Introduction

Iron is an important micronutrient for all aerobic bacteria, including mycobacteria. However, they face conditions of iron limitation due to the poor solubility of the iron at biological pH. The mammalian host further limits the iron for pathogenic bacteria by holding the iron in a protein-bound form. Bacteria however, have adapted to low iron conditions by elaborating novel iron acquisition machinery. Both the siderophore-mediated and direct acquisition systems are well studied in a number of bacteria. Iron levels not only regulate the bacterial iron acquisition machinery but also control the expression of virulence determinants. Thus, the ability to acquire iron is one of the contributing factors to virulence.

Mycobacteria are unique in that they elaborate two kinds of siderophores, the intracellular mycobactin and the extracellular carboxymycobactin. The structure of mycobactin has been extensively studied in different mycobacterial species and the species-specific nature of the mycobactin has been used as a taxonomic marker. With the wealth of information from the genome analysis of *Mycobacterium tuberculosis*, the causative organism for tuberculosis, experimental approaches have made possible the understanding of the biosynthetic machinery of mycobactin. It is synthesized by the polyketide pathway, catalysed by proteins from the *mbt* cluster in the genome. The ability to synthesise mycobactin is considered a virulence determinant in *M. tuberculosis* as the *mbtB*-deficient mutant of *M. tuberculosis* failed to multiply inside macrophages. There is increasing evidence to show that this pathogen is subjected to conditions of iron limitation *in vivo* and hence understanding of the host-pathogen interactions with respect to iron acquisition will help in a significant manner towards control measures of tuberculosis.

Cell surface receptors for the uptake of ferri-siderophore are well characterized in a number of bacterial systems. In mycobacteria, the picture is not complete and the role of the iron-regulated envelope proteins (IREPs) is the focus of study by researchers interested in mycobacterial iron acquisition machinery. A 29 kDa protein is a ferri-exochelin receptor in *Mycobacterium smegmatis*. In *Mycobacterium tuberculosis*, a cell wall associated iron-regulated protein HupB, showing coordinate regulation with the
expression of the two siderophores mycobactin and carboxymycobactin is one of the areas of research interests in our lab. The clinical significance of the HupB was evident by the detection of anti-HupB antibodies in the serum of patients with tuberculosis.

Tuberculosis is a disease of major concern and concerted efforts are being made globally to develop new drugs against *M. tuberculosis* and / or an effective vaccine to control the disease. This is necessitated due to the large number of deaths each year, detection of increasing numbers of infected individuals and more important the development of multi-drug resistance. There is an urgent need for the identification of novel drug targets and detection of novel proteins as vaccine candidates.

The secretory proteins of *M. tuberculosis* probably play an important role in host-pathogen interactions and help in the establishment of infection and the development of the disease. It is well known that the cell mediated immunity play an important role in tuberculosis. There is a complex interplay of the immune cells via the host of cytokines produced by them in response to the release of the mycobacterial antigens by the macrophage. The proteins secreted by *M. tuberculosis* have been the focus of study and proteins like the ESAT-6, expressed only by *M. tuberculosis* has gained considerable importance in the light of its role as a virulence determinant and as a diagnostic antigen.

Thus the spent growth medium of *in vitro* cultures have been analysed and culture filtrate proteins (CFPs) have been studied extensively and explored as potential vaccine candidates. They include the secretory / excretory proteins released into the immediate environment by the growing mycobacteria. There is a wealth of information on the CFPs from *M. tuberculosis* and the immuno stimulatory effect of these proteins in experimental animals including mice, guinea pigs and cattle as well in human patients with the disease. Challenge studies have shown the immuno-protective effect of some of the culture filtrate proteins.

The CFP profile is influenced by several factors, including temperature and time of growth. In this study, the two major objectives include understanding the influence of iron levels on the CFP profile of *M. tuberculosis* and analysis of the immune potential of these CFPs in human patients with tuberculosis.