Volume Number 10 || Issue Number 3 || Year 2025 || Page 11 || * Corresponding Author: lubnarasool@uosahiwal.edu.pk ||

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746

WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY

Research Manuscript www.sciplatform.com Peer review

Functional and physiochemical characterization of a hypothetical protein of cytochrome p450 in fungi

[a] Hina Saeed, [a] Abdur Rauf, [a] Lubna Rasool *, [a] Aqsa Afzal, [a] Zoha Saleem, [b] Hamid Manzoor

[a] Department of Chemistry, University of Sahiwal, Sahiwal 57000, Pakistan,
[b] Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan.

Contribution | **Saeed, H.** writing original draft, **A. Rauf:** review and editing, **L. Rasool**: supervision, **A. Afzal**: formal analysis, **Z. Saleem**: visualization, **H. Manzoor**: project administration.

Cytochrome P450 belongs to membrane-bound hemoprotein generally present in the all domains system such as animals, plants, fungi, protists, bacteria, and archaea. They play most fascinating roles as in bio-catalysis in different chemical reactions like bio-remediation, bio-degradation, detoxification, bio-conversions as well as in the metabolism of drugs. Current study has focused on structure and function analysis of CYP in *Fusarium oxysporum* through computation tools. The structural analysis showed that primary structure contained more hydrophobic amino acids such as leucine (10.5%), alanine (7.9%), valine (7.1%) and highest 45.26% of α-helix of secondary structure. Procheck analysis shows that ramachandran favoured regions are 95.09%. While the functional analysis showed that intracellular and the topology of beta-barrel transmembrane protein and alpha-helical CYP proteins is globular nature. Evolutionary aspect studies were conducted clustal omega, *F. oxysporum* CYP 51 is more similar to *A. alliaceus* because both of species share same ancestor. The protein-protein interactions investigation showed that *F. oxysporum* CYP shows closely relations with squalene synthase and terpene cyclase.

Keywords: *Fusaruim oxysporum*, protein structure analysis, phylogenetic tree, domain analysis, ramachandran model

INTRODUCTION: Cytochromes are redox active proteins that consist of a heme group, occurring in the living system and classified as binding to different types of cytochromes i.e. cytochromes a, b, c and d, oxidases , and cytochrome P450 (CYP) (Breskvar *et al.*, 1991). Merely approximately 40% of the sequences in the CYP family are homologous, whereas 55% of the sequences in the subfamilies are identical (Nelson *et al.*, 1996). The cysteinyl residue's Sulphur atom and heme iron core connect protoporphyrin IX of heme-thiolate proteins to the apoprotein in a hydrophobic pocket. The CO used to restrain the majority of P450-catalyzed reactions and using 450nm light to reverse this restriction. This inactive P450–CO complex is the source of the name P450 (Kahn and Durst, 2000). Out of ten, three classes of P450 mono-oxygenase systems found in fungi, fundamental for the survival in their capacity to quickly modify their metabolism (Hannemann *et al.*, 2007). The ubiquitous enzyme CYP is one of the most intriguing targets for bio-catalysis because of extensive substrate, catalytic adaptability, and unconventional kinetics. It is used in biotechnology, medicine, bioconversions, detoxifications, and bioremediation (van Gorcom *et al.*, 1998; Bernhardt and Urlacher, 2014). Isoforms contribute for the degradation of over 80% of pharmaceutical medications and associated with the CYP2, CYP3 and CYP1 families (Ingelman-Sundberg, 2004). It is known as P450-foxy, which was lately confined to the fungus *F. oxysporum* (Shoun and Tanimoto, 1991; Nakayama *et al.*, 1996) involved in lipid metabolism of fatty acid ω-1-ω-3 hydroxylation. P450s belonging to fungal classes II, VIII, and IX are engaged in the production of primary metabolites (Aoyama *et al.*, 1984; Aoyama *et al.*, 1989), secondary metabolites (Črešnar and Petrič, 2011). CYP protein also helpful for the detoxification and/or degradation of xenobiotic (Lisowska *et al.*, 2006), hydroxylation of fatty acids (Hardwick, 2008), de-nitrification processes (Nakahara *et al.*, 1993), poly aromatic hydrocarbons (PAHs) (Moorthy *et al.*, 2015), lignin (Syed and Yadav, 2012), signaling networks. The worldwide three types of industrial pollutants that are categorized as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins (PCDDs) (Safe, 1990), and polychlorinated biphenyls (PCBs) are degraded by fungi. It have been reported that *Aspergillus niger* SK 9317 was also used to study the degradation of PAHs and pyrene with 4 rings (Wunder *et al.*, 1994). With divers metabolic functioning and range, pesticides as well as insecticides have been detoxified by fungal CYP (Wolfand *et al.*, 2016). It is a target for antifungal drugs to control pest and plant pathogenic (Song *et al.*, 2018). Fan *et al.* 2013 examined that azole fungicides inhibit the CYP51-encoded P450 cytochrome 14α-demethylase sterol, necessary for the production of sterols. It has been reported that ecosystem sustainability fungi and other microbes play a significant role in bioremediation, fungal metabolism. Furthermore, the adjustment to particular ecological niches are facilitated by cytochrome P450 proteins (CYPs) (Moktali *et al.*, 2012).

OBJECTIVES: The current study has focused on the structure and function analysis through computation tools. Preliminary analysis has been based on physiochemical characterization of P450 of *Fusarium oxysporum*. Structural modelling has been performed, to predict tertiary structure and ramachandran plot of P450 protein. Furthermore, protein-proteins interaction, motif analysis and functional domain analysis has also been determined.

MATERIALS AND METHODS: **Physiochemical characterization:** Protein FASTA sequence accession no: KAJ9418597.1 obtained from National Centre for Biotechnology Information, (https:/www.ncbi.nlm.nih.gov). ExPASy–ProtParam tool (http:// web.expasy.org/protparam/), the physical parameters of Fungal Cytochrome p450 are investigated. These include the composition and no. of an amino acids, aliphatic index, theoretical pI, molecular weight, and extinction coefficient, grand average of hydrophobicity (GRAVY) and instability index of amino acids.

Multiple FASTA sequence alignment and phylogenetic tree: Clustal omega tool was used to analyze different sequences to obtain the best possible sequence matching of three FASTA sequences of fungal P450. Furthermore, phylogenetic tree constructed by comparing multiple fungal proteins (https://www.ebi.ac.uk/jdispatcher/msa/clustalo).

Protein-protein interaction networks: The structure is formed from computational predictions, STRING database (https://string-db.org/) was used for interactions between fungal CYP and relationships accumulated from others (primary) databases, direct (physical) and indirect (functional) linkages (Szklarczyk *et al.*, 2015).

Structural modelling: Primary structure of fungal CYP has been analyzed by using ExPASy-Protparam tool (https://web.expasy.org/cgi-bin/protparam/protparam). Secondary structure analysis has been constructed through PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/), showing particular secondary structures including alpha helix(H), random coil (C), beta strand (E) (Jones, 1999). SOPMA was used to obtain the accurate %age of alpha helix, extended sheets, beta turns and random coils (https://npsa-prabi.ibcp.fr/cgi-bin/npsa). Swiss-Model QMEAN (Qualitative Model Energy Analysis) which is used for examining the quality of 3D protein structure model and Ramachandran plot (such as bond length, dihedral angles) (https://swissmodel.expasy.org/interactive) of Fungal CYP (Mahgoub and Bolad, 2013) constructed protein models, supplying a quick and comprehensive estimation of their reliability. PROCHECK was used to analyse the protein structure's stereo-chemical ability and its validation (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/).

Functional characterization of putative fungal CYP: Functional characterization of fungal CYP has been predicted by different computational tools to facilitate functional investigations. In this research paper, multiple software have been used such as; Inter-ProScan used for the estimation of domains (www.ebi.ac.uk/interpro/search/sequence-search), Deep-TMHMM 2.0 used for topology (https://dtu.biolib.com/DeepTMHMM), NetNGlyc-1.0 used for the analysis of N-glycosylation (https://services.healthtech.dtu.dk/services/NetNGlyc-1.0/), signal peptides are analyzed by Signalp-5.0 (https://services.healthtech.dtu.dk/services/SignalP-5.0/). The computational software

helpful for the prediction of CYP structure, sequence similarly, structural arrangement and interactions between different proteins.

.**RESULT AND DISCUSSION: Determination of physiochemical parameters of fungal CYP:** *Fusarium oxysporum* family 51 CYP *(*accession no KAJ9418597.1), contains amino acids (506) in the cytochrome p450 protein's peptide chain. Some common physical parameters of proteins by utilizing ExPASy–ProtParam tool included a list of some key features that can be used to conclude any enzyme and protein molecule's functionality: molecular weight, pI value, no. of amino acids, instability index, extinction coefficient, aliphatic index or average hydrophobicity (Gasteiger *et al.*, 2005). This selected protein contains isoelectric point (pI) which is indicated as the pH where the quantity of amino acids that are negatively (acidic) and positively charged (basic) in a protein is equal, resulting in a protein having no net electrical charge. The pI can range from around 3 to 12, with most proteins having a pI between 4 and 7 (Bano *et al.*, 2023). Theoretically, Fungal (CYP) P450 contains a pI value of 7.23. The average molecular weight of CYP protein is 56902.39 Da and its protein structure is considered stable. It is expected that a protein would be stable if its II is less than 40. It is predicted that the protein will be unstable if the II is more than 40.95 (Wilkins *et al.*, 1999). The calculated value of the instability index (II) is 47.44. Total numbers of positively and negatively charged residues are 54. An indicator of a protein's relative aliphatic amino acid content is the aliphatic index, which includes valine, leucine, isoleucine, and alanine (Falquet *et al.*, 2002). With an aliphatic index value of 91.73, the protein is known to be stable over a broad temperature range. The protein is more thermo stable the higher its aliphatic index is. Hydropathicity refers to the hydrophobic (water-repelling) or hydrophilic (water-attracting) nature of a molecule, such as a protein or peptide. The extinction coefficient, which quantifies the intensity of a particular wavelength of light absorbed by a protein, functions as a proportionality constant in accordance with the Beer-Lambert equation (Herzog *et al.*, 2018). In this research, extinction coefficient values of protein are measured at 280 nm in the water having units of M-1 cm-1. The extinction coefficient contains calculated value are 686759 [Abs 0.1% (=1 g/l) 1.207] for the all Cys residual pairs to produce cysteine and 68300 value [Abs 0.1% (=1 g/l) 1.200] for the reduction in all Cys residual pairs. It is a measure of the affinity of a molecule for water. The negative value of GRAVY (Grand average of hydrophobicity) represents the non-polar nature of protein and should be hydrophilic nature for the better bonding with H_2O. The value of Grand average of hydropathicity (GRAVY) is -0.147 (Kyte, 1993).

Multiple FASTA sequence alignment and phylogenetic tree: Clustal omega is used to provide graphical identification of alignment and helps to identify conserved regions, gaps, and mis-matches. Three fungal CYP species have been selected for sequence alignment, i.e. *Fusarium oxysporum* family 51 (KAJ9418597.1), *Aspergillus pseudonomiae* (AB8266292.1), *Aspergillus alliaceus* (ALG03239.1). The dash (-) indicates the gaps. Throughout all sequences, a conserved-substitutions represented by colon (:), semi-conserved substitutions indicated by a dot (.). Non-conserved sections were shown by blank spaces; these were areas where an amino acid from one protein is substituted for another with entirely different chemical characteristics. Conserved sections indicate that an amino acid with the same chemical characteristics has been substituted for the original amino acid in one or two proteins. Amino acids with semi-conserved regions exhibit both conserved and non-conserved behavior in figure S1. For the phylogenetic tree, it is illustrated that the 9 distinct fungal protein sequences have been analysed. There are gaps across each position and missing data has been removed. It is feasible to investigate the role of fungal CYP proteins from a number of common ancestors by using the tree's branch pattern. The phylogenetic analysis shows that *F. oxysporum* family 51 (KAJ9418597.1) is more similar to *Aspergillus alliaceus* (ALG03239.1) in figure 2(c). Since both species share same ancestor.

Protein-protein interaction networks: String illustrates the functional relationships of proteins in a network of direct physical and indirect functional linkages and genome-wide connectivity, weighted and integrated, and with a confidence quantity (Kandel and Lampe, 2014). The *F. oxysporum* family 51 P450 belongs to sterol 14-demethylase identified as FOXG_11545. Total numbers of interacted proteins are 10 in figure S2, and identified as A0A0D2XUJ8, FOXG_02348, A0A0D2Y3A6, FOXG_05977, A0A0D2XUD3, A0A0D2XEJ3 A0A0D2YA17, FOXG_00394, FOXG_08223 and FOXG 06061.

Structural modelling: Structural studies and composition of different amino acids of proteins have displayed the characteristics of all the fungal CYP proteins. It was found that the five fundamental amino acids are leucine (10.5%), alanine (7.9%), valine (7.1%), serine (6.3%), and proline (6.1%) in figure 1. The highest amount of hydrophobic amino acids, such as leucine, alanine, and valine, indicates that the nature of the protein may be intracellular. In general, extracellular regions tend to have a higher proportion of hydrophilic and charged amino acids, which helps them interact with water and other polar molecules, such as serine (6.3%), threonine (5.3%) and lysine (5.1%). However, since glycine is an amino acid without a side chain and is frequently present on the protein's surface, this provides the polypeptide chain a great degree of flexibility. Given that its structure is polar and charged, aspartate tends to remain on the protein surfaces. When it becomes submerged within proteins, it additionally transforms into a salt bridge. During the development of higher order tertiary structures, histidine and lysine are the illustrations of positively charged amino acids that favor the side chains of proteins in order to form salt bridges.

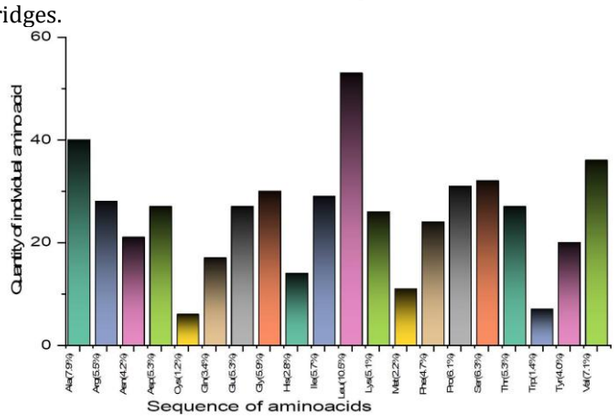

Figure 1: A column graph that shows how the P450 enzyme's predominant amino acids are contributed by the main structure.

By using PSIPRED and SOPMA online software, the secondary structure of the fungal CYP 450 has been identified and the percentage composition of these proteins was predicted in figure 2(a). The mean value of the a-helix is 45.26% relates that the a-helix region of P450 protein prefers its secondary structure, obeyed by random coils of 40.32%. Ultimately, there is a reduced quantity of the extended sheet region (11.26%) and the least amount of beta-turn (3.16%) in figure 2(b).

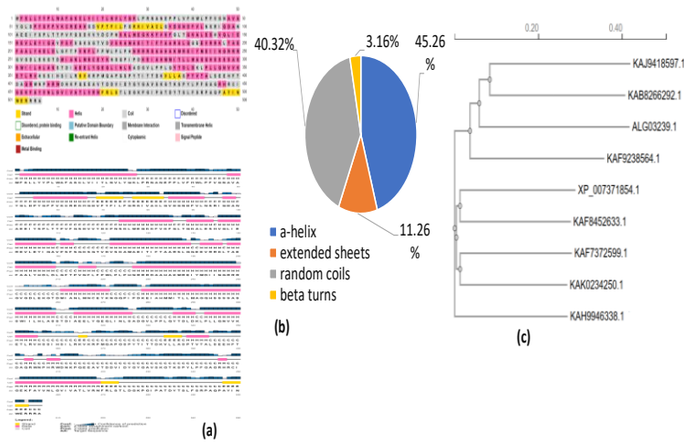

Figure 2: (a) Prediction of secondary structures (Psipred) of fungal P450 (b) pie graph that indicates which secondary structures in fungal cytochrome P450 (SOPMA) are prominent. (c) phylogenetic tree Illustrating the evolutionary interactions among multiple fungal species by using CLUSTAL OMEGA.

To assist with the establishment of models of homology of protein with different degrees of complexity, the SWISS-MODEL workplace used to develop the protein's tertiary structure that results from the spatial arrangement of secondary structures (Arnold *et al.*, 2006). According to research, the protein is effectively folded into small 3D region, as shown by the 3D structure of the protein displayed by QMEAN PDB in figure 3(a) (Mahgoub and Bolad, 2013).

The sequence identity of protein is 60.81%. The amount of non-bounded residues in the structure of P450 protein has studied with distance of 2.38 Å by using X-rays between different pairs of atoms.

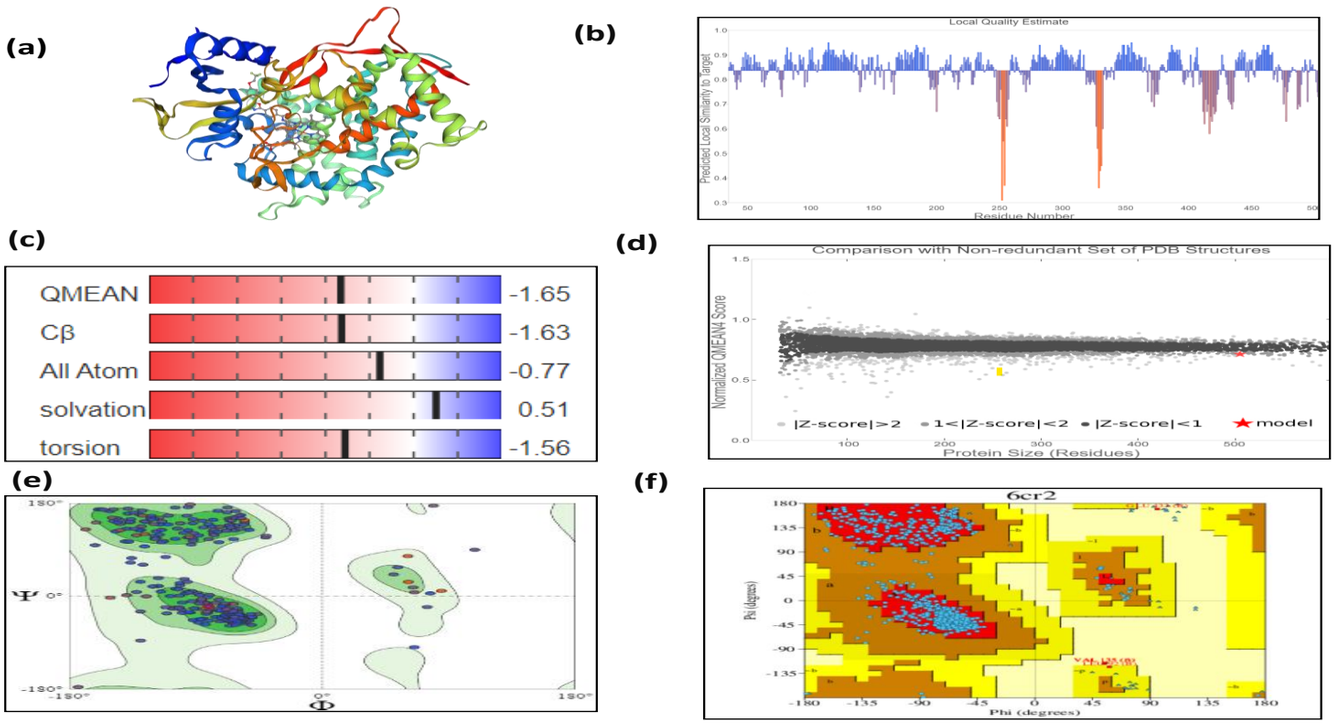

Figure 3: (a) QMEAN PDB 3D model (b) QMEANDisCo Global results; (c) & (d) Z-score value of QMEAN PDB 3D structure (e) Ramachandran plot of fungal cytochrome P450 *Fusarium oxysporum,* (f) Pro-check predicted the no. of residues in favoured, allowed, and outlier regions.

The value of QMEANDisCo Global is 0.84 ± 0.05 in figure 3(b), and QMEAN Z scores also explained in graph form on the basis of protein size (residues) in figure 3 (c & d). Sequence similarity is 0.49 and GMQE value is 0.82, range is 35-504 amino acids and total coverage is 0.92. SWISS-Model program verifies protein 3D structure determined through crystallography (Waterhouse *et al.*, 2018). The *Aspergillus fumigatus* enzyme 14-alpha demethylase sterol (CYP51B) connected with the VNI derivative N-(1-(2,4-dichloroph enyl)-2-(1H-imidazol-1-yl) ethyl)-4-(5-(2-fluoro-4-(2,2,2tri-fluoroe thoxy) phenyl)-1,3, 4-oxadiazol-2-yl) with regard to the tertiary structure, benzamide (1,3, 4-oxadiazol-2-yl) was discussed. The Ramachandran plot generated by SWISS-Model interactive workspace tool is the polypeptide chain has two torsion angles that prefers the percentage of residues in each amino acid found in a polypeptide chain's favored region, outlier areas, and allowed, as described by the (φ) and (ω) plots in figure 3(e) (Rose, 2019). Protein stability is influenced by the number of residues in the favoured region. The plot shows several results such as MolProbity score (1.41) and clash score (2.83). Ramachandran favoured regions are 95.09%. Ramachandran outliers (A495 GLN, A414 PRO, A118 VAL, A330 VAL, A326 SER) favoured 1.07%. Rotamer outliers (A367 LYS, A396GLU, A271LYS, A370 MET) are 0.99.

C-Beta deviations (A327 ALA, A44 PHE, A269 GLU, A489 LEU) are 4. Bad bonds (A306 HIS) are 2 / 3810. The regions that are most favored, A, B, and L have 744 residues with 91.2%, whereas the additional regions that are allowed (a, b, l, and p) have 68 residues with 8.3%. Moreover, just 1 residue present in the allowed region [l, ~a, ~p, ~b] with 0.1%, but only 3 residues with 0.4%* are present in the disallowed regions [XX]. The non-glycine *and* non-proline residue counts are 816 at 100%. There are 4 end-residues presents (Gly and Pro excluded). Proline has 60 residues, whereas glycine has 58. There are 938 residues in all. With the R-factor of no more than 20.0, resolution of 2.0 angstroms, 118 structures were analysed in figure 3(f).

Parameters		Score	Average
1- Dihedral angles	Phi-psi distribution	-0.01	
	Chi1-chi2 distribution	-0.23	
	Chi1 only	0.03	-0.18
	Chi3 & chi4	0.34	
	Omega	-0.55*	
2-Main chain covalent forces	Main-chain bond lengths	0.58	
	Main-chain bond angles	0.44	0.50
3-Overall average			0.09

Table 1: The measurement of the Ramachandran plot was conducted by using Pro-check analysis.

The most favoured regions [B, A, L] should contain more than 90% of high-quality models. Values below -0.5* are regarded as rare, while values below -1.0** are extremely rare in table 1.

Functional characterization of putative fungal CYP: By using the Inter-ProScan tool the output results of the reference protein were predicted. It gives information about parameters that are described below which includes families and predicts domains and important sites, functional sites, subfamilies, conserved sites in figure S3 (Jones *et al.*, 2014). The fungal protein belongs to the family of cytochrome P450 with accession numbers (IPR001128), (IPR002403), (IPR050529). Class E cytochrome P450 proteins (IPR002403), which belong to sequence cluster group IV, are represented by this entry. Despite having no discernible functional similarity, the cholesterol 7-alpha-hydroxylase (CYP7) and lanosterol 14-alpha-demethylase (CYP51) families and this make up Group IV, have a high degree of sequence similarity. Members of this family (IPR050529) are monooxygenases that catalyze the removal of methyl groups from sterol substrates, including eburicol and lanosterol which are necessary for the bile acid synthesis, steroid hormones as well as the composition of membranes. Azole medications, which block the action of sterol 14-alpha demethylase, which is essential for the growth of fungal species and synthesis of ergosterol, also target this family. The homologous superfamily containing accession ID IPR036396 and the range of residues is 25-506, the second superfamily of P450 has an accession ID SSF48264 and the range of residues is 25-504 and the CATH-Gene3D homologs superfamily of P450 has residues in the range of 32–506, which is integrated by IPR036396. The conserved sites of fungal CYP have accession ID IPR017972 and it contains the range of residues from 442-451. Another conserved site P450 cysteine heme-iron ligand has an accession ID PS00086 containing the same residues ranging from 442-451. The domain CDD type has an accession ID cd11042 and the range of residues from 62-501. Sterol 14alpha-demethylase, also known as cytochrome P450 51 (CYP51) and several similar cytochrome P450s, make up this family. When it comes to the manufacture of important sterols, CYP51 is the only cytochrome P450 enzyme that functions similarly in fungi, plants, and animals. The other features such as PHOBIUS non-cytoplasmic domain are the region of a membrane-bound proteins predicted to be outside the membrane, in the extracellular region having the range of

residues from 1-5 and 54-506. The portion of a membrane-bound protein known as the TMHMM-TMhelix is expected to be embedded in membrane and has residue ranges ranging from 4–25 to 38–60. This online webserver, NetNGlyc-1.0, is employed for estimating N/glycosylation in figure S4(b). Even though they might contain potential motifs, proteins lacking the signal peptide are uncertain to be targeted to the N-glycosylation machinery and may not be glycosylated. Red highlights indicate asparagines that are expected to be N-glycosylated in Table S1. *Fusarium oxysporum* family 51 CYP shows the glycosylation at 11 positions. It contains a threshold value of 0.5. If values of peaks are low from the threshold value then it will not be stable. Signal peptides are used to find out the cleavage site prediction in amino acids, protein localization, secretion, membrane integration, and mitochondrial targeting, detected by online server Signalp 5.0. For fungal CYP, there is no cleavage in the amino acid sequence. Signal peptide (Sec/SPI) having value 0.1248 in figure S4(a). The most accurate and reliable methodology to estimate the topology of beta-barrel transmembrane protein and alpha-helical at this moment is Deep-TMHMM. The topology of fungal CYP is globular explained in figure S4(c).

CONCLUSION: Cytochrome P450 (CYP450) is a group of enzymes that are involved in the metabolism of various substances, including drugs, hormones, and toxins. One of the most significant functions is mono-oxygenase, participating in different metabolic processes. The selected organism *F. oxysporum* is a versatile fungal specie that plays a beneficial role in biodegradation, bio-remediation and hydroxylation processes. Computational parameters are used to analyse the structural and functional analysis of fungal species that provide great information in a short time. Theoretically, the pI value of fungal Cytochrome P450 is 7.23 which indicate that it shows basic. The protein has an average molecular weight of 56902.39 Da. Research indicates that the values higher than 40 indicate the instability of the protein structure. The selected protein's instability index (II) is 47.44. The protein's primary structure reveals that it could have been intracellular in origin because hydrophobic amino acids notably leucine, alanine, and valine are present in large concentrations. The secondary structure indicates a-helix mean value of 45.26% predominantly relates to the secondary structure of protein P450 followed by a-helix region and also helps to build a 3D structure with sequence identity of protein at 60.81% along with Ramachandran-favoured regions at 95.09%. The ω-hydroxy fatty acid's production, which are valuable in the industrial sector, enhanced their efficiency because of these fungal cytochrome P450s having a wide range in commercial and pharmaceutical developments. The *F. oxysporum* flexibility and enzymatic capabilities make it a more effective participant in environmental clean-up and waste management by degradation processes.

ACKNOWLEDGEMENT: All authors highly acknowledge the University of Sahiwal, Sahiwal and Bahauddin Zakariya University, Multan for technical support.

CONFLICT OF INTEREST: All the authors declared no conflict of interest.

LIFE SCIENCE REPORTING: In current research article no life science threat was reported

ETHICAL RESPONSIBILITY: This is original research, and it is not submitted in whole or in parts to another journal for publication purpose.

INFORMED CONSENT: The author(s) have reviewed the entire manuscript and approved the final version before submission.

REFERENCES:

Aoyama, Y., Y. Yoshida and R. Sato, 1984. Yeast cytochrome p-450 catalyzing lanosterol 14 alpha-demethylation. Ii. Lanosterol metabolism by purified p-450(14)dm and by intact microsomes. Journal of biological chemistry, 259(3): 1661-1666.

Aoyama, Y., Y. Yoshida, Y. Sonoda and Y. Sato, 1989. Deformylation of 32-oxo-24,25-dihydrolanosterol by the purified cytochrome p-45014dm (lanosterol 14 alpha-demethylase) from yeast evidence confirming the intermediate step of lanosterol 14 alpha-demethylation. Journal of biological chemistry, 264(31): 18502-18505.

Arnold, K., L. Bordoli, J. Kopp and T. Schwede, 2006. The swiss-model workspace: A web-based environment for protein structure homology modelling. Bioinformatics, 22(2): 195-201.

Bano, S., L. Rasool, A. Rauf and S. Hussain, 2023. In silico characterization of heat shock protein hsp27 in mammals. World journal of biology and biotechnology, 8(3): 1-4.

Bernhardt, R. and V. B. Urlacher, 2014. Cytochromes p450 as promising catalysts for biotechnological application: Chances and limitations. Applied microbiology and biotechnology, 98(14): 6185-6203.

Breskvar, K., B. Črešnar, A. Plaper and T. Hudnik-Plevnik, 1991. Localization of the gene encoding steroid hydroxylase cytochrome p-450 from rhizopus nigricans inside a hindiii fragment of genomic DNA. Biochemical biophysical research communications, 178(3): 1078-1083.

Črešnar, B. and Š. Petrič, 2011. Cytochrome p450 enzymes in the fungal kingdom. Biochimica et Biophysica Acta (BBA)-proteins and proteomics, 1814(1): 29-35.

Falquet, L., M. Pagni, P. Bucher, N. Hulo, C. J. Sigrist, K. Hofmann and A. Bairoch, 2002. The prosite database, its status in 2002. Nucleic acids research, 30(1): 235-238.

Gasteiger, E., C. Hoogland, A. Gattiker, S. e. Duvaud, M. R. Wilkins, R. D. Appel and A. Bairoch, 2005. Protein identification and analysis tools on the expasy server. Springer.

Hannemann, F., A. Bichet, K. M. Ewen and R. B. A.-G. S. Bernhardt, 2007. Cytochrome p450 systems biological variations of electron transport chains. Biochimica et biophysica Acta (BBA) – general subjects, 1770(3): 330-344.

Hardwick, J. P., 2008. Cytochrome p450 omega hydroxylase (cyp4) function in fatty acid metabolism and metabolic diseases. Biochemical pharmacology, 75(12): 2263-2275.

Herzog, B., A. Schultheiss, J. J. P. Giesinger and Photobiology, 2018. On the validity of beer–lambert law and its significance for sunscreens. Photochemistry and photobiology, 94(2): 384-389.

Ingelman-Sundberg, M., 2004. Human drug metabolising cytochrome p450 enzymes: Properties and polymorphisms. Naunyn Schmiedebergs Arch pharmacol, 369(1): 89-104.

Jones, D. T., 1999. Protein secondary structure prediction based on position-specific scoring matrices. Journal of molecular biology, 292(2): 195-202.

Jones, P., D. Binns, H.-Y. Chang, M. Fraser, W. Li, C. McAnulla, H. McWilliam, J. Maslen, A. Mitchell and G. Nuka, 2014. Interproscan 5: Genome-scale protein function classification. Bioinformatics, 30(9): 1236-1240.

Kahn, R. A. and F. Durst, 2000. Function and evolution of plant cytochrome p450. In: Recent advances in phytochemistry. Elsevier: pp: 151-189.

Kandel, S. E. and J. N. Lampe, 2014. Role of protein–protein interactions in cytochrome p450-mediated drug metabolism and toxicity. Chemical research in toxicology, 27(9): 1474-1486.

Kyte, J., 1993. A simple method for displaying the hydropathic character of a protein. Journal of molecular biology, 268: 10558-10563.

Lisowska, K., J. Szemraj, S. Różalska and J. J. F. m. l. Długoński, 2006. The expression of cytochrome p-450 and cytochrome p-450 reductase genes in the simultaneous transformation of corticosteroids and phenanthrene by cunninghamella elegans. 261(2): 175-180.

Mahgoub, E. O. and A. Bolad, 2013. Correctness and accuracy of template-based modeled single chain fragment variable (scfv) protein anti-breast cancer cell line (mcf-7). Open journal of genetics, 3(03): 183.

Mahgoub, E. O. and A. J. O. J. o. G. Bolad, 2013. Correctness and accuracy of template-based modeled single chain fragment variable (scfv) protein anti-breast cancer cell line (mcf-7). Open journal of genetics,3(03): 183.

Moktali, V., J. Park, N. D. Fedorova-Abrams, B. Park, J. Choi, Y.-H. Lee and S. J. B. g. Kang, 2012. Systematic and searchable classification of cytochrome p450 proteins encoded by fungal and oomycete genomes. BMC genomics, 13(1): 1-13.

Moorthy, B., C. Chu and D. J. Carlin, 2015. Polycyclic aromatic hydrocarbons: From metabolism to lung cancer. Toxicological sciences, 145(1): 5-15.

Nakahara, K., T. Tanimoto, K.-i. Hatano, K. Usuda and H. J. J. o. B. C. Shoun, 1993. Cytochrome p-450 55a1 (p-450dnir) acts as nitric oxide reductase employing nadh as the direct electron donor. The Journal of biological chemistry, 268(11): 8350-8355.

Nakayama, N., A. Takemae and H. Shoun, 1996. Cytochrome p450foxy, a catalytically self-sufficient fatty acid hydroxylase of the fungus fusarium oxysporum. The journal of biochemistry, 119(3): 435-440.

Nelson, D. R., L. Koymans, T. Kamataki, J. J. Stegeman, R. Feyereisen, D. J. Waxman, M. R. Waterman, O. Gotoh, M. J. Coon and R. W.

Estabrook, 1996. P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics genomics, 6(1): 1-42.

Rose, G. D., 2019. Ramachandran maps for side chains in globular proteins. Proteins: structure, function, and bioinformatics, 87(5): 357-364.

Safe, S., 1990. Polychlorinated biphenyls (pcbs), dibenzo-p-dioxins (pcdds), dibenzofurans (pcdfs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (tefs). Critical reviews in toxicology, 21(1): 51-88.

Shoun, H. and T. Tanimoto, 1991. Denitrification by the fungus fusarium oxysporum and involvement of cytochrome p-450 in the respiratory nitrite reduction. Journal of biological chemistry, 266(17): 11078-11082.

Song, J., S. Zhang and L. Lu, 2018. Fungal cytochrome p450 protein cyp51: What we can learn from its evolution, regulons and cyp51-based azole resistance. Fungal biology reviews, 32(3): 131-142.

Syed, K. and J. S. Yadav, 2012. P450 monooxygenases (p450ome) of the model white rot fungus phanerochaete chrysosporium. Critical reviews in microbiology, 38(4): 339-363.

Szklarczyk, D., A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos and K. P. Tsafou, 2015. String v10: Protein–protein interaction networks, integrated over the tree of life. Nucleic acids research, 43(D1): D447-D452.

Van Gorcom, R. F., C. A. van den Hondel and P. J. Punt, 1998. Cytochrome p450 enzyme systems in fungi. Fungal genetics and biology, 23(1): 1-17.

Waterhouse, A., M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, F. T. Heer, T. A. P. de Beer, C. Rempfer and L. Bordoli, 2018. Swiss-model: Homology modelling of protein structures and complexes. Nucleic acids research, 46(W1): W296-W303.

Wilkins, M. R., E. Gasteiger, A. Bairoch, J.-C. Sanchez, K. L. Williams, R. D. Appel and D. F. Hochstrasser, 1999. Protein identification and analysis tools in the expasy server. 2-D proteome analysis protocols: 531-552.

Wolfand, J. M., G. H. LeFevre and R. G. Luthy, 2016. Metabolization and degradation kinetics of the urban-use pesticide fipronil by white rot fungus trametes versicolor. Environmental science: processes & impacts, 18(10): 1256-1265.

Wunder, T., S. Kremer, O. Sterner and H. Anke, 1994. Metabolism of the polycyclic aromatic hydrocarbon pyrene by aspergillus niger sk 9317. Applied microbiology and biotechnology, 42(4): 636-641.