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3D structure prediction of lignolytic enzymes lignin peroxidase and manganese peroxidase based on homology modelling

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ABSTRACT

Lignolytic enzymes have great biotechnological value in biopulping, biobleaching, and bioremediation. Manganese peroxidase (EC 1:11:1:13) and lignin peroxidase (EC 1:11:1:14) are extracellular and hem-containing peroxidases that catalyze H_2O_2-dependent oxidation of lignin. Because of their ability to catalyse oxidation of a wide range of organic compounds and even some inorganic compounds, they got tremendous industrial importance. In this study, 3D structure of lignin and manganese peroxidase has been predicted on the basis of homology modeling using Swiss PDB workspace. The physicochemical properties like molecular weight, isoelectric point, Grand average of hydropathy, instability and aliphatic index of the target enzymes were performed using Protparam. The predicted secondary structure of MnP has 18 helices and 6 strands, while LiP has 20 helices and 4 strands. Generated 3D structure was visualized in Pymol. The generated model for MnP and LiP has Z-score Qmean of 0.01 and -0.71, respectively. The predicted models were validated through Ramachandran Plot, which indicated that 96.1 and 95.5% of the residues are in most favored regions for MnP and LiP respectively. The quality of predicted models were assessed and confirmed by VERIFY 3D, PROCHECK and ERRAT. The modeled structure of MnP and LiP were submitted to the Protein Model Database.

Key words: 3D structure, homology, lignin peroxidases, manganese peroxidases

Introduction

Lignin-degrading enzymes from white-rot fungi have been intensively studied for potential biotechnological applications such as biopulping, biobleaching, and bioremediation (Breen & Singleton, 1999; Hatakka, 2001). Lignin-degrading enzyme system consists of laccases, lignin peroxidases, manganese peroxidases, and manganese-independent peroxidases, and H_2O_2-generating oxidases (Hatakka, 1994). Manganese peroxidase (MnP; EC 1:11:1:13) and lignin peroxidase (LiP; EC 1:11:1:14) were firstly reported in *Phanerochaete chrysosporium* (Tien & Kirk, 1983; Glenn et al., 1983). MnP and LiP are extracellular and hem-containing peroxidases that catalyze H_2O_2-dependent oxidation of lignin (Glenn et al., 1983; Schoemaker, 1990) and related compounds (Kirk et al., 1986; Higuchi, 1985).

Manganese peroxidase is considered one of the key enzymes involved in the lignin degradation caused by white rot fungi. Contribution of the Mn^{2+}/Mn^{3+} redox system as mediator of substrate oxidation is the main feature of catalytic cycle of MnP (Glenn et al., 1987). The Mn^{3+} formed diffuses away from the active site of MnP (Wariishi et al., 1989) and takes part in the oxidation of organic substrates as a redox mediator (Pasczynski et al., 1986). The reducing substrate of MnP and LiP are different (Mn^{2+} and nonphenolic aromatic), even though MnP shows a high sequence resemblance to LiP. LiP characterized by its ability to oxidize veratryl alcohol to veratryl aldehyde in presence of H_2O_2. On the other side, the MnP is unable to oxidize veratryl

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alcohol, which is non phenolic lignin model, because it requires a free phenolic group on the aromatic ring as a substrate and manganese as a cofactor. H_2O_2 is required by MnP to carry out catalytic reaction. Both phenolic and non-phenolic compounds were oxidised by LiP in presence of H_2O_2 (Rai et al., 1997).

Knowledge of 3D structure of laccase, LiP and MnP can aid us to reveal the ambiguity of how lignolytic enzymes have attained such vast functional diversity. The 3D structure of protein is very crucial for experimentally discovering its functionality. The information of 3D structure can be gathered by advance techniques like X-Ray crystallography or NMR spectroscopy. These experimental techniques are very cumbersome and do not always succeed in determining structure for all proteins especially membrane proteins (Johnson et al., 1994). Besides the rate of accumulation of protein sequence data is far more than the structural information available, forming a gap between experimentally solved structures and sequences available. This gap can be reduced with the use of advanced computational tools like homology modeling (Nokthai et al., 2010), docking studies and structure-function activity. Homology modeling methods are based on the fact that evolutionary related proteins share a similar structure, as the existing proteins result from the continuous evolution of previously existing ones. Due to this, proteins can be clubbed together in groups known as families where they share similar folds. If the structure of single member of the family is known, the structure of other members of family can be predicted by a technique termed as 'Homology Modeling'. Thus, models of a protein with unknown structure (target) can be built on the basis of an alignment of known protein structure (template). It involves following steps: (1) identification of homologs, which can be used as template(s) for modeling; (2) alignment of the target sequence to the template(s); (3) building a model for the target based on alignment (s) information and (4) model evaluation. All these four steps are usually repeated until a satisfactory model is obtained (Sanchez & Sali, 1997; Marti-Renom et al., 2000).

Although *Ganoderma lucidium* and *Phlebia radiata* have enormous potential in delignification, till date the 3D structure of MnP and LiP enzymes for these organisms have not been established. The aim of this study was to generate predicted 3D structure of lignolytic enzymes viz lignin and manganese peroxidase by using comparative homology. The homology based modeling of the lignolytic enzyme laccase have been already reported by us (Kale et al., 2015) and the modeled structure of lacasse from *Rigidoporus microporus* was submitted to Protein Model Database (PMDB ID PM0079954). *Ganoderma* sp. has isolated and evaluated its lignolytic potential (data not shown) and deposited in Genebank with accession number KT210090.

Materials and Methods

Retrieval of target sequence

The amino acid sequence of enzyme manganese peroxidase (*Ganoderma lucidum*, ACA48488.1) and lignin peroxidase (*Phlebia radiata*, AAW71986.1) were retrieved from NCBI database in FASTA format. The physicochemical properties of the enzymes were obtained by submitting the sequences to protparam server (Gasteiger et al., 2005). Subcellular localization was predicted by using CELLO v.2.5. (Yu et al., 2004; 2006). Profunc server was used to predict the secondary structure, sequence motifs, structural matches, ligand binding templates and active site template for target enzymes (Laskowski et al., 2005).

Selection of template and 3D model building

Basic Local Alignment Search Tool (BLAST) analysis (Altschul & Gish., 1997) against protein database (PDB) was used to find the template for the enzymes (MnP and LiP). Multiple sequence alignments were performed with ClustalW (Figures 1 and 2). Swiss model server (ProMod Version 3.70.) was used to build of 3D structures by homology modeling under automated mode with energy minimization parameters (Lovell et al., 2002).

Model validation

The RAMPAGE server and ERRAT were used to check quality and reliability of the models (Colovos & Yeates, 1993; Arnold et al., 2006). Prosa-Web server was used for stereo-chemical analysis of protein (Wiederstein & Sippl, 2007). To calculate the interaction energy of the model VERIFY 3D server was used (Eisenberg et al., 1997; Gasteiger et al., 2005). The CASTp (Computed Atlas of Surface Topography of proteins) web tool was used to predict active sites with their respective volume and area. The Combfunc server was used to recognize the likely biochemical function of a protein from its three-dimensional structure (Forslund & Sonnhammer, 2008). PMDB IDs were obtained by submitting predicted protein structures to Protein Model Database (PMDB).

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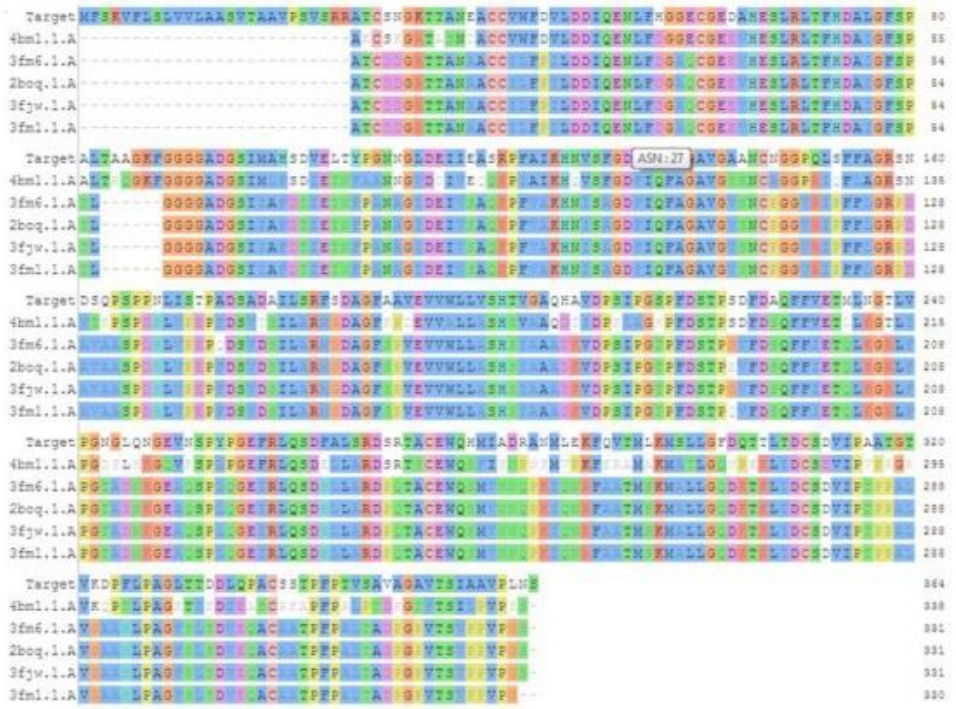

Figure 1. *Pairwise sequence alignment of target manganese peroxidase sequence (ACA48488.1) with PDB entries.*

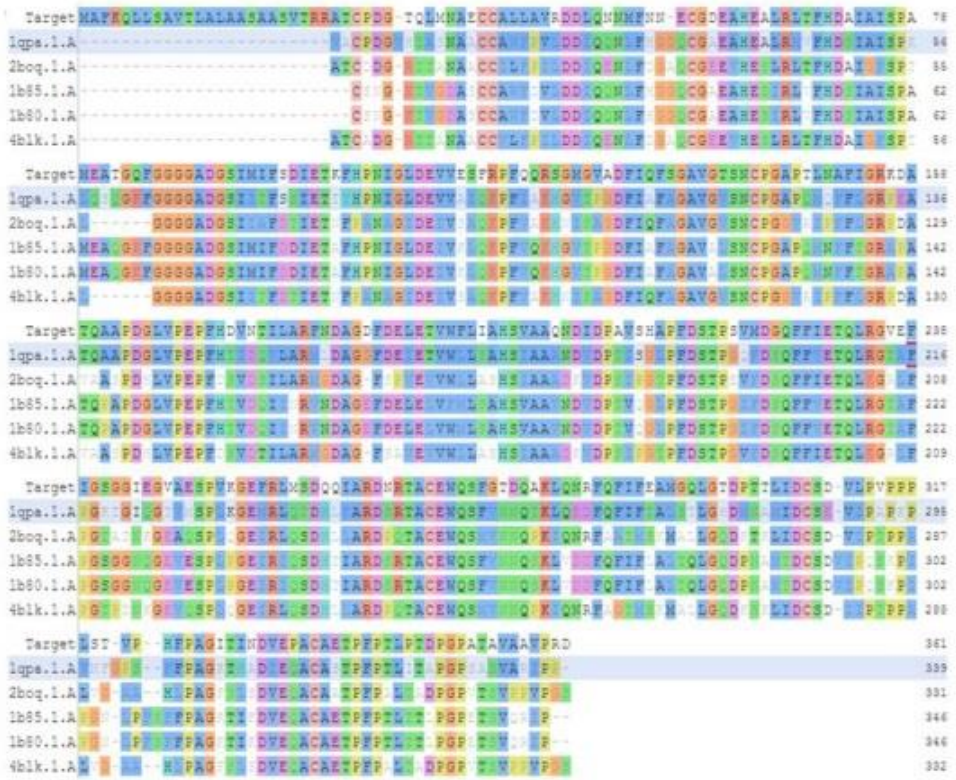

Figure 2. *Pairwise sequence alignment of target lignin peroxidase sequence (AAW71986.1) with PDB entries.*

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Results and Discussion

Manganese peroxidase

The physicochemical properties of the query enzyme (MnP) were obtained using Protparam and results were shown in Table 1. It contains 364 amino acids having molecular weight of 38110.5 Da. MnP has isoelectric point of 4.42 that specifies that enzyme is likely to precipitate in acidic buffers. The highest fungal Lip and versatile peroxidase activity were observed at pH 3 (Martínez et al., 2009). The total number of negatively charged residues (Asp + Glu, 40) was found higher than the total number of positively charged residues (Arg + Lys, 17). It is soluble in hydrophobic solvent (+0.031), which was recognized through GRAVY index. The instability index (Ii) of 43.72 is little higher showing the predicted protein model is slightly unstable. A high aliphatic index (Ai) indicates that a protein may possibly stable over a wide range of temperatures. Subcellular localization prediction of target proteins was carried out using CELLO v.2.5, which revealed that both proteins are extracellular. The predicted secondary structure of MnP from *Ganoderma lucidium* represents 18 helices and 6 strand (Figure 3A). In MnP, ProFunc analysis showed that 7 motifs matched in scan against PROSITE, PRINTS, PFam-A, TIGRFAM, PROFILES and PRODOM motifs, 327 and 50 matching sequences were found in FASTA and BLAST search respectively, 465 significant structural matches, 7 nest located in the structure, 20 ligand binding templates and 2 significant hit out of 584 enzyme active site template were identified. While in gene neighbor analysis, homologus genome location data were not found in both enzymes.

Manganese peroxidase from *Ganoderma lucidum* (ACA48488.1) was used as a query sequences against PDB database in pBLAST. Chain B, *Pleurotus ostreatus* MnP4 (PDB: 4BM1) having 92% of query coverage with 62% of maximum identity was selected as a template molecule for MnP (Table 2). 3D structure of protein helps to understand function and active sites of proteins and also facilitate drug design. As compare to computational methods, X -ray crystallography or NMR spectroscopy are difficult and costly methods (Arnold et al., 2006). In the Swiss Model Workspace, model selection was performed through the value of QMEAN Z-score value. The Z-score is pinpointing of overall model quality and is used to check whether the input structure is within the range of scores typically found for native proteins of similar size (Benkert et al., 2011). QMEAN6 score is a combination of 6 statistical potentials comprising of C-beta atoms for residual level implementation, all-atom energy for capturing the model, solvation energy for burial status of residues and torison angle for local geometry, SSE and ACC (Benkert et al., 2009). The reliability estimates varies between 0 and 1 with higher values for better models. The generated model for MnP has 61.79% sequence identity with template [4BM1] with Z-score QMean of -0.01 (Figure 4A). Ramachandran plots of φ (phi); ψ (psi) pairs were created to evaluate them to a predicted distribution. The backbone conformation of the modeled structure was calculated by analyzing the φ and ψ torsion angles. Rampage derives φ / ψ plots for Gly, Pro, Pre-Pro and other residues (Ramachandran et al., 1963). The predicted models were validated using RAMPAGE through Ramachandran plot.

Table 1. *Physiochemical properties of the enzymes.*

Properties	**Values**	
	Manganese peroxidase from *Ganoderma lucidum*	**Lignin peroxidase from *Phlebia radiata***
Number of amino acids	364	361
Molecular weight	38110.5	38439.0
Theoretical Isoelectric point	4.42	4.29
Formula	$C_{1680}H_{2580}N_{448}O_{536}S_{15}$	$C_{1692}H_{2604}N_{456}O_{535}S_{17}$
Total number of negatively charged residues (Asp + Glu)	40	48
Total number of positively charged residues (Arg + Lys)	17	19
Computed instability index (II)	43.72	44.32
Aliphatic index	78.32	76.32
Grand average of hydropathy (GRAVY)	0.060	-0.047

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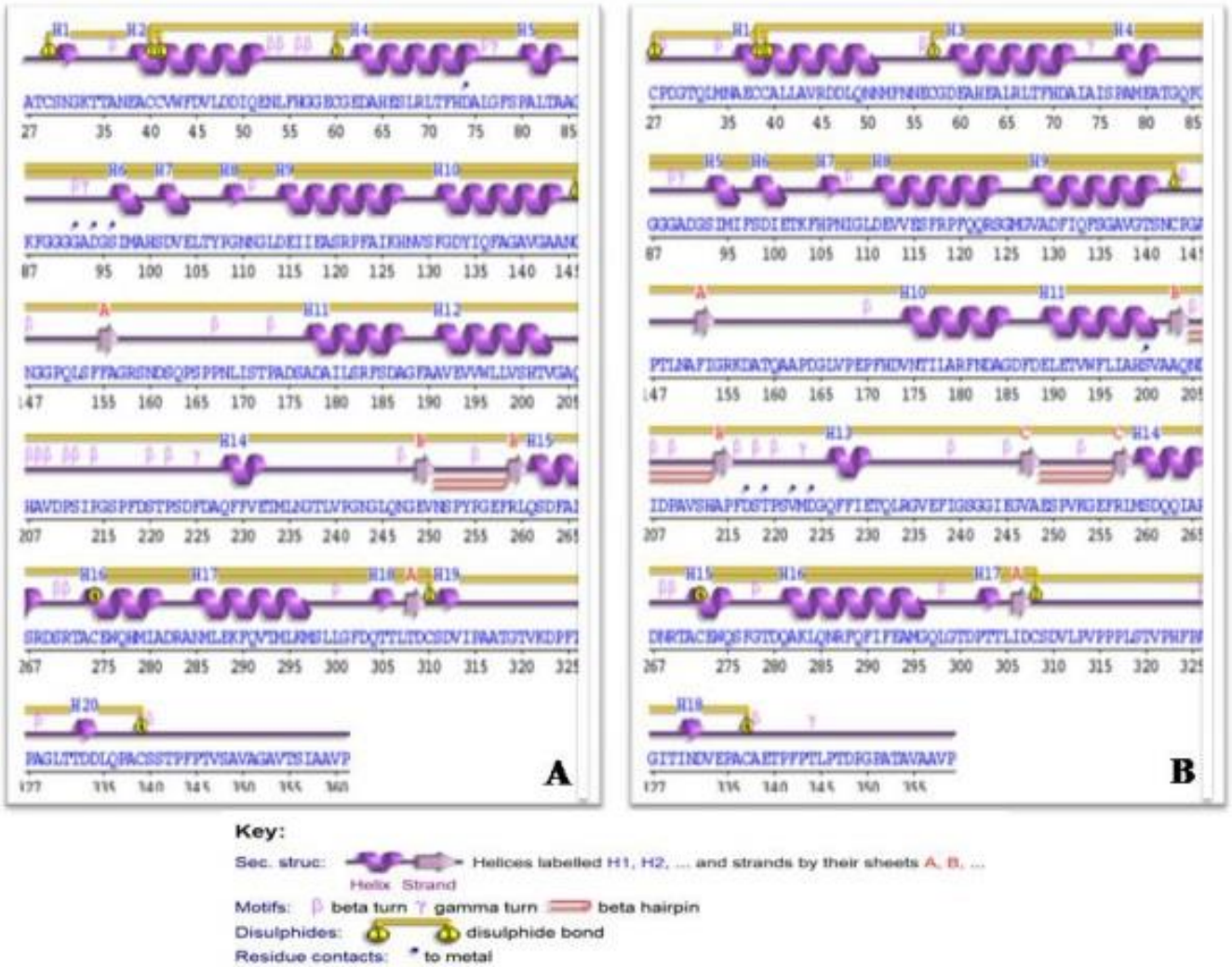

Figure 3. *Secondary structure prediction of manganese peroxidase (**A**) and lignin peroxidase (**B**).*

In case of Ramchandran plot, if a model has more than 90% residues in favorable region, then it is considered as good quality model. Accordingly, model showed 96.1% of the residues in most favored regions, 3.6% residue in allowed region and 0.3% residue in outlier region (Figure 5). ERRAT is a protein structure verification algorithm that analyzes statistics of non-bonded interactions between different atom types based on characteristic atomic interaction (Colovos & Yeates, 1993). The ERRAT overall quality factor value of predicted structure was 84.59, showing the validity of structure (Figure 6A). Prosa Z score for model (-8.68) and template (-8.14) suggest similarity between the two structures (Figure 7A). VERIFY 3D used for assessing and confirmation of quality of predicted model for MnP (98.21%) and reliable results were obtained as shown in Figure 8.1.

From the result of CASTp server, the active sites of the enzymes were identified. MnP have 51 active site covered area about 1010.2Å2 and volume of about 1463.3Å3 (Figure 9a). As per the Combfunc analysis it was found that, *Ganoderma lucidum* MnP performs various functions such as oxidoreductase activity, metal ion binding, L-ascorbate peroxidase activity, catabolism of lignin, hydrogenperoxide, aromatic compound, phenylpropanoid and organic substance.

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Table 2. *PDB sequences that produces significant alignment in BLASTp against Ganoderma lucidum (ACA48488).*

PDB ID	Description	Max score	Total score	Query cover	E value	Identity
4BM1	Chain A, Crystal structure of manganese peroxidase 4 from *Pleurotus ostreatus* - Crystal form I	419	419	92%	3e-145	62%
3FM6	Chain A, Crystal structure analysis of fungal versatile peroxidase from *Pleurotus eryngii*	396	396	92%	2e-136	57%
2BOQ	Chain A, Crystal structure of versatile peroxidase	396	396	92%	3e-136	57%
3FM1	Chain A, Crystal structure analysis of fungal versatile peroxidase from *Pleurotus eryngii*	396	396	92%	3e-136	57%
3FJW	Chain A, Crystal structure analysis of fungal versatile peroxidase from *Pleurotus eryngii*	395	395	92%	4e-136	57%
3FM4	Chain A, Crystal structure analysis of fungal versatile peroxidase from *Pleurotus eryngii*	395	395	92%	4e-136	57%
3FKG	Chain A, Crystal structure analysis of fungal versatile peroxidase from *Pleurotus eryngii*	394	394	92%	9e-136	57%
4BLK	Chain A, Crystal structure of fungal versatile peroxidase I from *P. ostreatus* - crystal form I	394	394	92%	2e-135	57%
3FMU	Chain A, Crystal structure analysis of fungal versatile peroxidase from *Pleurotus eryngii*	390	390	92%	3e-134	56%
4G05	Chain A, Crystal structures of several mutants of *Pleurotus eryngii* versatile peroxidase	386	386	88%	1e-132	57%

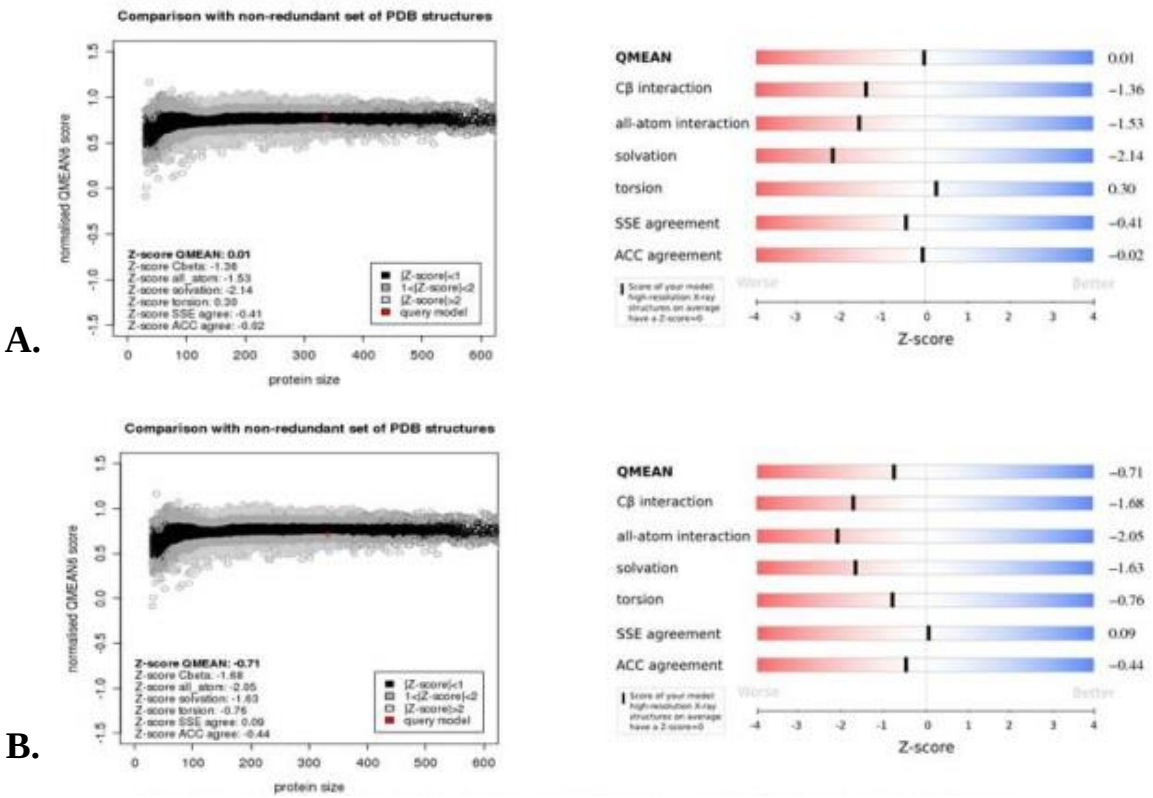

Figure 4. *Estimation of quality of predicted model by calculating Z score Qmean: (**A**) manganese peroxidase and (**B**) lignin peroxidase.*

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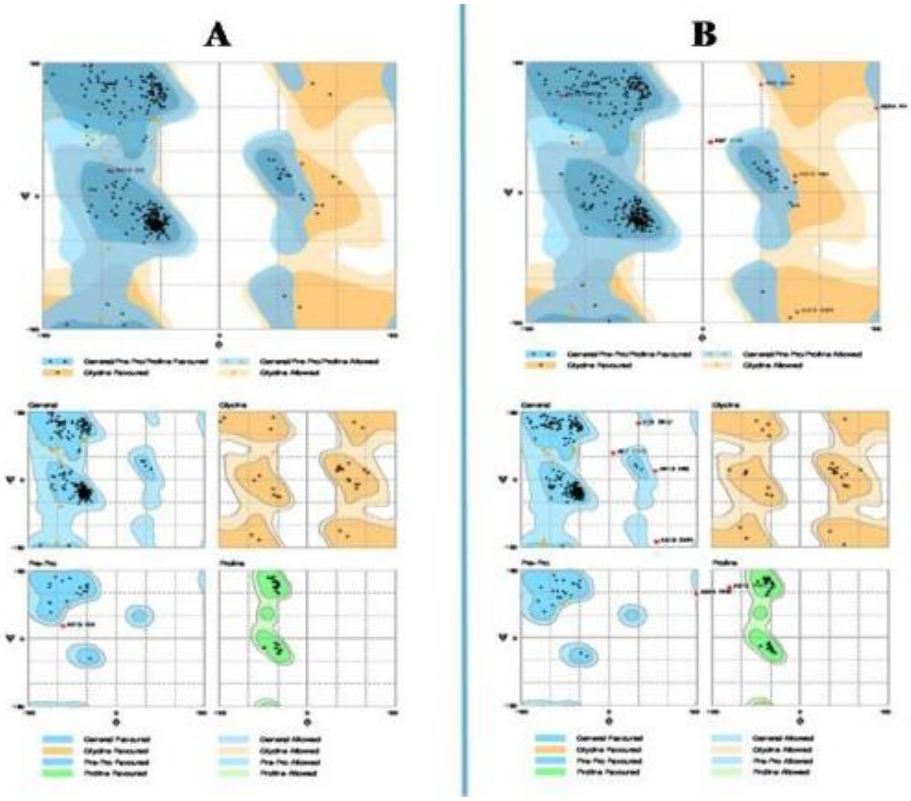

Figure 5. *Ramachadran plot value of modelled manganese peroxidase (**A**) and lignin peroxidase (**B**).*

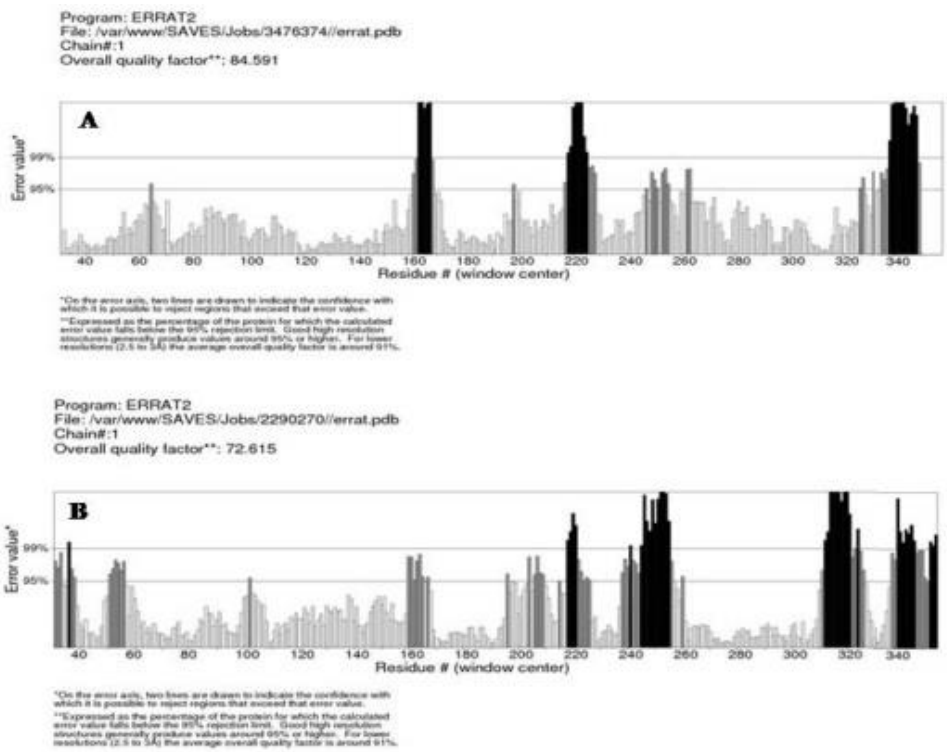

Figure 6. *Errat results obtained for modelled manganese peroxidase (**A**) and lignin peroxidase (**B**).*

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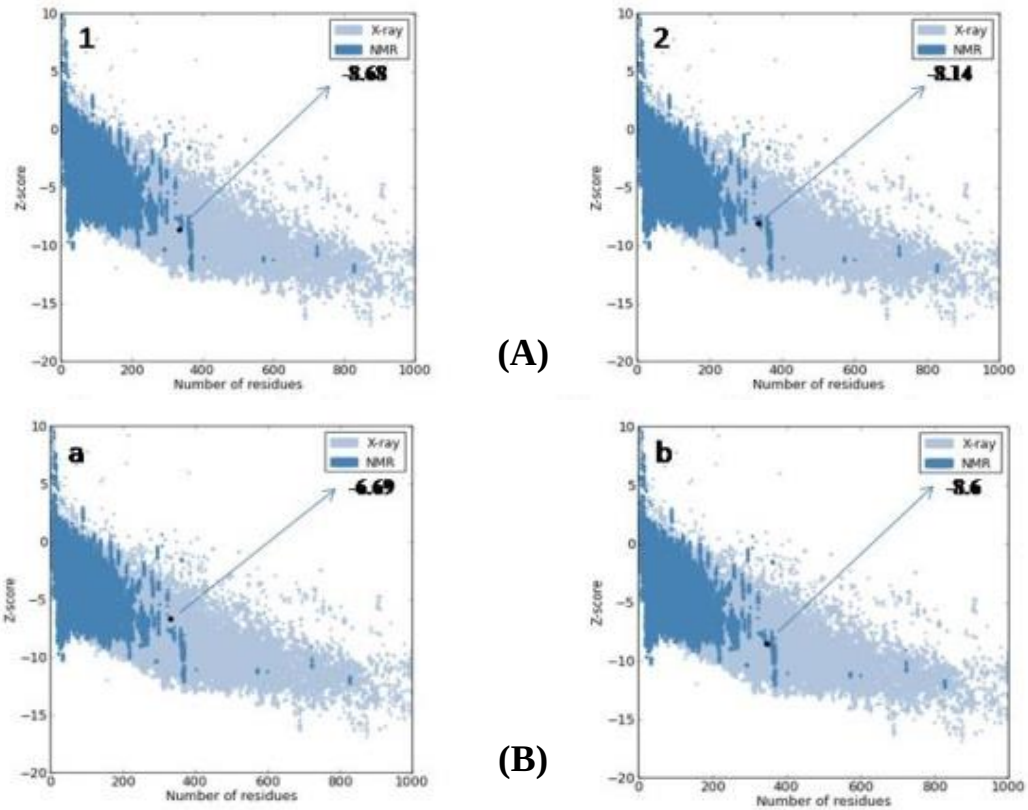

Figure 7. *Prosa plot: (**A**) for manganese peroxidase, 1 - Model; 2 - Template (4BM1); (**B**) for lignin peroxidase, a - Model; b - Template (1B80).*

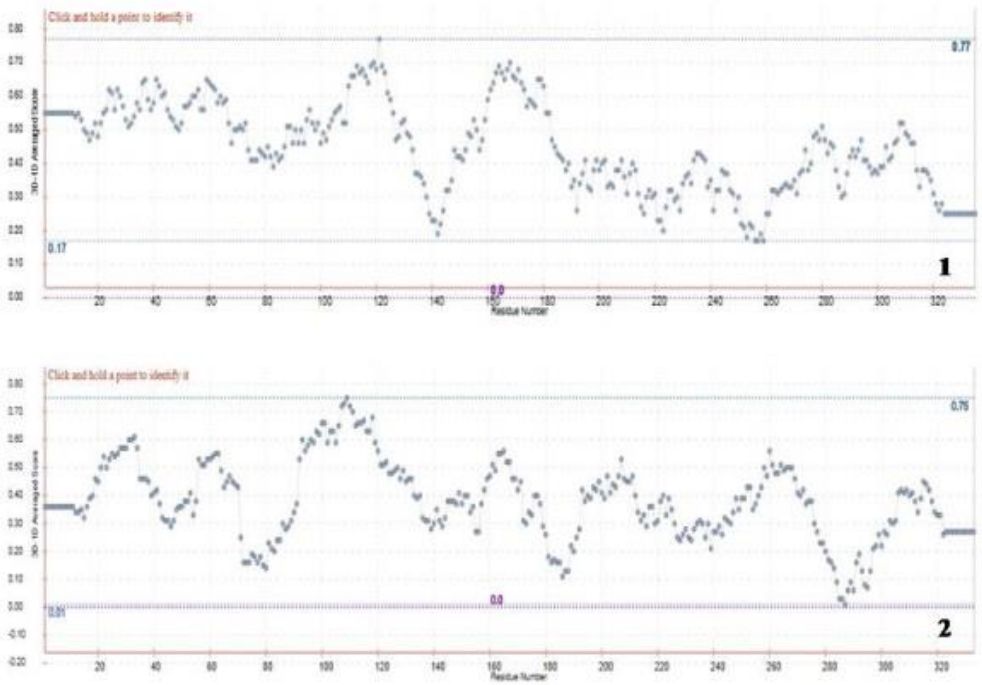

Figure 8. *VERIFY 3D plot analysis modelled manganese peroxidase (1) and lignin peroxidase (2).*

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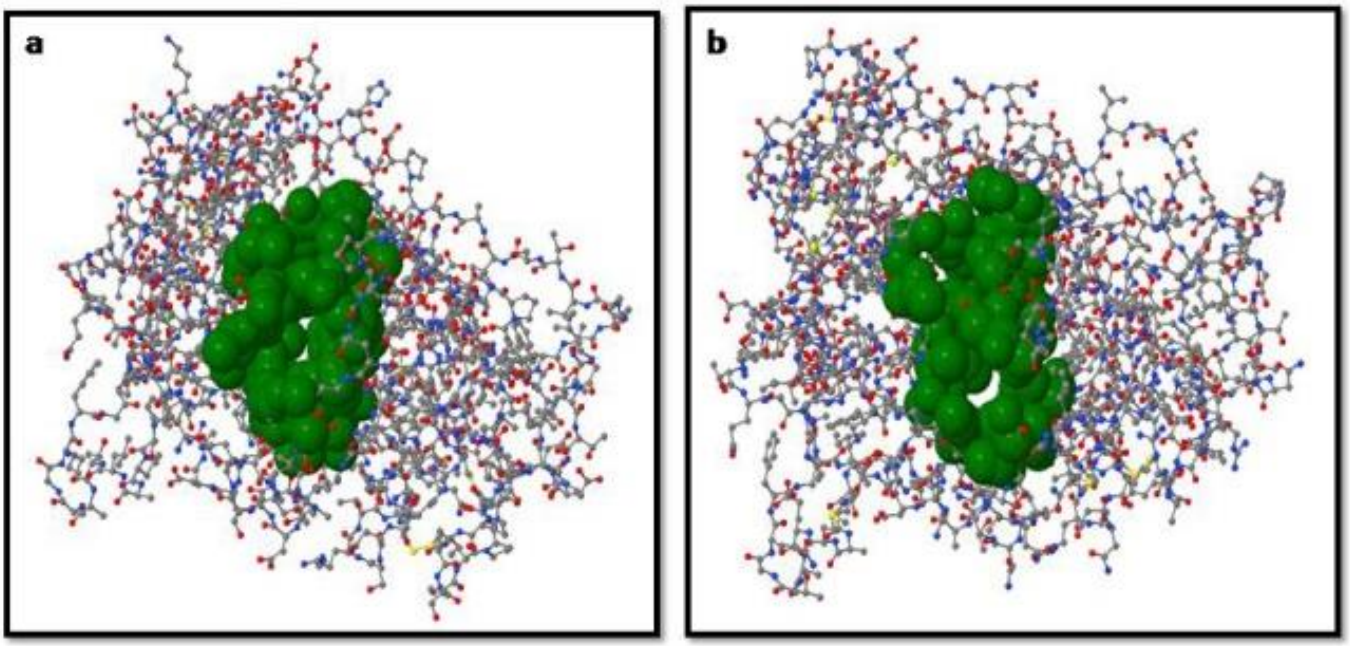

Figure 9. *Castp based analysis of manganese peroxidase (a) and lignin peroxidase (b) for prediction of active sites.*

Lignin peroxidase

After evaluating physicochemical properties of query LiP sequence (Table 1), it was observed that LiP contains 361 amino acids with 38439 dalton molecular weight and have acidic isoelectric point (4.29). As in case of MnP, the total number of negatively charged residues was found higher than the total number of positively charged residues. GRAVY index attesting to solubility of LiP in hydrophilic solvent (-0.047). Ii of 44.32, signifying that enzyme would be stable in solution. Ai value of 76.32 confirmed the thermostability of enzyme. 20 helix and 4 strands have been predicted in secondary structure of LiP from *Phlebia radiata* (Figure 3B). ProFunc analysis showed that 7 motifs matched in scan against PROSITE, PRINTS, PFam-A, TIGRFAM, PROFILES and PRODOM motifs, 86 and 50 matching sequences were found in FASTA and BLAST search respectively, 419 significant structural matches, 6 nest located in the structure, 20 ligand binding templates and 2 significant hit out of 584 enzyme active site template were identified.

LiP from *Phlebia radiata* (AAW71986.1) was used as a query sequences against PDB database in pBLAST. Chain B, Recombinant Lignin Peroxidase H8 (PDB: 1B80) was selected as a template molecule with 94% of query coverage and 63% of maximum identity (Table 3). Modelled structure for LiP has 63.36% sequence identity with template [1B80] with Z-score QMean -0.71 (Figure 4B). LiP showed 95.5 % of the residues in most favored regions, 2.7% residue in allowed region and 1.8% residue in outlier region as predicted in Ramachandran plot (Figure 5B). The ERRAT overall quality factor value of predicted structure was 72.61 (Figure 6B). Prosa Z score was 6.69 & 8.6 for model and template respectively (Figure 7B). VERIFY 3D used for assessing and confirmation of quality of predicted model for LiP (90.1%) and reliable results were obtained as shown in Figure 8.2.LiP have 61 active site covered area of 1024.8Å2 and volume of 1308.3Å3 (Figure 9b). In addition to lignin catabolic process, LiP from *Phlebia radiata* involved in metal ion binding, catalytic, antioxidant, diarylpropane peroxidase, oxidoreductase activity as predicted through Combfunc server. Final 3-D structures were generated and visualized in Pymol (Figure 10).

Submission of models to PMDB

There The modelled structure of MnP from *Ganoderma lucidum* and LiP from *Phlebia radiata* were submitted to the Protein Model Database with ID number PM0080099 and PM0080125, respectively.

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Conclusion

In this study, the 3D model of predicted manganese and lignin peroxidase were generated by using homology modeling with SWISS-MODEL. The validity of the models were confirmed with RAMPAGE, VERIFY 3D and ERRAT. The resulted 3D structure can be exploited further through Molecular Docking Approach to gain more insight of its interaction with lignin, mediators, various aromatic compounds including dyes and pollutant to enhance its activity.

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