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Computational Approach to Study Catalytic Properties of Dioxygenase Enzyme in Bioremediation

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ABSTRACT

Bioremediation is a degradation or transformation of contaminants into nonhazardous or less-hazardous substances using microorganism. A large number of enzymes from bacteria, fungi, and plants have been reported to be involved in the biodegradation of toxic organic pollutants. *In silico* approach uses variety of computational tools to predict best binding sites for pollutant degradation which permits to explore the potential of microorganisms in cleaning up the particular compound from the environment. Its broad substrate specificity offers a wide opportunity for screening pollutants in order to predict potential targets for degradation. We identified potent bacteria that can be used to accomplish bioremediation i.e. *Pseudomonas putida.* This study aimed to evaluate the *catechol 1, 2- dioxygenase* enzyme which comes in class of *oxidoreductase* and in sub- class of *oxygenase* binding affinity with the pollutant and to explore its target potential sites using docking software. The chosen pollutants were Catechol, Endosulfan, Carbaryl, Xylene, Toluene and Acrylamide. In this *Pseudomonas putida* wild type enzyme showed good results by endosulfan pollutant with -6.68 KJ/mol binding energy while mutated enzyme have shown better result with endosulfan with binding energy of -6.73 KJ/mol.

Keywords: Bioremediation, *catechol 1, 2-dioxygenase*, *Pseudomonas putida*

INTRODUCTION:

The rapidly growing industrialization along with an increasing population has resulted in the accumulation of a wide variety of chemicals. Thus, the frequency and widespread use of man-made "xenobiotic" chemicals has led to a remarkable effort to implement new technologies to reduce or eliminate these contaminants from the environment. Commonly- used pollution treatment methods for the remediation of contaminated sites have also had adverse effects on the environment, which can lead to the formation of toxic intermediates [1]. Bioremediation, a biodegradation process in which sites contaminated with xenobiotics are cleaned up by means of bacterial bio-geochemical processes, preferably in situ, exploits the ability of microorganisms to reduce the concentration and toxicity of a large number of pollutants [2].

The detoxification of toxic organic compounds by various bacteria and fungi and higher plants through oxidative coupling is mediated with oxidoreductases [3]. Microbes extract energy via energy-yielding biochemical reactions mediated by these enzymes to cleave chemical bonds and to assist the transfer of electrons from a reduced organic substrate (donor) to another chemical compound (acceptor). During such oxidation reduction reactions, the contaminants are finally oxidized to harmless compounds. The oxidoreductases participate in the humification of various phenolic substances that are produced from the decomposition of lignin in a soil environment [4]. In the same way, oxidoreductases can also detoxify toxic xenobiotics, such as phenolic or anilinic compounds, through polymerization, copolymerization with other substrates, or binding to humic substances. Microbial enzymes have been exploited in the decolorization and degradation of azo dyes [5].

The use of enzyme catechol dioxygenases for bioremediation has been relatively little explored, although, there is a great potential to use these enzymes mainly associated with the use to bioreactors, to clean high amounts of wastewater contaminated with phenol, benzoate, fluorocatecol, bromocatecol, cholorocathecol, methilcathecol, herbicides (diuron), polychlorinated biphenyls, chloroethanes and others[6, 7, 8]. The environment is polluted by a lot of aromatic compounds such as chlorophenols, creosols or nitrophenols which can substrate for catechol 1, 2- dioxygenase [9]. This free enzyme can undergo deactivation in biodegradation and industrial processes so it is very important to obtain highly stable enzymes [10]. The enzymes catechol dioxygenases add two oxygen atoms to the aromatic ring, disrupting chemical bonds and allowing opening this ring [11]. The catechol 1,2-dioxygenase (C12O) (EC 1.13.11.1) contains Fe^{+3} as prosthetic group and belongs to the enzymes that make cleavage of catechol as intradiol (or ortho cleavage), producing cis-cis muconic acid [12].

This study aimed to predict the best affinity between known pollutants with catechol 1, 2- dioxygenase enzymes as a receptor in *Pseudomonas putida* involved in bioremediation implementing *In silico* based method. Activity of catechol 1, 2-dioxygenase enzyme of *Pseudomonas putida* was used to predict the potential of this bacteria to degrade various pollutants with the help of molecular ligand docking.

MATERIALS AND METHODS:

Source of Enzyme:

Enzyme Catechol 1, 2- dioxygenase protein structure was retrieved from RCSB pdb database of *Pseudomonas putida.*

Pollutants:

Pollutants list were selected from Environmental Protection Agency (EPA) and those pollutants that were found in underground water area, water disposal and land disposal area were chosen. The pollutants were Catechol, Endosulfan, Carbaryl, Xylene, Toluene and Acrylamide.

Computational Study:

Protein structure of *Pseudomonas putida* (2AZQ) was retrieved from RCSB pdb database and structure was edited in UCSF Chimera [13]. Ligand was drawn using ChemDraw software [14] and converted into 3D structure using Chem3D software. Blind docking is performed by using GUI interface of Autodock [15] and software was downloaded from the Scripps portal [16]. The prepared receptors of microorganisms and pollutants as ligands were blind docked to predict the best affinity between each of them by noting their lowest binding energies in the range of -5 to -15 Kcal/Mol [17].

After the completion of docking processes their binding sites were viewed in PyMOL [18] software tool and their sites were noted down for mutation. Mutation was brought about using SPDBV/Deep viewer tool [19] and these mutated proteins were again docked by using Autodock tool.

RESULTS AND DISCUSSION:

The 3D structure of catechol 1, 2- dioxygenase from *Pseudomonas putida* was retrieved from RCSB pdb database whose pdb entry no. 2AZQ and edit in UCSF Chimera (figure 1).

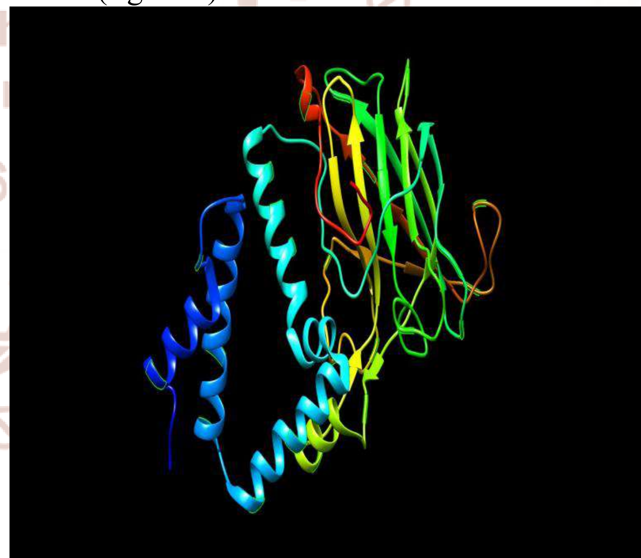

Figure 1. 2AZQ (*Pseudomonas putida*)

Pseudomonas putida docking results are as follows with different pollutants and table 1 shows the grid box coordinates:

Table 1: The grid box coordinates *Pseudomonas putida* protein

Protein	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
2AZQ	60	58	58	0.511	26.621	8.754	-12.177

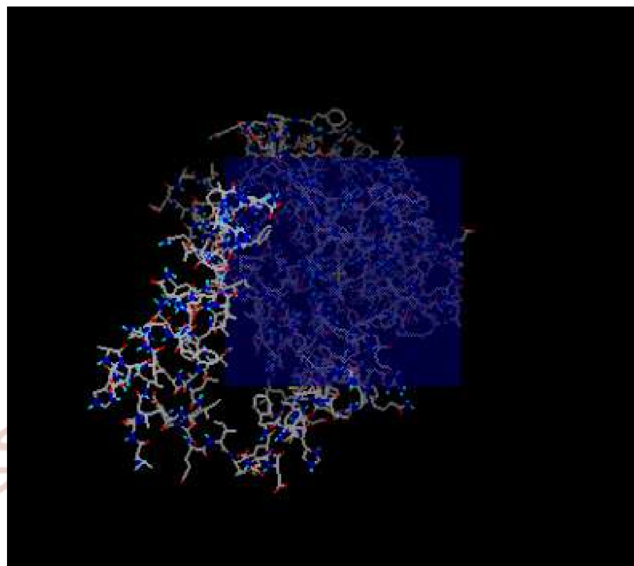

Figure 2. Grid box used for covering all binding residues involved in binding of ligand

After getting these binding sites, mutation was done with SPDBV and on the basis of energy those amino acids were selected which showed best minimum energy after mutation at that particular site. The grid box coordinates were not changed. Here, table 2, shows the binding energy of wild type protein with pollutants and table 3, shows the binding energy of mutated protein with pollutants.

Table 2: The binding energy of wild type protein with pollutants

S. No.	Name of pollutant (Ligand)	Binding Sites	Binding energy KJ/mole
1	Catechol	TYR16 & HIS162	-5.36
2	Endosulfan	GLY160 & GLY197	-6.68
3	Carbaryl	ARG303 & PRO304	-5.66
4	Xylene	ARG303 & PRO304	-4.81
5	Toluene	ASN68, GLY71 & TYR109	-4.75
6	Acrylamide	PRO304	-3.66

Table 3: The binding energy of mutated protein with pollutants

S. No.	Name of pollutant (Ligand)	Mutated Sites	Binding energy KJ/mole
1	Catechol	HIS 162 → SER162	-5.48
2	Endosulfan	GLY160 → ALA160	-6.73
3	Carbaryl	PRO304 → ARG304	-5.86
4	Xylene	PRO304 → LEU304	-5.00
5	Toluene	GLY71 → ALA71	-4.75
6	Acrylamide	PRO304 → ALA304	-3.76

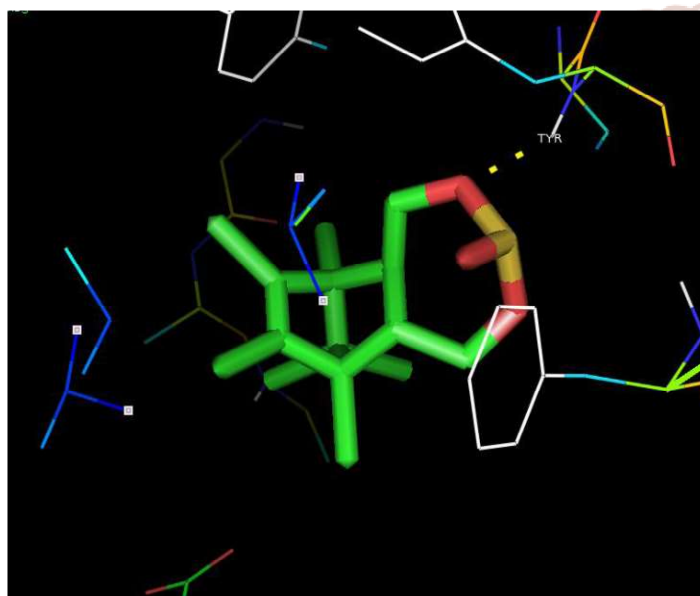

Figure 3. PYMOL view showing new binding sites of mutant enzyme of *Pseudomonas putida* with endosulfan

In our present study, the original bacterial protein have shown higher binding affinity towards the selected pollutant as seen in the docking results table 2. *Pseudomonas putida* wild type enzyme showed best binding energy with endosulfan i.e. -6.68 KJ/mol. On observing the docking results *Pseudomonas putida* of mutated protein figure 3 against the taken pollutant endosulfan the best binding energy was shown by *Pseudomonas putida* having energy of about -6.73KJ/mol .

Here, lower the negative value of binding energy (KJ/mol) more stable is molecule and as a result the interaction between catechol 1, 2- dioxygenase mutant enzyme with pollutant catechol, carbaryl and Xylene showed medium binding affinity by *Pseudomonas putida*. While in case of pollutant toluene and acrylamide it was observed that least binding affinity was shown by *Pseudomonas putida* mutant enzyme

CONCLUSION

Biological degradation by organisms (fungi, bacteria, viruses, protozoa) can efficiently remove hazardous chemical from the environment, especially organochlorines, organophosphates and carbamates used in agriculture and industries. The enzymatic degradation of synthetic pesticides with microorganisms represents the most important strategy for the pollutant removal, in comparison with non-enzymatic processes. The binding affinity of catechol 1, 2- dioxygenase enzyme as receptor shown by wild type bacteria in *Pseudomonas putida* endosulfan pollutants shows -6.68 KJ/mol, carbaryl -5.66 KJ/mol, catechol -5.36 KJ/mol, xylene -4.81 KJ/mol, toluene -4.75 KJ/mol and acrylamide -3.66 KJ/mol. Hence it is concluded that in *Pseudomonas putida* best binding affinity was shown by endosulfan pollutant.

On inducing mutation in wild type enzyme protein catechol 1, 2- dioxygenase the *Pseudomonas putida* mutated enzyme have shown better result with endosulfan with binding energy of -6.73 KJ/mol but medium affinity with carbaryl -5.66 KJ/mol, catechol -5.86 KJ/mol, xylene -5.00 KJ/mol. *Pseudomonas putida* shows lower binding affinity with toluene -4.75 KJ/mol and acrylamide -3.76 KJ/mol. Finally, it is concluded from our research that *Pseudomonas putida* mutant enzyme act as optimal bioremediation agent for pollutant endosulfan only.

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