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Homology Modeling and *In silico* Approach for Identification of Probable Substrates for the Enzyme Laccase from *Pleurotus ostreatus* for Bioremediation

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Phenol oxidase (benzenediol O_2:oxidoreductase [EC 1.10.3.2], namely, Laccase) *Pleurotus ostreatus* catalyzes the oxidation of various aromatic compounds (mono-, di, and polyphenols, aminophenols, and diamines) by reducing molecular oxygen to water through an oxidoreductive multicopper system. It is one of the well studied enzyme used for bioremediation of xenobiotics. Its broad substrate specificity offers a wide opportunity for screening pollutants inorder to predict potentical targets for degradation. Present study utilizes protein – ligand docking as tool to achieve it. Homology modeling of the enzyme Laccase from *Pleurotus ostreatus* has been performed based on the best hits from NCBI BLAST against PDB database. The crystal structures of Laccase of *Trametes versicolor* (PDB ID 1GYC_A) *and Rigidoporus lignosus* (PDB ID 1KYA_A, 1V10_A) from Protein Data Bank were identified as suitable templates which revealed high level of sequence identities respectively. A comparative assessment of secondary structure using SSM web server revealed greater percentage of residues in beta sheets. A three-dimensional model was generated using MODELLER9v7 based on multiple templates (PDB ID's 1GYC_A, 1KYA_A, 1V10_A). Protein sequence alignment was performed using CLUSTALW 2.0.8. With the aid of molecular mechanics method using force field AMBER the final model was obtained. Inspection and analysis of the final model was made by PROCHECK, VERIFY3D graph, PROVE Program. After the prediction of 3-dimensional model of Laccase, the possible active site of Laccase was determined using CASTp web server. The three dimensional structures of ligands (substrates and pollutants) including all hydrogen atoms, were built and minimized using the Dundee PRODRG2 Server. Toxicity studies were carried out using the web server OSIRIS Property Explorer in which the Mutagenic, Tumorigenic irritation and reproductive effects of the ligands were analysed. The Docked complexes were validated based on the GOLD Scoring function to pick out the best substrates for Laccase, suggesting that it might be able to oxidize these pollutants.

Keywords: Laccase, Homology Modeling, Docking, Toxicity risk.

Abbreviations: ABTS, 2,20-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid; AMBER, assisted model building with energy refinement; E.C., enzyme commission number; GOLD, genetic optimisation for Ligand docking; NCBI, National Center for Biotechnology Information; PDB, Protein Data Bank; RMSD, root mean square deviation, SSM, Secondary Structure Matching; CASTp, Computed Atlas of Surface Topography of protein.

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Laccase (p-diphenol: oxygen oxido reductases (EC 1.10.3.2)) from *Pleurotus ostreatus (P.ostreatus)* belongs to a group of polyphenol oxidases containing copper atoms in the catalytic centre which are usually called multicopper

oxidases. Laccase is a dimeric or tetrameric glycoprotein, which usually contains four copper atoms per molecule of enzyme (monomer) distributed in three different copper binding (redox) sites. These are crucial for numerous reactions in cell like pathogenicity; immunity and morphogenesis of organisms and in the degradation of various aromatic compounds (e.g.nitroaromatics, chloroaromatics, polycyclic aromatics, biphenyls and components of oil) with the eventual goal of exploiting their metabolic potential for the bioremediation of contaminated sites[1-3].

The copper atoms allow the proteins to perform electron transfer reactions, because copper atoms are able to switch their oxidation states between Cu^{1} and Cu^{n}. Therefore, many researchers have studied both the degradation as well as the removal of environmental pollutants by these enzymes. Among the blue copper oxidases, laccases are a sub class of comparatively broader substrate specificity enzymes known to degrade several xenobiotics such as phenols, anilines, benzenethiols, etc.[4]. Consequently, laccases have evoked particular interest in biotechnological applications, ranging from biopulping[5] to remediation of wastewater[6]. Most of these enzymes are extra cellular in nature. In some species, they occur as isozymes in both extra- and intracellular environments.

Laccases have been reported in fungi [7], in plants[8] and in bacteria[9]. The catalytic properties of Laccase have had a great impact on the development of biosensors. Advances in research have widened the variety of xenobiotics that can be degraded by laccases from simple phenols, anilines and benzenethiols to polycyclic aromatic hydrocarbons, and organophosphorus insecticides[10]. Their role in synthesis and/or degradation of the biopolymer lignin is well known. X-ray crystal structure studies over the past decade have enabled the elucidation of a significant number of structural and functional aspects of these enzymes. Due to their comparatively broader substrate specificity, Laccases share a sequence pattern that can distinguish them as a specific subgroup of multi-copper oxidase family[11]. As the substrate specificity differs from one Laccase to other, Laccase from different sources can be utilized for degrading different pollutants. Although docking has been successfully used for drug screening[12].

Three Dimensional Structure of the enzyme Laccase from *P.ostreatus* is not available in the public domain databases as predicted by X – ray crystallography and NMR. An attempt has been made to predict the three dimensional structure of the same using homology modeling**.**

MATERIAL AND METHODS

Protein family and domain Prediction

The protein sequence of Laccase from *P. ostreatus* (accession number: Q12729) was retrieved from Swissprot database. The positions of the protein functional family and domains of *P.ostreatus* Laccase were predicted using PFAM[13] and INTERPROSCAN[14].

3D model building

The query sequence of *P. ostreatus* Laccase was searched to find out the related protein structures to be used as templates by the NCBI BLAST (Basic Local Alignment Search Tool)[15] program against PDB[16]. Sequences that showed maximum identity with high score and less *E*-value were aligned and were used as reference structures to build 3D model for *P.ostreatus* Laccase. A model of *P.ostreatus* Laccase was generated using multiple templates[17]. The initial model of *P.ostreatus* Laccase was built by using homology-modeling methods and the MODELLER 9v7 [19]. The parameters set for MODELLER 9v7 is shown in Table 1. The pdfs restrain C^{α}–C^{α} distances, main-chain N–O distances, and main-chain and side-chain dihedral angles. Hetero atoms (Cu) present in the reference structure (template with highest similarity) were transferred into the generated model by setting the program '*env.io.hetatm=True*' (Table 2). The 3D model of a protein was obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non hydrogen atoms. The co-ordinates for the structurally conserved regions (SCRs) for *P.ostreatus* Laccase were assigned from the templates using multiple sequence alignment

(using ClustalW program [18]) as shown in Fig.1. Energy minimization of the developed model was performed using AMBER force field[20]. A comparative assessment of secondary structure was done by using protein structure comparison service Secondary structure Matching (SSM)[30]. The final structure obtained was analyzed by Ramachandran plot using PROCHECK (a program to check the stereo chemical quality of protein structures)[21], and environment profile using VERIFY-3D (structure evaluation server) [22], and PROVE program [23]. This model was used for the identification of active site and for docking studies.

Binding-site analysis

The Binding-site of *P.ostreatus* Laccase was identified using CASTP server[24]. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities, and the area and circumference of mouth openings. The results obtained were used for protein–ligand docking.

Docking

Nearly 600 phenolic compounds (substrates and pollutants) were selected as ligands for Docking Studies from NCBI Pubchem Database. Three dimensional structures of these compounds were built in mol2 format using the Dundee PRODRG2 Server. Docking of each Ligand with the protein *P.ostreatus* Laccase was performed using GOLD 3.0 [Genetic Optimisation for Ligand Docking][25], which was set to 30 cycles of run. A point was defined representing the center of the active site residues, from the knowledge of the crystal structure of protein (taken from the mean of the X, Y, and Z coordinates of all the active site molecules). From this point, the search was carried out in 10 Å radius (since this was found to be the optimum). The annealing parameters for Van der Waal's and hydrogen bonding were set to 4.0 Å and 2.5 Å respectively.

The parameters used for genetic algorithm were population size (100), selection pressure (1.1), number of operations (1,00,000), number of islands (5), niche size (2), migrate (10), mutate (95) and cross-over (95) (all default values).

Prediction of Toxicity Risk of the compounds used for Docking Studies

OSIRIS Property Explorer[26] a program that calculates very useful parameters like cLogP, solubility, and molecular weight. In the present work Molecular weight, Mutagenic property, tumorigenicity, irritant effects, effects on reproduction and Aqueous solubility (logS Value) predicted results were included in the Table 5.

RESULTS AND DISCUSSION

The homology model of *P.ostreatus* Laccase enzyme was generated using three templates *(PDB ID:* 1GYC_A) from *Trametes versicolor*[27], *(PDB ID:* 1KYA_A) [28] and 1V10_A)[29] from *Rigidoporus Lignosus*. They had a high percentage of sequence identity (66%, 64% and 60%) respectively as shown in Fig 1.

The final model was thus generated using the above mentioned templates as indicated in Fig .2

The amino acids interacting with Cu atoms were observed to be HIS , CYS, ILE, at different positions as indicated in Table 2.

Table 1. Options used in MODELLER 9v7 program

```
alnfile = 'alignment.ali',    # alignment filename
knowns  = '1 GYC, 1KYA, 1V10', # codes of the templates
sequence = 'Laccase')         # code of the target
```

Table 2. Cu atom interactions inside the generated model

S.No	Atom	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4
1	Cu	HIS402	CYS459	HIS464	ILE461
2	Cu	HIS120	HIS405	HIS458	-
3	Cu	HIS73	HIS118	HIS460	-
4	Cu	HIS73	HIS75	HIS405	HIS407

Validation of Laccase Model

Validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The phi/psi angles of 90.5% residues are found to be in favored regions, 9.1% residues are in the additional allowed

Table 3. The RMSD Values of superimposed structures

Structure	RMSD in A°			
	Model	1GYC_A	1KYA_A	1V10_A
Model		0.568A°	0.582A°	0.698A°
1GYC_A	0.568A°		0.402A°	0.696A°
1KYA_A	0.582A°	0.402A°		0.744A°
1V10_A	0.698A°	0.696A°	0.744A°	

Table 4. GOLD average fitness scores for predicted targets and few known substrates. * Marked compounds are known targets

Pubchem CID No.	Common Name or IUPAC Nomenclature of the Compounds	Gold Score
6386307	Thiodicarb	63.37
70424	1-Phenyl-2-trityl diazene	51.09
344999	2,5-bis[(benzyl amino) methyl] benzene-1,4-diol	48.73
91864	tris(2,4,6-trimethyl phenyl) phosphate	48.14
3627083	2-[[2-[(2-hydroxy-4-methyl phenyl)methylamino] ethyl amino] methyl]-5-methylphenol	48.09
8272	Bromo phenol blue*	47.24
10545677	2,6-bis(4-methoxy phenyl)-3,4,5-trimethyl phenol	46.51
11427473	4-(5,6,7-trimethyl-2-phenyl pyrazolo [1,5-a] pyrimidin-3-yl)phenol	45.19
5373218	3-methoxy-5-methyl-2-[(2E,6E)-3,7,11-trimethyl dodeca-2,6,10-trienyl] phenol	44.84
327968	Furanoterpene	43.97
79628	2-[4-(carboxy methyl)-2,5-dihydroxy phenyl] acetic acid	43.47
11826323	3,4,5-trimethyl-2,6-bis(2-phenyl phenyl) phenol	43.32
413487	2-(2H-1,3-benzoxazol-3-ylmethyl)-5-[(cyclo hexyl amino) methyl] benzene-1,4-diol	42.64
11792728	4-[4-(4-hydroxy-2,3,5-trimethyl phenoxy) butoxy]-2,3,6-trimethyl phenol	42.10
3791690	Bromo phenol blue sodium salt*	44.48
10853902	4-[2-(4-hydroxy-2,3,5-trimethyl phenoxy) ethoxy]-2,3,6-trimethyl phenol	41.82
8606	Captan	41.74
5809575	4-[(E)-2-(3,5-dimethoxy phenyl) ethenyl]-2-methoxyphenol	41.65
5375758	[3-methoxy-5-methyl-2-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyl]phenyl] acetate	41.30
11833070	3,4,5-trimethyl-2-(2-phenyl phenyl) phenol	40.66
5468719	Rietone 1	40.10
9932727	2,4,5-trifluoro-6-nitro-3-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienoxy] phenol	39.77
96672	3,6-bis(morpholin-4-yl methyl) benzene-1,2-diol	39.25
5385086	5-[(E)-2-(3,5-dimethoxy phenyl) ethenyl]-2-methoxyphenol	37.96
10631638	(4-hydroxy-2,3,6-trimethyl phenyl) thiocyanate	37.90
5464076	ABTS*	37.27
361118	Trimethyl 3,3',3"-methane triyl tris(6-hydroxy-5-methylbenzoate)	37.60
67929	Trityl fluoride	36.91
10608543	4-chloro-2,3,5-trimethyl-6-prop-2-enylphenol	36.06
67215	leuco malachite green	35.86
10797865	4-benzhydryl-N,N-dimethyl aniline	35.75
10742	syringic acid*	35.10

```
1GYC                    AIGPAASLVVANAPVSPDGF-LRDAIVVNG---------VFPSPLITGKKGDRFQLNVVD 50
1KYA_A                  GIGPVADLTITNAAVSPDGF-SRQAVVVNG---------GTPGPLITGNMGDRFQLNVID 50
sp|Q12729|LAC1_PLEOS    AIGPTGDMYIVNEDVSPDGF-TRSAVVARSDPTTNGTSETLTGVLVQGNKGDNFQLNVLN 59
1V10                    -ATVALDLHILNANLDPDGTGARSAVTAEG---------TTIAPLITGNIDDRFQINVID 50
                            . .: : *  :.***   *.*:....            . *: *: .*.**:**::

1GYC                    TLTNHTMLKSTSIHWHGFFQAGTNWADGPAFVNQCPIASGHSFLYDFHVPDQAGTFWYHS 110
1KYA_A                  NLTNHTMLKSTSIHWHGFFQKGTNWADGPAFINQCPISSGHSFLYDFQVPDQAGTFWYHS 110
sp|Q12729|LAC1_PLEOS    QLSDTTMLKTTSIHWHGFFQSGSTWADGPAFVNQCPIASGNSFLYDFNVPDQAGTFWYHS 119
1V10                    QLTDANMRRATSIHWHGFFQAGTTEMDGPAFVNQCPIIPNESFVYDFVVPGQAGTYWYHS 110
                         *:: .* ::********** *:.  *****:***** ...**:*** **.****:****

1GYC                    HLSTQYCDGLRGPFVVYDPKDPHASRYDVDNESTVITLTDWYHTAARLGPRFPLGADATL 170
1KYA_A                  HLSTQYCDGLRGPFVVYDPNDPAADLYDVDNDDTVITLVDWYHVAAKLGPAFPLGADATL 170
sp|Q12729|LAC1_PLEOS    HLSTQYCDGLRGPFIVYDPSDPHLSLYDVDNADTIITLEDWYHVVAPQNAVLPT-ADSTL 178
1V10                    HLSTQYCDGLRGAFVVYDPNDPHLSLYDVDDASTVITIADWYHSLS---TKAPPAPDTTL 167
                        ************.*:****.**  . ****: .*:**: ****  :   .  *  .*:**

1GYC                    INGLGRSASTPTA-ALAVINVQHGKRYRFRLVSISCDPNYTFSIDGHNLTVIEVDGINSQ 229
1KYA_A                  INGKGRSPSTTTA-DLSVISVTPGKRYRFRLVSLSCDPNYTFSIDGHNMTIIETDSINTA 229
sp|Q12729|LAC1_PLEOS    INGKGRFAGGPTS-ALAVINVESNKRYRFRLISMSCDPNFTFSIDGHSLQVIEADAVNIV 237
1V10                    INGLGRNSANPSAGQLAVVSVQSGKRYRFRIVSTSCFPNYAFSIDGHRMTVIEVDGVSHQ 227
                        *** ** .. .::  *:*:.*  .******::* ** **::****** : :**.*.::

1GYC                    PLLVDSIQIFAAQRYSFVLNANQTVGNYWIRANPNFGTVGFAGGINSAILRYQGAPVAEP 289
1KYA_A                  PLVVDSIQIFAAQRYSFVLEANQAVDNYWIRANPNFGNVGFTGGINSAILRYDGAAAVEP 289
sp|Q12729|LAC1_PLEOS    PIVVDSIQIFAGQRYSFVLNANQTVDNYWIRADPNLGSTGFDGGINSAILRYAGATEDDP 297
1V10                    PLTVDSLTIFAGQRYSVVVEANQAVGNYWIRANPSNGRNGFTGGINSAIFRYQGAAVAEP 287
                        *: ***: ***.****.*::***:*.******:*. *  ** *******:** **.  :*

1GYC                    TTTQTTSVIPLIETNLHPLARMPVPGSPTPGGVDKALNLAFNFNGTN--FFINNASFTPP 347
1KYA_A                  TTTQTTSTAPLNEVNLHPLVATAVPGSPVAGGVDLAINMAFNFNGTN--FFINGASFTPP 347
sp|Q12729|LAC1_PLEOS    TTTSSTST-PLEETNLVPLENPGAPGPAVPGGADININLAMAFDVTNFELTINGSPFKAP 356
1V10                    TTSQNSGT-ALNEANLIPLINPGAPGNPVPGGADINLNLRIGRNATTADFTINGAPFIPP 346
                        **:..:.. .* *.** **    .** ...**.*  :*: :  : *.  : **.:.* .*

1GYC                    TVPVLLQILSGAQTAQDLLPAGSVYPLPAHSTIEITLPATALAPGAPHPFHLHGHAFAVV 407
1KYA_A                  TVPVLLQIISGAQNAQDLLPSGSVYSLPSNADIEISFPATAAAPGAPHPFHLHGHAFAVV 407
sp|Q12729|LAC1_PLEOS    TAPVLLQILSGATTAASLLPSGSIYSLEANKVVEISIP--ALAVGGPHPFHLHGHTFDVI 414
1V10                    TVPVLLQILSGVTNPNDLLPGGAVISLPANQVIEISIP-----GGGNHPFHLHGHNFDVV 401
                        *.******:**. .. .***.*:: .* ::  :**::*      *. ******** * *:

1GYC                    RSAGSTTYNYNDPIFRDVVSTGTPAAGDNVTIRFQTDNPGPWFLHCHIDFHLEAGFAIVF 467
1KYA_A                  RSAGSTVYNYDNPIFRDVVSTGTPAAGDNVTIRFRTDNPGPWFLHCHIDFHLEAGFAVVF 467
sp|Q12729|LAC1_PLEOS    RSAGSTTYNFDTPARRDVVNTGTDAN-DNVTIRFVTDNPGPWFLHCHIDWHLEIGLAVVF 473
1V10                    RTPGSSVYNYVNPVRRDVVSIG--GGGDNVTFRFVTDNPGPWFLHCHIDWHLEAGLAVVF 459
                        *:.**:.**:  *  ****. *  .  ****:** ***************:*** *:*:**

1GYC                    AEDVADVKAANPVPKAWSDLCPIYDGLSEANQ 499
1KYA_A                  AEDIPDVASANPVPQAWSDLCPTYDARDPSDQ 499
sp|Q12729|LAC1_PLEOS    AEDVTSITAP---PAAWDDLCPIYD------- 495
1V10                    AEDIPNIPIANAISPAWDDLCPKYNANN---- 487
                        ***:..:  .   . **.**** *:
```

Fig. 1. Multiple sequence alignment of Laccase from *P. ostreatus* with Laccase of Trametes *versicolor* (PDB ID 1GYC_A) *and Rigidoporus lignosus* (PDB ID 1KYA_A, 1V10_A) done using CLUSTALW server that was subsequently submitted to MODELLER. A hyphen (" - ") indicates a one-residue gap. The conserved regions are indicated by '*'

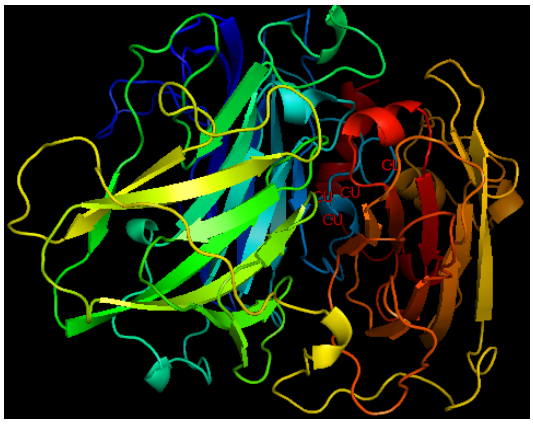
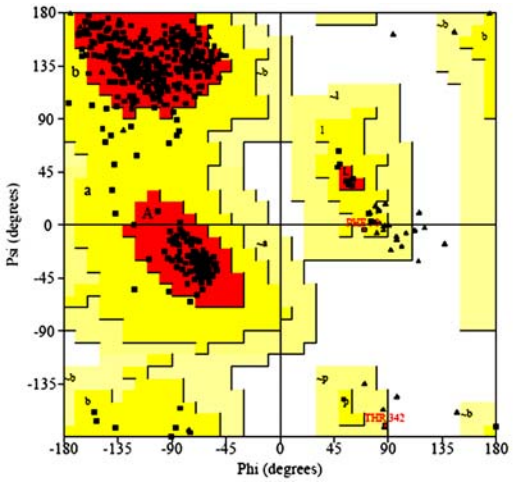


Fig. 2. The final 3D structure of the generated model along with the hetero atoms (Cu) of the enzyme *P. ostreatus* Laccase

regions 0.5% in the generously allowed regions. No Residues are in the disallowed conformations (Fig.3).

The RMSD (Root Mean Square deviation) deviation for covalent bonds relative to the standard dictionary was - 0.32 Å and for the covalent angles was - 0.15 A°. The overall PROCHECK G-factor was - 0.20. The score indicates that the modeled structure is acceptable as value is greater than the acceptable value of –



Plot statistics

Residues in most favoured regions [A,B,L]	379	90.5%
Residues in additional allowed regions [a,b,l,p]	38	9.1%
Residues in generously allowed regions [~a,~b,~l,~p]	2	0.5%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	419	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	39	
Number of proline residues	35	
Total number of residues	495	

Fig. 3. Ramachandran's map of Laccase enzyme built using MODELLER software. The plot calculations on the 3D model of Laccase enzyme were computed with the PROCHECK program

Fig. 4. Secondary structure matching of final model (*P. ostreatus* Laccase) and templates

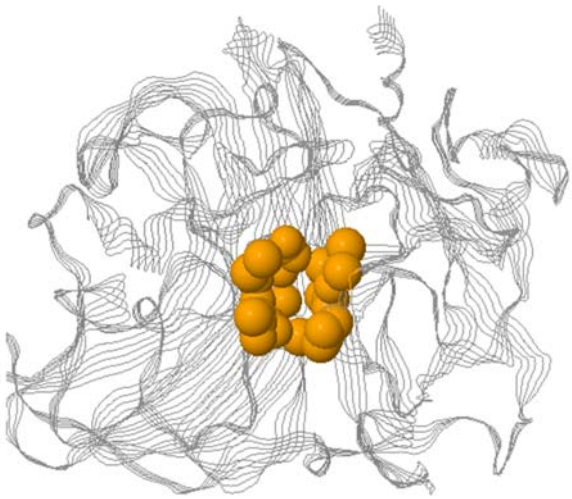


Fig. 5. The Active site of *P.ostreatus* Laccase enzyme from generated homology model

0.50. From the VERIFY_3D analysis, it was found that 82.65% of the residues scored more than 0.2, meaning that 93.75% of the residues complemented with the 1D-3D profile. Analysis of the entire structure calculated from PROVE program gave Z-score RMS of 1.509. Z-score above 4.0 and below - 4.0 represents the occurrence of many errors in the structure in terms of the packing of the buried atoms.

Superimposition and Secondary structure matching of Templates with Laccase Model

Superimposition of the model obtained with the templates and Secondary Structure matching were done by using with the protein structure comparison service (SSM). The structural superimposition of C^{α} trace of templates and Model is shown in Fig. 4 & 5. The weighted root mean square deviation of C^{α} trace between the

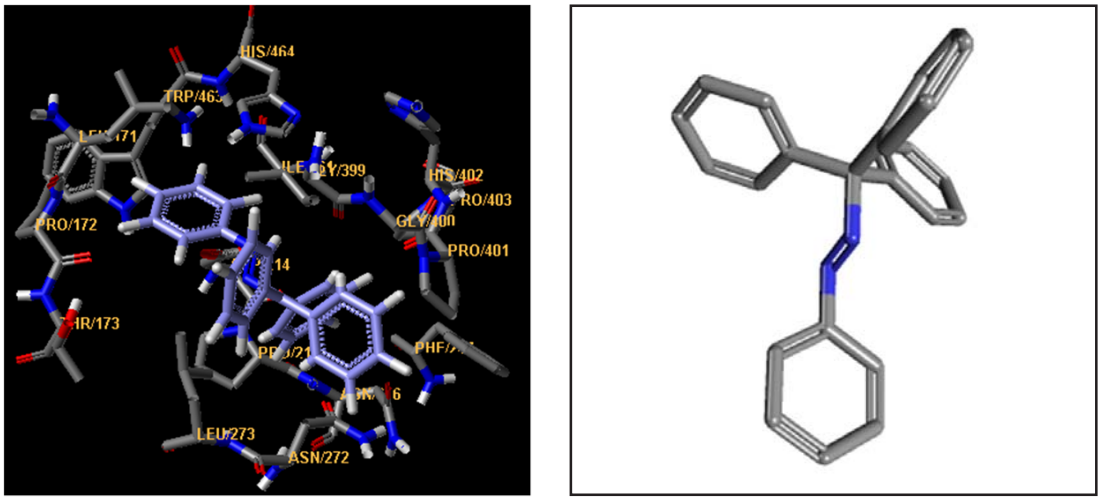


Fig. 7. A. 3D structure of the predicted target (through Hypothesis) 1-Phenyl-2-trityl diazene. B. Binding of 1-Phenyl-2-trityl diazene (predicted target) in the active site of Laccase enzyme. Active site residues are represented in Blue color and the predicted target (1-Phenyl-2-trityl diazene) is represented in white color.

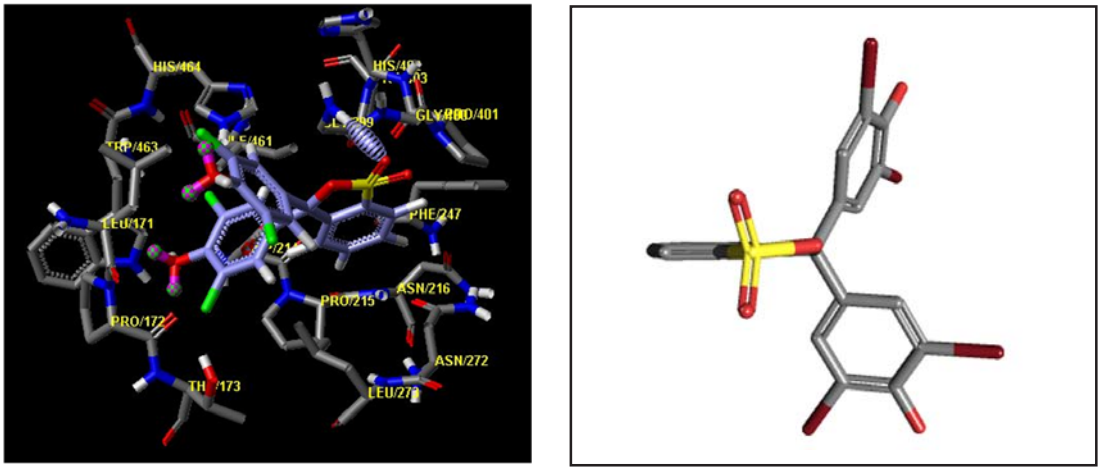


Fig. 8. A. 3D structure of the substrate (Known) Bromophenol Blue. B. Binding of (substrate) Bromophenol Blue in the active site of Laccase enzyme. Active site residues are represented in Blue color and the substrate (Bromophenol Blue) is represented in white color

templates and final refined model were 0.568Å, 0.582 Å, 0.698Å (Table 3). The secondary structures of templates, final Laccase model are found to be highly conserved.

Active site identification of Laccase enzyme

After the final model was built, the possible binding sites of *P.ostreatus* Laccase enzyme were searched using the CASTp Web Server. The binding site obtained was compared with the active site of the template. It was found that residues in the active site , namely LEU171, ALA174, ASP214, PRO215, ASN216, PHE247, ALA248, ASN272, LEU273, GLY399, PRO401, Pro403, ILE461 and HIS464 are conserved as shown in Fig.6.

It was observed that 214Asp and 464His within the defined ligand binding site cavity of *P.ostreatus* Laccase were conserved. These finding correlated with the structural data of 1KYA in which 206Asp and 458His contribute to the ligand binding site of laccase by the formation of hydrogen bonding with ligand 2, 5-xylidine (XYD)[31]. Hence in the present study 214 ASP and 464 HIS were chosen as the most favored site to dock the ligand site.

Table 5. Prediction of Toxicity Risk of the compounds used for Docking Studies

Pubchem CID No of	Molecular weight (g/mol) Activity Solubility the compound	Mutagenic Property effects	Tumourogenic Property	Irritant	Reproductive	Aqueous (logs value)
6386307	354.00000	High Risk	High Risk	Absent	Medium Risk	-3.54
70424	348.43970	High Risk	High Risk	No Risk	No Risk	-5.28
344999	348.40000	No Risk	No Risk	No Risk	No Risk	-3.42
91864	452.52228	Medium Risk	Absent	High Risk	No Risk	-7.64
3627083	300.39536	High Risk	High Risk	Medium Risk	No Risk	-2.7
8272	669.96070	No Risk	No Risk	No Risk	No Risk	-5.42
10545677	348.43486	No Risk	No Risk	No Risk	No Risk	6.56
11427473	329.39506	No Risk	No Risk	No Risk	No Risk	6.09
5373218	342.51486	No Risk	No Risk	Medium Risk	No Risk	4.57
327968	340.45592	No Risk	No Risk	Medium Risk	No Risk	-4.44
11826323	440.57482	High Risk	Medium Risk	Medium Risk	Absent	-10.7
413487	354.44274	High Risk	High Risk	Medium Risk	Absent	4.53
11792728	358.47120	No Risk	No Risk	No Risk	No Risk	4.9
3791690	691.94253	High Risk	No Risk	No Risk	No Risk	-
10853902	330.41804	No Risk	No Risk	No Risk	No Risk	-4.36
8606	300.58932	High Risk	Tumourogenic	No Risk	R.effective	-4.8
5809575	286.32242	No Risk	No Risk	No Risk	High Risk	-3.51
5375758	384.55154	No Risk	No Risk	Medium Risk	No Risk	-4.85
11833070	288.38290	High Risk	Medium Risk	Medium Risk	No Risk	-6.52
5468719	416.50728	No Risk	No Risk	Medium Risk	No Risk	-3.27
9932727	413.43065	No Risk	No Risk	Medium Risk	No Risk	-5.43
96672	308.37280	No Risk	No Risk	No Risk	No Risk	-0.19
5385086	286.32242	No Risk	No Risk	No Risk	High Risk	-3.51
10631638	193.26544	No Risk	No Risk	Medium Risk	No Risk	-2.96
5464076	514.61872	No Risk	No Risk	No Risk	No Risk	-4.05
361118	508.51652	No Risk	No Risk	No Risk	No Risk	-4.08
67929	262.32080	No Risk	No Risk	No Risk	No Risk	-3.74
10608543	210.69990	No Risk	No Risk	No Risk	No Risk	-3.80
67215	330.46594	High Risk	High Risk	No Risk	No Risk	-3.93
0797865	313.34776	Medium Risk	No Risk	No Risk	No Risk	-3.96
10742	198.17270	Mutagenic	No Risk	No Risk	No Risk	-1.37

Docking Results with GOLD:

Docking studies were performed using GOLD 3.0 [Genetic Optimisation for Ligand Docking]. The differences in the best five docking solutions (RMSD < 1.5 A°) GOLD scores are given in Table 4. GOLD results indicate that the substrates and pollutants listed in Table 4 can be oxidized by Laccase which correlated with the experimental data[33,34].

Toxicity Studies

The predicted toxicity risks of the ligands used for docking studies are shown in Table 5. It shows the compound Properties with high risks of undesired effects like mutagenicity, tumorigenicity, irritant effects, and effects on reproduction. Most of the Ligands used for Docking studies have aqueous solubility and the logS Values are greater than -4, when these compounds are used in Research laboratories and industries, they will contaminate the fresh water when they are released in to the water bodies (lakes, ponds, and Rivers), which will affects plants and organisms living in these bodies of water . There will be a high risk for water-related diseases. This work can be considered for determining the putative targets for bioremediation using *P.ostreatus* laccase. After the discovery of oxidizing reaction pollutant range of laccases, their biotechnological importance showed a marked increase[35]. Their importance could be further extended by the use of docking. Similar kind of work would be helpful to find putative pollutants for other biodegradative enzymes.

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