IITB-Monash Research Academy

An Indian-Australian research partnership

Project Title:	Structural characterisation of enzymes for biotechnological application in microbial degradation of water pollutants	
Project Number	**IMURA0449**	
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IITB Department:	Department of Chemistry	

Research Academy Themes:

Highlight which of the Academy's Theme(s) this project will address?

(*Feel free to nominate more than one. For more information, see www.iitbmonash.org)*

1. Advanced computational engineering, simulation and manufacture
2. Infrastructure Engineering
3. Clean Energy
4. **Water**
5. Nanotechnology
6. **Biotechnology and Stem Cell Research**

The research problem

Structural and biochemical studies of catabolic enzymes which have significance in bioremediation with emphasis on biodegradation of group of pollutants, that includes mixture of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatics derived from sediment and soil contaminated with petroleum, aromatic derivatives having functional groups such as alkyls, halogens and nitro groups obtained from industrial source etc. They pose to be environmental threat because of their high toxicity and persistency as many of these compounds can't be degraded or utilized by most organisms, thus creating severe water pollution. A plethora of bacteria, having wide metabolic diversity and genetic plasticity, can adapt to limiting nutrient supplies and hostile environments. Hence, a numbers of bacterial species acquire catabolic ability to biodegrade a variety of water and soil based aromatic pollutants into simple, non-toxic tricarboxylic acid (TCA) cycle intermediates by using the pollutants as their sole carbon and energy source.

We have chosen biodegrading bacterium, *Pseudomonas putida* which can efficiently metabolize various type of aromatic hydrocarbons such as phenol and its methylated derivatives, m, p- and o-cresol, and 3,4 dimethyl-phenol via the *meta* cleavage pathway of catchol (or methylated catchol). In addition to phenols, *P. Putida* can utilize a wide variety of aromatic compounds as the sole source of carbon and energy, including benzolate, protocatechuate, p-hydroxybenzoate and m-toluate.

We have targeted catabolic enzymes from the bacterium in order to understand mechanism of their action in catabolic process for developing strategies to remove these pollutants from the environment. In order to achieve this goal as a first step in this proposal we plan to clone, purify, crystallize and determine their 3D structures with the aim of deciphering their functions. Subsequently we plan to develop high sensitivity bioassays and also engineer the protein of interest in order to design efficient degrading process of the pollutant.

Project aims

The various soil and water-borne aromatic pollutants after being detected and captured by the transcriptional regulatory proteins need to be degraded via specific catabolic pathways, which subsequently convert them into simpler non-toxic TCA cycle intermediates. This entire process involving both the regulatory and the catabolic proteins can help in achieving the ultimate aim of making the surrounding environment free from the toxic aromatics. Analysis of the entire genome of *P.putida* proposes that the catabolism of the various aromatic compounds occur mainly via four major metabolic pathways: the protocatechuate (pca genes) and the catechol (cat genes) pathways both of which are two parallel branches of a common beta-ketoadipate pathway, the homogentisate (hmg genes) pathway and the phenylacetate (pha genes) pathway.

In this proposal, we have targeted catabolic enzymes from the protocatechuate (pca genes) pathway of *Pseudomonas putida* for which most of the clones are available and a number of enzymes has been expressed and purified and a few of them have crystallisation hits.

The objectives are (i) expression, purification, biochemical/biophysical characterization and crystallization of enzymes from the pca pathway, (ii) X-ray diffraction data collection, phasing, model building and refinement (iii) Structure analysis, (iv) determination of enzyme-substrate/product complex, mutagenesis of key residues of the enzymes and dechiphering its mechanism.

X-Ray crystallographic structure determination of the catabolic enzymes from the pca pathway provides unique chance of performing metabolic engineering in order to catabolizing large number of toxic aromatic compounds. The structure/function work will provide a basis for improving enzyme stability, substrate specificity and kinetic properties that can utimately lead to develop biological process for destroying toxic compound from water.

Expected outcomes

While the short-term goal is to understand function of the catabolic enzymes and its mode of action with its substrate, the long-term goal is to perform metabolic engineering in order to catabolize a large number of toxic aromatic compounds in a productive manner. The aim is to disseminate research information through a wide range of publications, mainly in high quality international scientific journals. The results from the project will be communicated by presenting results in international conferences within the field.

How will the project address the Goals of the above Themes?

Aromatic compounds are one of the wide-spread pollutants in the environment. The elimination of a wide range of such pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Therefore understanding catabolic pathways and mechanisms and responsible enzymes is an effective means to define important factors for efficient cleanup of pollutants and this feature makes a valuable research for a future development of industrial-water cleaning technologies.

Capabilities and Degrees Required

The proposed projects represent a unique opportunity for a PhD student to acquire deep insights into molecular biology, biochemistry and structural biology using a variety of biochemical/biophysical techniques. The duties/tasks and responsibilities of the PhD candidate are to lead the proposed project(s) under the supervision of his/her supervisors at IITB, and in close collaboration with colleagues at Monash University and Australian Synchrotron. By the end of the PhD study period, the candidate should have acquired a deep knowledge in molecular biology and biochemistry as well as a broad knowledge in structural biology. The PhD candidate should demonstrate excellent ability to design, perform, interpret, critically assess and contextualize generated data from experimental work. Furthermore, he/she should demonstrate aptitude to discuss and spread research results to both the national and international scientific community as well as to the non-scientific community.

The eligible candidate must hold a Master´s degree in Biotechnology, Biochemistry, Chemistry, biophysics, or equivalent. Previous experience in molecular biology and/or biochemistry as well as basic computational knowledge is desirable. Structural biology knowledge is desirable but not absolutely required. We are seeking a highly motivated, creative person with good technical skills. The successful applicant should be fluent in English, with excellent communication capacity combined with the ability to interact effectively and work productively in a team. Emphasis will be placed on personal suitability as well as genuine enthusiasm for the topic.

Potential Collaborators

Please visit the IITB website www.iitb.ac.in OR Monash Website www.monash.edu to highlight some potential collaborators that would be best suited for the area of research you are intending to float.

1. Prof. Ruchi Anand, Department of Chemistry, Indian Institute of Technology, Bombay
2. Prof. Matthew Wilce, Department of Biochemistry and Molecular Biology, Monash University, Australia
3. Dr. Santosh Panjikar, Department of Biochemistry and Molecular Biology, Monash University, Australia and Australian Synchrotron, 800 Blackburn Road, Clayton, Australia

Please provide a few key words relating to this project to make it easier for the students to apply.

Protein crystallography, Biosensor, Water pollutant, Enzyme kinetics and mechanism