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Computational Modeling and Docking studies showed that Nitrogen containing pollutants are putative inhibitors of *Pseudomonas* KNK003A Carbamoylase.

KEYWORDS: Carbamoylase, bioremediation, Pseudomonas KNK003A, modeling, docking

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ABSTRACT *A large number of enzymes from Pseudomonas have been reported to be involved in the biodegradation of toxic organic pollutants. Bioremediation is a nature friendly technology that is powered by microbial enzymes. The mechanisms of bioremediation related enzymes hydrolases have been extensively studied. Carbamoylase is an enzyme of class hydrolases. It is an interest to generate carbamoylase structure model from Pseudomonas from bioremediation point of view. This paper attempts to provide information via in silico studies, on the various inhibitors of the enzyme, generously present in various polluted effluents. These may be declining the efficiency of enzymatic biodegradation. Pretreatment of effluents can be recommended for efficient bioremediation.*

Introduction:
Bioremediation is a cost-effective technique for treatment of polluted environment and it involves the usage of microorganisms for pollutants degradation .Some members of the genus *Pseudomonas* are able to metabolize chemical pollutants in the environment. Bioremediation technologies rely on the activity of microbial enzymes involved in the metabolic and cometabolic transformation of a variety of organic substrates. Many xenobiotic compounds can be degraded by intracellular enzymes and thereby undergo detoxification. To date, contaminants have been exposed to enzymatic degradation primarily by stimulating microbial growth in contaminated areas. There are a number of enzymes involved in the mechanism of Bioremediation like aminopeptidases, lipases, oxidoreductases, laccases, hydrolases etc.

Carbamoylase, an enzyme of class hydrolases, from *Pseudomonas sp.* and other bacteria have been shown to hydrolyze and detoxify aromatic hydrocarbons and nitrogenous compounds. These compounds are major representatives of non-metal pollutants found in many contaminated soils by municipal, industrial and agricultural wastewater sites. Their removal from polluted environmental niches depends to a great extent on microbial degradation, which can also be applied on several technological applications. Elevated levels of nitric compounds not only complicate the use of natural resources, but also have a negative influence on the ecological state and on the activity of various bioremedial enzymes of microbes.

Inhibition of carbamoylases has been reported by substrates, substrate analogs, reaction products, and reaction product analogs. *Agrobacterium sp.*carbamoylase was inhibited by ammonium, one of the spontaneous decomposition products of the reaction [Louwrier A & Knowles, 1996]. Hence, the prevention of nitrogen pollution by the removal of ions by pretreatment of contaminants is a necessary stage of a great importance. The most widely used methods for removing ammonium ions are air stripping, ion exchange, breakpoint chlorination, adsorption by activated coal, chemical coagulation and biological nitrification, denitrification etc.

Till date, crystal structure of *Pseudomonas* KNK003A carbamoylase is not present in public repository databases, so determining the 3D structure provides a new opportunity for the discovery of more potent inhibitors of bioremediation from various polluted sites. To this end, an approach has been taken that combines identification of 3D structure of *Pseudomonas* KNK003A carbamoylase and computational docking process to identify a series of potent inhibitors from Pubchem Database.

Methodology:
Template Selection:
The amino acid sequence of *Pseudomonas* KNK003A carbamoylase was obtained from the NCBI protein database (Accession number: BAD00008.1) [http://www.ncbi.nlm.nih.gov/protein]. Template selection was done using BLASTp [Altshul SF *et al,* 1997] for the query sequence against PDB database [http://www.rcsb.org/]. Crystal structure of Chain A, of N-Carbamoyl-D-Amino-Acidamidohydrolase was taken from the protein data bank (PDB ID: 1FO6) and used as the template for building the initial 3D model.

Sequence Analysis:
The sequence alignment of carbamoylase with the template was accomplished using ALIGN 2D.

Homology Modeling:
The Modeller 9v6 [Sali A & Blundell TL, 1993] program was employed to generate the initial 3D models of carbamoylase. Modeller generates the 3D models by optimization of molecular probability density functions. The optimization process consists of applying the variable target function as well as conjugated gradients and molecular dynamics with simulated annealing. A set of five models of carbamoylase were produced based on the resulting alignment obtained above. The outcomes were ranked based on the DOPE scoring function of MODELLER.

Homology models validation:
The model with highest score was validated by the UCLA SERVER and Procheck [Laskowski RA *et al., 1993*]. The model was further refined by energy minimization. The energy minimization was performed using the SPDBV package. The optimized model was subjected to quality assessment with respect to its geometry and energy and was then subjected to molecular docking. PROCHECK was utilized for geometric evaluation. The five inhibitor molecules NH^{4+} ,EDTA, imidazole, aniline and diamine ethoxylate were downloaded from Pubchem database of NCBI , and converted to 3D structure with OPENBABEL 2.3.1 software. These substrates were geometrically optimized for further use in docking.

Analysis of Ligand binding sites and pockets:
Ligand binding sites were predicted by QSITE FINDER at

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http://www.modelling.leeds.ac.uk/qsitefinder/ [Lovell SC *et al.*, 2003].

Protein-ligand docking:
The docking of ligands to the carbamoylase was performed using AutoDock [Morris GM *et al.*, 2009] software. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Using the software, polar hydrogen atoms were added to carbamoylase protein and its nonpolar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The grid box with a dimension of 126 x 126x 126 points was used to cover the entire enzyme binding site and accommodate ligands to move freely. The best conformation was chosen with the lowest docked energy, after the docking search was completed. The protein – ligand interactions were viewed by PyMOL viewer.

Discussion:
The enzyme carbamoylase is 312 amino acid residues long. Sequence search using BLASTp identified the crystal structure with PDB ID: 1FO6. The enzyme showed 61% sequence identity to the query sequence with an E- value of 5e-130

Figure 1 : Pairwise alignment of carbamoylase from *Pseudomonas* KNK003A and the template(PDB ID : 1FO6).

>pdb|1FO6|A Chain A, Crystal Structure Analysis Of N-Carbamoyl-D-Amino-Acid

Amidohydrolase

Score = 377 bits (967), Expect = 5e-130, Method: Compositional matrix adjust.

Identities = 185/303 (61%), Positives = 218/303 (72%), Gaps = 0/303 (0%)

Query 1 MTRIVNAAAAQMGPISRSETRKDTVRRLIALM-REAKARGSDLVVFTELALTTFFPRWVIE 60

MTR + A Q GPI+R+ETR+ V RL+ ++ A +RG + +VF ELALT-TFFPRW

Sbjct 1 MTRQMILAVGQQGPIARAETREQVVGRLLDMLT-NAASRGVNFIVFPELALTTFFPRWHFT 60

Query 61 DEAELDSFYEKEMPGPETQPLFDEAKRLEIGFYLG-YAELAEEGGRKRRFNTSILVDRSGR 120

DEAELDSFYE EMPGP +PLF+ A L IGF LGYAEL EGG KRRFNTSILVD+SG+

Sbjct 61 DEAELDSFYETEMPGPVVRPLFETAAELGIGFNLG-YAELVVEGGVKRRFNTSILVDKSGK 120

Query 121 IVGKYRKVHLPGHKEPQPGRKHQHLEKRYFEPG-DLGFGVWRAFDGVMGMCICNDRRWPET 180

IVGKYRK+HLPGHKE + R QHLEKRYFEPGDLGF V+ MGM ICNDRRWPET

Sbjct 121 IVGKYRKIHLPGHKEYEAYRPFQHLEKRYFEPGDL-GFPVYDVDAAKMGMFICNDRRWPET 180

Query 181 YRVMGLQGVEMVMLGYNTPYDHTGHDDID-SLTQFHNHLSMQAGAYQNSTWVIGTAKCGTE 240

+RVMGL+G E++ GYNTP + D LT FH+ LSMQAG+YQN W K G E

Sbjct 181 WRVMGLKGAEIICGGYNTPTHNPPVPQHDHLTS-FHHLLSMQAGSYQNGAWSAAAGKVGME 240

Query 241 EGSKMVGQSVIVAPSGEIVAMACTIEDEII-TARCDLDMGKRYRETIFDFARHREPDAYRL 300

EG ++G S IVAP+GEIVA+ T+EDE+ITA DLD + RE IF+F HR+P Y L

Sbjct 241 EGCMLLGHSCIVAPTGEIVALTTTLEDEVITAALDL-DRCRELREHIFNFKAHRQPQHYGL 300

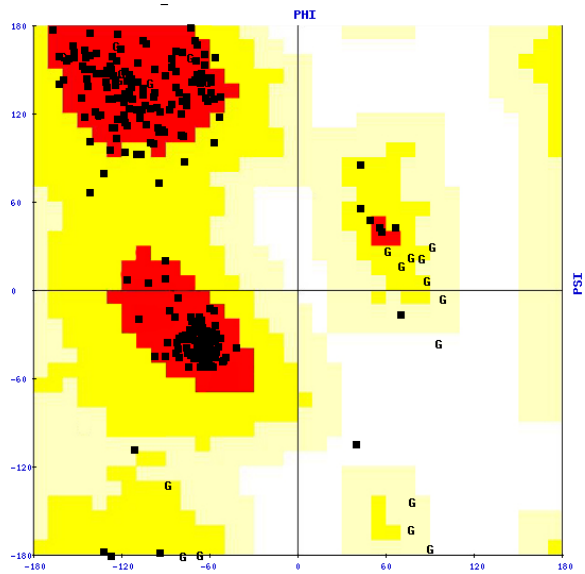
Query 301 IVE 303

I E

Sbjct 301 IAE 303

We then developed structures for carbamoylase using MODELLER. Five models were generated using this procedure. The model with the lowest DOPE (Discrete Optimized Protein Energy) with the score of -34114.02(Table1) was considered to be thermodynamically stable and chosen for further refinement and validation. The stereochemical quality and accuracy of the selected model was evaluated using Ramachandran Plot and Procheck.

Figure 2: Ramachandran plot of carbamoylase 3D model obtained by PROCHECK.



The Ramachandaran plot revealed 91.4% core, 7.5% allowed, 0.8% generously allowed, 0.4% disallowed.#

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Q – site Finder predicted 10 different sites. The residues that find the binding sites were identified as THR-2; VAL-5,44,115, 166,192,231;ASN-6;ALA-7,162;ASP-41,116,164;LEU-42,276;ILE-90,232;GLY-91,165;PHE-92,163;ARG-117;MET-167,191,193;GLU-190;TRP-230;CYS-274.

This structure (Figure3) was used for docking.

Figure 3: Carbamoylase structure model produced using MODELLER 9v6.

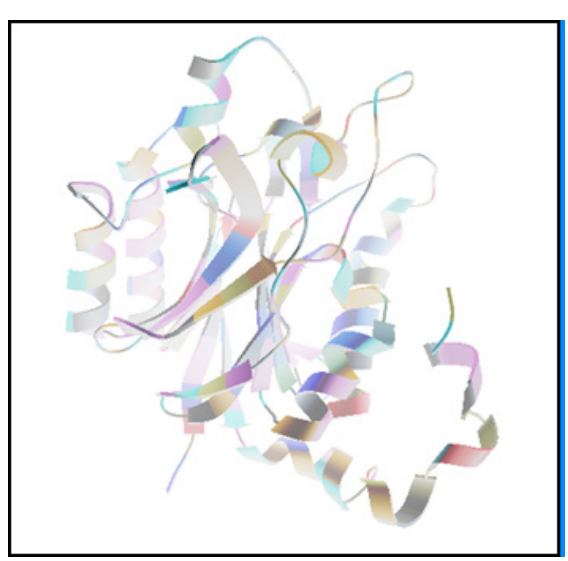

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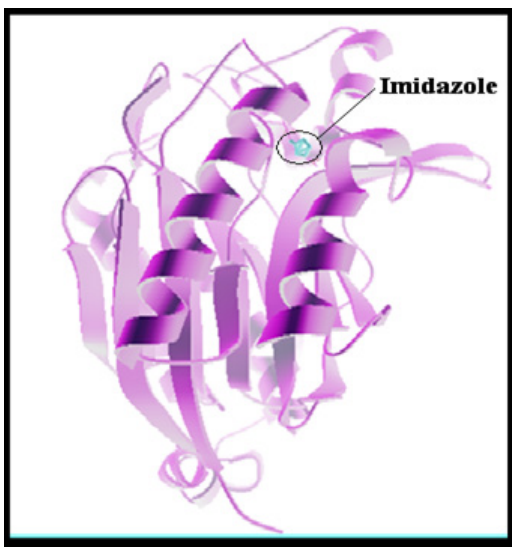
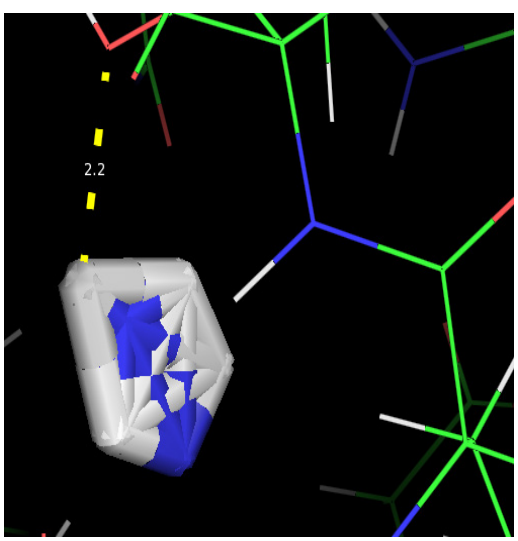
Figure 4: Binding mode of carbamoylase with Imidazole(1-A),Aniline (2-A) and EDTA(3-A). Images by PyMOL Viewer are shown in (1-B),(2-B),(3-B) respectively .

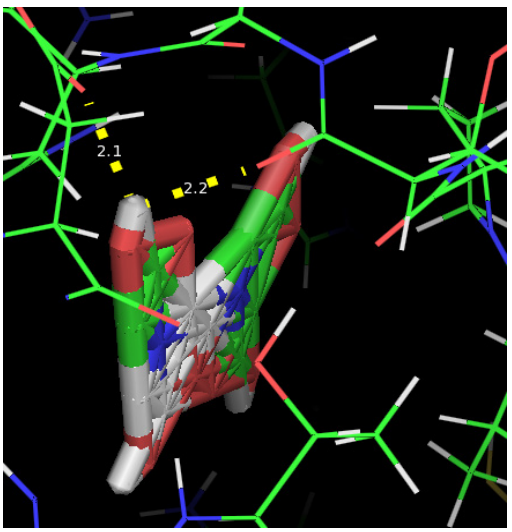


1-A



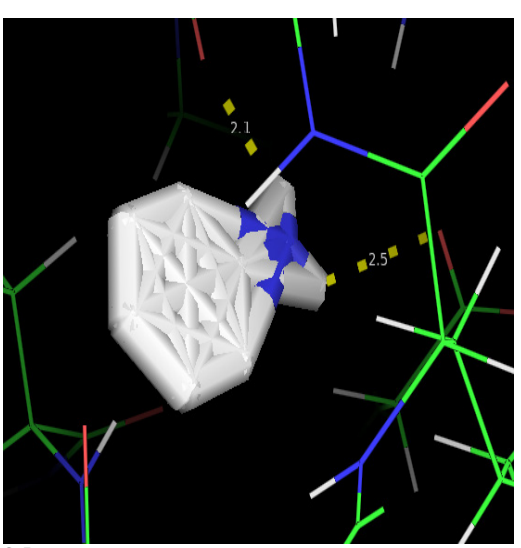
1-B

2-A



2-B

3-A



3-B

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The five ligands (Table2) were docked using Autodock software (Figure 4).The binding energies were obtained in the range from -4.74 to +3960 kcal/mol (Table3).The results indicate that diamine ethoxylate does not provide any stable conformational binding due to high torsional strain. EDTA and aniline are strong inhibitors.

Conclusion:

The aim of study was to identify the most accurate 3D model for carbamoylase. The enzyme acts as the bioremediation tool against various pollutants comprising aromatic hydrocarbons. Docking results indicated that the various forms of nitrogenous compounds generously available in industrial effluents act as potent inhibitors of carbamoylase. Further analysis can be carried out in wet lab. In preview of *in silico* studies , pretreatment of industrial effluents containing nitrogenous compounds should be carried out for efficient bioremediation by various *Pseudomonas sp*.

Table 1: Dope score of the constructed models

S. No.	DOPE SCORE OF MODELS
1.	-34114.02
2.	-33132.47
3.	-33961.01
4.	-33956.66
5.	-34058.99

Table 2:Structure of ligands

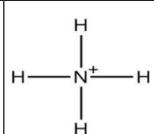
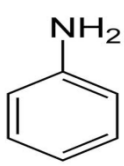
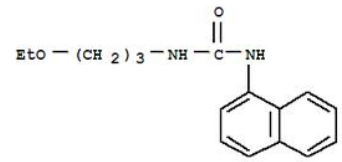
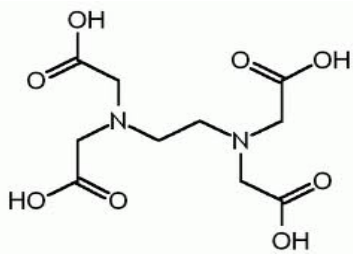
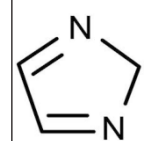
S.No.	LIGAND	STRUCTURE
1.	Ammonium ions	
2.	Aniline	
3.	Diamine ethoxylate	
4.	EDTA	
5.	Imidazole	

Table3: Binding energy of docked ligands.

S.No.	LIGANDS	BINDING ENERGY (KCal/mol)
1.	AMMONIUM IONS	-2.44
2.	ANILINE	-3.22
3.	DIAMINE ETHOXYLATE	3960
4.	EDTA	-4.74
5.	IMIDAZOLE	-3.07

REFERENCE Altshul, S.F. et al. Nucleic Acids Res 1997; 25:3389[PMID:9254694] | http://www.ncbi.nlm.nih.gov/protein. | http://www.rcsb.org/ | Laskowski, R.A., et al. J Applied Crystallography 1993;26:283 | Louwrier, A., & Knowles, C.J. The purification and characterization of a novel D(-)-specific carbamoylase enzyme from an Agrobacterium sp. Enzyme MicrobTechnol 1996; 19:562-571. | Lovell, S.C. et al. Proteins 2003; 50:437 [PMID: 12557186] | Morris, G.M. et al., (2009). J Comput Chem 2009; 30:2785 | Sali, A., & Blundell, T.L. J Mol Biol 1993; 234:779 [PMID:8254673].