant aminoacids which are involved in its catalysis. These results can be further applied for designing and developing recombinantly efficient laccases having wide range applications in clinical, chemical, environmental and industrial sectors.

2. Materials and methods

2.1. Selection and retrieval of laccase protein sequences

Laccase protein sequences of six different fungi viz., *Phlebia brevispora* HHB-7030 SS6 v1.0 [20], *Dichomitus squalens* CBS463.89 (White rot), *Fomitopsis pinicola* FP-58527 SS1 [21], *Wolfiporia cocos* MD-104 SS10 [21] (Brown rot) and *Chaetomium globosum* v1.0 [22], *Cadophora* sp. DSE1049 (Soft rot), were retrieved from JGI (Joint Genome Institute) MycoCosm database. The *D. squalens* CBS463.89 and *Cadophora* sp. DSE1049 laccase protein sequences (Dsqual_59186 and Cadophora_560981) were produced by the "US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/in collaboration with the user community". We have used CAZy (Carbohydrate active enzymes), KOG (Eukaryotic orthologous groups) and GO (Gene Ontology) tools of JGI MycoCosm database during the retrieval of laccase protein sequences. Initially, we have retrieved a total of 56 laccase protein sequences (*P. brevispora* (5), *D. squalens* (12), *F. pinicola* (6), *W. cocos* (4), *C. globosum* (6) and *Cadophora* sp. (22)) respectively, from JGI MycoCosm database. All the retrieved laccase protein sequences from each organism was queried through BLAST against protein data bank (PDB) database using PSI-BLAST algorithm a variation of BLAST (sensitive to low-similarity, provides biologically relevant sequences and three times faster than regular BLAST) [23]. Laccase protein sequences showing highest sequence similarity and query coverage was designated as the template for the comparative modeling studies.

2.2. Phylogenetic analysis

All the retrieved laccase protein sequences of each organism were aligned using ClustalW algorithm (fast, accurate, and robust method, which uses a residue comparison matrix and position specific gap penalties to align sequences) of MEGA v7 software [24]. The ClustalW aligned sequences were considered for the construction of phylogenetic trees using Neighbour Joining method and Bootstrap resampling of 1000 replicates parameters were used for the estimation of phylogenetic tree topologies [25]. The phylogenetic trees were constructed for both intra and inter organism level to determine the laccase target sequences which are closely related to the template during the evolution.

2.3. Physico-chemical properties of selected laccases

Physico-chemical properties of above selected laccase protein sequences were determined using the ExPASy ProtParam tool [26]. Our analysis included the parameters such as aminoacid composition, number of positively (+R) and negatively (-R) charged aminoacid residues, predicted molecular weight, theoretical isoelectric point (pI), extinction coefficient (EC) [27], instability index (Ii) [28], aliphatic index (Ai) [29] and GRAVY (grand average hydropathicity) [30].

2.4. Structural and functional properties of laccases

The above selected laccase protein sequences were studied for their structural and functional properties for which we have used SOPMA (Self-optimized prediction method with alignment) tool for determining secondary structure elements [31]. We have used Motif Scan web server to identify the well-known motif sequences using the motif sources such as PeroxiBase, HAMAP, PROSITE patterns and profiles, More profiles, Pfam HMM (both local and global) profiles [32]. To understand the cellular localization of selected laccases, the protein sequences were subjected CELLO v2.5 web server [33]. We have used EDBCP (Ensemble-based Disulfide Bonding Connectivity Pattern) for understanding the presence of cysteine residues and to predict the most possible disulfide (S—S) bonds [34]. To predict the location and presence of signal peptide cleavage sites the protein sequences of laccases were analyzed using SignalP v4.1 web server [35]. And to predict the presence of transmembrane helices we have analyzed the selected protein sequences using TMHMM v2.0 web server (http://www.cbs.dtu.dk/services/TMHMM/). Acetylation of the selected fungal laccase proteins are assessed using the NetAcet v1.0 web server [36].