In silico docking studies on the aromatic hydrocarbon degradation protein (AHDP) of *Citrobacter freundii* for the degradation of hydrocarbons

Kalaivani M.[1], Selvakumar S.[2*] and Balu Prakash[3*]
1. PG and Research Department of Microbiology, Kandaswami Kandar's College, P. Velur, Namakkal - 638182, Tamil Nadu, INDIA
2. Department of Microbiology, The Kavery College of Arts and Science for women, Mechery, Salem - 636453, Tamil Nadu, INDIA
3. Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram - 600117, Tamil Nadu, INDIA
*drselvamicro@gmail.com; prakazbt@gmail.com

Abstract

Crude oil spills and pollutants containing mixtures of components with varying volatility and solubility pose a significant hazard to the environment. Conventional physical and chemical methods for pollutant removal are often ineffective, unsafe and not cost-efficient. In this study, the biodegradation potential of crude oil using microbial intervention was evaluated, focusing on the aromatic hydrocarbon degradation protein (AHDP) of Citrobacter freundii. The efficiency of this bacterial protein in degrading crude oil was analyzed through molecular docking studies involving F6H10 hydrocarbon and Methanol-NaOH, using various in silico tools and databases.

The molecular interaction analysis revealed that the AHDP protein has a strong affinity toward F6H10 hydrocarbon, indicated by a docking score of 4556, suggesting its potential role in effective biodegradation.

Keywords: Aromatic hydrocarbon degradation protein (AHDP), *Citrobacter freundii*, Hydrocarbon, Biodegradation, *In silico*, Molecular docking.

Introduction

Crude oils, which are mostly made up of aliphatic and aromatic hydrocarbons, are frequently released into the environment from subsurface reserves. Because petroleum hydrocarbons occur naturally in all marine settings, many different microbes have evolved the ability to utilise hydrocarbons as sources of carbon and energy for growth. Oil-degrading microorganisms are ubiquitous, yet they may only make up a small part of the microbial community before the spill. Hundreds of bacteria, archaea and fungi have been found to break down petroleum. The biodegrading capabilities of local microbial populations or exogenous microorganisms utilised as inoculants are critical to the effectiveness of bioremediation technologies applied to hydrocarbon-polluted settings[8,18].

Hydrocarbon-exposed populations become acclimated, demonstrating selection enrichment and genetic alterations[3,11]. Adapted microbial communities can respond to hydrocarbon pollution within hours[3] and have higher biodegradation rates than communities that have never been exposed to hydrocarbons[11]. As a result, the capacity to isolate large numbers of specific oil-degrading microorganisms from an environment is often interpreted as proof that those microbes are the most active oil degraders in that environment[3] and can be employed in bioremediation of petroleum oil-polluted locations.

Since the hydrocarbon mixtures differ markedly in volatility, solubility and susceptibility to degradation and the necessary enzymes cannot be found in a single organism, a mixed culture of microbial communities is required to complete biodegradation of oil pollutants[1]. Various researchers found that individual microorganisms can only metabolise a limited range of hydrocarbon substrates and because crude oil is made up of a variety of compounds, biodegradation requires a combination of different bacterial groups or consortia working together to degrade a wider range of hydrocarbons[2,4].

Oil spills and pollution in the water environment have posed a significant threat to the ecosystem and human health by introducing harmful organic compounds into the food chain including polycyclic aromatic hydrocarbons (PAHs)[18]. Polycyclic aromatic hydrocarbons (PAHs) are important environmental contaminants found in soil and water and the majority of these PAHs are recalcitrant in nature. Physical and chemical approaches such as volatilization, photooxidation, chemical oxidation and bioaccumulation[21] are rarely efficient in removing and cleaning up PAHs[15] and they are also not as safe or cost-effective as microbial bioremediation. Bacteria have long been thought to be one of the most common free-living and ubiquitous hydrocarbon degrading agents found in the environment[7].

Petroleum hydrocarbons are major sources of energy for industry and everyday living. Petroleum, on the other hand, is a major pollutant of the environment[13]. Petroleum has the ability to cause a variety of harmful consequences due to its complex makeup.

Depending on the exposure, dosage and organism exposed, it can produce acute deadly toxicity, sub-lethal chronic toxicity, or both. Some petroleum components have the ability to bioaccumulate within vulnerable aquatic creatures and be transmitted up the food chain via trophic transfer[9,14]. The biodegrading capabilities of local microbial populations

or exogenous microorganisms utilised as inoculants are critical to the effectiveness of bioremediation technologies applied to hydrocarbon-polluted settings[8,18]. The most critical need for oil bioremediation is the existence of microorganisms with the proper metabolic capabilities[19]. Several studies have found hydrocarbon-degrading microorganisms such as *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Acinetobacter lwoffi* in various ecological studies[2,11]. Various researchers have found that *C. freundii* is an efficient oil-degrading bacterium[10].

In this study, we carried out molecular docking studies of aromatic hydrocarbon degradation protein (AHDP) of *Citrobacter freundii* with F6H10 hydrocarbon and methanol NaOH using various *in silico* tools and databases.

Material and Methods

Target protein sequence retrieval: The aromatic hydrocarbon degradation protein (AHDP) of *Citrobacter freundii* was retrieved from NCBI database with the ID PZR26169.1 (https://ncbi.nlm.nih.gov.in).

Protein structure prediction: The protein sequence was submitted to an automated homology modelling server called Swiss model (https://swissmodel.expasy.org/) in order to predict the 3D structure. Swiss model is a server for automated comparative modelling of three-dimensional (3D) protein structures. The modelled protein 3D structure was validated using ProCheck server (https://saves.mbi.ucla.edu/) and viewed with the help of molecular visualization software called Discovery studio software.

Cheminformatics Drug 3D prediction: Drug molecules such as methanol NaOH, (CID: 23721981) and F6H10 hydrocarbon (CID: 197559) were chosen and retrieved from NCBI –PubChem (https://pubchem.ncbi.nlm.nih.gov/) in order to perform molecular drug docking studies. The 2D drugs were converted into 3D structure using Cheminformatics protocols.

Molecular Drug Docking: Molecular drug docking studies were performed using an automated molecular drug docking server called PatchDock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) on AHDP protein of *Citrobacter freundii* with the drug molecules, methanol NaOH and F6H10 hydrocarbon in order to identify the molecular binding affinities between the chemical molecules and the protein target.

Results and Discussion

The protein AHDP of *Citrobacter freundii* was retrieved from NCBI database in FASTA format. The length of the nucleotide sequence was 1629 bp and its corresponding` amino acid length was 542 aa (Fig. 1). The 3D structure of the protein AHDP of *Citrobacter freundii* (Fig. 2) predicted by Swiss model was viewed with the molecular visualization tool. The assessment of Ramachandran Plot for the predicted protein structure of AHDP (*Citrobacter freundii*) is shown in fig. 3. Protein structure validation results of AHDP (*Citrobacter freundii*) are depicted in table 1.

>PZR26169.1 aromatic hydrocarbon degradation protein [*Citrobacter freundii*]
MDTRITDTRITRIKRTRITRKGLSRLGFIACFSLTANALFAQIPEDVLKYSWQPVNGTARINAVGGAMGS
LGGDISATFTNPAGLAFYKTGDLVISPGYNFLNNKSTFRGTPGKDKDNTFNLGASGYVAGWGSDRGKWKS
QAFGIAVTRTANFNNTVYYTGQNDFSSGAEQYAAEAASSGVSLEDMPYSNRVSFGTRMAAWNYLIDSASL
PGHTGQDVISMSMWDALKNGGNFLVNQSQLIETSGGITEIALGYAGNKNDKFYLGGSLGIPILNYQKNTR
FREEDATNNSDNNFGFYELNETFKTKGVGFNLKLGAIMKPAEFIRVGLAVHSPTWYALEDSYFGRMSVNL
DKYRTVPGTTTVTSDQLVQNGAFPNYKYQLMTPWRFMVSGSYVLREAEDVRQQKGFITADVEYVTYKSNK
YASAEEYNDDTYYDGLNSVIKQYYKNAFNFRVGGELKFTTIMTRLGFSYYGNPYADPELKANKMFVSGGL
GYRNNGIFIDLTYTHAIQKDVDFPYRLPDKANTFANLKGTGSNIMLTFGIKI

Fig. 1: Protein sequence of aromatic hydrocarbon degradation protein (AHDP) of *C. freundii*

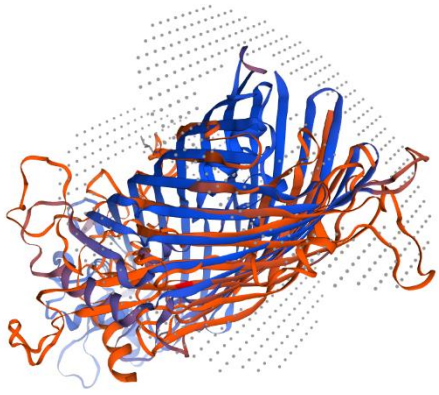


Fig. 2: 3D structure of AHDP (*Citrobacter freundii*) modelled by SWISS model

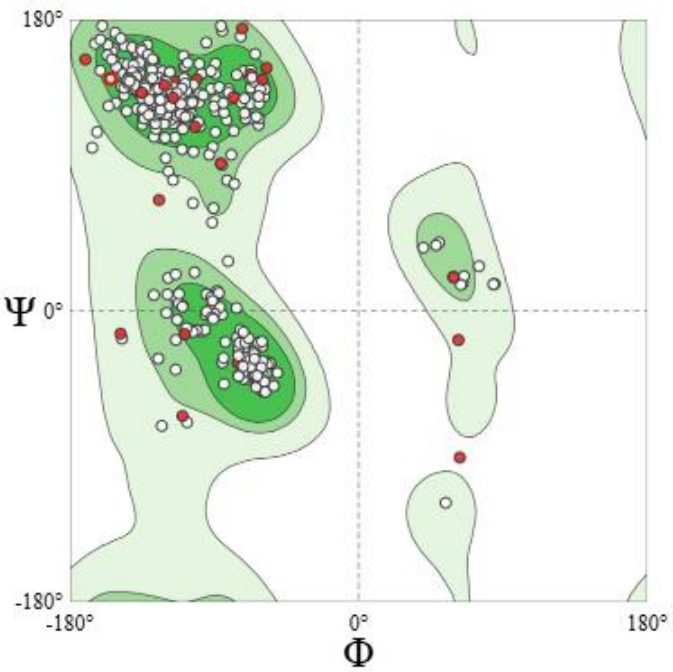
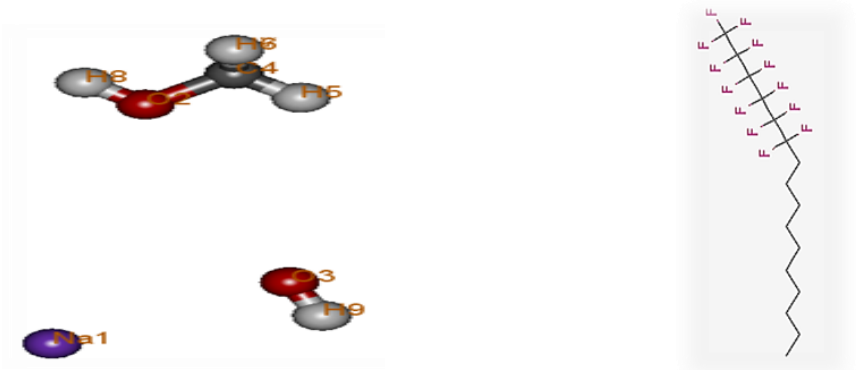


Fig. 3: Protein Structure Validation of AHDP (*Citrobacter freundii*) by Ramachandran plot.

Table 1
Protein Structure Validation Results of AHDP (*Citrobacter freundii*)

Validation Parameter	Value	Ideal Threshold / Goal	Remarks
Ramachandran Plot			
– Favored regions	496 residues (95.94%)	>98%	Slightly below ideal
– Allowed regions	15 residues (2.9%)	-	Acceptable
– Outliers	2 residues (0.39%)	<0.05%	Slightly above ideal
Rotamer Quality			
– Poor rotamers	0 (0.00%)	<0.3%	Excellent
– Favored rotamers	413 (97.87%)	>98%	Near ideal
Geometry Checks			
– Bad bonds	0 / 4148 (0.00%)	0	Excellent
– Bad angles	23 / 5620 (0.41%)	<0.1%	Slightly above threshold
– Cβ deviations >0.25 Å	4 (0.86%)	0	Minor deviation
– Cis non-Prolines	1 / 501 (0.20%)	<0.05%	Slightly above threshold
Other Validation Metrics			
– CaBLAM outliers	7 (1.4%)	<1.0%	Slightly above ideal
– CA geometry outliers	2 (0.39%)	<0.5%	Acceptable
– Ramachandran Z-score	1.35 ± 0.36		Z



(a) **(b)**

Fig. 4: a) 3D Structure of Methanol NaOH. b) 3D Structure of F6H10 Hydrocarbon.

The 3D structure of methanol NaOH with coloured atoms: Grey-Carbon, Blue-Nitrogen, Purple-Sodium and White-Hydrogen using Discovery Studio Software is shown in fig. 4a. The 2Dstructure of F6H10 obtained from PubChem compound database is shown in fig. 4b. The 3D structure of F6H10 hydrocarbon with coloured atoms: Grey-Carbon, Blue-Nitrogen, Purple-Sodium and White-Hydrogen using Discovery Studio Software is shown in fig. 5. The patchdock result page showing the molecular interaction between the AHDP of *Citrobacter freundii* and methanol-NaOH with a high drug docking score value of 1922 and ACE (Atomic contact energy) value of -17.01 Kcal/mol is shown in table 2. The docked complex structure is shown in fig. 6.

The aminoacids involved in interaction between the AHDP protein (*Citrobacter freundii*) and the existing drug molecule - methanol NaOH structure were labelled using Discovery Studio Software and are shown in fig. 7. The PatchDock result page showing the molecular interaction between the modelled protein target, AHDP (*Citrobacter freundii*) and F6H10 hydrocarbon with a drug docking score value of 4556 and ACE (Atomic Contact Energy) value of 86.77 Kcal/mol (Table 2) and the interaction are shown in fig. 8.

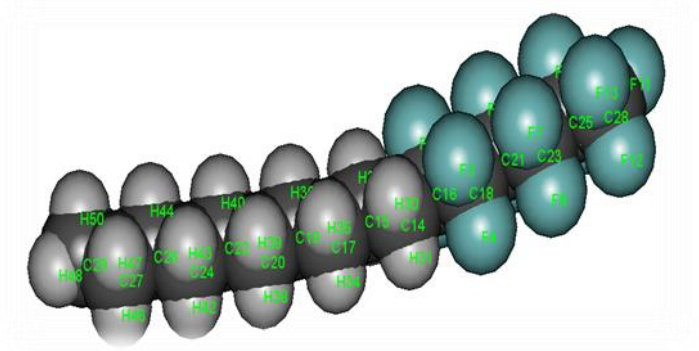


Fig. 5: 3D Structure of F6H10 Hydrocarbon

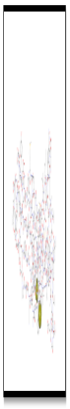


Fig. 6: Molecular interaction between AHDP with Methanol-NaOH

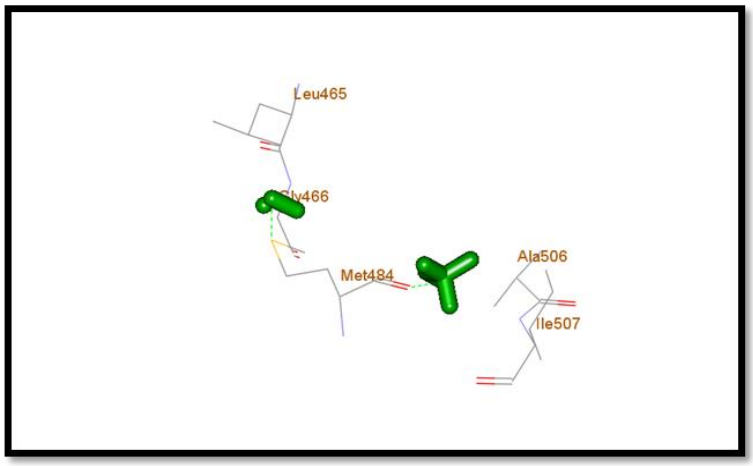


Fig. 7: Molecular interaction of amino acids in AHDP-Methanol NaOH complex. Green colour indicates Methanol NaOH in Stick model.

Table 2
Molecular docking analysis of AHDP with F6H10 Hydrocarbon

Solution No.	Score	Area	ACE
1	4556	501.40	86.77
2	4554	591.00	11.12
3	4360	495.20	-35.56
4	4358	594.90	-46.68
5	4318	575.50	13.00
6	4305	569.50	8.24
7	4260	539.90	68.96
8	4256	569.80	62.62
9	4204	581.80	36.14
10	4189	514.00	-27.63
11	4184	543.20	-47.48
12	4140	560.00	127.62
13	4120	484.80	70.95
14	4118	524.20	67.56
15	4108	485.00	20.37
16	4014	521.60	-18.50
17	3996	458.30	-51.24
18	3976	466.90	33.41
19	3964	468.90	-9.55
20	3964	563.40	-9.55

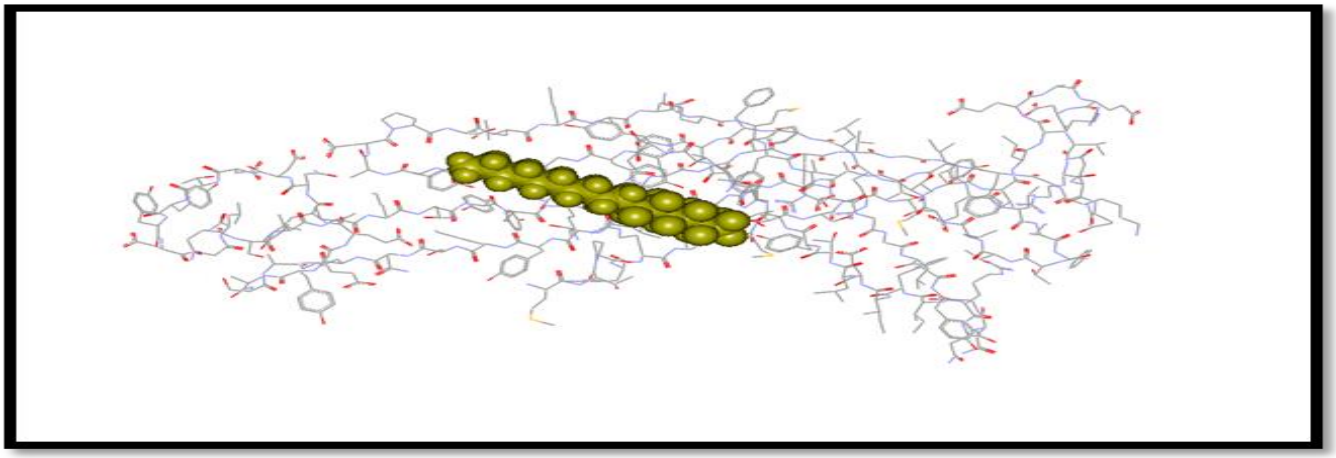


Fig. 8: Molecular interaction between AHDP and F6H10 Hydrocarbon

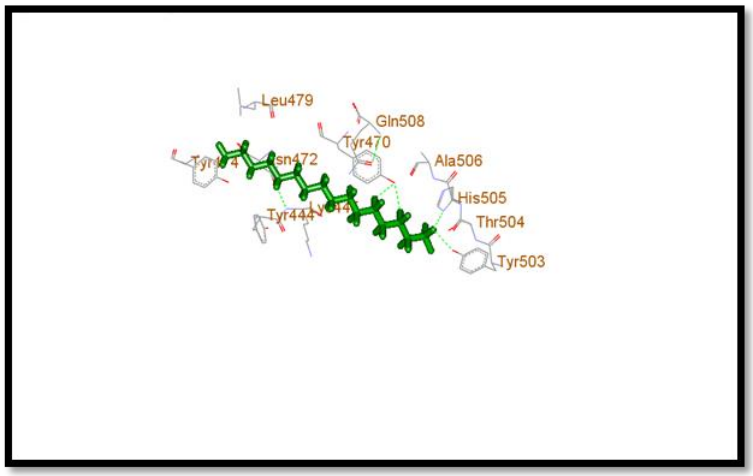


Fig. 9: Molecular interaction of amino acids in AHDP-F6H10 complex. Green colour indicates F6H10 Hydrocarbon in Stick model

Table 3
Aminoacid interactions found in the docked complexes

Complex	Amino acids involved in binding	No. of amino acids involved in interaction
AHDP-Methanol NaOH	LEU465, GLY466, MET484, ALA506, ILE507	5
AHDP-F6H10 hydrocarbon	TYR144, TYR444, LYS445, ASN472, TYR470, LEY479, TYR503, THR504, HIS505, ALA506, GLN508	11

The amino acids involved in the interaction between the protein AHDP (*Citrobacter freundii*) with F6H10 hydrocarbon were labelled using Discovery Studio Software and are shown in fig. 9. Amino acid interactions found in the docked complexes are illustrated in table 3. It is clear that based on the molecular drug docking scores, the AHDP protein of *Citrobacter freundii* efficiently binds with the selected F6H10 hydrocarbon molecule when compared to methanol NaOH. Amino acids involved in H-bond interaction between the protein AHDP and the existing drug molecule methanol NaOH were LEU465, GLY466, MET484, ALA506 and ILE507, whereas in the AHDP–F6H10 hydrocarbon complex, they were TYR144, TYR444, LYS445, ASN472, TYR470, LEY479, TYR503, THR504, HIS505, ALA506 and GLN508; results are shown in table 3.

The biodegradation rates of a few chemicals found in crude oils, such as resins, hopanes, polar molecules and asphaltenes, are nearly unnoticeable. Lighter crudes, like the oil from the BP Deepwater Horizon accident, have a higher proportion of simpler lower molecular weight hydrocarbons which are more easily biodegraded than heavier crudes like the Exxon Valdez oil. Although PAHs are a tiny component of crude oils, they are most harmful to plants and animals. Bacteria can entirely convert PAHs to biomass, CO_2 and H_2O, although they usually need to add O_2 first via dioxygenase enzymes. Petroleum hydrocarbons can also be degraded anaerobically, though at far slower rates. Petroleum hydrocarbons can biodegrade at temperatures ranging from 0 to 80^0C. Other components are required for microorganisms to grow.

The quantities of these components in marine environments, most notably nitrates, phosphates and iron, can slow down the biodegradation of oil. When large amounts of hydrocarbons are discharged into the marine environment, having an appropriate supply of these rate-limiting nutrients is crucial for controlling biodegradation rates and thus the permanence of potentially detrimental environmental impacts. Bioremediation, which was employed extensively in the Exxon Valdez spill, entailed adding nitrogen (N) nutrients to fertilisers to speed up oil biodegradation rates[17]. The majority of petroleum hydrocarbons are water-insoluble. Biodegradation of hydrocarbons occurs at the hydrocarbon–water interface. As a result, the oil's surface area-to-volume ratio has a substantial impact on its biodegradation rate. Dispersants, such as Corexit9500, which were deployed during the BP Deepwater Horizon spill, increase the available surface area, which could speed up biodegradation rates[17]. Because crude oil is made up of a variety of compounds and individual microorganisms can only metabolise a limited number of hydrocarbon substrates[2,5], biodegradation of crude oil necessitates a combination of bacterial groups or consortia that can degrade a wider range of hydrocarbons[2,4]. Molecular docking studies are helpful in studying the biodegradable mechanism of various organic pollutants[12].

Conclusion

The present molecular interaction study between the AHDP protein of *Citrobacter freundii* and the drug molecules namely methanol-NaOH and F6H10 hydrocarbon revealed that the binding affinity between the AHDP protein and F6H10 hydrocarbon was greater when compared to Methanol-NaOH as shown by its docking score (4556) and the number of aminoacids (11) involved. Thus F6H10 hydrocarbon could efficiently be degraded by AHDP protein of *Citrobacter freundii*. These results can be correlated with the results of wet lab studies.

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