Introduction
Over 6.5 million cases of tuberculosis, resulting in ~2.5 million deaths every year make tuberculosis one of the most lethal infectious diseases. Currently, one-third of the world’s population is infected with the tubercle bacilli (Murray & Salomon, 1998). *Mycobacterium tuberculosis* (*Mtb*), the etiological agent of tuberculosis is a gram positive, aerobic, non-motile rod shaped bacterium that enters the human host by inhalation of infectious aerosols. The characteristic features of the tubercle bacillus include its slow growth, dormancy, complex cell envelope, intracellular pathogenesis and genetic homogeneity. These features contribute to the chronic nature of the disease, imposing lengthy treatment regimens and pose a formidable obstacle for researchers.

Upon entry into the host, *Mtb* encounters the alveolar macrophages. These cells are both, the host and the first line of defense against the pathogen. Primary pulmonary infection with *Mtb* induces long term protective immunity in the majority of infected subjects. Life time risk of reactivation of latent infection is less than 5% in immunocompetent individuals. According to the prevailing paradigm, protection relies on type 1 cell-mediated immunity involving interaction between *Mtb*-specific CD4 and CD8 T-lymphocyte and cells of the monocyte macrophage (MO/MA) lineage (Kaufmann and van Embden, 1993; Rook *et al.*, 1996; Rich, 1996).

Despite the availability of effective short-course chemotherapy (DOTS) and the Bacille Calmette Guerin (BCG) vaccine, the tubercle bacillus continues to claim more lives than any other single infectious agent (Snider and La Montagne, 1994). β-lactam antibiotic appears to be active *in vitro* (Casal *et al.*, 1987; Sorg & Cynomon, 1987; Wong *et al.*, 1988) and *in vivo* (Nadler *et al.*, 1991). However, *Mtb* historically has been regarded as intrinsically resistant due to the presence of one or more β-lactamases (Hackbarth *et al.*, 1997; Voladri *et al.*, 1998). Recent years have seen increased incidence of tuberculosis in both developing and industrialized countries, wide spread emergence of drug resistance
strain and a deadly synergy of the disease with the human immunodeficiency syndrome (HIV). There is an immediate need to search for new molecules that can be used as future drug targets.

The state of dormancy in which the bacillus remains quiescent within infected tissue may reflect metabolic shutdown resulting from the action of a cell-mediated immune response that can curtail but not eradicate the infection. As immunity wanes, through ageing or immune suppression, the dormant bacteria reactivate, causing an outbreak of disease often many decades after the initial infection. The molecular basis of dormancy and reactivation remains obscure but is expected to be genetically programmed and involve intracellular signaling pathways. The cell envelope of *M. tuberculosis* contains an additional layer beyond the peptidoglycan that is exceptionally rich in unusual lipids, glycolipids and polysaccharides. Novel biosynthetic pathways generate cell-wall components such as mycolic acids, mycocerosic acid, phenolthiocerol, lipoarabinomannan and arabinogalactan, and some of these may contribute to mycobacterial longevity, trigger inflammatory host reactions and act in pathogenesis. Little is known about the mechanisms of the pathogen survival within macrophage, or the extent and nature of the virulence factors produced by the bacillus and their contribution to disease.

The genome size of *Mtb* H37Rv is 4,411,529 base pairs (bp) with a G+C content of 65.6%. The genome is rich in repetitive DNA, particularly insertion sequences, and in new multigene families and duplicated housekeeping genes. The G+C content is relatively constant throughout the genome. *M. tuberculosis* shares over 99.9% identity at the DNA level with the other members of the tubercle complex (*M. bovis*, *M. microti* and *M. africanum*). From the genome sequence, it is clear that the tubercle bacillus has the potential to synthesize all the essential amino acids, vitamins and enzyme co-factors, though some of the pathways involved may differ from that of other bacteria. *M. tuberculosis* can metabolize
a variety of carbohydrates, hydrocarbons, alcohols, ketones and carboxylic acids. It is apparent from genome inspection that, in addition to many functions involved in lipid metabolism, the enzymes necessary for glycolysis, the pentose phosphate pathway, and the tricarboxylic acid and glyoxylate cycles are all present (Cole et al., 1998).

Based on growth studies in vitro, Mtb virulence is correlated with a shift to anaerobic metabolism from a strict aerobic respiratory mode of metabolism. This effect is enhanced in vivo as manifested by other anaerobic metabolism characteristics, such as inability to oxidize substrates, a dissociated tricarboxylic acid cycle, presence and induction of glyoxylate cycle, a reduced state of cytochrome system, and uncoupled oxidative phosphorylation. This shift towards a glycolytic mode of metabolism occurs as the pathogen adapts itself to the host tissue conditions. In vivo studies indicate that up to 70% of the glucose is metabolized through the EMP pathway (Ramakrishnan et al., 1962). Thus glycolysis is central to the organisms' survival and consequently a potential drug target.

However, since similar enzyme set for the glycolytic pathway is also present in the human host, differences at either the biochemical or structural level between the pathogen (M. tuberculosis) and the host (H. sapiens) need to be elucidated. Characterization of glycolytic enzymes of Mtb and elucidation of primary structure of proteins and their arrangement into a three dimensional functionally active structure is important because of the need to find potential bypasses and species-specific enzyme sets. Identification and utilization of the differences in the metabolism of the pathogenic and host organisms is one of the strategies for developing new drugs to control microbial pathogens. In the past, the biochemical characterization of metabolic enzymes has been exploited for the development of potential drugs for various pathogens like Plasmodium falciparum, Trypanosomes, HIV etc. The enzymes of the glycolytic pathway, being central to energy metabolism have been well characterized from almost all the living organisms. Comparative analysis of metabolic
pathways in different genomes has revealed surprising plasticity of the glycolytic pathway. Across 17 species of bacteria analyzed, no two species encode the same pattern of glycolytic enzymes. Therefore, blocking seemingly central enzymes might not be effective as a drug target because alternate substrate fluxes have to be considered (Dandekar et al., 1999).

Traditionally, antibiotic agents have largely been targeted at processes that are essential for the growth of the target organism under optimal conditions. Enzymes and pathways that are required for growth and survival under the nutritionally restrictive conditions present in the phagosome represent attractive alternative targets for new anti-TB therapies that can also target latent infection of the bacterium. Genome sequencing of *Mtb* H37Rv has identified the regions of *Mtb* genome, which contain the probable genes for glycolysis by homology search, thereby aiding the process of characterization of the pathway that will help increase our knowledge about the basic metabolic pathways and their critical importance in the development of the disease. This study was undertaken with the aim of analyzing such differences.

**Aims and objectives:**

1. Cloning, expression and purification of glycolytic pathway genes encoding phosphoglucose isomerase, triosephosphate isomerase and enolase of *Mycobacterium tuberculosis*.
2. Biochemical characterization of the enzymes.
3. Elucidation of structure function relationship by site-directed mutagenesis.
4. Attempts to crystallize the recombinant proteins.