# *UNLOCKING THE BIOCATALYTIC EFFICIENCY OF FACTOR-INDEPENDENT URATE HYDROXYLASE FROM Bacillus subtilis IN PETROCHEMICAL HYDROCARBON DETOXIFICATION*

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## ABSTRACT

Petrochemical hydrocarbons are toxic, persistent environmental pollutants that pose serious ecological and health risks. Bioremediation using microbial enzymes offers a sustainable and effective alternative for their degradation. This study presents an in-silico analysis of Factor-independent urate hydroxylase from *Bacillus subtilis* to evaluate its potential in hydrocarbon biodegradation. The enzyme's physicochemical properties revealed moderate stability and hydrophilicity, favoring activity in aqueous environments. Post-translational modification analysis predicted multiple regulatory sites, suggesting adaptability to environmental conditions. Structural modeling and validation confirmed a high-quality 3D structure suitable for molecular docking. Nine petrochemical hydrocarbons were selected for virtual screening. Docking results showed strong binding affinities, particularly with 1,2,3,4,7,8-hexachlorodibenzofuran (-7.4 kcal/mol), crystal violet (-7.3 kcal/mol), and dioxins, with key residues (e.g., ARG207, VAL211, PHE289) mediating interactions. Toxicity predictions indicated high neurotoxicity and hepatotoxicity among the compounds, highlighting the urgency for effective remediation tools. The study concludes that Factor-independent urate hydroxylase demonstrates promising interaction with harmful hydrocarbons and play a key role in microbial bioremediation. These computational findings provide a foundation for future experimental validation and potential application in cleaning up petrochemical-contaminated environments.

**Keywords:** Toxicity Analysis, Petrochemical Hydrocarbons, Computational Biodegradation, Factor-Independent Urate Hydroxylase, Biocatalytic Pathway Analysis, PTM.

## INTRODUCTION

Petrochemical hydrocarbons are a diverse group of organic compounds derived primarily from the processing of crude oil and natural gas. These hydrocarbons form the backbone of a vast range of industrial products, including fuels (such as gasoline, diesel, and jet fuel), lubricants, solvents, plastics, and synthetic fibers (Matar and Hatch, 2001). Despite their indispensable role in modern society, the release of petrochemical hydrocarbons into the environment, whether through accidental spills, industrial discharge, or improper waste management, has posed a major ecological and public health threat. These compounds are known for their persistence, toxicity, and bio-accumulative nature, which make them formidable (Kubičková and Kubička, 2010).

Hydrocarbons create poisonous slicks in aquatic environments that obstruct sunlight, lower oxygen levels, and disturb marine life, which causes bioaccumulation in the food chain. By preventing microbial activity and plant growth, they reduce soil fertility on land. Polycyclic aromatic hydrocarbons (PAHs) and benzene are examples of volatile organic compounds (VOCs) that evaporate into the atmosphere and cause pollution, respiratory illnesses, and cancer in people (Kim et al., 2013). Because these substances can cause oxidative stress, DNA mutations, and cellular malfunction, prolonged exposure to them has been connected to neurological conditions, cancer, and liver damage. Furthermore, because of their sluggish biodegradation, they cause environmental contamination over time, making sustainable remediation techniques necessary to lessen their effects. When volatile hydrocarbons, like benzene, are inhaled or absorbed by people, they transform into reactive epoxides that cause oxidative stress, the production of DNA adducts, and the development of cancer (Chang et al., 2006). In adipose tissues, lipophilic hydrocarbons build up, rupturing cell membranes and interfering with enzymatic processes. As endocrine disruptors, chlorinated hydrocarbons (like PCBs) can linger for decades. Meanwhile, inefficient hydrocarbon combustion exacerbates cardiovascular and respiratory conditions by producing ozone and fine particulate matter (PM2.5). By activating into electrophilic intermediates through cytochrome P450, they cause cytotoxicity, organ damage, and long-term ecological devastation by alkylating proteins and nucleic acids (Naveed et al., 2025).

Among various bioremediation approaches, microbial degradation has gained considerable attention due to its cost-effectiveness and eco-friendliness. Certain microorganisms have evolved metabolic pathways capable of degrading complex hydrocarbon structures, converting them into less harmful or benign end products. One such promising microbial species is *Bacillus subtilis*, a gram-positive, spore-forming bacterium commonly found in soil (Haritash and Kaushik, 2009).

*Bacillus subtilis* is renowned for its robustness, genetic adaptability, and the production of a wide array of enzymes that contribute to the biodegradation of organic pollutants, including petrochemical hydrocarbons (Wilcke, 2000). Unlike other hydroxylases, Factor-independent urate hydroxylase is a special microbial enzyme that catalyzes the oxidative breakdown of uric acid into allantoin without the need for coFactors like NAD(P)H or metal ions. The enzyme uses a radical-based method to break the urate ring, most likely a process mediated by reactive oxygen species (ROS), in which molecular oxygen is directly activated. Urate is subjected to a two-electron oxidation during catalysis, resulting in a peroxide intermediate that breaks down into 5-hydroxyisourate, which subsequently spontaneously reorganizes into allantoin. This enzyme is energetically efficient for microbial uricolysis since it does not require external electron donors, in contrast to dependent hydroxylases (Keenan, 2020). It works by carefully placing the substrate in the active site, which makes it easier for oxygen to be directly inserted at urate's C5 position. Urate, a strong pro-oxidant connected to oxidative stress in pathogens, is prevented from building up by this process. The coFactor independence of the enzyme indicates that bacteria such as Bacillus subtilis have evolved to recycle nitrogen in low-nutrient settings. Applications for treating gout or the biodegradation of pollutants high in purines may benefit from an understanding of its chemistry (Hyndman et al., 2016).

The Factor-independent urate hydroxylase from *Bacillus subtilis* is hypothesized to contribute significantly to the initial steps of hydrocarbon oxidation, potentially facilitating ring-cleavage reactions critical for the breakdown of stable aromatic structures present in petrochemical pollutants (Roh et al., 2009). However, the structural and functional attributes of this enzyme in the context of hydrocarbon biodegradation remain largely unexplored, especially from a computational and in silico perspective. This study aims to perform an in-silico analysis of the Factor-independent urate hydroxylase from *Bacillus subtilis* to evaluate its potential role in the biodegradation of petrochemical hydrocarbons. By employing computational modeling, structural prediction, active site analysis, and molecular docking techniques, we seek to uncover the enzyme's interaction patterns with representative hydrocarbon pollutants (Naveed et al., 2023). The findings will contribute to a deeper understanding of the enzyme's biodegradative capacity and may support the development of microbial strategies for environmental remediation of petrochemical-contaminated sites.

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## METHODOLOGY

### Sequence Retrieval and Physicochemical Characteristics Prediction

The amino acid sequence of the Factor-independent urate hydroxylase enzyme from *Bacillus subtilis* was retrieved from the National Center for Biotechnology Information (NCBI) protein database (https://www.ncbi.nlm.nih.gov/). The retrieved FASTA sequence was then subjected to physicochemical characterization using the ExPASy ProtParam tool (https://web.expasy.org/protparam/). This analysis provided insights into the enzyme's molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and grand average of hydropathicity (GRAVY), which are essential for understanding its biochemical behavior (Schoch et al., 2020).

### Post Translational Modification Analysis

The MusiteDeep tool (https://www.musite.net/) was employed to predict potential post-translational modification (PTM) sites in the Factor-independent urate hydroxylase sequence. This deep learning-based platform accurately identifies various PTMs, such as phosphorylation, glycosylation, and ubiquitination, providing valuable insights into the enzyme's functional regulation and interaction potential (Wang et al., 2017).

### Pathway Analysis of enzyme

Pathway analysis of the Factor-independent urate hydroxylase was conducted using the PATRIC database (https://ngdc.cncb.ac.cn/databasecommons/database/id/230) to identify its involvement in metabolic and biodegradation pathways (Wattam et al., 2014). To perform pathway analysis, genomic or metagenomic data (e.g., FASTA sequence) to the platform was uploaded to generate maps of annotated genes to KEGG or MetaCyc pathways. The system generates interactive pathway maps with enzyme annotations, allowing you to visualize metabolic potential and compare across strains.

### Secondary and Tertiary Structure Prediction

The secondary structure of the Factor-independent urate hydroxylase was predicted using the PSIPRED tool (http://bioinf.cs.ucl.ac.uk/psipred/), which provided insights into the arrangement of alpha-helices, beta-sheets, and coils within the protein (Buchan and Jones, 2019). For tertiary structure prediction, the Swiss-Model server (https://swissmodel.expasy.org/) was utilized to generate a 3D model based on homology modeling. The quality and reliability of the predicted protein structure were further assessed through structural validation tool i.e. MolProbity (http://molprobity.biochem.duke.edu) ensuring its suitability for downstream analysis (Schwede et al., 2003).

### Selection of Compounds

Nine different petrochemical hydrocarbons were selected for virtual screening to evaluate their potential interactions with the Factor-independent urate hydroxylase. These compounds were chosen based on their environmental relevance and structural diversity. The 3D structures and molecular weights of these hydrocarbons were retrieved from the (https://pubchem.ncbi.nlm.nih.gov/) in SDF format, ensuring accurate molecular representations for docking studies (Kim et al., 2016).

### Molecular Docking and Interaction analysis

Molecular docking was performed via PyRx software between the Factor-independent urate hydroxylase and the nine selected petrochemical hydrocarbons to assess their binding affinities and interaction patterns(Dallakyan and Olson, 2014). The docking simulations were conducted to predict the most favorable binding conformations and energy scores. Post-docking interaction analysis was conducted using Discovery Studio Visualizer, which provided detailed visualization and identification of key residues involved in hydrogen bonding, hydrophobic interactions, and other molecular forces stabilizing the enzyme-ligand complexes.

### Toxicity Analysis

The toxicity profiles of the selected petrochemical hydrocarbons were evaluated using the ProTox-II web server (https://tox.charite.de/protox3/). This tool predicted various toxicity parameters, including $LD_{50}$ values, toxicity class, hepatotoxicity, carcinogenicity, immunotoxicity, and cytotoxicity, providing a comprehensive understanding of the potential health and environmental risks associated with each compound (Banerjee et al., 2018).

## RESULTS

### Sequence Retrieval and Physicochemical Characteristics Prediction

The amino acid sequence of the Factor-independent urate hydroxylase enzyme was retrieved from the NCBI database under the accession number MFP6330316.1. The protein comprises 494 amino acids with a molecular weight of 56,710.75 Da and a theoretical isoelectric point (pI) of 5.46. The enzyme has more negatively charged residues (74) than positively charged ones (59), indicating an overall acidic nature. The instability index of 39.44 suggests that the protein is marginally stable under in vitro conditions. Its aliphatic index of 74.48 indicates moderate thermostability, while the negative GRAVY value (-0.552) reflects a hydrophilic nature, suggesting good interaction with aqueous environments.

### Post translational modification analysis

A protein's tight regulatory role in different cellular processes is suggested by MusiteDeep's prediction of many PTM sites (phosphorylation, glycosylation, ubiquitination, acetylation, and palmitoylation) along with their potential amino acids as shown in table 1. Glycosylation suggests extracellular connections or protein stability, whereas phosphorylation sites suggest possible signaling cascades or kinase/phosphatase control. Acetylation may control metabolic activity (e.g., in enzymes) or gene expression (if on histones), while ubiquitination marks may target the protein for breakdown or alter protein-protein interactions. Palmitoylation sites imply trafficking regulation or membrane interaction. These PTMs' co-occurrence suggests a highly dynamic protein that is regulated by many levels of post-translational regulation, perhaps combining structural, metabolic, and signaling roles. These changes and their interaction in certain biological situations require additional experimental validation (such as mass spectrometry).

**Table 1:** Post translational modification analysis

| Post-translational modification site | Amino Acid residues |
|---|---|
| Phosphorylation | SER41<br>SER43<br>SER78<br>SER81<br>SER177<br>SER317 |
| Glycosylation | ASN374<br>ASN442 |
| Ubiquitination | LYS92<br>LYS197<br>LYS298<br>LYS320 |
| SUMOylation | - |
| Acetylation | LYS167<br>LYS180<br>LYS191<br>LYS320 |
| Methylation | - |
| Pyrrolidone carboxylic acid | - |
| Palmitoylation: | CYS490 |
| Hydroxylation | - |

### Pathway Analysis

The enzymatic breakdown of caffeine and related methylxanthines in microbes and plants is described in KEGG pathway map 00232 (Caffeine Metabolism). Theobromine (3,7-dimethylxanthine) or paraxanthine (1,7-dimethylxanthine) are produced by N-demethylating caffeine (1,3,7-trimethylxanthine) using enzymes such as NdmA (a cytochrome P450) or XanA (xanthine dehydrogenase) (Figure 1). These are then further demethylated to xanthine.

After that, xanthine dehydrogenase (XDH) oxidizes xanthine to uric acid, a crucial step that enters purine breakdown pathways (for example, via urate hydroxylase to allantoin). CdxA in certain bacteria (like Pseudomonas putida) directly cleaves caffeine into trimethyluric acid. The process demonstrates how microorganisms have adapted to metabolize caffeine as a source of carbon and nitrogen, which may find use in bioremediation or decaffeination technologies.

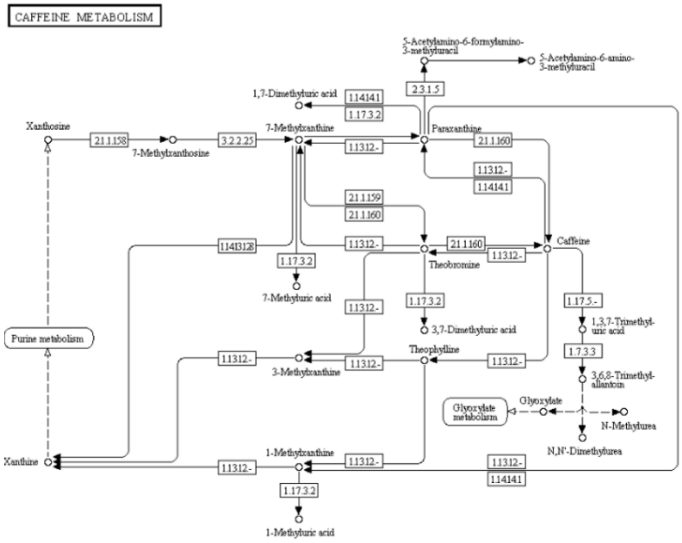



**Figure 1:** Pathway analysis of an enzyme

**Secondary Structure Prediction**

The secondary structure of the urate hydroxylase enzyme predicted by PSIPRED reveals a balanced composition of alpha-helices and beta-strands. This structural arrangement suggests a stable and functionally active conformation (Figure 2).

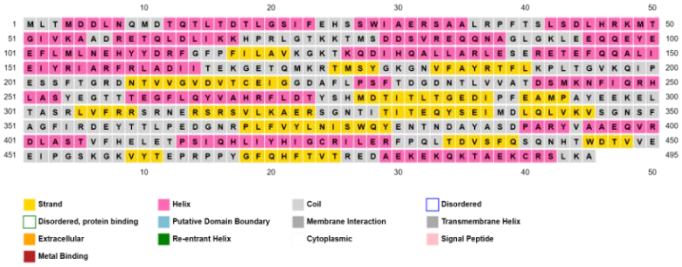



**Figure 2:** Secondary structure prediction

**Tertiary structure prediction**

The tertiary structure of the urate hydroxylase enzyme was predicted using SWISS-MODEL, generating a high-quality 3D model (Figure 3). Validation through MolProbity Ramachandran analysis showed 97.6% of residues in favored regions and 99.4% in allowed regions, with only three outliers (Thr76, Lys167, and Met172), indicating a reliable and well-validated structural model (Figure 4).

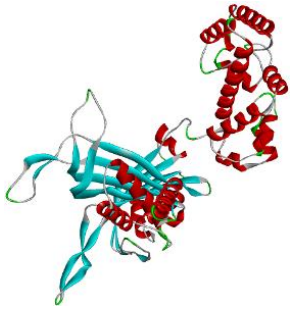


**Figure 3:** Tertiary structure prediction using swiss model

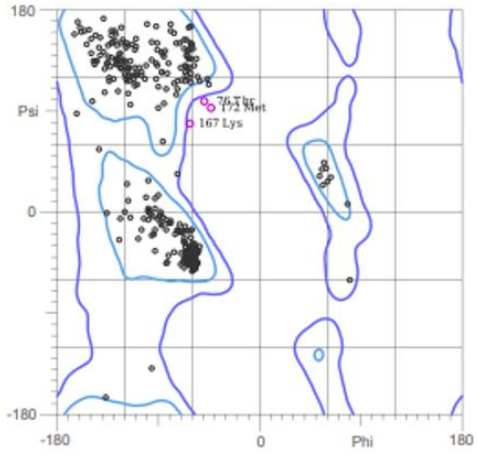
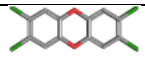
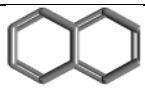
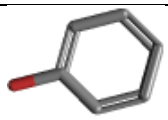
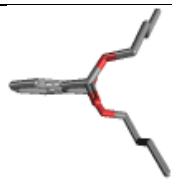
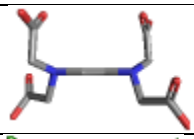
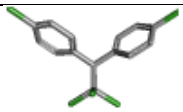
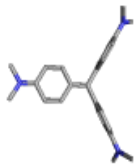
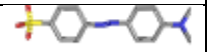
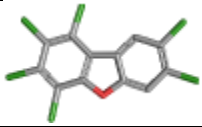



**Figure 4:** Ramachandran plot of protein

**Selection of compounds**

The list of nine petrochemical hydrocarbons retrieved from the PubChem which are given below in table 2 comprises of their PubChem ID, molecular weight and its 3D structure. The compounds were selected according to their detrimental effects known in literature.

**Table 2:** Petrochemical hydrocarbons retrieved from PubChem

| Petrochemical Hydrocarbons | PubChem ID | MW | 3D structure |
|---|---|---|---|
| 2,3,7,8-Tetrachlorodibenzo-P-Dioxin | 15625 | 322 g/mol |  |
| naphthalene | 931 | 128.169 g/mol |  |
| Phenol | 996 | 94.11 g/mol |  |
| Dibutyl Phthalate | 3026 | 278.34 g/mol |  |
| Edetic Acid | 6049 | 6049 |  |
| Clofenotane | 3036 | 354.5 g/mol |  |
| Crystal violet | 11057 | 408 g/mol |  |
| Methyl orange | 23673835 | 327.34 g/mol |  |
| 1,2,3,4,7,8-Hexachlorodibenzofuran | 51130 | 374.9 g/mol |  |

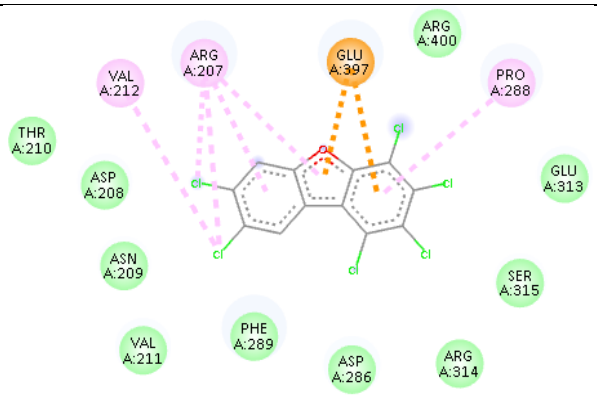
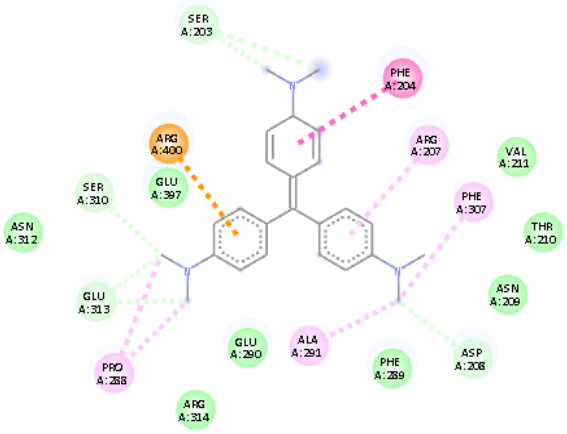
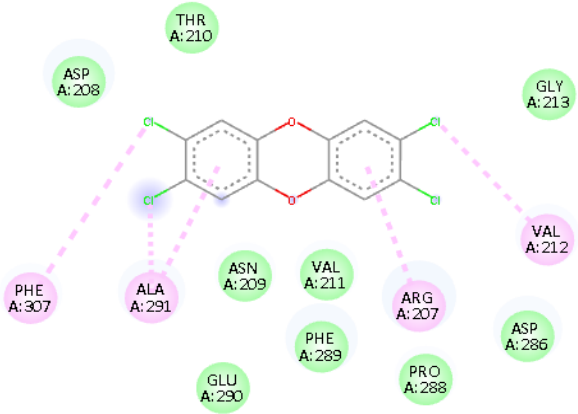
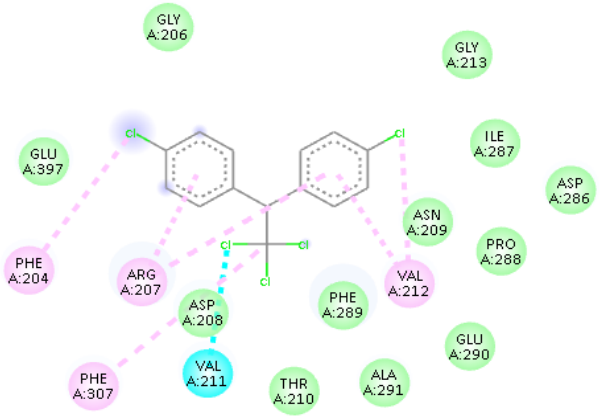
**Molecular Docking and Interaction Analysis**

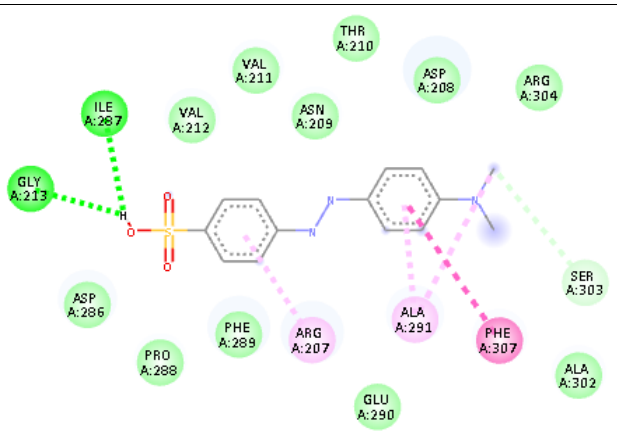
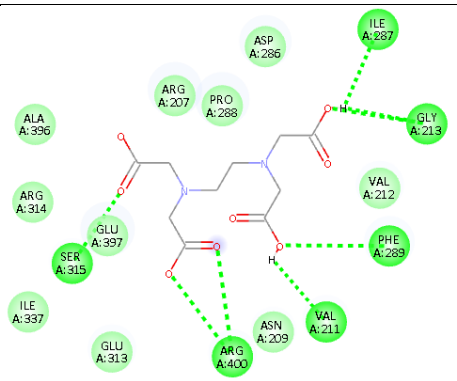
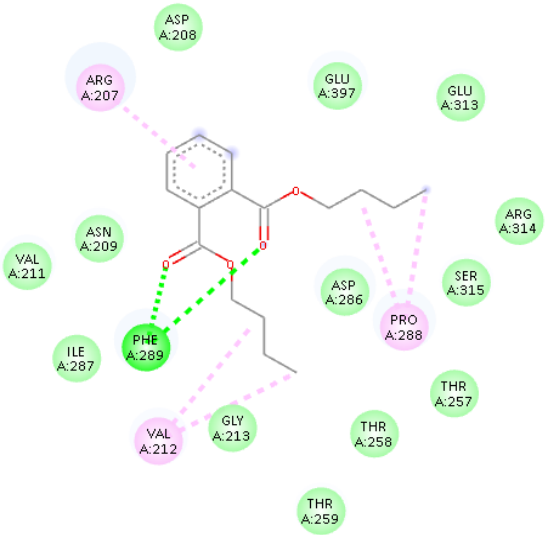
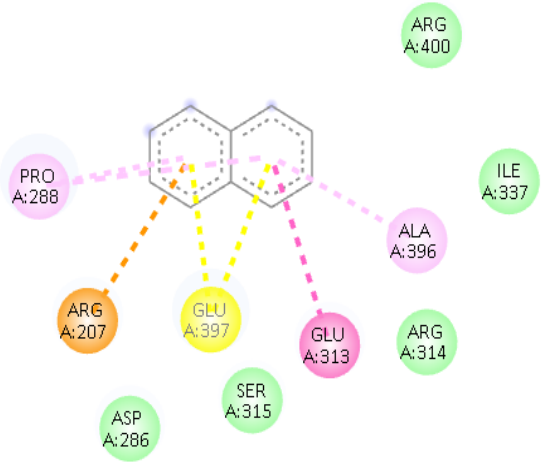
Molecular docking through PyRx revealed the interaction between the enzyme and its substrate. The docked complexes were obtained each with their specific binding energy ranging from -7.4 to -4.0 Kcal/mol in descending order as illustrated in table 3. The highest binding energies showed the formation of stable enzyme-substrate complexes which fully interact with the active site residues of Factor-Independent urate hydroxylase, primarily with 1,2,3,4,7,8-Hexachlorodibenzofuran which infers the best interaction i.e. -7.4. All the nine compounds showed stable and functional degradation of these noxious hydrocarbons with binding affinities greater than -5.0 except for the benzene which showed a lesser interaction with the amino acid residues as compared to others. The interaction analysis figures has been depicted in table 4.

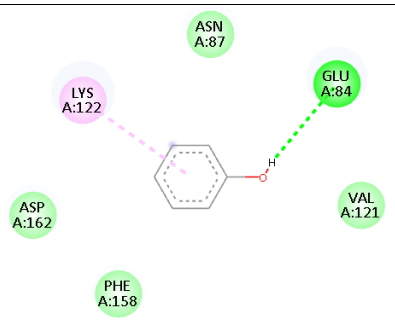
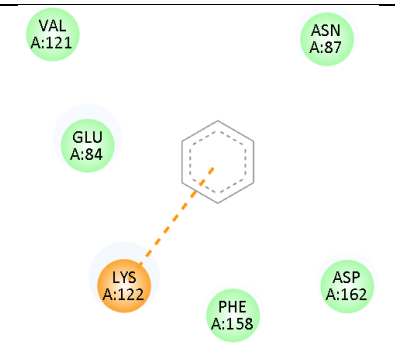
**Table 3:** Molecular docking and interaction analysis

| Petrochemicals | Binding energies (Kcal/mol) | Interacting Residues | Bond interactions |
|---|---|---|---|
| 1,2,3,4,7,8-Hexachlorodibenzofuran | -7.4 | VAL212, ARG207, THR210, ASP208, ASN209, VAL211, PHE289, ASP286, ARG314, ER315, GLU313, PRO288, ARG400, GLU397 | Van der Waals, Pi-Alkyl Pi-Anion |
| Crystal violet | -7.3 | SER203, PHE204, ARG207, VAL211, THR210, ASN209, ASP206, PHE289, GLU290, ARG314, GLU313, ASN312, SER310, GLU397, PHE307, ALA291, PRO288, ARG400 | Van der Waals, PI-Pi T- shaped Pi-Alkyl Pi-Cation Carbon Hydrogen bond |
| 2,3,7,8-Tetrachlorodibenzo-P-Dioxin | -7.00 | ASP208, THR210, GLY213, ASP286, PRO288, VAL211, PHE289, ASN209, GLU290, VAL212 | Van der Waals, Pi-Alkyl |
| Clofenotane | -7.00 | GLY213, ILE287, ASP286, PRO288, GLU290, ALA291, THR210, ASN209, PHE289, ASP208, GLU397, GLY206, VAL212, ARG207, PHE307, PHE204 | Van der Waals, Alkyl Pi-Alkyl Halogen |
| Methyl orange | -6.9 | GLY213, ILE287, VAL212, VAL211, ASN209, THR210, ASP208, ARG304, SER303, ALA302, GLU290, PHE289, PRO288, ASP286, PHE307, ALA291, ARG207 | Van der Waals, Conventional Hydrogen bond PI-Pi T- shaped Pi-Alkyl Carbon Hydrogen bond |
| Edetic Acid | -6.00 | ILE287, GLY213, PHE289, VAL211, ARG400, SER315, ASP286, VAL212, ASN209, GLU313, ILE337, GLU397, ARG314, ALA396, ARG207, PRO288, ASP286 | Van der Waals, Conventional Hydrogen bond |
| Dibutyl Phthalate | -5.9 | ARG207, PRO288, VAL212, PHE289, ASP208, GLU397, GLIU313, ARG314, SER315, ASP286, THR257, THR259, GLY213, ILE287, ASN209, VAL211, ASN209 | Van der Waals, Conventional Hydrogen bond, Alkyl, Pi-Alkyl |
| naphthalene | -5.2 | ARG400, ILE337, ARG314, SER315, ASP286, ALA396, GLU313, GLU397, ARG207, ALA396, PRO288 | Van der Waals, Pi-Alkyl Pi-Cation Pi-Anion Amide- Pi Stacked |
| Phenol | -4.5 | GLU84, ASN87, VAL121, PHE158, ASP162, LYS122 | Van der Waals, Conventional Hydrogen bond, Pi-Alkyl |
| Benzene | -4.00 | VAL121, ASN87, ASP162, PHE158, GLU84, LYS122 | Van der Waals, Pi-Alkyl Pi-Cation |

**Table 4:** Interaction analysis of petrochemical hydrocarbons with Factor-Independent Urate hydroxylase

| Petrochemicals | Binding energies (Kcal/mol) |
|---|---|
| 1,2,3,4,7,8-Hexachlorodibenzofuran |  |
| Crystal violet |  |
| 2,3,7,8-Tetrachlorodibenzo-P-Dioxin |  |
| Clofenotane |  |

| | |
|---|---|
| Methyl orange |  |
| Edetic Acid |  |
| Dibutyl Phthalate |  |
| naphthalene |  |

| | |
|---|---|
| Phenol |  |
| **Benzene** |  |

**Toxicity Analysis**

Toxicity analysis of all petrochemical hydrocarbons revealed that they possess toxic properties, including potential neurotoxicity and hepatotoxicity. These findings indicate that the compounds also pose harmful effects on the nervous system, highlighting the need for careful environmental and health risk assessment. The summary for this analysis has been depicted in Table 5.

**Table 5:** Toxicity analysis by PROTOX3.

| Petrochemical hydrocarbons | Hepatotoxicity | Neurotoxicity | Nephrotoxicity | Respiratory toxicity | Cardiotoxicity |
|---|---|---|---|---|---|
| 1,2,3,4,7,8-Hexachlorodibenzofuran | Inactive | Active | Inactive | Inactive | Inactive |
| Crystal violet | Inactive | Active | Inactive | Inactive | Inactive |
| 2,3,7,8-Tetrachlorodibenzo-P-Dioxin | Active | Active | Inactive | Active | Inactive |
| Clofenotane | Active | Active | Inactive | Active | Inactive |
| Methyl Orange | Active | Active | Inactive | Active | Inactive |
| Edetic Acid | Active | Active | Inactive | Active | Inactive |
| Dibutyl Phthalate | Active | Active | Inactive | Active | Inactive |
| naphthalene | Active | Active | Inactive | Active | Inactive |
| Phenol | Active | Active | Inactive | Active | Inactive |
| Benzene | Active | Active | Inactive | Active | Inactive |

## DISCUSSION

The increasing environmental burden posed by petrochemical hydrocarbons has drawn considerable attention toward developing sustainable and efficient bioremediation strategies (Singh et al., 2017). Microbial enzymes, due to their specificity and environmental compatibility, have emerged as promising tools in this regard. Among the various enzymes studied, urate hydroxylase typically associated with purine metabolism has gained interest for its potential role in xenobiotic degradation, especially in Factor-independent forms that do not require accessory proteins for functionality (Virk, 2022). Bacillus subtilis, known for its robustness and enzymatic versatility, serves as an ideal candidate for harboring such a multifunctional enzyme. The current study utilized an in silico approach to explore the structure and functional capabilities of the Factor-independent urate hydroxylase from Bacillus subtilis with a focus on its interaction with nine environmentally relevant petrochemical hydrocarbons (Bai et al., 2021). The physicochemical analysis of the enzyme highlighted its moderate thermostability and hydrophilic nature, both of which support its functionality in aqueous environmental conditions. This is consistent with the biochemical profiles reported for other pollutant-degrading enzymes such as dioxygenases and monooxygenases, which are commonly active under similar conditions (Pérez-Pantoja et al., 2012).

Post-translational modification (PTM) analysis revealed multiple sites for phosphorylation, ubiquitination, and glycosylation, among others, indicating potential regulation and adaptation mechanisms for different environmental stimuli. Comparable observations have been reported in environmental stress-responsive enzymes where PTMs modulate activity and substrate specificity(Macek et al., 2009). Notably, phosphorylation at key serine residues may enhance enzyme-substrate binding or catalytic efficiency, a property valuable in biodegradation contexts. Because Bacillus subtilis has a low native capacity to digest methylxanthines, its metabolism of caffeine deviates from the classic plant/microbial routes (KEGG:00232). B. subtilis can metabolize caffeine-derived intermediates through xanthine dehydrogenase (Xdh), a crucial enzyme in its purine degradation pathway, even though it lacks specific caffeine-demethylating enzymes like NdmA or CdxA seen in Pseudomonas.

Urate hydroxylase (controlled by the puc operon) oxidizes xanthine (produced from the breakdown of exogenous caffeine) to uric acid, which is subsequently transformed into allantoin. Glyoxylate and urea are produced by further breakdown and contribute to central carbon/nitrogen metabolism. Significantly, strains of B. subtilis that have been modified to express heterologous ndmA (from P. putida) exhibit improved breakdown of caffeine, indicating the possibility of bioremediation. Because of its ecological niche, the organism's natural process favors recycling uric acid over directly using caffeine (Bai et al., 2021).

The predicted 3D model demonstrated excellent stereochemical quality (with 97.6% of residues in favored regions), supporting its structural reliability for molecular docking studies. Structural features such as the abundance of binding grooves and accessibility of active residues provide a plausible explanation for its broad interaction with diverse hydrocarbon structures (Shi et al., 2023). Similar structural compatibility has been observed in laccases and peroxidases that interact with various xenobiotic compounds (Couto and Herrera, 2006). Molecular docking studies provided insight into the enzyme's affinity toward selected hydrocarbons. The highest binding affinities were observed for 1,2,3,4,7,8-hexachlorodibenzofuran (-7.4 kcal/mol), crystal violet (-7.3 kcal/mol), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (-7.0 kcal/mol), with key residues such as ARG207, VAL211, and PHE289 repeatedly participating in hydrophobic and hydrogen bonding interactions. These findings align with previous docking-based studies of pollutant-binding proteins where similar residues often form the core of the binding pocket (Jain et al., 2019). Compared to enzymes such as cytochrome P450s or peroxidases, the urate hydroxylase displayed comparable or higher binding affinities with some hydrocarbons, underscoring its potential as an alternative biocatalyst for environmental detoxification.

Toxicity analyses through ProTox-II confirmed that many of the selected hydrocarbons exhibit significant neurotoxicity, hepatotoxicity, and respiratory toxicity, reiterating their hazardous nature and the pressing need for effective degradation strategies (Naveed et al., 2024c, Naveed et al., 2024b). The ability of the enzyme to bind strongly with these toxicants suggests a possible role in initial detoxification stages. While previous studies have predominantly emphasized physical or chemical methods for pollutant removal, our findings advocate a bio-based approach that could complement or substitute existing methods in low-resource or ecologically sensitive regions (Naveed et al., 2024a).

## CONCLUSION

In conclusion, this study reveals that the Factor-independent urate hydroxylase from Bacillus subtilis possesses desirable physicochemical characteristics and structural features conducive to interaction with a wide range of petrochemical hydrocarbons. The enzyme's ability to stably bind to several hazardous compounds with high affinity positions as a promising candidate for microbial bioremediation applications. Future in vitro and in vivo studies will be essential to validate these computational insights and to further elucidate its catalytic mechanisms under environmentally relevant conditions.

## DECLARATIONS

**Ethical Approval:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Availability of data and materials:** All the data generated in this research work has been included in the manuscript.

**Author Contributions:** Conceptualization, Muhammad Naveed; methodology, Hajra Sohail; software, Walaa F Alsanie; validation, Abdulhakeem S Alamri; formal analysis, Hajra Sohail; investigation, Hajra Sohail; resources, Tariq Aziz; data curation, Hajra Sohail.; writing—original draft preparation, Hajra Sohail.; writing—review and editing, Tariq Aziz; visualization, Majid Alhomrani; supervision, Tariq Aziz and Muhammad Naveed.; project administration, Muhammad Naveed; funding acquisition, Tariq Aziz

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