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# Molecular level biodegradation of phenol and its derivatives through dmp operon of *Pseudomonas putida*: A bio-molecular modeling and docking analysis

*Sujay Ray*[1, 2,*], *Arundhati Banerjee*[3]

1. *Department of Biochemistry and Biophysics, University of Kalyani, Kalyani, 741245 Nadia, West Bengal, India*
2. *Department of Biotechnology, Bengal College of Engineering and Technology, Shahid Sukumar Sarani, Bidhannagar, Durgapur-713212, West Bengal, India*
3. *Department of Biotechnology, National Institute of Technology, Mahatma Gandhi Avenue, Durgapur 713209, West Bengal, India*

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ABSTRACT

Participation of *Pseudomonas putida*-derived methyl phenol (*dmp*) operon and DmpR protein in the biodegradation of phenol or other harmful, organic, toxic pollutants was investigated at a molecular level. Documentation documents that *P. putida* has DmpR protein which positively regulates *dmp* operon in the presence of inducers; like phenols. From the operon, phenol hydroxylase encoded by dmpN gene, participates in degrading phenols after *dmp* operon is expressed. For the purpose, the 3-D models of the four domains from DmpR protein and of the DNA sequences from the two Upstream Activation Sequences (UAS) present at the promoter region of the operon were demonstrated using discrete molecular modeling techniques. The best modeled structures satisfying their stereo-chemical properties were selected in each of the cases. To stabilize the individual structures, energy optimization was performed. In the presence of inducers, probable interactions among domains and then the two independent DNA structures with the fourth domain were perused by manifold molecular docking simulations. The complex structures were made to be stable by minimizing their overall energy. Responsible amino acid residues, nucleotide bases and binding patterns for the biodegradation, were examined. In the presence of the inducers, the biodegradation process is initiated by the interaction of phe50 from the first protein domain with the inducers. Only after the interaction of the last domain with the DNA sequences individually, the operon is expressed. This novel residue level study is paramount for initiating transcription in the operon; thereby leading to expression of phenol hydroxylase followed by phenol biodegradation.



* *Corresponding author*. E-mail: raysujay@gmail.com (Sujay Ray).

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## Introduction

Phenol ($C_6H_5OH$) being a mildly acidic, volatile, organic pollutant is appreciably soluble in water (Weber et al., 2004). So, it is essential to completely remove phenol and its derivatives by biodegradation. A gram negative bacterium, *Pseudomonas putida* (*P. putida*), is found to be capable of consuming toxic compounds such as phenol, as their only carbon and energy source with optimum growth conditions being 30°C and pH = 6.8 (Şeker et al., 1997). Aerobic biodegradation of phenol is performed by gene encoding the N-fragment in *Pseudomonas putida*-derived methyl phenol operon (*DmpN* gene) that codes for phenol hydroxylase (Nordlund et al., 1990; Movahedyan et al., 2009).

*Pseudomonas putida* (CF-600) has DmpR protein, which acts as a regulator, positively controlling the expression of entire *dmp* operon (*dmpKLMNOPQBCDEFGHI*). This operon carries genes encoding certain enzymes necessary to breakdown phenols into pyruvate and acetyl-CoA (intermediates for Citric Acid Cycle) (Shingler et al., 1992; Sarand et al., 2001). DmpR protein comprises four domains namely Domain-A (the effector-sensing domain), Domain-B (a linker domain), Domain-C (transcriptional activator domain) and finally Domain-D (DNA binding domain). It is well documented through wet-laboratory research that in the presence of inducers like phenols, domain A interacts with inducers (Shingler and Pavel, 1995; Gupta et al., 2012). Then domain C interacts with domain A with the help of domain B and thereafter domain D binds to DNA structures present in two UAS in promoter (Shingler and Moore, 1994; Shingler and Pavel, 1995; Gupta et al., 2012). Interaction of DNA and domain D is aided by a DNA binding protein, Integration Host Factor (IHF) that introduces a sharp bend (>160°) in DNA, facilitating interaction between components in nucleoprotein array (Goosen and van de Putte, 1995). After these sequential interactions being successful, the operon opens up releasing phenol hydroxylase for biodegradation. It was also efficiently validated in the present study.

However, till date, detailed structural information regarding molecular level interactions between these proteins has not been dealt with. Although to understand thiosulfate oxidation, several molecular docking and interaction studies were examined in sox operon (*soxVWXYZABCDEFGH*) (Bagchi and Ghosh, 2011; Bagchi, 2011, Bagchi, 2012; Ray and Bagchi, 2013).

Present study is basically focused on residue level and molecular basis of degradation of toxic substances like phenols/phenolics with the involvement of dmp operon from *P. putida*, which thereby causes participation of gene encoding enzymes for the biodegradation. Current study therefore, includes analysis with description of 3D structures of four domains and two DNA structures of DmpR by discrete molecular modeling processes. Binding patterns and molecular level interactions were properly analyzed by performing several respective molecular docking simulations among domain A and inducers, among domains A–B–C, and domain D with two DNA structures independently. Involvement of the responsible amino acid residues and nucleotide bases was properly predicted and analyzed.

This is most probably an unexplored and novel description where the probe provides clear information regarding residue level interactions between domains of DmpR protein in the presence of inducers. It serves as a prime necessity for initiating transcription and thereby understanding biodegradation to create a sustainable environment.

## 1. Materials and methods

### 1.1. Sequence analysis and molecular modeling of domains A, B, C and D from DmpR protein of P. putida

#### 1.1.1. Sequence analysis

The amino acid sequence of DmpR protein of *P. putida* was obtained from NCBI nucleotide database (Accession No.: X68033.1) and were verified from UniProtKB too. Pfam was used to identify the conserved domains (highly conserved regions especially responsible for protein functionality) (Punta et al., 2011). BLAST validated the results. Domains often serve as an important interaction site for proteins too (Jones et al., 1998; George and Heringa, 2002). Four domains were identified as XylR_N, V4R, Sigma54_activat and HTH_8 for domains A, B, C and D respectively. Total lengths of domains A, B, C and D were found to be 103, 62, 168 and 42 amino acid residues long respectively. Amino acid sequences of these four domains were identified. These amino acid sequences were used separately to build homology models by MODELLER.

#### 1.1.2. Homology modeling of domains C and D

The amino acid sequences of these two domains were used separately to build homology models by MODELLER. For homology modeling, the widely accepted prerequisite lies that the sequence identity of the templates with target protein should be more than 30% (Sander and Schneider, 1991; Xiang, 2006). Results from HH-Pred (Söding et al., 2005) inferred that domains C and D had their templates in X-ray crystal structure from *Salmonella typhimurium* (PDB code: 1OJL, chain A) and *Escherichia coli* (PDB code: 1ETO, chain A); sharing 55% and 38% sequence identity respectively. BLAST against PDB corroborated the results from HH-Pred. HHpred is highly sensitive method for homology detection or structure prediction and quite often allows to make inferences from more remotely homologous relationships (Söding et al., 2005). Root Mean Square Deviations (RMSD) are then used to study globular protein conformation by superimposing $C_\alpha$ atoms of modeled structures on each of their respective original crystal templates. PyMOL (a *MO*Lecular viewer tool using *P*ython Language) yielded 0.691 Å and 0.977 Å RMSD for domains C and D respectively.

#### 1.1.3. Modeling of domains A and B

No suitable templates were found for domains A and B from HH-Pred as well as from BLAST against PDB. Raptor-X (Källberg et al., 2012) was used to perform remote homology modeling for them. Remote homology modeling from protein sequence poses a challenging feature as structure is typically