

## Abstract

Thesis title: **Structural and functional studies of enzymes of Proline biosynthesis pathway in *Mycobacterium tuberculosis***

Tuberculosis is the second largest killer disease on global scale after HIV-AIDS. The rapid evolution of *Mycobacterium tuberculosis* into drug resistant strains and the toxicity of these drugs are the major constraints in prevention and cure of this disease. One strategy for drug discovery can be to target the metabolic pathway in *Mycobacterium*, to design a broad range of drugs that target key metabolic pathways related to lipid metabolism, carbohydrate metabolism, amino acid metabolism, energy metabolism, vitamin and cofactor biosynthetic pathways and nucleotide metabolism.

It has been already reported that proline biosynthesis pathway is essential for the survival of *Mycobacterium* in macrophage. Disruption of proC gene from this pathway is lethal for the bacteria. Therefore, understanding the structural and functional details of this protein is a major step towards drug design.

In *Staphylococcus*, inactivation of a global regulator, ccpA, leads to utilization of proline in arginine biosynthesis pathway as a substrate via urea cycle [19]. The highly conserved pathway of arginine biosynthesis which uses glutamate as a substrate remains no longer active.

Here, our broad objective is to design inhibitors against proline biosynthesis pathway targeting proC enzyme of *M. tuberculosis*, the connecting enzyme of both proline and putative alternate pathway for arginine biosynthesis. However, inhibiting a single enzyme in a biosynthetic pathway may or may not prevent the growth of the bacterium and hence targeting multiple enzymes in this pathway hold a better premise. A similar pathway for proline synthesis from arginine may occur in *M. Tuberculosis*. Hence, our study also included characterization of ProA and ProB, which catalyzes the initial two steps of the pathway. The ProB gene was cloned successfully but it could not be expressed.

In the work presented in my thesis, the cloning, expression and purification of proA and proC was successfully done. Initial characterization was also performed. The crystallization for both the recombinant proteins was carried out in collaboration with Dr.Kalaivani Raja.

The crystals for proA diffracted at a resolution of 3.5 Angstrom. The MtproA crystals belonged to the P3 space group but there is high mosaicity and B-factor is yet poor.MtproA is a tetramer with a melting temperature of 72 degree celsius. The urea concentration of 1.4M denatured half of the protein in solution.

MtproC was determined to be a decamer with a melting temperature of 65 degree celsius and the secondary structure is mostly alpha-helical. It is a stable protein and needle shaped crystals were obtained and reproducibility was confirmed in 9 conditions. The desired resolution was not obtained. Crystals diffracted at 8.5 Å mostly and the best resolution attained was 6.5 Å. The kinetic parameters for both forward and reverse reaction were done and kinetic parameters were successfully determined.