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Research Article

Structural-functional analyses of textile dye degrading azoreductase, laccase and peroxidase: A comparative in silico study

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ABSTRACT

Background: Textile industry not only plays a vital role in our daily life but also a prominent factor in improving global economy. One of the environmental concern is it releases huge quantities of toxic dyes in the water leading to severe environmental pollution. Bacterial laccase and azoreductase successfully oxidize complex chemical structure of nitrogen group-containing azo dyes. Additionally, the presence of textile dye infuriates bacterial peroxidase to act as a dye degrading enzyme. Our present study deals with three textile dye degrading enzymes laccase, azoreductase, and peroxidase through analyzing their structural and functional properties using standard computational tools.
Result: According to the comparative analysis of physicochemical characteristics, it was clear that laccase was mostly made up of basic amino acids whereas azoreductase and peroxidase both comprised of acidic amino acids. Higher aliphatic index ascertained the thermostability of all these three enzymes. Negative GRAVY value of the enzymes confirmed better water interaction of the enzymes. Instability index depicted that compared to laccase and preoxidase, azoreductase was more stable in nature. It was also observed that the three model proteins had more than 90% of total amino acids in the favored region of Ramachandran plot. Functional analysis revealed laccase as multicopper oxidase type enzyme and azoreductase as FMN dependent enzyme, while peroxidase consisted of α-β barrel with additional haem group.
Conclusion: Present study aims to provide knowledge on industrial dye degrading enzymes, choosing the suitable enzyme for industrial set up and to help in understanding the experimental laboratory requirements as well.
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1. Introduction

Worldwide, rapid industrialization and urbanization is strongly based on the connection between environmental pollution and public welfare [1]. Large quantities of textile dyes are released through effluents every year throughout the world. Textile industry effluents contain a significant quantity of dye with many toxic metals. Approximately, 10–15% unbound azo dye are released as liquid waste every year [1]. Azo dye is the main component of textile industry that is used in dying process. However, the release of untreated or partially treated dye containing effluents, have detrimental impact on environment in terms of salinity, decreasing Biological Oxygen Demand (BOD), varying pH, increasing chemical oxygen demand (COD) and upsurge of suspended solids in the water body [1,2]. In recent times, bacterial degradation of wastewater is known to be a successful process to clean up the environment [3]. Biological treatment is mainly based on the enzymes synthesized by the respective microorganisms. There are several microorganisms which have the ability to degrade reactive azo dyes by reducing azo bonds using flavin containing NADH or NADPH dependent azoreductase [4,5]. The enzymatic degradation of azo dye is very advantageous as enzymes can increase the reaction rate due to its high catalytic activity. Also, the very quantity of enzymes is required to alleviate water pollution. In addition to that, enzymatic treatment is environment friendly as well as cost effective [6]. There are mainly two enzymes known to be involved in the breakdown of industrial

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azo dyes, namely, azoreductase and laccase [3,7]. Furthermore, the presence of synthetic azo dye, peroxidase is also competent to degrade synthetic azo dye up to a certain extent [8,9].

The experimental determination of protein structure through X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy is very expensive and time-dependent. In the growing field of proteomics, computational characterization of enzymes has major significance for the data accumulation and analysis to reveal the structure/function of the enzymes in day to day life [10]. Azoreductase are of two types: membrane bound and intracellular. Intercellular azoreductase which are thermostable and hydrophilic in nature able to degrade azo dye most efficiently in general. The present study performs computational prediction of three important dye degrading enzymes: viz., laccase, azoreductase and peroxidase, using various computational tools. This study helped in decoding better protein structure, its validation and functional characters.

2. Materials and methods

2.1. Sequence retrieval

RCSB-PDB is a protein data bank containing experimentally determined sequence and structure of proteins. Three protein sequences of industrially important dye degrading enzymes laccase, azoreductase AzrA and peroxidase was retrieved from RCSB-PDB database [http://www.rcsb.org] in both FASTA and PDB format.

2.2. Physiochemical characterization

Computational analysis of physiochemical characters gives an idea on the nature of proteins. Physicochemical characterization of the retrieved sequences was performed using in silico ExPASy-ProtParam tool [http://web.expasy.org/protparam/] that firms the physical and chemical parameters of the protein sequences. Different parameters, viz., number of amino acids, theoretical isoelectric point (pI), grand average of hydropathicity (GRAVY), molecular weight, aliphatic index and instability index, were computed.

2.3. Primary structure analysis

In primary structure, amino acids are joined by peptide bonds forming a polypeptide chain. For wet lab experiments, different qualitative and quantitative methods like Biuret test, ion exchange chromatography is normally used [11]. In this case of computational analysis, a web-based server, GPMAW [http://www.alphalyse.com/customer-support/gpmaw-lite-bioinformatics-tool/buy-gpmaw/] was used to analyze the primary structure of the retrieved sequences. Most common contributing amino acids and their nature had been assessed using this computational tool.

2.4. Secondary structure analysis

Normally in laboratory practice, the structure of protein is predicted by NMR (Nuclear Magnetic Resonance) spectroscopic study [11]. In this scenario, computational secondary arrangement of the retrieved protein sequences were predicted in terms of α-helices, β turn, β-sheets percentage present in the protein using an online web-based server SOPMA (Self-Optimized Prediction Method with Alignment) [https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html] [12].

2.5. Tertiary structure analysis, homology modeling and validation

Tertiary structure of the proteins is structurally elucidated by x-ray crystallography but computationally it may be evaluated easily using some web servers. A web-based bioinformatics tool ESBRI [http://bioinformatica.isa.cnr.it/ESBRI/input.html] was used to detect the combination of salt bridges present in the tertiary structure of the retrieved protein sequences. In this study, SAVES server [http://servicesn.mbi.ucla.edu/SAVES/] was used to obtain high resolution 3D structure of the proteins. Homology 3D modeling of the retrieved proteins were performed using SWISS-MODEL QMEAN (Qualitative Model Energy Analysis) [https://swissmodel.expasy.org/qmean/] [13]. Three different scoring approaches were available under Swiss model workspace, viz., QMEAN, QMEANDisCo and QMEANBrane. Therefore, it was estimated to be the most suitable and best matched protein structure depending on model quality. The different geometric aspects of protein structure were mainly observed through QMEAN, whereas QMEANDisCo improved the local quality of QMEAN by computing the pairwise residue–residue distances with assembles of distance constraints (DisCo) extracted from homologous structures of the proposed model. QMEANBrane is a version of QMEAN which estimated the local quality of membrane protein model in their different segments, viz., membrane, interface and soluble. The protein model quality was further analyzed using ANOLEA (Atomic Non-Local Environment Assessment) and GROMOS [http://swissmodel.expasy.org/workspace/?func=tools_structureassessment1] [14]. The crystallographic protein structures of the retrieved sequences were confirmed using ERRAT server [http://services.mbi.ucla.edu/ERRAT/], a computational-based tool that analyses the statistics of non-bonded interactions among different atoms of a protein sequence [15]. The 3D models of the retrieved proteins were validated through the generation of Ramachandran plot, constructed using PROCHECK [http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/] to find the energetically allowed regions for backbone dihedral angles ψ against φ of the amino acid present in protein structure [16]. The percentages of amino acids residues in favored region, allowed region and outlier region was evaluated by RAMPAGE [http://mordred.bioc.cam.ac.uk/~rapper/rampage.php] [17].

2.6. Functional analysis and protein–protein interactions

To recognize the functional motifs present in the retrieved proteins, MEME (Multiple Extraction-Maximization for Motif Elicitation) tool [http://meme-suite.org/] [18,19] was used. In order to find out the functional ligand binding sites in the target proteins, 3DLigandSite [http://www.sbg.bio.ic.ac.uk/3dligandsite/] [20] generated the heterogens present in the protein structure facilitating functional binding. A web-based server, EXPASY-PSORT [http://www.psort.org/psortb/index.html] predicted the sub-cellular location of the proteins. All the three selected dye degrading proteins were analyzed through InterProScan [http://www.ebi.ac.uk/interpro/] and were classified according to their families by predicting the important functional domains. Co-expression of three query proteins laccase, azoreductase and peroxidase with other related proteins were studied with the help of a computational tool, STRING v10.5 [https://string-db.org]. STRING revealed protein–protein association with other closely associated proteins compared to the query sequence and generated the functional networks among them.

3. Results

3.1. Sequence retrieval

Sequences of the three dye degrading proteins, viz., laccase, azoreductase and peroxidase (Laccase 2XU9, Azoreductase AzrA 3W77 and Peroxidase 5GT2), were retrieved from the RCSB-PDB protein database in both FASTA and PDB format. In origin these enzymes are from *Thermus thermophilus* HB27 (**2XU9**), *Bacillus* sp. B29 (**3W77**) and *Escherichia coli* K12 (**5GT2**), respectively.

3.2. Physicochemical characterization

Computational analysis of the physicochemical qualities of a protein in turn gives theoretical overview on the behavior and nature of the protein. In our study, the three important dye degrading proteins were primarily characterized by basic physicochemical parameters such as the number of amino acids, pI, instability index, molecular weight, GRAVY and aliphatic index (Table 1). As a result, this analysis confirmed that laccase was chiefly composed of basic amino acids while azoreductase and peroxidase were acidic amino acid consisting proteins. The instability index estimated the following stability of a protein: the value lower than 40 are more stable and higher than 40 may be unstable. In this context, our data revealed that azoreductase (instability index 23.95) was more stable compared to laccase and peroxidase. The aliphatic index determined the thermostable nature of globular proteins based on alanine, valine and leucine amino acids; the higher aliphatic index specified more thermotolerance. Therefore, the higher value of aliphatic index ascertained that all proteins were thermostable in nature. Thermostable enzymes have high specificity and potentiality for many industrial applications [21]. Moreover, all these three proteins were of hydrophilic character as the negative GRAVY value confirmed the better interaction between protein and water [22].

3.3. Primary structure analysis

The primary structure analysis revealed that the aliphatic amino acids leucine and alanine were commonly present in all the three proteins. Compositional difference and the percentage of top five contributing amino acids of these three proteins were represented in Fig. 1a. Aliphatic amino acid valine was present in both laccase and azoreductase whereas cysteine existed in lowest percentage in all the three proteins (0.2%, 0.0% and 1.4% in laccase, azoreductase and peroxidase, respectively). The total number of negatively charged amino acid residues (Asp + Glu) was much higher than the total number of positively charged amino acid residues (Arg + Lys) in all three proteins (Fig. 1b). In general, all the three proteins were rich in aliphatic amino acids with net negative charges and contain no or very low numbers of cysteine residues.

3.4. Secondary structure analysis

Based on primary structure, secondary structure is generally determined in wet lab using various prediction techniques [23]. In computational study, the characteristic features of secondary structure of laccase, azoreductase and peroxidase determined through SOPMA server [12] was presented in Fig. 1d. All of these proteins were commonly rich in random coils, which probably played an important function to predict protein's flexibility and conformational changes like enzymatic turnover. This was in line with our result, where higher percent of amino acid residues in random coils indicated that the proteins had true enzymatic functions. In addition, high percentage of α-helices were found in azoreductase (46.15%) and peroxidase (36.81%) (Fig. 1d), signifying the thermal resistance of the proteins based on their intrinsic stability [24,25].

3.5. Tertiary structure analysis, homology modeling and validation

The numbers of salt bridges present in the tertiary structure of all the three proteins were computed through ESBRI web server and mean distance of the amino acid residues are shown in Fig. 1c. Salt bridge is a connection between a positive (side chain nitrogen in Arg, Lys or His) and a negative atom (side chain oxygen in Asp or Glu) with an interatomic distance less than 7.0 Angstrom [26]. The dominating salt bridges found to be Lys-Asp followed by Arg-Glu. Salt bridges having an important function in stabilizing and destabilizing protein structure [27] with the interatomic mean distances (Fig. 1c) revealed that all the three proteins were energetically favorable stabilized by salt bridges. The Structure Analysis and Verification Server (Saves V 5.0) computed the quality of standard X-ray crystallographic structures of proteins. By analyzing model quality score investigated by different computational tools like ERRAT, Whatcheck, Procheck, and PROVES; this server verified the excellence of protein structure (Fig. 2). Saves server is very useful to evaluate protein structure both by quality and quantity (PROVE program). It also provided a score based on 3D-1D structure ratio (Table 1). Based on the SAVES scores, it may be concluded that all the three proteins of interest had stable crystallographic structure (Fig. 3a, Fig. 3b, Fig. 3c). For homology modeling, global model quality was estimated using SWISS-MODEL QMEAN tool. The targeted protein structures were compared with non-redundant PDB structures and the predicted results represented in Fig. 2. QMEAN Z-score of laccase, azoreductase and peroxidase were 1.85, 1.82 and −0.53, respectively (Fig. 2a, Fig. 2b and Fig. 2c). The desirable QMEAN score and Z-score of a protein is should optimally be within 0–1 [28] and <1 [29]. This is in comparison with a non-redundant set of PDB structures to obtain a high-quality model. Local model quality is important for practical application in structure interpretation which was evaluated through SWISS-MODEL and the target-template alignment is predicted in Fig. S1. The graphs accurately showed local model reliability with the residues along the sequences of laccase, azoreductase and peroxidase (Fig. 2a, Fig. 2b and Fig. 2c). In order to evaluate the packaging quality of the model proteins, ANOLEA program was used that calculated the energy of amino acid chains by estimating the "Non-Local Environment" (NLE) of each heavy atom present in protein molecule. The analysis of bio-molecular conformation obtained from computer-based simulation, was performed through a molecular dynamics computer simulation package GROMOS. The Fig. S2a, Fig. S2b, Fig. S2c1 and Fig. S2c2 represented the result of ANOLEA and GROMOS in which the y-axis of the plot symbolized the energy for each amino acid of the protein chains. Favorable and unfavorable energy environment for a given amino acid were represented by the negative and positive energy values respectively (Fig. S2a, Fig. S2b, Fig. S2c1, and Fig. S2c2). Protein structures were further validated through ERRAT server that analyzed error regions in crystallographic proteins by investigating the statistics of non-bonded atomic interactions [15]. ERRAT quality factor of azoreductase and peroxidase (99.495 and 95.959 respectively) was found to be very satisfactory. The error value

Table 1
Comparative physicochemical characteristics and quality assessment scores of all the three dye degrading enzymes.

Serial No.	Physicochemical characters							Quality assessment scores			
	Enzymes	No of AA	Theoretical PI	MW (KDa)	II	AI	GRAVY	3D-1D score (%)	ERRAT Quality factor	QMEAN Z-score	AA in FR of Ramamchandran plot (%)
1	Laccase 2XU9	439	7.09	48.727	41.29	96.83	−0.146	94.76	85.273	1.85	90.2
2	Azoreduactase AzrA 3W77	436	4.80	47.880	23.95	87.98	−0.060	92.51	99.495	1.82	93.9
3	Peroxidase 5GT2	1291	5.40	143.541	75.82	75.82	0.420	83.28	95.959	−0.53	90.3

Where AA – Amino Acids; MW – Molecular Weight; II – Instability Index; AI – Aliphatic Index; GRAVY – Grand Average Hydropathy; FR – Favorable region.

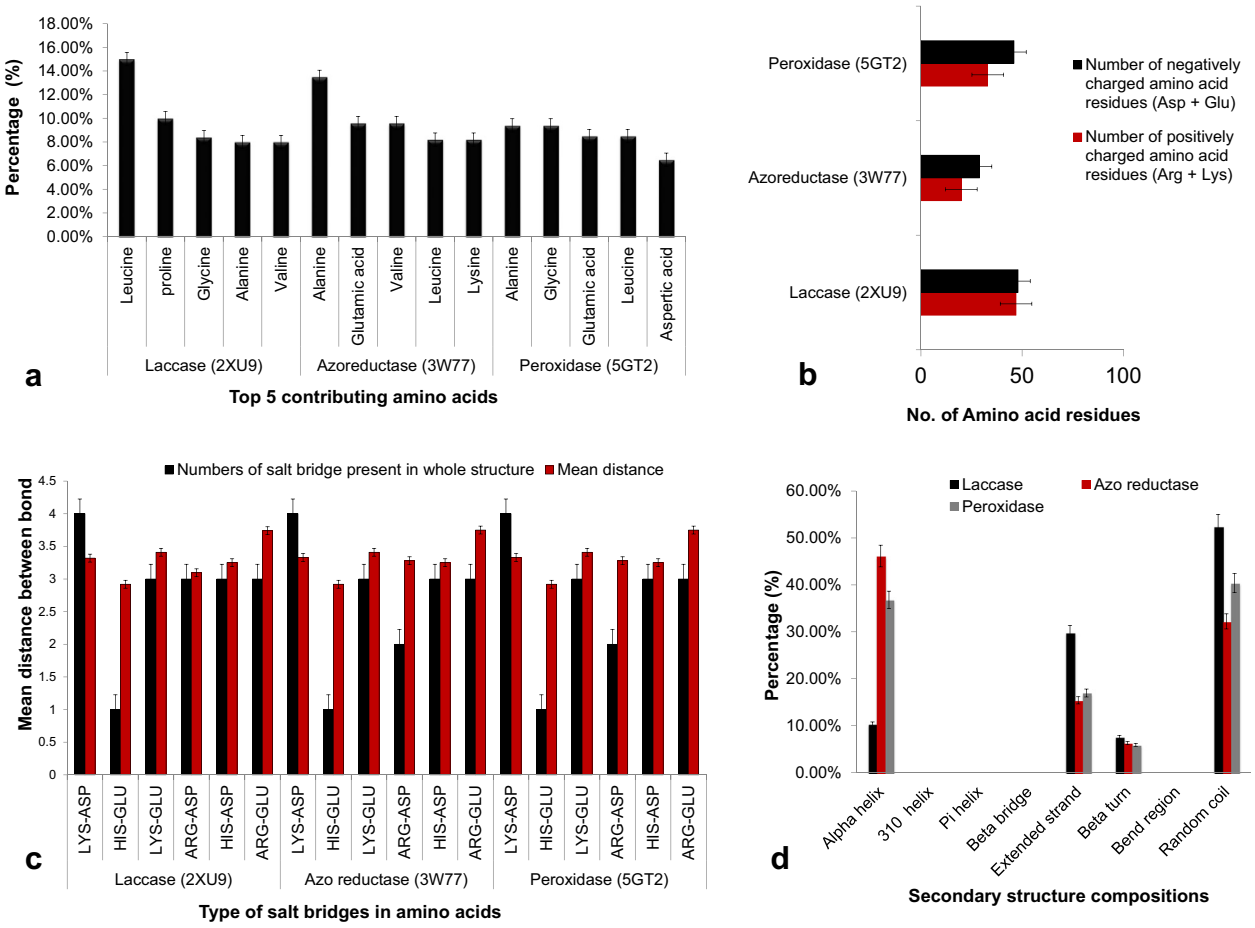

Fig. 1. Graphical representation of primary, secondary and tertiary structure analyses of textile dye degrading laccase (2XU9), azoreductase (3W77) and peroxidase (5GT2); where (a) Amino acid compositions, (b) Comparison in composition of positively and negatively charged amino acids, (c) Salt bridge composition, and (d) Comparison of secondary structures.

of more than 95 exhibited better crystallographic structure of the protein model [15]. The stereo chemical validation of the protein structures were performed through PROCHECK [16] that generated the Ramachandran plots where φ-ψ torsion angles of the amino acid residues were plotted against each other. In quadrant 1 of the Ramachandran plots, some conformations in the allowed region were rare left handed α-helices. Quadrant 2 contained the most favorable sterically allowed conformations of β-strands. Quadrant 3 of the plot contained right-handed α-helices, while the conformation with disfavored region dealt with the quadrant 4 in the plot. In this study, Ramachandran plots (Fig. 3d, Fig. 3e and Fig. 3f) of laccase, azoreductase and peroxidase models showed that 90.2%, 93.9% and 90.3% residues respectively were in the most favored regions. It again confirmed that the protein models had satisfactory quality. Moreover, three of these models were reliable as they had no or very less residues in disallowed region (Fig. 3d, Fig. 3e and Fig. 3f). The stereo chemical properties of laccase, azoreductase and peroxidase were predicted through RAMPAGE tool where it generated a number of plots in a PostScript format [30]. The plot analysis exhibited that model protein laccase had 97.7% residues in favored region, 2.1% in allowed region and only 0.2% in outlier region. In case of azoreductase model, the plot had 97.8% residues in favored region, 2.2% in allowed region and nothing in outlier region. Finally, the peroxidase model revealed 96.0% residues in favored region, 3.7% in allowed region and 0.3% in outlier region.

3.6. Functional analysis and protein–protein interactions

InterPro scan analysis had predicted the domain position in the proteins. Laccase had three domains: the region from 29–135 with multicopper oxidase- type 3 activity, region 174–262 with multicopper oxidase- type 1 activity and region 325–438 with multicopper oxidase- type 2 activity [http://www.ebi.ac.uk/interpro/]. The protein sequences of laccase represented a Cu-binding site conserved among multicopper oxidases. InterProscan analysis also revealed that the region of 3–203 residues of azoreductase represented a flavodoxin-like fold domain and an open twisted/α-β structure. This structure consisted of five parallel β-sheets surrounded by α-helices, while peroxidase exhibited detail identified dimeric α-β barrel domains. MEME tool takes a number of unaligned DNA/protein sequences similar to the target DNA/protein sequences as input and gives output as motifs of that target DNA/protein. The result of MEME showed the location of motifs in the protein structures [31,32]. The distribution and location of the three motifs for each protein structure are represented in Fig. S3. The ligand binding sites in the targeted protein sequences were predicted through 3DLigandSite server that predicted the ligands present in homologous structures. The ligand binding sites in the protein sequences of laccase, azoreductase and peroxidase enzymes are represented in Fig. S3. This program also computed the heterogens present in the ligand cluster used for prediction. In case of laccase, copper is the heterogen. Whereas, there were two heterogens present in the binding site of azoreductase, namely, Flavin adenine dinucleotide (FAD) and Flavin mononucleotide (FMN). In case of peroxidase, FMN, FAD and Zn were present as heterogens. The prediction of subcellular localization of the proteins was performed through EXPASY-PSORT that predicted that all the three proteins resided in cytoplasm. Protein–protein association as observed from STRING analysis (Fig. 3g, Fig. 3h and Fig. 3i), that is the multicopper oxidase enzyme laccase showed considerable first shell

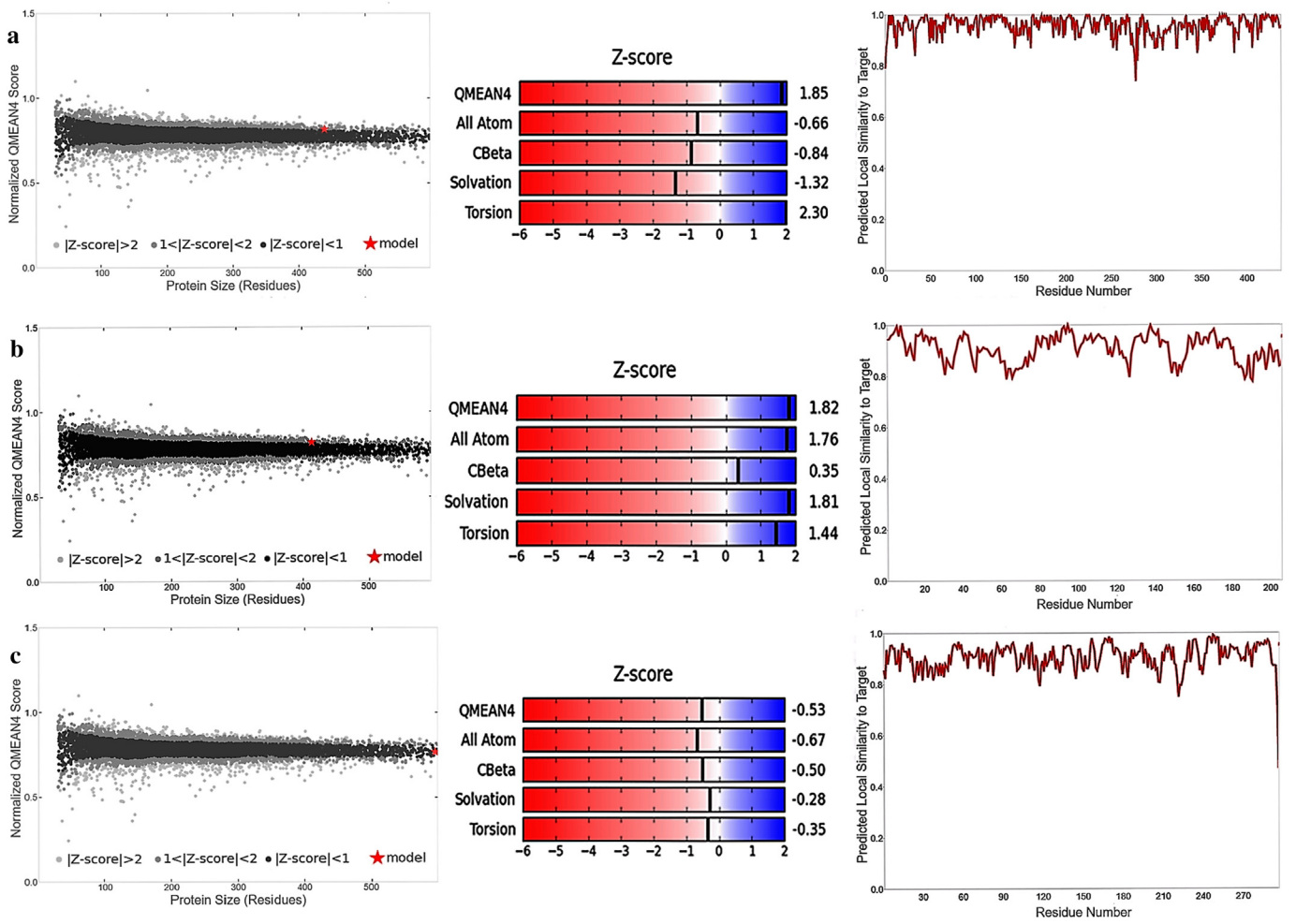

Fig. 2. Quality comparison, evaluation of Z-score and quality estimation of dye degrading proteins; where (a) Laccase, (b) Azo reductase, and (c) Peroxidase.

interaction with other proteins of the same organism having unknown 3D structure. It had also shown putative homologous co-expression with thiosulfate reductase precursor. For azoreductase, all proteins which were exhibiting interactive network with query protein had unknown 3D structure. Finally, peroxidase enzyme exhibited most number of first and second shell co-expression with different proteins of *E. coli* compared to other two proteins of interest. Thus, by analyzing the interaction networks, peroxidase was seen to have better functional activities.

4. Discussion

In this study, choices of the enzymes were made on two bases: availability of native sequences without addition of any ligands and its common origin from thermotolerant bacteria. Cellular components of thermotolerant organisms are also thermostable. Apart from high temperature, they are also able to withstand acidic and alkaline denaturants. Thermostable enzymes are highly specific and have considerable potential for many industrial applications [21] which was the principle reason behind choosing the three dye degrading enzymes of thermotolerant bacterial origin. Presence of very low number of cysteine residue and high number of aliphatic amino acids in primary structure indicated that laccase, azoreductase, and peroxidase are intracellular proteins. Since cysteine is hydrophobic and polar; hence, these proteins were also non-polar and hydrophilic in nature. Due to the presence of high percentage of α-helix, azoreductase and peroxidase were considered as more thermostable than laccase. Relationship of α-helix and thermostability is already earlier supported by many reports where structurally stable thermophilic proteins have abundant α-helix region [24,25,33]. In all the three models, more than 90% of amino acids were in the favored region of the Ramachandran plot indicating that the models were stable in nature [22,30]. This result was also supported by the SAVES score where all the three proteins had stable crystallographic structures. From the analyses of SWISS-MODEL QMEAN, ANOLEA and ERRAT, it was also found that all the three protein models were of good quality. Interestingly, overall quality of azoreductase and peroxidase was again found to be more acceptable as detected by ERRAT (99.495 and 95.959 respectively). Salt bridges play a key function in stabilizing and destabilizing protein structures and through this study, all the three proteins were found to be energetically favorable and stabilized by the salt bridges. Overall from the structural analyses, it may be established that azoreductase and peroxidase enzymes have better thermal stability and superior model quality while azoreductase with maximum α-helices content is most thermostable out of the three dye degrading enzymes. InterProScan analysis delivered enzyme family classifications and important functions of the three dye degrading enzymes. It scanned the positions of three domains in laccase; out of which the multicopper oxidases [34] comprised of three different copper centers namely, type 1/blue, type 2/normal and type 3/coupled binuclear. Moreover, this analysis reported that laccase is a 3-domain enzyme which oxidizes different phenols and diamines. InterProScan analysis also revealed that the region of 3–203 residues of azoreductase represents a flavodoxin-like fold domain which is an open twisted/α-β structure. This structure comprised of five parallel β-sheets surrounded by α-helices.

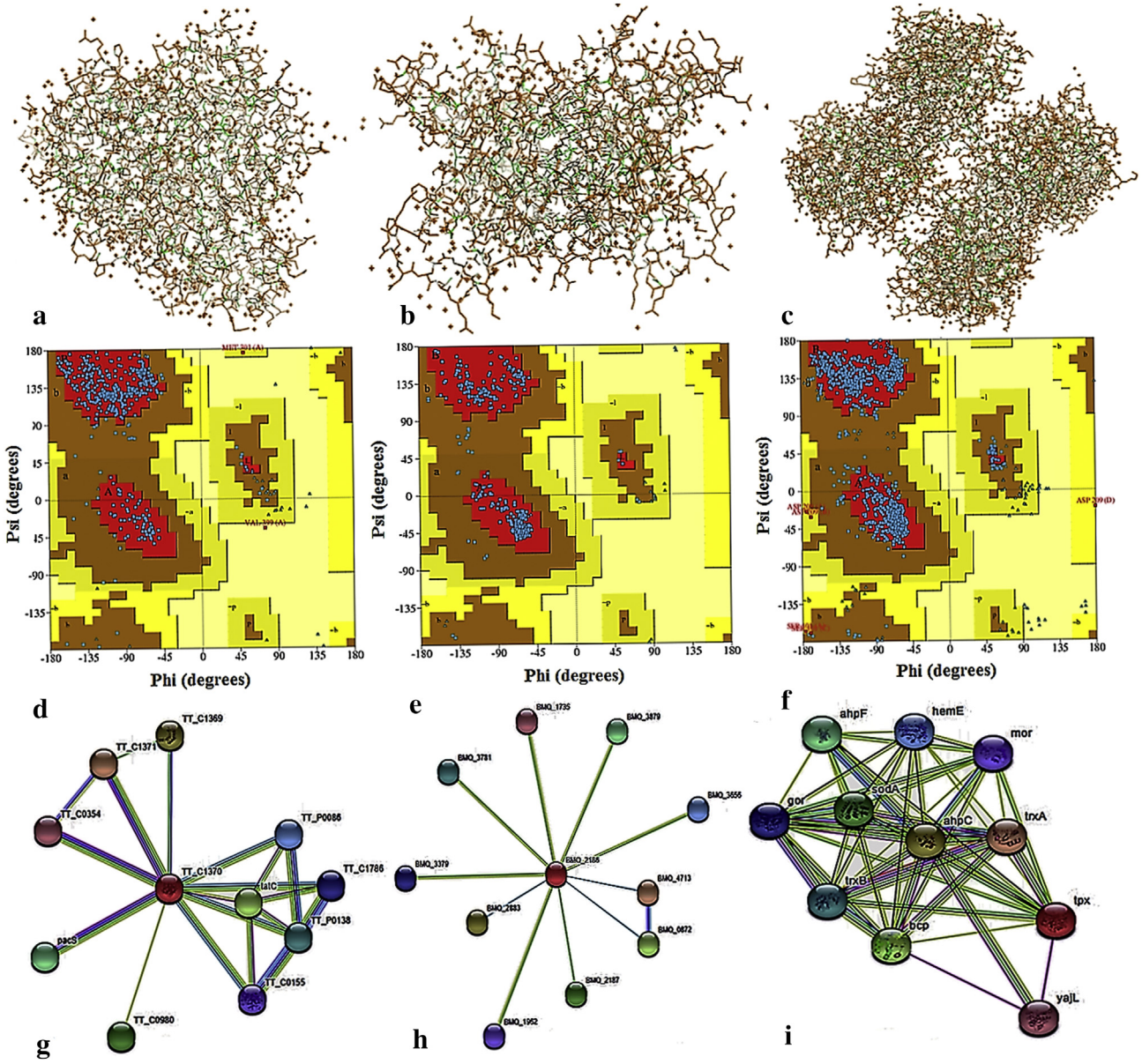

Fig. 3. Structural comparison of three dye degrading enzymes by Predicting 3D structures of (a) Laccase (b) Azoreductase, and (c) Peroxidase; validation of the 3D structures of (d) Laccase, (e) Azoreductase, and (f) Peroxidase by plotting them in Ramachandran plots; Functional comparison by generating the protein–protein interaction networks of (g) Laccase, (h) Azoreductase, and (i) Peroxidase.

Azoreductase is a flavodoxin-containing protein. Furthermore, the analysis also showed that azoreductase contained NADH as an electron donor. This cleaved azo bond and gets reduced into aromatic azo compounds. Peroxidase also exhibited detail identities with dimeric α-β barrel domains, and α + β sandwich fold along with an antiparallel β sheet. This resulted in the formation of a closed barrel. Additionally, the analysis classified the peroxidase enzyme in DyP (for dye de-colorizing peroxidase) family, which is comprised of a novel haem peroxidase [35] involved in degradation of peroxidase substrates as well as several dye-derived anthraquinones. From STRING analysis, it was defined that most of the interacting proteins to the query organisms (laccase and azoreductase) had unknown 3D structure, but they were still showing much interconnecting networks, possibly due to gene neighborhood interaction or gene co-occurrence expression [36]. Overall from the functional analysis, it may be articulated that peroxidase being a universal enzyme has known co-expressive interaction with the identified proteins whereas, laccase and azoreductase may have particularly focused enzymatic activity on dye degradation resulting into co-expressive interactions mostly with the unrecognized proteins. Bacterial origin azoreductase is earlier reported to be functionally stable for azo dye degradation (methyl red, amaranth, and methyl orange) [37]. Whereas, microbial fuel cell containing laccase enzyme are described to treat azo dyes feeding through cathode chamber in a sustainable way [38].

5. Conclusion

Our study suggests that model protein structures of all the three dye degrading enzymes, viz. laccase, azoreductase, and peroxidase, stood of good quality having stable crystallographic structures. The physicochemical characterization also revealed that these three dye degrading enzymes were intracellular, hydrophilic and thermostable in nature residing in cytoplasm. All the three enzymes were also found to be energetically favorable stabilized by salt bridges. Peroxidase is already known as a universal enzyme and present in almost all microorganisms. Interestingly, in the present in silico study,

peroxidase was recognized almost structurally and functionally similar to the other two-dye degrading enzymes viz., Laccase and azoreductase. Being a universal enzyme, peroxidase has a wide substrate specificity which may be preferred for degrading a broad range of azo dye, but remarkably, azoreductase was observed to be the most interesting enzyme with highest α-helix content directly indicating best thermal resistance. Also, its maximum ERRAT value represented the best protein model quality among all the three dye degrading enzymes. Therefore, it may be concluded that the enzyme azoreductase has the most favorable structural quality among the other dye degrading enzymes which can be further studied for likely industrial applications. This study will also help researchers to understand the important structural-functional properties of industrially important textile dye degrading enzymes and may also help in the necessary laboratory set up to experimentally confirm its activities.

Conflict of interest

The authors declare no competing interests.

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Supplementary material

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References

[1] Roy U, Sengupta S, Das P, et al. Integral approach of sorption coupled with biodegradation for treatment of azo dye using *Pseudomonas* sp.: batch, toxicity, and artificial neural network. 3 Biotech 2018;8:192. https://doi.org/10.1007/s13205-018-1215-1.
[2] Sarkar S, Banerjee A, Halder U, et al. Degradation of Synthetic Azo Dyes of Textile Industry: A Sustainable Approach Using Microbial Enzymes. Water Conservation Science and Engineering 2017;2:121–31. https://doi.org/10.1007/s41101-017-0031-5.
[3] Singh RL, Singh PK, Singh RP. Enzymatic decolorization and degradation of azo dyes-A review. Int Biodeterior Biodegradation 2015;104:21–31. https://doi.org/10.1016/j.ibiod.2015.04.027.
[4] Chen H, Feng J, Kweon O, et al. Identification and molecular characterization of a novel flavin-free NADPH preferred azoreductase encoded by azoB in *Pigmentiphaga kullae*K24. BMC Biochem 2010;11:13. https://doi.org/10.1186/1471-2091-11-13.
[5] Dehghanian F, Kay M, Kahrizi D. A novel recombinant AzrC protein proposed by molecular docking and *in silico* analyses to improve azo dye's binding affinity. Gene 2015;569:233–8. https://doi.org/10.1016/j.gene.2015.05.063.
[6] Neifar M, Chouchane H. MahjoubiJA, et al. *Pseudomonas extremorientalis* BU118: a new salt-tolerant laccase-secreting bacterium with biotechnological potential in textile azo dye decolourization. 3. Biotech 2016;6:107. 10.1007%2Fs13205-016-0425-7.
[7] Singh D, Sharma KK, Jacob S, et al. Molecular docking of laccase protein from *Bacillus safensis* DSKK5 isolated from earthworm gut: a novel method to study dye decolorization potential. Water Air Soil Pollut 2014;225:2175. https://doi.org/10.1007/s11270-014-2175-7.
[8] Santos A, Mendes S, Brissos V, et al. New dye-decolorizing peroxidases from *Bacillus subtilis* and *Pseudomonas putida* MET94: towards biotechnological applications. ApplMicrobiol Biotechnol 2014;98:2053–65. https://doi.org/10.1007/s00253-013-5041-4.
[9] Falade A, Mabinya L, Okoh A, et al. Peroxidases Produced by New Ligninolytic *Bacillus* strains Isolated from Marsh and Grassland Decolourized Anthraquinone and Azo Dyes. Pol JEnvironmental Stud 2019;28:3163–72. https://doi.org/10.15244/pjoes/92520.
[10] Nadeem MS, Al-Ghamdi MA, Khan JA, et al. Recombinant production and biochemical and *in silico* characterization of lactate dehydrogenase from *Geobacillus thermodenitrificans DSM-465*. Electron JBiotechn 2018;35:18–24. https://doi.org/10.1016/j.ejbt.2018.06.003.
[11] Pramanik K, Kundu S, Banerjee S, et al. Computational-based structural, functional and phylogenetic analysis of Enterobacterphytases. 3 Biotech 2018;8:262. https://doi.org/10.1007/s13205-018-1287-y.
[12] Geourjon C, Deleage G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Bioinformatics 1995;11(6):681–4. https://doi.org/10.1093/bioinformatics/11.6.681.
[13] Arnold K, Bordoli L, Kopp J, et al. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 2006;22:195-201 https://doi.org/10.1093/bioinformatics/bti770
[14] Melo F, Feytmans E. Assessing protein structures with a non-local atomic interaction energy. J MolBiol 1998;277:1141–52. https://doi.org/10.1006/jmbi.1998.1665.
[15] Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 1993;2:1511–9. https://doi.org/10.1002/pro.5560020916.
[16] Laskowski RA, MacArthur MW, Moss DS, et al. PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr 1993;26:283-91https://doi.org/10.1107/S0021889892009944
[17] Lovell SC, Davis IW, Arendall III WB, et al. Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. Proteins 2003;50:437–50. https://doi.org/10.1002/prot.10286.
[18] Bailey TL, Boden M, Buske FA, et al. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res 2009;37:W202–8. https://doi.org/10.1093/nar/gkp335.
[19] Bailey TL, Johnson J, Grant CE, et al. The MEME Suite. Nucleic Acids Res 2015;43:W39–49. https://doi.org/10.1093/nar/gkv416.
[20] Wass MN, Kelley LA, Sternberg MJ. 3DLigandSite: predicting ligand-binding sites using similar structures. Nucleic Acids Res 2010;38:W469–73. https://doi.org/10.1093/nar/gkq406.
[21] Haki GD, Rakshit SK. Developments in industrially important thermostable enzymes: a review. BioresourTechnol 2003;89:17–34. https://doi.org/10.1016/S0960-8524(03)00033-6.
[22] Pramanik K, Soren T, Mitra S, et al. *In-silico* structural and functional analysis of *Mesorhizobium* ACC deaminase. ComputBiol Chem 2017;68:12–21. https://doi.org/10.1016/j.compbiolchem.2017.02.005.
[23] Li Z, Wang J, Zhang S, et al. A new hybrid coding for protein secondary structure prediction based on primary structure similarity. Gene 2017;618:8–13. https://doi.org/10.1016/j.gene.2017.03.011.
[24] Petukhov M, Kil Y, Kuramitsu S, et al. Insights into thermal resistance of proteins from the intrinsic stability of their α-helices. Proteins 1997;29:309–20.
[25] Kumar S, Tsai CJ, Nussinov R etal. Factors enhancing protein thermostability Protein Eng 2000;13:179–91. https://doi.org/10.1016/j.gene.2017.03.011.
[26] Kumar S, Nussinov R. Salt bridge stability in monomeric proteins. J MolBiol 1999;293:1241–55. https://doi.org/10.1006/jmbi.1999.3218.
[27] Costantini S, Colonna G, Facchiano AM. ESBRI: a web server for evaluating salt bridges in proteins. Bioinformation 2008;3(3):137.
[28] Berman HM, Westbrook J, Feng Z, et al. The protein data bank. Nucleic Acids Res 2000;28:235–42. https://doi.org/10.1093/nar/28.1.235.
[29] Benkert P, Künzli M, Schwede T. QMEAN server for protein model quality estimation. Nucleic Acids Res 2009;37:W510–4. https://doi.org/10.1093/nar/gkp322.
[30] Notredame C, Higgins DG, Heringa J. T-coffee: a novel method for fast and accurate multiple sequence alignment. J MolBiol 2000;302:205–17. https://doi.org/10.1006/jmbi.2000.4042.
[31] Dutta B, Banerjee A, Chakraborty P, et al. *In silico* studies on bacterial xylanase enzyme: Structural and functional insight. J Genet EngBiotechnol 2018;16:749–56. https://doi.org/10.1016/j.jgeb.2018.05.003.
[32] Kanchan S, Sharma P, Chowdhury S. Evolution of endonuclease IV protein family: an *in silico* analysis. 3 Biotech 2019;9:168. https://doi.org/10.1007/s13205-019-1696-6.
[33] Escobedo A, Topal B, Kunze MB, et al. Side chain to main chain hydrogen bonds stabilize apolyglutamine helix in a transcription factor. Nat Commun 2019;10(1):2034. https://doi.org/10.1038/s41467-019-09923-2.
[34] Kadam SK, Tamboli AS, Sambhare SB, et al. Enzymatic analysis, structural study and molecular docking of laccase and catalase from *B. subtilis* SK1 after textile dye exposure. EcolInform 2018;48:269–80. https://doi.org/10.1016/j.ecoinf.2018.10.003.
[35] Sugano Y, Muramatsu R, Ichiyanagi A, et al. DyP, a unique dye-decolorizing peroxidase, represents a novel heme peroxidase family ASP171 replaces the distal histidine of classical peroxidases. J BiolChem 2007;282:36652–8. https://doi.org/10.1093/nar/gkw937.
[36] Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2016;45:D362-D368 https://doi.org/10.1093/nar/gkw937
[37] Verma K, Saha G, Kundu LM, et al. Biochemical characterization of a stable azoreductase enzyme from Chromobacterium violaceum: Application in industrial effluent dye degradation. Int J Biol Macromol 2019;121:1011–8. https://doi.org/10.1016/j.ijbiomac.2018.10.133.
[38] Mani P, VT F, Bowman K, et al. Degradation of azo dye (Acid orange 7) in a microbial fuel cell: comparison between anodic microbial-mediated reduction and cathodic laccase-mediated oxidation. Front Energy 2019;7(101). https://doi.org/10.3389/fenrg.2019.00101.