

# Homology Modelling and *in silico* Characterization of Laccase from *Lentinula edodes*

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## ABSTRACT

**Introduction:** Laccases are phenol oxidases which belong to the superfamily of multicopper oxidases. Laccases are found in almost all wood-rotting fungi. There is evidence that laccases can play an important role in lignin degradation, fruiting body formation, pigment formation during asexual development, competitor interactions and pathogenesis. Laccase from *Lentinula edodes* is used in variety of applications like to reduce toxicity, partial decolorization of effluent water and decolorization of chemically different dyes like Remazole brilliant blue R, bromophenol blue, methyl red and naphthol blue black. The objectives of this study include prediction of three-dimensional (3D) structure of laccase from *Lentinula edodes* using homology modelling, *in silico* characterization and analysis of laccase from this organism using computational methods. **Methods:** The sequence of laccase from *Lentinula edodes* was retrieved from UniProt database and sequence analysis was carried out using BLAST for the selection of template. The protein 3D structure was modelled using ModWeb server. The obtained 3D model of the laccase from *Lentinula edodes* was visualized and analyzed using RasMol. The quality of the 3D structure of protein was verified by its energy and stereochemical properties. The erred regions were remodelled by loop modelling using SWISS PDB viewer. Further, the *in silico* characterization of the laccase from *Lentinula edodes* was computed. **Results:** The 3KW7 A of Trametes

Sp AH28-2 is used as template for model building of laccase from *L. edodes*. The atom model obtained in PDB format showed unstable region in the model. These unstable regions were selected and remodelled by loop modelling. The remodelled structure was further evaluated by its stereochemical quality and energy. The quality of the remodelled structure was found to be improved. **Conclusion:** Evaluated 3D structure of laccase from *L. edodes* shows that predicted model was of good quality because maximum residues are present in favoured region which indicates that stereochemical quality of predicted 3D structure was reasonably good. It suggests that this model can be used to understand molecular interaction of this laccase with the other proteins.

**Key words:** Laccase, *Lentinula edodes*, Homology modelling, *In silico*, BLAST, MODWEB, Swiss PDB Viewer.

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## INTRODUCTION

Laccases are copper containing oxidase enzymes found in many plants, fungi and micro-organisms. Laccases were also found in various basidiomycetous and ascomycetous fungi.<sup>1</sup> The first report of a bacterial laccase was from the Gram-negative soil bacterium *Azospirillum lipoferum*<sup>2</sup> and the enzyme was believed to be involved in melanisation.<sup>3</sup> These enzymes can be used for textile finishing or textile dyeing, teeth whitening and also it has industrial, environmental, diagnostic and synthetic uses.<sup>4</sup> Laccases can also be used in bioremediation.<sup>5</sup> *Lentinula edodes* (shiitake) is one of the world's second largest cultivated medicinal and edible mushrooms used as functional foods. It is used in the treatment of tumors, flu, heart diseases, high blood pressure, obesity, problems related to sexual dysfunction and ageing, diabetes, liver ailments, respiratory diseases, exhaustion and weakness.<sup>6</sup> *L. edodes* is considered to be one of the most valuable medicinal mushrooms.<sup>7,8</sup> The experimental methods to determine the protein 3D structure like X-ray crystallography, nuclear magnetic resonance spectroscopy are technically demanding, time consuming and may not keep with which new protein sequences are being discovered by genomics research. Although a large number of genes are being discovered, the number of protein structures being solved by experimental methods is limited.

Alternative strategies for structure prediction and modelling of proteins are computational methods. The major computational methods for predicting the structure of proteins are *ab initio* methods and homology

modelling. Homology modelling remains the most accurate prediction method.<sup>9</sup> It helps to bridge the gap between the available sequences and structural information by providing reliable and accurate protein models. Homology modelling is a technique for predicting or generating detailed 3D structures of proteins based on coordinates of known homologues.

The main steps to create a Homology model are as follows: 1) Identification of structural homologues. 2) Selection of structural homologues used as templates for modeling. 3) Alignment of templates with the protein sequence to be modelled. 4) Model building. 5) Evaluation and refinement of the model.

The objectives of this present study are to predict the three-dimensional (3D) structure of laccase from *Lentinula edodes* using homology modelling, *in silico* characterization and analysis of laccase from this organism using computational methods.

## MATERIALS AND METHODS

### Retrieval of laccase sequence of *Lentinula edodes* from UniProt database

The sequence details of the laccase from *Lentinula edodes* was retrieved from UniProt database. The UniProt Knowledgebase (UniProtKB) is the central hub for the collection of functional information on proteins, with accurate, consistent and rich annotation.<sup>10</sup>

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## Identification of Template

For getting the homologous templates, BLAST<sup>11,12</sup> was used. In bioinformatics, Basic Local Alignment Search Tool (BLAST) is a sequence similarity search program that can be used via a web interface or as a stand-alone tool. There are several types of BLAST to compare all combinations of nucleotide or protein queries with protein databases. BLAST is a heuristic method that finds short matches between two sequences and attempts to start alignments from these hotspots and also provides statistical information to help decipher the biological significance of the alignment.<sup>13</sup>

## Secondary structure prediction

SOPMA was used for secondary structure prediction of laccase from *L. edodes*. SOPMA (Self - Optimized Prediction Method with Alignment) is an improvement of SOPM method.<sup>14</sup> SOPMA correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database containing 126 chains of non-homologous (less than 25% identity) proteins.<sup>15</sup>

## Model building

For building the models, ModWeb server was used.<sup>16</sup> ModWeb is a comparative modelling webserver for protein structure modelling. ModWeb accepts one or many sequences in the FASTA format and calculates their models using ModPipe based on the best available templates from the Protein Data Bank (PDB). ModWeb includes a database of annotated comparative protein structure models, containing models for more than 3.8 million unique protein sequences. ModWeb is developed in the laboratory of Andrej Sali at UCSF.<sup>17</sup> ModWeb models are also accessible through the Protein Model Portal.

## Loop modelling

Modelling of the errored loops in laccase structure from *L. edodes* was carried out using Swiss-PDB Viewer ver 4.1. Swiss-PDB Viewer is an application that provides a user-friendly interface that allows the analysis of several proteins at the same time. These regions were selected using control panel and suitable loop was selected from the database which assures the stability of the selected loop including the overall structure.<sup>18</sup>

## Visualization of the model

The modelled 3D structure of laccase from *L. edodes* was visualised and analysed using Rasmol.<sup>19</sup> RasMol is a computer program written for molecular graphics visualization and used mainly to depict and explore biological macromolecule structures, such as those found in the Protein Data Bank. It was originally developed by Roger Sayle in the early 1990s.

## Evaluation of the model

The evaluation of the modelled 3D structure for laccase from *L. edodes* was carried out using Verify 3D program. The three-dimensional (3D) profile of a protein structure is a table computed from the atomic coordinates of the structure that can be used to score the compatibility of the 3D structure model with any amino acid sequence. Three-dimensional profiles computed from correct protein structures match their own sequences with high scores. An incorrectly modelled segment in an otherwise correct structure can be identified by examining the profile score in a moving-window scan.<sup>20,21</sup> The stereo-chemical quality of the modelled laccase structure from *L. edodes* was analyzed by Ramachandran plot<sup>22</sup> using the software RAMPAGE.<sup>23</sup>

## Computation of Physical and Chemical Properties of Laccase from *L. edodes*

The physical and chemical properties of laccase from *L. edodes* was computed by ProtParam,<sup>24</sup> a tool which allows the computation of

various physical and chemical parameters of a given protein sequence. The computed parameters include the molecular weight, theoretical pI, extinction coefficient, estimated half- life, instability index, aliphatic index and grand average of hydropathicity.

## RESULTS

### Sequence analysis

The sequence of the laccase from *L. edodes* was retrieved from UniProt KB, which is given in FASTA format (Figure 1). This laccase sequence consists of 524 aa and it has a mass of 55,357 Da.

### Identification of template

The sequence which is obtained from the Uniprot KB was used as the input in BLAST-P server to find out the suitable template for the model building. It was seen that the Lac b from *Trametes* sp AH28-2 (3KW7A) had 63% identity among the homologous sequences resulted from the BLAST-P server. It shows the strongest match with the laccase from *L. edodes*. Resolution of this laccase structure is 3.44 Å. Sequence length is 502.

### Secondary structure prediction of target protein

The secondary structure of laccase from *L. edodes* was predicted by the improved self-optimized prediction method (SOPMA). The protein sequence of the laccase was given as input and four conformational states, including helices, sheets, turns and coils were analyzed. The predicted secondary structure of the laccase from *L. edodes* is shown in Figure 2.

### Model building

The refined sequence-sequence alignment as obtained by BLAST- P (Figure 3) was used to construct 3D model of laccase from *Lentinula edodes* using MODWEB server.

### Model evaluation

A full atom model in PDB format of the laccase from *Lentinula edodes* was obtained by ModWeb. The model generated from ModWeb was submitted to Verify 3D program and the graph was obtained (Figure 4). Analysis of the graph revealed that some of the regions in the model were not stable and such regions corresponded to the regions of insertion and deletion. These regions (Table 1) were considered for Loop modelling. The stereochemical quality of the model was evaluated using Ramachandran plot (Figure 5).

While loop modelling, for each loop region (Table 1) anchor residues were carefully selected and the loop database of SPDBV was scanned. Of the loops obtained from the database, one was selected on its stereo chemical compatibility and its side chains interaction with the rest of the structure. Loops selected were added to the model one at a time and all the selected loop regions were remodelled. After remodelling the loop regions, the model was subjected to energy minimization using Swiss PDB Viewer.

```
>tr[E1CGD5][E1CGD5_LENED Laccase lcc6 OS=Lentinula edodes
GN=lcc6PE=3SV=1MNFVTALPLIAQLIGTALAAIGPVTDLHVVNKFIQPDGFNRSYI
LAEGVFPGLISGNKGDNFQINVINELTNDTMLLSTSIHWHGLFQGGTNWADGPAF
INQCPAAGNSFLYNFNVPDQAGTFWYHSHLATQYCDGLRGLPVVYDPQDPYLSL
YDVEDDSTVITLSDLYHVPAPLIVGAATSDATLINGLGRYTNGPATAPLAVISVTLG
KRYRFRLVISICEPNFVFSIDGHTFTVIEVDGVSHDPVVADSIQIFASQRYSFVLNAN
QIVGNYPWIRANPSVGTGTGFTGGINSAILRYVGPVADPVTVSTISINPLETSLHPLV
SPGAPGSATLSGVDVLDLRLVLGFNAGSELVNGVKFVPPVTVLLQILSGATTAASL
LPAGSVYTLPLNSTIQLSFDASAVAAIGGPHPFHLHGHNFVDVVRPAGSTTYNYANPI
RRDTVSTGAATDNVTIRFTTNNAGPWFLHCHIDWHLEAGFAIVLAEDAPGVASAN
PTTDAWNDCPIYDALTSaelGGGGGPTS
```

**Figure 1:** Laccase sequence obtained from UniPort KB in FASTA format of from *Lentinula edodes*.



**Extinction coefficients** Extinction coefficients are in units of  $M^{-1} \text{ cm}^{-1}$ , at 280 nm measured in water.

Ext. coefficient 64080

Abs 0.1% (=1 g/l) 1.158, assuming all pairs of Cys residues form cystines

Ext. coefficient 63830

Abs 0.1% (=1 g/l) 1.153, assuming all Cys residues are reduced

**Estimated half-life** The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hr (mammalian reticulocytes, *in vitro*).

>20 hr (yeast, *in vivo*).

>10 hr (*Escherichia coli*, *in vivo*).

**Instability index** The instability index (II) is computed to be 25.78

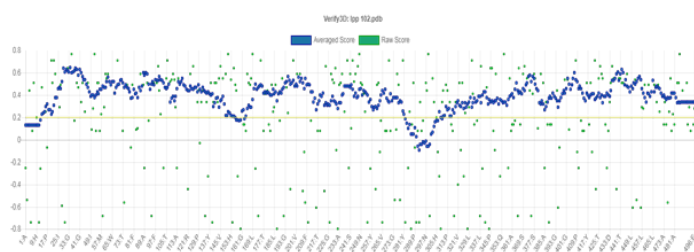
This classifies the protein as stable.

**Aliphatic index:** 97.71

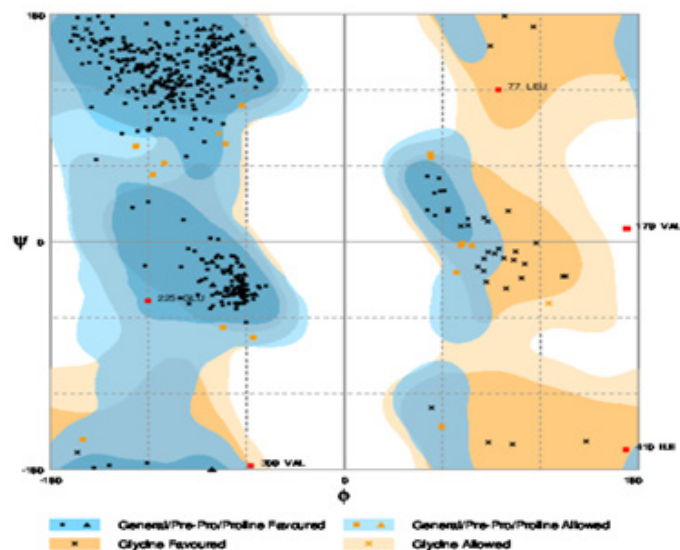
**Grand average of hydropathicity (GRAVY):** 0.230

## DISCUSSION

Laccases have the ability to synthesize the products which may be highly valuable from pharmaceutical point of view. In pharmaceutical industry, laccases have two major roles- either they are directly involved in the synthesis of new medicinally valuable compounds by different synthesis methods or applicable for the bioremediation of by-products



**Figure 4:** Verify 3D graph of laccase from *L. edodes* before loop modelling.

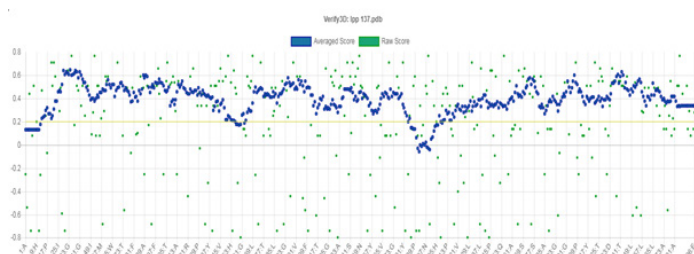


**Figure 5:** Ramachandran plot of laccase from *L. edodes* before loop modelling.

Number of residues in favoured region (~98.0% expected): 469 (94.9%)

Number of residues in allowed region (~2.0% expected): 20 (4.0%)

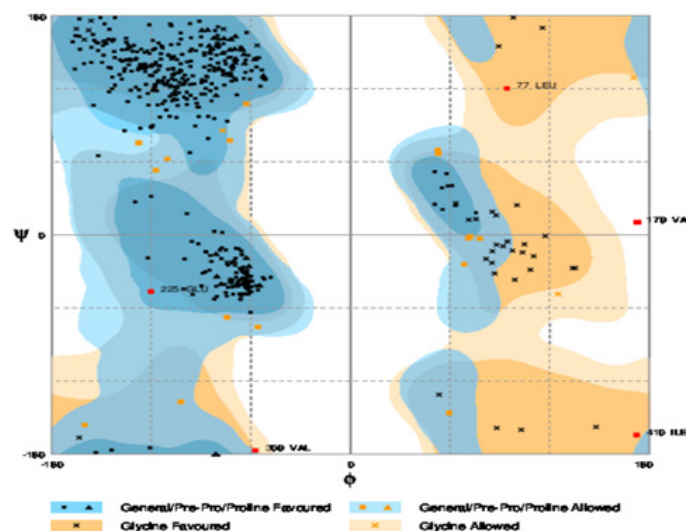
Number of residues in outlier region: 5 (1.0%)



**Figure 7:** Verify 3D graph of laccase from *L. edodes* after loop modelling.



**Figure 6:** 3D structure of laccase from *L. edodes* after loop modelling as viewed in RasMol.



**Figure 8:** Ramachandran plot of laccase from *L. edodes* after loop modelling.

Number of residues in favoured region (~98.0% expected): 470 (95.1%)

Number of residues in allowed region (~2.0% expected): 19 (3.8%)

Number of residues in outlier region: 5 (1.0%)

**Table 1: Regions selected for loop modelling.**

Loop number	Amino acid residue
1	290 – 293
2	293 – 296
3	296 – 300

or hazardous organic pollutants released from the pharmaceutical industries.<sup>25</sup> Mikolasch *et al.* synthesized the different penicillins through novel coupling process using laccase.<sup>26</sup> Laccases may be valuable in cancer treatment. In recent years, laccases from different sources have been used in the development of new anticancer medicines and inhibition of proliferation of different types of cells. Authors have discussed prospective roles of laccases in the above mentioned applications.<sup>27,28</sup> In addition, laccases have been utilized in deactivation study of HIV and hepatitis C virus that are responsible for acquired immunodeficiency syndrome (AIDS) and hepatitis C respectively.<sup>29,30</sup> Hosny *et al.* utilized the laccase of *R. vernicifera* in the formation of two new catechin- hydroquinone adducts.<sup>31</sup> These catechins have antioxidant activities and also act as free radical scavenger due to which they have protective roles against cancer, inflammatory and cardiovascular diseases.<sup>32,33</sup> Lateef *et al.* synthesized the silver nanoparticles (AgNPs) using crude laccase obtained from an edible mushroom *Lentinus edodus*.<sup>34</sup> According to the report, silver nano particles have been utilized in burns treatment, as dental materials, treatment of water, textile fabrics and sunscreen lotions.<sup>35</sup> In addition, Erb- Downward *et al.* studied the role of laccase in the production of prostaglandin by *C. neoformans*.<sup>36</sup>

In this study, the sequence of the laccase from *L. edodes* was retrieved from UniProt KB in FASTA format (Figure 1). BLAST- P server was used to identify the template for laccase from *L. edodes*. The Lac b from *Trametes* sp AH28-2 (3KW7A) with 63% identity was used as template. The secondary structure was predicted by the improved self- optimized prediction method (SOPMA). The results revealed that the proportion of random coils,  $\beta$  turns,  $\alpha$  helices and extended strands ( $\beta$  folds) accounted for 36.26%, 13.17%, 18.32% and 32.25% of the secondary structure, respectively (Figure 2).

The refined sequence – sequence alignment as obtained by BLAST- P (Figure 3) was used to construct 3D model of laccase from *Lentinula edodes* using MODWEB server. The model generated from ModWeb was submitted to Verify 3D program and the graph was obtained (Figure 4). Analysis of the graph revealed that some of the regions in the model were not stable and such regions corresponded to the regions of insertion and deletion. These regions were considered for Loop modelling (Table 1). The stereo chemical quality of the model was evaluated using Ramachandran plot (Figure 5). The number of residues in favoured region was 469 (94.9%), the number of residues in allowed region was 20 (4.0%) and the number of residues in outlier region was 5 (1.0%).

Each of the selected loops (Table 1) was remodelled using Swiss PDB Viewer. After loop modelling (Figure 6), verify 3D graph (Figure 7) and stereo chemical quality of the structure by Ramachandran plot (Figure 8) were analyzed. The regions of the troughs in the graph (Figure 4) were found to be improved. The number of residues in favoured region is 470 (95.1%), the number of residues in allowed region is 19 (3.8%) and the number of residues in outlier region is 5 (1.0%). These numbers indicate that the stereo chemical quality of predicted 3D structure is reasonably good. In addition, the analysis of physical and chemical properties of the laccase from *L. edodes* was carried out using ProtParam tool.

## CONCLUSION

Laccase from *L. edodes* has important applications like lignin degradation, pigment biosynthesis, fruiting body formation, detoxification, morphogenesis, as well as pathogenesis. It is also used in hair colouring systems, it has many other agricultural, industrial and medicinal applications. In this study, we have reported the three- dimensional homology model of the laccase from *L. edodes* I, the sequence of which retrieved from Uniprot KB database. The results indicate that the stereo chemical quality and verify 3D profile of the laccase model is reasonably good. It suggests that this modelled protein can be used to understand the structural and functional properties of laccase from *L. edodes*. This enzyme has potential for the synthesis of several useful drugs such as anticancerous, antioxidants, synthesis of hormone derivatives because of its high value of oxidation potential. Hence, understanding the three-dimensional structure of this enzyme by the above study will enable us to explore the options of above-mentioned drugs. In addition, this modelled protein can also be helpful in exploring the molecular interactions of this laccase with the other proteins.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**3D:** Three Dimensional; **UniprotKB:** Uniprot Knowledgebase; **BLAST:** Basic Local Alignment Search Tool; **SOPMA:** Self- Optimised Prediction Method with Alignment; **PDB:** Protein Data Bank; **SPDBV:** Swiss PDB Viewer; **GRAVY:** Grand Average of hydropathicity; **HIV:** Human Immuno Virus; **AIDS:** Acquired immune deficiency syndrome; **AgNps:** Silver Nanoparticles.

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