IITB-Monash Research Academy

Indian Institute of Technology Bombay | MONASH University

An Indian-Australian research partnership

Project Title: **Investigation of Butyrolactone like Small Molecules in Regulation of Antibiotic Production in Streptomyces**

Project Number **IMURA0241** *(will be inserted by The Academy)*

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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

Define the problem: Small molecules possessing the butyrolactone like scaffold have been reported to regulate antibiotic production and differentiation in various Streptomyces species. These molecules diffuse into the extracellular medium much like the quorum sensing molecules and synchronize the initiation of antibiotic production via a genetic switch. This genetic switch comprises of transcription factors/receptors that have specific binding sites for the butyrolactone ligand molecule. The receptors also have a binding site for a specific DNA regulatory sequence as well as a specific butyrolactone. The antibiotic production is controlled in response to the dose of the butyrolactone which is hypothesized to cause a structural change resulting in the dislodging of the receptor from the DNA. In the case of *Streptomyces coelicolor*, transcription factor ScbR has been shown to bind a specific butyrolactone molecule SCB1 and control the downstream production of a polyketide cluster in addition to the actinorhodin and undecylprodigiosin antibiotic gene cluster. The recognition of the small molecule butyrolactone moiety by the receptor is very specific and tight. Small changes in stereochemistry of the recognition molecule abolishes binding. However, the structural basis of these interactions and factors modulating them is largely unknown. In *S. coelicolor* apart from ScbR, two other gene products CprB and CprA are also present whose functions are unknown, neither is the identity of the small molecule recognized by these class of receptors known. Thus we plan to investigate the role played by these receptors in antibiotic production and whether there is any type of cross talk or feedback regulation that exists in this system.

An analogous system regulated by butyralactone molecules has also been reported in an alternate commercially important species, *Streptomyces fradiae*. This pathway has a complex array of transcription regulators, which are hypothesized to be interdependent and regulate the production of the antibiotic tylosin. The TylP TylQ, TylS, TylR and TylU gene cluster possesses regulatory genes that either positively or negatively control tylosin production via a set of butralactone type molecules. However, the identity of these molecules is also not known. We aim to better understand the factors that control this metabolic network and the fine tunning of this pathway by the butyrolactones.

Project aims

Define the aims of the project

1. Identification of the small molecule via mass spectrometry, that regulates the putative butyrolactone like transcription regulators, CprB and CprA in Streptomyces coelicolor

2. *In vitro* and the *in vivo* characterization of the function of CprB and CprA and the role played by them in regulation of the antibiotic production in Streptomyces.
    a.) Identification of DNA binding site(s) for CprB and CprA.

    b.) Genomic expression profiling of CprB and CprA mutants under various growth conditions to identify their physiological role.

    c.) X-ray Structure characterization of CprB and/or CprA with its cognate molecules*.

3. Identification of the analogous butyrolactone like small molecule that regulates the tylosin antibiotic production pathway via mass spectrometry in *streptomyces fradiae*

4. Characterization of the transcription regulatory network that are regulated by the identified molecules.

* **access to beamtime at the Monash Synchrotron will be required for proper execution of this aim.**

Expected outcomes

Highlight the expected outcomes of the project

The expected outcome of the project is that the study on this system will lead to understanding of the role played by CprB and CprA in antibiotic production and its regulation. It will provide insights into the regulation of the butyralactone based receptor systems. We will be able to conclude whether the CprA, CprB and ScbR cluster work in synergy or are independent antibiotic production control paths which are either separately activated under specific growth conditions or times. In addition supporting Mass spectrometric, structural and biochemical work will shed light into the mode of binding of the small molecule and cognate DNA. Parallel study on the tylosin gene cluster in *Streptomyces fradiae* will help in broadening our understanding of the butyralactone regulatory network and help in understanding the evolution of this system across species. The work will result in high quality publications in peer review journals and shed light on the process of antibiotic production.