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Exploration of alcohol dehydrogenase EutG from *Bacillus tropicus* as an eco-friendly approach for the degradation of polycyclic aromatic compounds

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Polycyclic aromatic compounds (PACs) are pervasive environmental contaminants derived from diverse sources including pyrogenic (e.g., combustion processes), petrogenic (e.g., crude oil), and biological origins. They are commonly found in gasoline, coal, and crude oil, reflecting their prevalence and varied origins in natural and anthropogenic activities. The aim of this study is to use *Bacillus tropicus* which is a spore-forming, gram-positive and facultative anaerobic bacteria, containing a gene for PACs degradation. In this study bacterial sample was collected from women's vaginal discharge through streaking and spreading techniques. The DNA was extracted from bacterial culture and then the bacterium was identified through 16S rRNA which appeared to be *B.tropicus*. Then the computational analysis was conducted where the sequence similarity and functional analysis of alcohol dehydrogenase EutG protein from *B.tropicus* was analyzed through PSI-BLAST and SMART tool, respectively. The PSI-BLAST showed 100% query coverage score and 9 domains of alcohol dehydrogenase EutG protein were predicted through SMART tool. The quality of the protein was also assessed through ProQ server with a predicted LQ score of 8.091, a Maxsub score of -0.350 and a z score of -10.76. Then the phylogenetic analysis was conducted to know the evolutionary relationship and closely related taxa. The 3D structure of the protein was predicted through SWISS MODEL and its quality was predicted through ERRAT with overall quality factor of 98.708. The Ramachandran plot also predicted its quality and showed that 93.8% residues were in the most favored region. After this, 3D structure of PACs were obtained from PubChem and molecular docking of the protein was performed with each of the compound. The lowest energy of -10.3 was obtained with Indeno[1,2,3-cd] pyrene and the best docked complex was visualized through discover studio to analyze its binding residues. Lastly, *in-silico* site-directed mutagenesis studies were performed which showed that the EutG gene (codes for alcoholic dehydrogenase) obtained from *B. tropicus*, will not get altered or have any decreasing effect on the enzyme's stability if it goes through any mutations. This suggests that *B. tropicus* can act as an efficient, non-virulent, and reliable candidate for the eco-friendly and cost-effective bioremediation of PACs.

Keywords Alcohol dehydrogenase, *Bacillus tropicus*, Bioremediation, Molecular docking, PACs, Site-directed mutagenesis

Polycyclic aromatic compounds (PACs) are ubiquitous contaminants that are polluting the environment. There are wide varieties of PACs such as pyrene, naphthalene, anthracene, phenanthrene etc. which are causing adverse circumstances on the health of the environment as well as human beings¹. PACs is a broader term which includes

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polyaromatic hydrocarbons (PAHs), but the key difference is, PAHs consist of only carbon and hydrogen atoms in their ring structure, whereas, PACs consists of other atoms like nitrogen, sulfur, oxygen besides carbon and hydrogen². PACs can be produced as complex mixtures or as separate compounds and they naturally occur in fossil fuels such as petroleum, coal etc. Natural events like volcanic eruptions and wildfires emit PACs in large amounts. Burning of woods and garbage is also a major cause of its release into the environment³. The toxicity of these compounds affects mammals, aquatic life, terrestrial invertebrates and growth of plants. These compounds are difficult to degrade and can stay for a longer period of time in the environment, thereby, affecting the quality of the environment⁴.

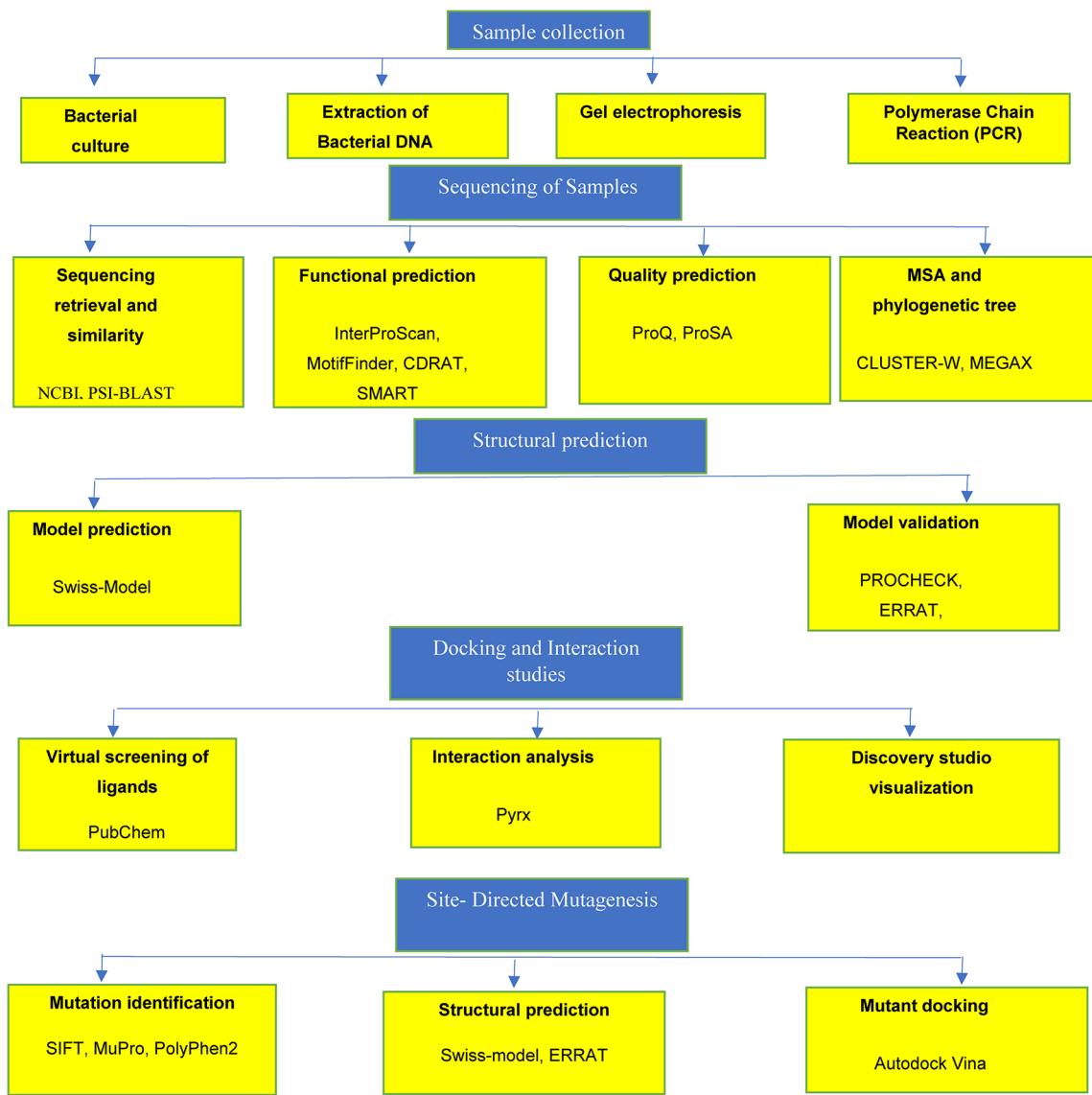
Humans are more frequently exposed to complex mixtures of PACs as compared to individual compounds. The major source of polycyclic aromatic compounds depends on various factors such as sunlight resulting in the deterioration of contaminated materials etc.⁵. There are many ways in which people are exposed to PACs like consuming grilled meat or smoked seafood⁶. Others include, inhaling polluted air, drinking tainted water, absorption of PACs through skin from the contaminated soil and exposure at places where PACs are released. Some PACs are associated with cancers and can also affect a person's immune system as well as reproductive system⁷.

There are four methods that are used for the removal of PACs from the environment which include physical, chemical, biological and combined methods⁸. Physical methods include membrane filtration, floatation and adsorption whereas chemical treatment method includes chemical precipitation, chemical oxidation, electrochemical technologies etc. These treatment methods are not easily available as they require high cost and work efficiency⁹. Biological methods include bioremediation which involve the enzymatic pathways and genetic regulations of microorganisms for the degradation of PACs. This is a much safer and effective approach to remediate and clean the environment by the utilization of microorganisms¹⁰. Currently many bacterial species such as *Pseudomonas putida*, *Pseudomonas stutzeri*, *Sphingomonas*, *Sphingobium*, *Novosphingobium*, *Ralstonia* etc. are used for degrading PACs. However, these combined methods involves the combination of all useful techniques at the same time¹¹.

EutG alcohol dehydrogenase from *B. tropicus* possesses the capability to degrade PACs due to its enzymatic activity. The significance of EutG alcohol dehydrogenase lies in its potential as an effective agent for degrading PACs in the environment¹². This enzyme is adept at catalyzing the oxidation of alcohols, suggesting that it may also possess the ability to metabolize similar chemical structures found in PACs¹³. By focusing on EutG, this study aims to explore its enzymatic activity against PACs and assess its potential as a bioremediation tool for mitigating environmental pollution caused by these harmful compounds.

Specifically, the study focuses on isolating *B. tropicus* and investigating the capabilities of its enzyme, alcohol dehydrogenase EutG, in degrading PACs. Through in-silico techniques, the study examines the enzyme's activity against PACs and conducts structural analysis to identify key residues and active sites. Molecular dynamic simulations and mutational analysis demonstrate the enzyme's increased stability and efficacy, suggesting its potential for PAC degradation.

Methodology



Sample collection

All the samples were taken from the women's vaginal discharges that were at the peak of their reproductive years through sterilized cotton swabs enclosed in tubes (All methods were carried out in accordance with relevant guidelines and regulations. Study approval was obtained from the "Bioethical Committee of the Faculty of Science and Technology, University of Central Punjab. Informed consent was obtained from every participant). The samples were collected from Lady Aitchison Hospital located on Mayo Hospital Rd, Anarkali Bazaar, Lahore.

Bacterial culture preparation

The bacterial cells were inoculated using Luria Bertani (LB) agar media. Bacterial cells were cultured on LB agar under aerobic conditions at 37 °C for 24 h. LB agar media was prepared containing Tryptone, Sodium Chloride (NaCl), Yeast Extract, and Agar per litter. Pure cultures were isolated using spreading and streaking methods. Antibacterial activity against *B. tropicus* was assessed using the agar well technique. Petri plates containing LB agar were prepared and wells of 5 mm were created. The bacterial strain was spread on the plates and allowed to dry. Microbial dilutions were then added to the wells. After incubation, the diameter of the inhibition zones was measured in millimetres and compared with positive control Chlortetracycline (30 mcg) disks, as well as distilled water as a negative control.

Extraction of bacterial DNA

The molecular identification of *B. tropicus* strains involved amplifying the 16S rDNA gene using the Cetyl Trimethyl Ammonium Bromide (CTAB) method to extract genomic DNA. Universal prokaryotic primers (27F

and U1492R) were used for PCR, with a reaction mixture prepared and cycled through 35 rounds. After the completion of PCR, the products were stored at 4 °C. 1% (w/v) agarose gel electrophoresis was used to visualize the amplified 16S rRNA product under a BIORAD gel documentation system. The samples were sequenced using the commercial services provided by 1st BASE.

Biochemical testing to identify unknown bacterial strains

Biochemical tests were conducted to identify the unknown bacterial strains based on differences in biochemical activities such as carbohydrates/fats metabolism, production of certain enzymes/proteins and utilization of specific reagents. These biochemical tests included catalase test, gelatin test, sugar fermentation test and 6.5% NaCl test.

Computational analysis of alcohol dehydrogenase EutG protein against polycyclic aromatic compounds

Sequence similarity

PSI-BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) search with Protein Data Bank (PDB) database was carried out to identify structural homologs. A sequence resemblance search was performed against the non-redundant (nr) database of protein sequences. Sequences having coverage and percent similarity less than 90% were removed from the analysis and those having greater than 90% were considered homologs for further probable function identifications¹⁴.

Functional analysis of EutG protein

Different bioinformatics tools have been used for the prediction of functions of the alcohol dehydrogenase EutG protein. The family and Motif of the protein were identified using INTERPROSCAN and MotifFinder. INTERPROSCAN (<https://www.ebi.ac.uk/interpro/search/sequence-search>) is an online tool performed to obtain the functional analysis and classify the protein sequences into the families and domains as well as binding sites¹⁵. For predicting domains, the CDRAT and SMART online servers has been used. CDART (https://www.ncbi.nlm.nih.gov/Structure/structure_services.html) used protein domain profiles and group proteins similar to the query sequence for searching similar proteins and an architecture based score was assigned¹⁶. SMART (<http://smart.embl-heidelberg.de>) is a Simple Modular Architecture Research Tool that annotates and explores protein domain architecture¹⁷.

Quality prediction

This study used ProQ—Protein quality prediction (bioinfo.se) along with ProSA-web (ProSA-web—Protein Structure Analysis (sbg.ac.at). The ProQ (<https://proq.bioinfo.se/ProQ/ProQ.html>) server employs diverse measures to assess the quality of protein models and offers a confidence score based on statistical and machine learning methods. It is extensively utilized for validating and comparing models, assisting in the comprehension of protein structure and function¹⁸. ProSA (<https://prosa.services.came.sbg.ac.at/prosa.php>) web server is used for protein structure and function prediction and analysis. To assess the quality of protein structures, it computes numerous structural metrics such as energy profiles, solvation potential, and Z-score. The ProsA server is an excellent resource for protein structure prediction and validation, as well as protein engineering and drug creation¹⁹.

Multiple sequence alignment and phylogenetic analysis

The multiple sequence alignment and phylogenetic analysis are indispensable tools for studying molecular evolution, understanding biological diversity, predicting protein structure and function, and advancing various areas of biomedical and evolutionary research. The multiple sequence alignment (MSA) and phylogenetic analysis were predicted through MEGA version 5.1 (<https://www.megasoftware.net>) by using the position substitution and neighbour-joining algorithm. The MEGA predicts the conserved regions in the query sequences²⁰.

Structure prediction

Model prediction and validation

Swiss-Model (<https://swissmodel.expasy.org/>) was used for the generation of a three-dimensional structure of alcohol dehydrogenase EutG. Swiss-Model is an online tool for the homology modelling of protein and is accessible through Expasy web portal²¹. The predicted structure was chosen for further validation and experimental analysis. The predicted three-dimensional protein structure was verified through ERRAT (<https://www.doe-mbi.ucla.edu/errat/>) and Ramachandran plot by using PROCHECK (<http://services.mbi.ucla.edu/PROCHECK/>). ERRAT analyses the atomic interactions and plots the quality factor of the protein structure by comparing it with highly refined structures²², whereas, PROCHECK examines the stereochemical quality and residual geometry of the predicted protein structure²³.

Docking and interaction studies

Virtual screening of ligands and preparation of 3D structure of alcohol dehydrogenase

10 Polycyclic aromatic compounds which are hazardous to the environment were selected from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and downloaded. PubChem is the largest database that contains information about chemical molecules and their activities. It also gives information about the physical and chemical properties of a substance or molecule²⁴. Before Molecular Docking, the protein was prepared by utilizing Discovery Studio. It is a free molecular modelling software which can be used to view and analyse data on proteins and small molecules. All the water molecules and ligands of the protein were removed so that the protein is purified and ready for the docking analysis.

Molecular docking studies

Docking of the 10 polycyclic aromatic compounds with the alcohol dehydrogenase EutG protein was performed by using PyRx. It is a virtual screening software that can perform docking of multiple ligands at the same time which makes it more efficient and streamlined²⁵. The purified 3D structure was loaded on PyRx and selected as a macromolecule. All the ligands were uploaded one by one and their energies were minimized so that their atoms do not cause hindrance in interaction with the receptor protein. After this, the grid was set and docking was conducted. The results appeared in the form of energies and more negative it is, the better the results are. The interaction of the best model and protein was visualized through Discovery Studio software. The binding residues between the protein and ligands were analyzed. Moreover, the distance and types of amino acids of the docking complex were also examined.

Site-directed mutagenesis

Mutation identification and structure prediction

Mutational analysis is important to understand the stability and efficacy of the protein in the environment when it goes under mutational changes. NCBI BLAST was run and the mutations were found through multiple sequence alignment options. Mutation identification and its validation were established by using three different tools. SIFT (https://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html) was used to predict the possible changes in protein by SNP²⁶. MuPro (<https://www.ics.uci.edu/~baldig/mutation.html>) was used to check the consistency of protein after detecting the mutation²⁷. Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>) checked the potential effect of single amino acid substitution in the protein structure²⁸. After mutational identification, SWISS-MODEL was used to build the mutated structure of alcohol dehydrogenase EutG again.

Mutant docking and interaction studies

Docking analysis was again performed between the mutated alcohol dehydrogenase EutG and Indeno[1,2,3-cd] pyrene that show maximum interaction with non-mutated protein. Docking analysis of the mutated protein with the ligand was conducted by using Autodock Vina²⁹. The best resulting output was then visualized on Discovery Studio to determine its interaction and binding residues.

Results

Preparation of bacterial plates

The cotton swab containing the sample was streaked over LB agar plates. The isolated colonies with distinct morphological characteristics were used to get pure cultures.

Molecular identification of bacteria

The visualized image of the amplified 16S rRNA gene observed in the Gel Documentation System. Figures 1 and 2 displays the results of the amplification of the extracted DNA for the 16S rRNA gene using the 27F/1492R primer set. The chromatogram of the *B. tropicus* is shown in Fig. 3 which indicates that there is no distortion all the peaks exhibit uniformity.

Biochemical testing

The results of all the biochemical tests are given in Table 1. The results shows that all the biochemical tests are positive in *B. tropicus* strain such as catalase, gelatin, 6.5% NaCl tolerance, Dextrose fermentation test (DFT), Sucrose Fermentation test (SFT) and Lactose fermentation test (LFT).

Sequence similarity

The results of the BLAST-p and PSI-BLAST showed 100 homologous protein sequences with a coverage score of 100% and a percent (%) identity of 98.1–100%. This high identity and coverage score indicates that the entire length of the query protein was aligned with each of the homologous sequences and these homologous proteins

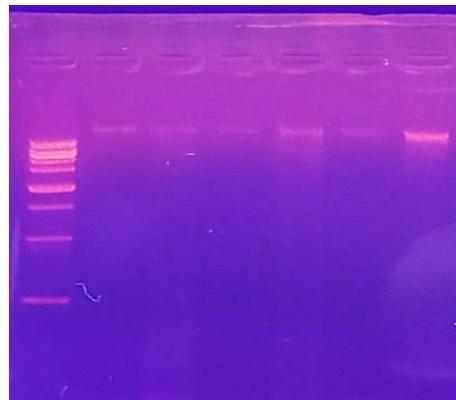


Fig. 1. DNA extraction of the *Bacillus tropicus* strain bands along with the DNA ladder.

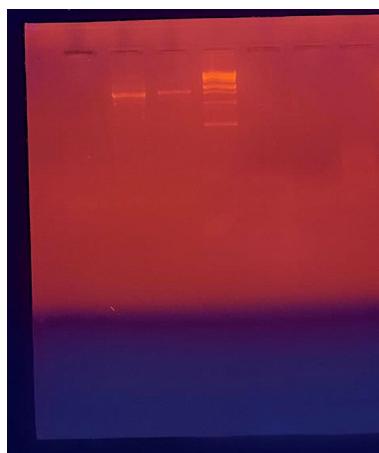


Fig. 2. 16S rRNA sequencing of the bacterial strain.

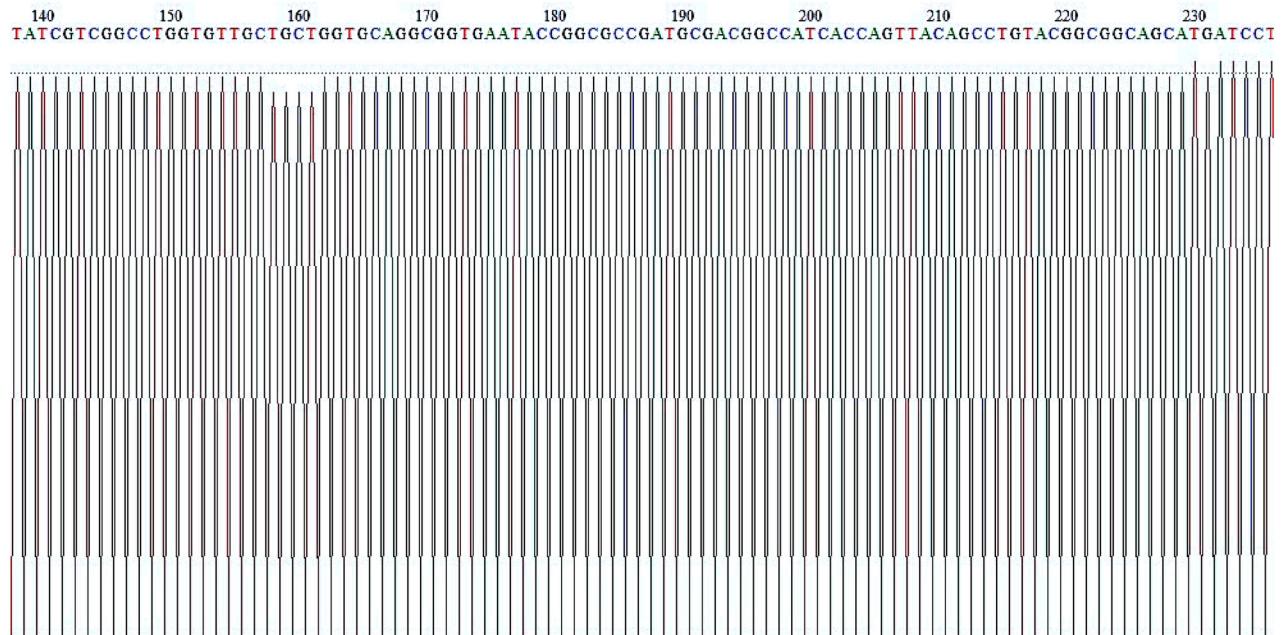


Fig. 3. The chromatogram of *B. tropicus* shows uniformity with no distortion, displaying all peaks clearly.

Sr. no	Test names	<i>B. tropicus</i>
1	Catalase test	+ive
2	Gelatin test	+ive
3	6.5% NaCl tolerance	+ive
4	Dextrose fermentation test (DFT)	+ive
5	Lactose fermentation test (LFT)	+ive
6	Sucrose fermentation test (SFT)	+ive

Table 1. Biochemical testing results are mentioned below.

are highly similar to the query protein. Furthermore, these sequences were appropriate for doing MSA to determine conserved regions and phylogenetic analysis to infer the evolutionary relationship.

Functional prediction of protein

The results of functional prediction of the proteins show that alcohol dehydrogenase EutG protein belongs to the iron-containing alcohol dehydrogenase family and domains as shown in Table 2. Findings from CDRAT revealed that alcohol dehydrogenase EutG belongs to the superfamily dehydroquinate synthase (DHQS) like also known as iron-containing alcohol dehydrogenase (Fe-ADH) family comprising Rossmann fold-like topology. In-depth sequence analysis explained its role in the catalytic activity and oxidoreductase activity which convert 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) to dehydroquinate (DHQ) in the second step of the shikimate pathway. The superfamily contains a dehydrogenate synthase-like protein structural fold and mostly contains iron.

Multiple sequence alignment and phylogenetic analysis

MSA of alcohol dehydrogenase EutG protein was performed through CLASTALW. MEGA5 was used all obtained alignments for phylogenetic analysis to assess function prediction. To establish an evolutionary relationship, the phylogenetic tree was constructed with protein sequences of alcohol dehydrogenase from different species using the Neighbour-joining method as shown in Fig. 4. The resulting phylogenetic tree revealed evolutionary relationship between *Bacillus cereus*, *Bacillus anthracis* and *Bacillus paranthracis*.

Model structure prediction

The best structural model of alcohol dehydrogenase EutG from *Bacillus tropicus* was generated using the Swiss-Model server. Based on the server's evaluation criteria, the model with the highest scoring was selected for further analysis. The result shows GQME 0.95 and QMEANDisCo Global 0.94 ± 0.05 . The query protein model shows 98.50% identity with the C2MK98.1. A template models. The generated 3D structure is shown in Fig. 5.

Structure quality prediction and validation

The quality of the protein was assessed using the ProQ server, which considered two measures: LGscore and Maxsub score. An excellent model was achieved for the alcohol dehydrogenase EutG protein, with a predicted LG score of 8.091 and a Maxsub score of -0.350 shown in Fig. 6A. The predicted high LGscore signifies that the protein model is of high quality, with a strong likelihood of accurately representing the protein's native structure. Furthermore, the ProsA server shows a z score of -10.76 as the more negative the z score better the predicted model (Fig. 6B). This z score suggests that the model is exceptionally good and closely resembles the native conformation of high-quality experimental protein structures.

Furthermore, ERRAT plot analysis in Fig. 6C represents the structure quality error values across the amino acid sequence of the protein. The overall quality factor for the EutG protein model was calculated to be 98.708. This high score indicates a very low frequency of structural errors, suggesting that the model is highly reliable. The graphical representation is shown in Fig. 6D. Secondly, the model was validated via the RAMACHANDRAN plot using the PROCHECK. The Ramachandran plot showed that 93.8% of residues were in the most favoured region, while 6.2% of residues were in the allowed region, verifying that the predicted 3D model of the protein is of good quality as shown in Fig. 6.

Docking and interaction studies

Virtual screening of ligands

The polycyclic aromatic compounds were downloaded from PubChem and are shown in Table 3. The three-dimensional structures and PubChem CID are also mentioned in Table 3.

Interaction analysis

Docking was performed between alcohol dehydrogenase EutG from *B. tropicus* and 10 polycyclic aromatic compounds. The results appeared in the form of energies and the best energy was with Indeno[1,2,3-cd] pyrene as shown in Table 4. The best model of Indeno[1,2,3-cd] pyrene was selected and visualized on Discovery Studio with the protein. The docked complex of alcohol dehydrogenase EutG and Indeno[1,2,3-cd] pyrene is shown

Sr. no	Domains	Name	Start	End
1	DNA polymerase alpha chain like domain	POLIIIAc	5	72
2	Cell division protein 48 (CDC48) domain 2	CDC48_2	22	83
3	Dihydrodipicolinate synthetase family	DHDPS	30	305
4	DNA topoisomerase IV	TOP4c	90	364
5	LeuA allosteric (dimerisation) domain	LeuA_dimer	126	260
6	Exonuclease domain in DNA-polymerase alpha and epsilon chain	EXOIII	133	296
7	Modified RING finger domain	Ubox	174	227
8	Alpha-macro-globulin thiol-ester bond-forming region	Thiol-ester_cl	269	293
9	Rho termination factor, N-terminal domain	Rho_N	319	363

Table 2. The domains predicted through the SMART online tool of the alcohol dehydrogenase EutG protein.

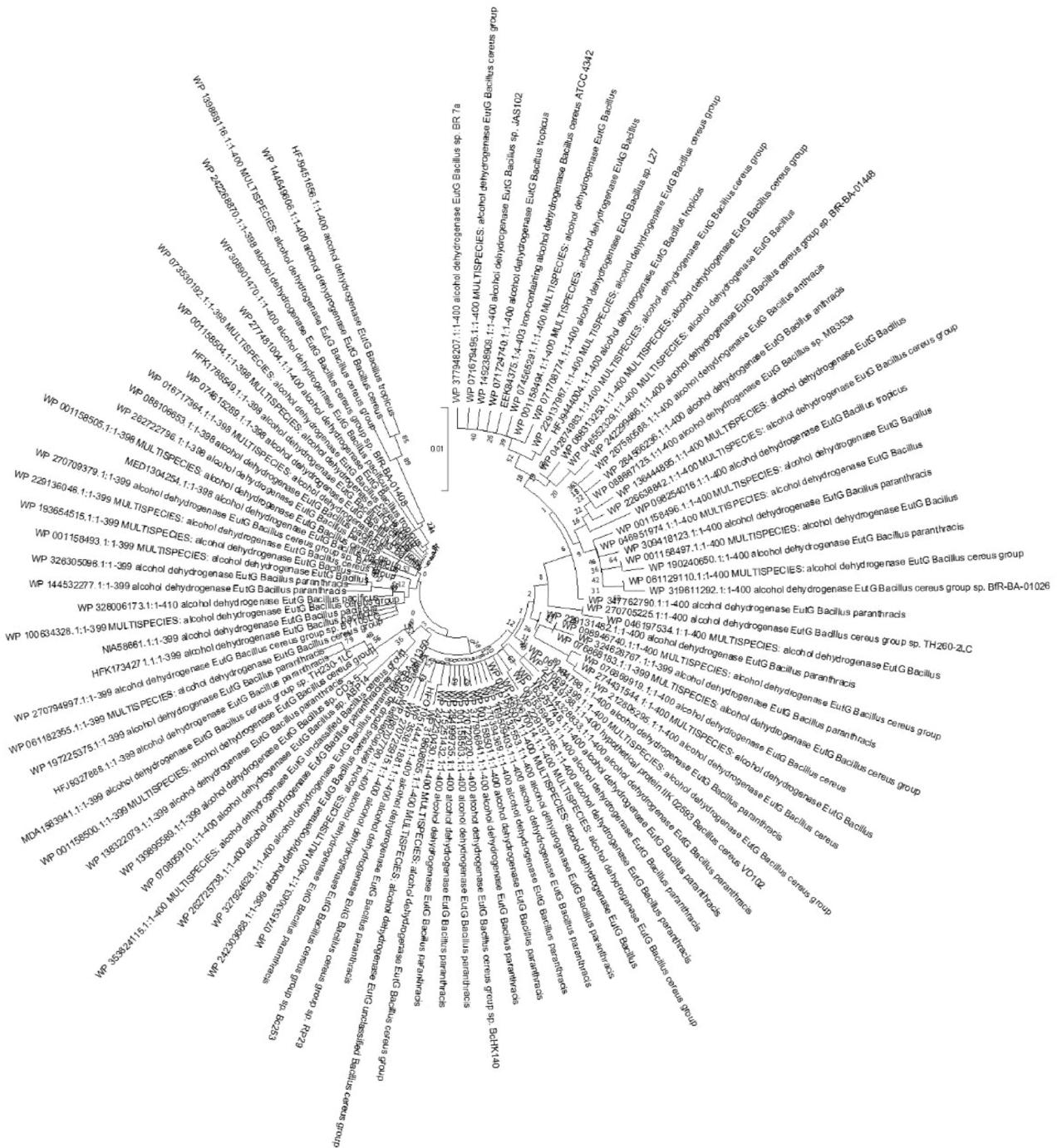


Fig. 4. The phylogenetic analysis of the alcohol dehydrogenase EutG protein of *B. tropicus* with other proteins constructed by MEGA.

in Fig. 7a. The binding residues of the complex are Asp102, His278, Met163, Val150 and Val152. These key residues play significant roles in stabilizing the docked complex through various interactions. The 2D diagram of the interaction between the protein and Indeno[1,2,3-cd] pyrene is shown in Fig. 7b. The electrostatic surface potential map of this docked complex is shown in Fig. 7c. The blue region in the surface map shows positive potential, which is associated with basic amino acids, whereas the red region shows negative potential which is associated with acidic amino acids and the white region indicates neutral potential which is linked with non-polar amino acids.

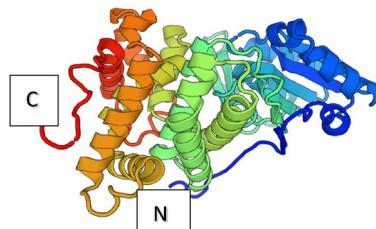


Fig. 5. Predicted 3D model of alcohol dehydrogenase EutG from SWISS-MODEL. The blue end shows the N-terminal of the protein structure and red end shows the C-terminal of the protein structure.

Site-directed mutagenesis

Mutation identification and structure prediction

The mutations identified by using different mutation prediction tools are shown in Table 5 and the mutated sequence that contained all negative mutations that cause instability in protein are highlighted in Fig. 8. The structure of the mutated protein predicted by SWISS-MODEL is shown in Fig. 9.

Mutant docking and interaction studies

Docking was performed between the Indeno[1,2,3-cd] pyrene and *B. tropicus* mutated enzyme alcoholic dehydrogenase EutG by Autodock vina that give better interaction results in -10.5 as shown in Table 6. This indicates that even after mutation, the complex is stable because no binding residues were affected. The docked complex is shown in Fig. 10 and the 2D diagram of the binding residues with the ligand is shown in Fig. 11. The electrostatic surface potential map of the docked complex indicating positive, negative and neutral potential as shown in Fig. 12.

Discussion

Bacteria, with a history spanning over three billion years, have evolved to utilize energy from a wide range of compounds, considered them as the reputation of being nature's ultimate scavengers³⁰. Their remarkable adaptability has made them valuable in the degradation and remediation of the environmental hazards. Numerous bacteria have been discovered with the ability to degrade polycyclic aromatic compounds (PACs), with particular emphasis on the degradation of naphthalene and phenanthrene, which has received extensive research attention³¹. Oxidative degradation of hydrocarbons and their derivatives in ecosystems have been done by various bacteria and fungi³². To explore the potential of *Bacillus specie*, computational methods were used to examine the binding affinity of polycyclic aromatic compounds. The study aimed to validate the organism's effectiveness and determine the enzyme's selectivity towards different PACs.

PAHs, which are aromatic compounds consisting of two or more bonded aromatic rings in various arrangements (linear, angular, or clustered), are known to be hazardous to living organisms and some are potential carcinogens³³. PAHs include naphthalene, fluorene, anthracene, phenanthrene, pyrene, benzo[a]pyrene and fluoranthene⁷. Due to their harmful nature, the United States Environmental Protection Agency (USEPA) has included 16 PACs in its list of priority pollutants³⁴. PAC pollution primarily arises from various human activities, including automobile emissions, fuel combustion, petroleum product spills, industrial discharges, and waste incineration. These activities release PACs into air, water, and soil, where they persist and accumulate due to their stable, hydrophobic nature. Understanding the specific sources and behaviours of PACs in different environments is critical for implementing targeted remediation efforts and minimizing exposure risks. Additionally, identifying these primary sources in developing countries will help to apply sustainable practices to reduce PAC emissions and protect ecosystems³⁵.

In the current study, the *B. tropicus* strain isolated from the vaginal discharge of the women was identified with respect to morphological and 16S RNA gene sequencing. Despite its known potential in recalcitrant compounds biodegradation, this bacterial strain of the genus *Bacillus sp.*, is reported for its ability to degrade PAHs and its derivatives. In a study conducted by Ni'matuzahroh et al.³⁶, the degradation of naphthalene and phenanthrene by *B. subtilis* 3KP was investigated. The degradation process involved the formation of 1-hydroxy-2-naphthoic acid, salicylic acid, and pyrocatechol as intermediates³⁶. Sonwani et al.³⁷ reported that *B. cereus* RKS4 was capable of degrading naphthalene. During this degradation process, 2-naphthol and catechol were formed as intermediate products³⁷.

The protein functional predictions show that ADH-EutG protein belongs to the iron-containing alcohol dehydrogenase (Fe-ADH) family comprising Rossmann fold-like topology and has a functional role in the catalytic activity and oxidoreductase activity. Furthermore, the quality prediction of the protein shows that LG score of 8.091 and a Maxsub score of -0.350 depicting that the protein is stable and ProsA shows a z score of -10.76 as the more negative the z scores best is the predicted model. The 3D structure of the ADH-EutG protein was predicted through Swiss-MODEL and validation was performed through POR-CHECK. The ERRAT value was 98.7 and the RAMACHANDRAN plot shows that 93.8% of residues were in the most favored region, while 6.2% of residues were in the allowed region, verifying that the predicted 3D model is of good quality.

Molecular docking analysis was performed through PyRx to find the interactions between protein ADH-EutG and pentachlorophenol derivatives. The results show that the Indeno[1,2,3-cd] pyrene exhibits the highest binding affinity of -10.3 with Root Mean Square Deviation RMSD 5.426. The RMSD determines whether the

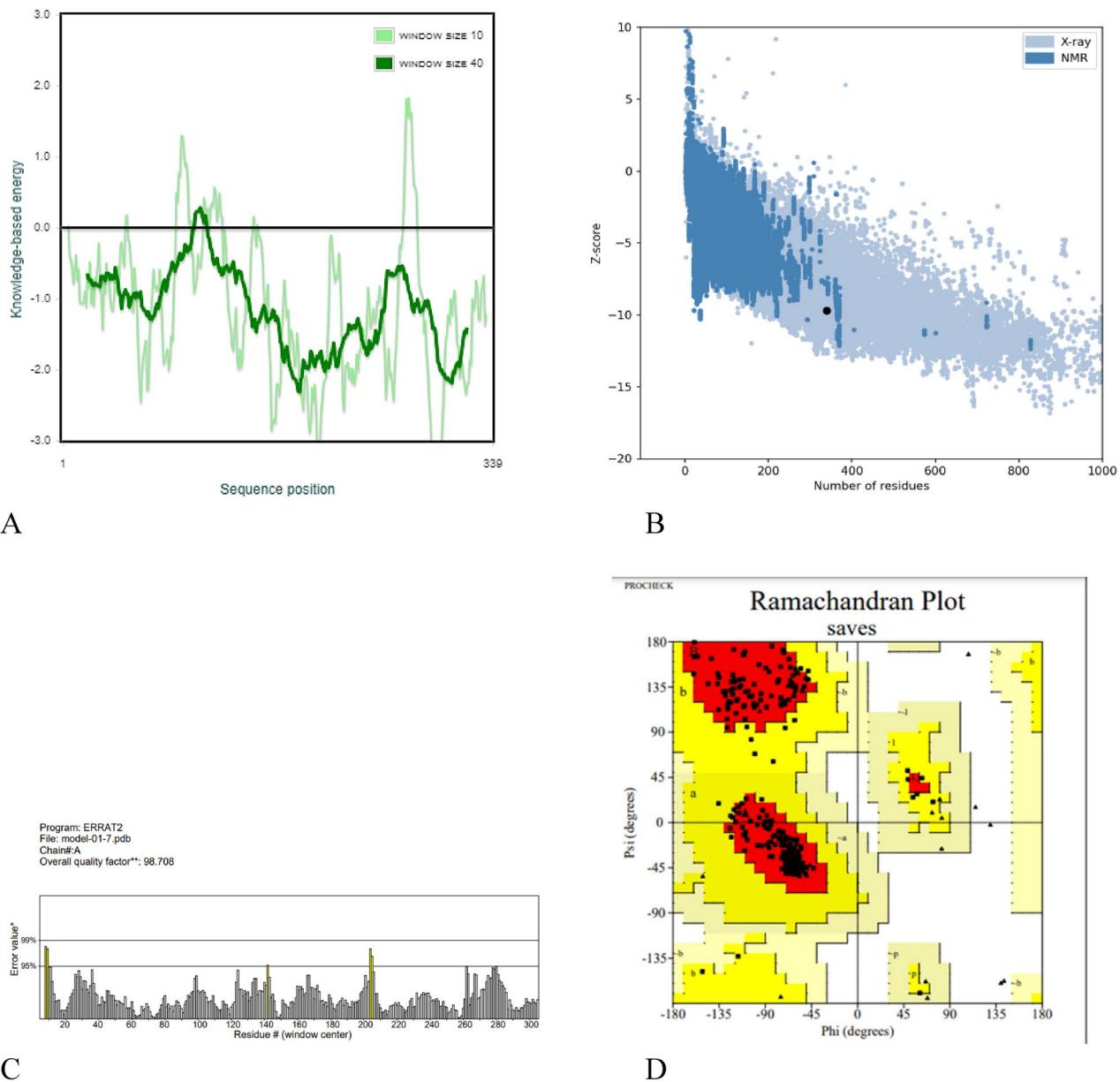


Fig. 6. This depicts the EutG protein structure quality prediction and validation of the protein. **(A)** The high quality of alcohol dehydrogenase EutG protein model predicted through the ProQ server. **(B)** The quality validation of the alcohol dehydrogenase EutG protein through ProsA server. **(C)** ERRAT plot for the alcohol dehydrogenase EutG [B. *tropicus*] model. Lower error values indicate better-quality regions. The nearly perfect score of 98.708 reflects that most regions of the protein have minimal errors, confirming the model's high quality. **(D)** RAMACHANDRAN plot of alcohol dehydrogenase EutG from *B. tropicus* obtained by PROCHECK. This graphically represents the distribution of residues in the favored, allowed, and disallowed regions. The concentration of residues in the most favored regions (Yellow) confirms the high quality of the predicted 3D model.

crystal structure and the predicted structures of the closed-docked complex were predicted or not. An RMSD value of ≤ 2 Å is considered indicative of a satisfactory match³⁸. A study was conducted to investigate the potential of compounds obtained through Gas chromatography–mass spectrometry in the crude oil sample of *Bacillus subtilis*. Among the compounds tested, straight chain hydrocarbons exhibited the lowest selection, with binding free energies ranging from -2.9 to -3.1 kcal/mol. The highest binding free energies (-3.8 kcal/mol to -5.1 kcal/mol) were observed for poly-branched hydrocarbons and cyclic compounds³⁹. Our findings demonstrated superior binding affinities compared to previous studies on the degradation of polycyclic aromatic compounds (PACs) using *Bacillus* species. This discovery holds significant importance as it suggests that our approach may be more effective in targeting and degrading these harmful compounds. By achieving better binding affinities, we have potentially identified a promising avenue for enhancing the remediation of PAC-

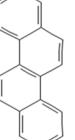
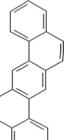
Sr.no	Compounds	2D-structure	PubChem CID	Molecular formula	Molecular weight (g/mol)	IC50 (nM)	Chemical properties
1	Pyrene		31,423	C16H10	202.25	8.70	Irritant, Environmental hazard
2	Anthracene		8418	C14H10	178.23	9.0	Irritant, Environmental hazard, Health hazard
3	Phenanthrene		995	C14H10	178.23	4.58	Irritant, Environmental hazard
4	Naphthalene		931	C10H8	128.17	4.68	Irritant, Environmental hazard, Health hazard
5	Chrysene		9171	C18H12	228.3	4.75	Environmental hazard, Health hazard
6	Fluorene		6853	C13H10	166.22	7.80	Environmental hazard
7	Benzo[a]pyrene		2336	C20H12	252.3	4.58	Irritant, Environmental hazard, Health hazard
8	Dibenz[a,h]anthracene		5889	C22H14	278.3	8.05	Environmental hazard, Health hazard
9	Dibenzothiophene		3023	C12H8S	184.26	7.95	Acute toxic, Irritant, Environmental hazard
10	Indeno[1,2,3-cd] pyrene		9131	C22H12	276.3	4.55	Health hazard

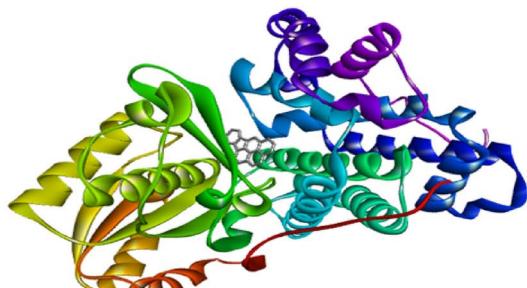
Table 3. The polycyclic aromatic compounds retrieved from PubChem.

Sr. no	Ligands	Binding affinity (Kcal/mol)	Rmsd/ub	Rmsd/lb	Ligand efficiency (kcal/mol/HA)
1	Indeno[1,2,3-cd] pyrene	-10.3	5.426	1.121	0.468
2	Benzo[a]pyrene	-10.0	5.452	1.096	0.50
3	Dibenz[a,h]anthracene	-9.2	7.364	0.015	0.418
4	Chrysene	-9.1	6.195	0.027	0.506
5	Pyrene	-8.4	4.096	0.111	0.525
6	Phenanthrene	-7.5	4.72	1.623	0.535
7	Anthracene	-7.4	2.125	0.069	0.528
8	Fluorene	-7.0	4.387	0.061	0.538
9	Dibenzothiophene	-6.8	4.469	0.642	0.523
10	Naphthalene	-5.7	4.015	0.011	0.57

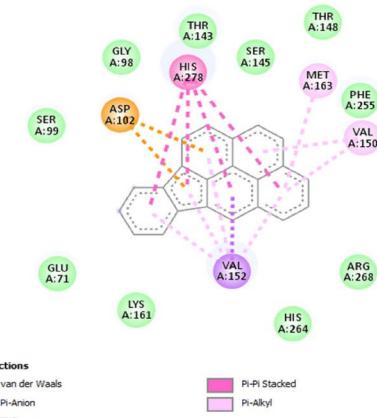
Table 4. The molecular docking results of the polyaromatic compounds with the alcohol dehydrogenase protein by PyRx.

contaminated environment using *Bacillus* species⁴⁰. This advancement could contribute to more efficient and environmentally-friendly strategies for addressing PAC pollution.

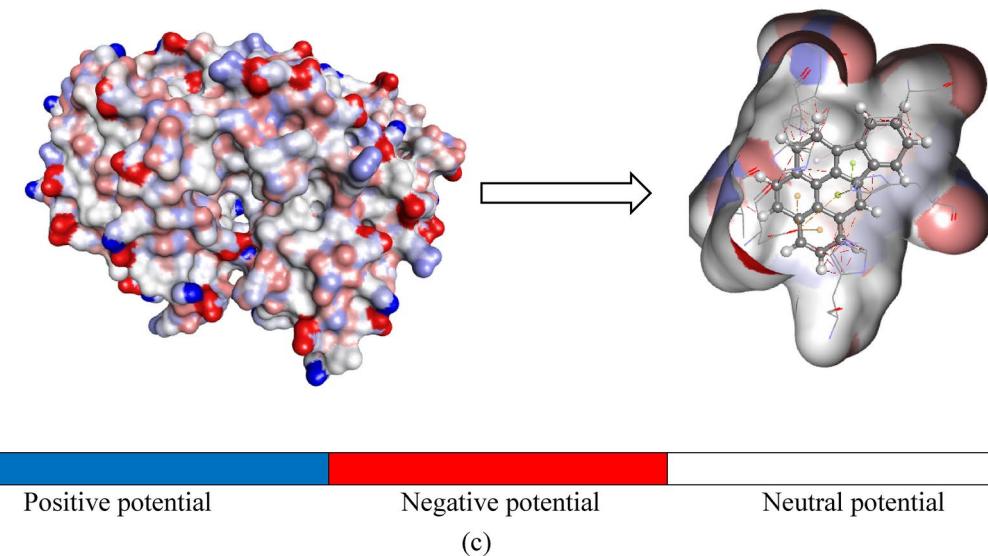
To investigate the impact of mutations on the ADH-EutG protein, we introduced the identified mutations into its primary structure. Through site-directed mutational analysis, we found that these mutations did not affect



(a)



(b)



(c)

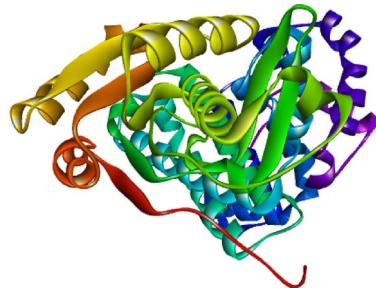
Fig. 7. The graphical representation of the docked complex of the Indeno[1,2,3-cd] pyrene with alcohol dehydrogenase EutG protein. **(a)** shows the 3D representation of the docked complex. **(b)** shows the 2D diagram of the interaction between the protein and Indeno[1,2,3-cd] pyrene. **(c)** shows the surface potential map of the docked complex.

the binding sites of the protein⁴¹. Subsequently, a model was generated, and docking analysis was performed comparing the non-mutated and mutant proteins. The results revealed a slight increase in binding score -10.5 kJ/mol of the mutant protein. This indicates that the conformational changes caused by the amino acid residue mutations did not significantly affect the binding pocket of the protein. The significance of these findings, from

SPECIES	Amino acid substituted	Mutation position	Amino acid replaced	Polyphen2	MU PRO results	SIFT
Alcohol dehydrogenase EutG	I	127	M	Probably damaging	Decrease	tolerated
Alcohol dehydrogenase EutG	E	234	D	Benign	Decrease	tolerated
alcohol dehydrogenase EutG [Bacillus sp. L27]	R	157	Q	Benign	Decrease	tolerated
alcohol dehydrogenase EutG [B. tropicus]	G	46	A	Probably damaging	Decrease	Not tolerated
Iron-containing alcohol dehydrogenase [Bacillus cereus ATCC 4342]	M	1	L	Benign	Increase	Not tolerated
alcohol dehydrogenase [Bacillus anthracis]	T	73	K	Probably damaging	Decrease	tolerated
alcohol dehydrogenase EutG [Bacillus cereus group]	N	400	D	Benign	Decrease	Not tolerated
alcohol dehydrogenase EutG [Bacillus sp. JAS102]	S	375	L	Possibly damaging	Decrease	Neutral
alcohol dehydrogenase EutG [Bacillus cereus]	G	31	E	Probably damaging	Decrease	Not tolerated
alcohol dehydrogenase EutG [Bacillus cereus group]	A	106	V	Probably damaging	Decrease	Tolerated
alcohol dehydrogenase EutG [Bacillus sp. MB353a]	M	125	R	Benign	Decrease	Not tolerated
alcohol dehydrogenase EutG [Bacillus cereus group]	Y	65	C	Probably damaging	Decrease	Not tolerated
alcohol dehydrogenase EutG [Bacillus cereus group sp. BfR-BA-01448]	E	234	D	Benign	Decrease	tolerated
alcohol dehydrogenase EutG [Bacillus cereus group]	V	208	D	Benign	Decrease	tolerated

Table 5. Mutations identified by different tools.

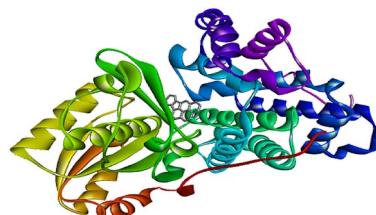
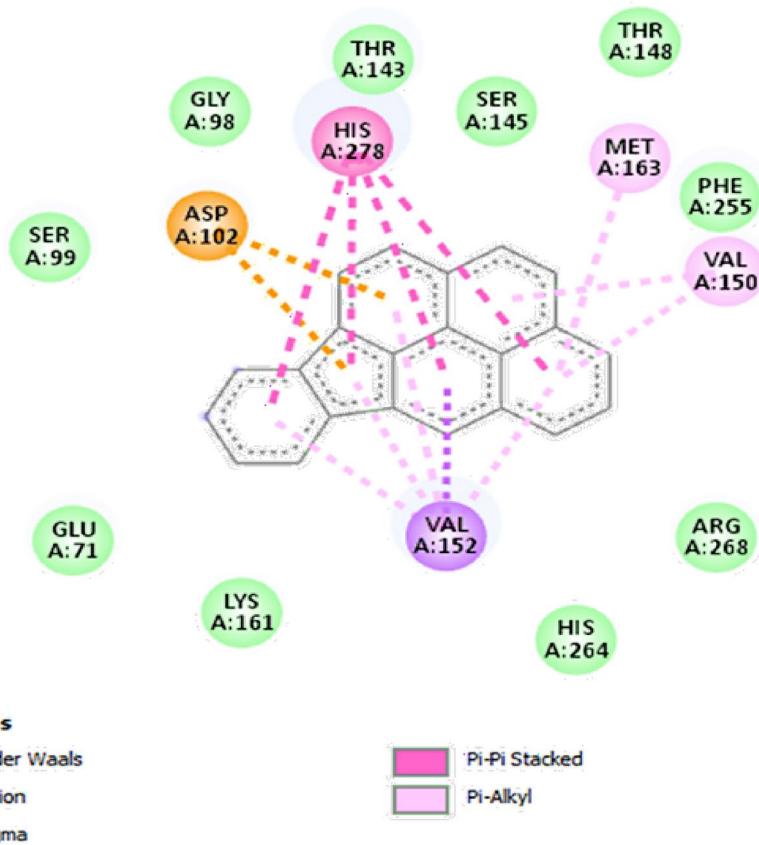
>QJE36766.1 alcohol dehydrogenase EutG [*Bacillus tropicus*]
 MQEVAEFRMPKSVLYGRNSLEKLGEQSKKFEKRAFIVTDTIMEDLAYVERCIQQQLNTKGITVITCNKVNA
 EPKNIHVLEALTICKEEKCDFIIGLGGGSCIDAALKVAVLYTNGGEVEDYVQKDREMDNDPLPLIAIPTT
 SG TGSEVTSAVITNKRTDVKMMM KHP SFTPQVAIDPILTRSVPPHITAATGIDALCHAVEAYISKVSQ
 SLTDVLALSAIESIMKYLRIA YEEGENMEAREAMMIASLQAGIAFSNASVTLVHGMSRPVGALFHVPHGI
 SNAILLPTVLEYTKGS AVKRLAEIGRCLN KDLYSYCDEEVADYTLMEIKKLCFDL RIPNLKEYGIGEVEF
 EKAISKMASDAIESGSPANNPRVPLYDEIKQLYREC FHYRYEDSIKTLGD

Fig. 8. The mutated sequence of alcohol dehydrogenase EutG from *B. tropicus*.**Fig. 9.** The mutated 3D model generated by SWISS-MODEL.

the perspective of molecular dynamic simulations, lies in understanding the stability and functional behavior of the mutant protein⁴². These insights contribute to our understanding of the protein's structure–function relationship and can aid in further exploration and optimization of its potential applications in various fields such as drug design and biotechnology. The study conducted by Naqvi et al.⁴³ provides valuable insights into the molecular behavior and implications of these mutations in the ADH-EutG protein.

The results obtained from molecular dynamic simulations of the docked complex between Indeno[1,2,3-cd] pyrene and the ADH-EutG protein indicate a satisfactory eigenvalue of 2.480069 e-04, confirming the stability of the complex. These findings highlight the significance of molecular dynamic simulations as a valuable tool for assessing the stability and behavior of protein–ligand complexes. However, it is crucial to note that further research and clinical trials are necessary to thoroughly evaluate the safety and efficacy of these novel therapeutic strategies⁴⁴. By conducting more in-depth investigations, gain in a comprehensive understanding of the complex's dynamics could be achieved and explore its potential applications in the development of effective and

Sr. no	Ligands	Binding affinity (Kcal/mol)	Rmsd/ub	Rmsd/lb	Ligand efficiency (kcal/mol/HA)
1	Indeno[1,2,3-cd] pyrene	-10.5	0.000	0.000	0.47
2	Benzo[a]pyrene	-10.3	5.399	1.108	0.515
3	Dibenz[a,h]anthracene	-10.3	3.333	0.806	0.468
4	Chrysene	-10.2	3.622	1.183	0.566
5	Pyrene	-10.1	6.359	1.520	0.631
6	Phenanthrene	-10.1	5.246	1.240	0.721
7	Anthracene	-10.1	3.306	1.304	0.721
8	Fluorene	-10.1	6.360	1.018	0.776
9	Dibenzothiophene	-9.5	3.343	1.630	0.730
10	Naphthalene	-9.4	3.283	1.492	0.94

Table 6. Mutant docking results by Autodock Vina.**Fig. 10.** Mutated complex alcohol dehydrogenase EutG from *B. tropicus* and Indeno[1,2,3-cd] pyrene.**Fig. 11.** 2D diagram of interaction between the mutated protein and Indeno[1,2,3-cd] pyrene.

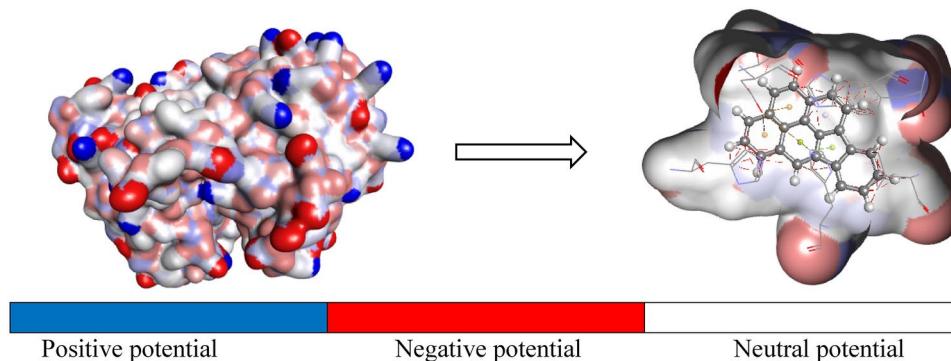


Fig. 12. Electrostatic surface potential map of the mutant docked complex.

safe therapeutic interventions⁴⁵. In conclusion, the alcohol dehydrogenase EutG protein has shown significant importance in the bioremediation of PACs. This enzyme has been found to play a critical role in the degradation of PACs, converting it into less toxic compounds. The ability of the EutG protein to efficiently degrade PACs and its derivatives makes it a valuable for environmental bioremediation applications. By harnessing the enzymatic activity of EutG, the harmful effects of PACs contamination can be mitigated, leading to the restoration of polluted environment. The use of EutG protein offers a promising approach for sustainable and eco-friendly remediation strategies targeting PACs.

Conclusion

In conclusion, this study sheds light on the molecular and structural characteristics of alcohol dehydrogenase EutG from *B. tropicus*. Different bacterial species, including various *Bacillus* strains, have been explored for their role in PAC degradation due to their resilient enzymatic pathways and metabolic flexibility. *Bacillus* species, through enzymes initiate the oxidation of PAC rings, facilitating further breakdown of these toxic compounds. In this study, EutG specifically demonstrated functional significance as an alcohol dehydrogenase capable of targeting PAC molecules, with the catalytic mechanism enhanced by site-specific binding that initiates PAC breakdown. This catalytic action creates intermediate products, leading to less toxic byproducts that contribute to reducing environmental pollution. The molecular docking analysis confirmed the formation of a stable complex between EutG and Indeno[1,2,3-cd] pyrene, therefore indicating stronger binding affinity and efficient degradation potential. The observed binding affinities were notable, demonstrating superior interaction strength compared to other PAC-degrading enzymes studied in *Bacillus* species, underscoring EutG's unique capacity for pollutant breakdown. Furthermore, through in-silico site-directed mutagenesis, it was demonstrated that the complex maintains its stability even after undergoing mutations. These mutations in alcohol dehydrogenase EutG indicate its structural resilience, allowing it to adapt to various environments. Targeted mutations at active-site residues could enhance binding affinity and catalytic activity, enabling more effective PAC degradation. These findings contribute to the broader understanding of alcohol dehydrogenase EutG and offer potential avenues for degrading PAC pollution in the environment. Future work could explore modifications to enhance substrate affinity and catalytic efficiency by combination of site-directed mutations and functional engineering, making EutG even more effective in PAC degradation. Moreover, field trials can be conducted field to assess the enzyme's performance in natural environments. Pilot studies in contaminated areas would help validate EutG's bioremediation potential, guiding adjustments for large-scale deployment. So, this study offers valuable insights to develop effective strategies for bioremediation in the environment contaminated with polycyclic aromatic compounds (PACs) and opens a gate in finding more potential strategies and bioremediating properties of other enzymes with several other toxic contaminants harming the ecosystem.

Data availability

All the data has been included in the manuscript.

Received: 21 August 2024; Accepted: 13 January 2025

Published online: 28 January 2025

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Acknowledgements

Authors are thankful to the Researchers Supporting Project number (RSPD2025R568), King Saud University, Riyadh, Saudi Arabia.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

All methods were carried out in accordance with relevant guidelines and regulations. Study approval was obtained from the “Bioethical Committee of the Faculty of Science and Technology, University of Central Punjab.

Consent to participate

All authors agreed to participate in this research. Informed consent was obtained from every participant.

Additional information

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