Introduction
Tuberculosis (TB) "the captain of death" kills more youth and adults than any other infectious disease. TB infects an individual every 4 seconds and kills the other every 10 seconds. The success of *Mycobacterium tuberculosis* (*M. tuberculosis*), the causative agent of tuberculosis, lies in its ability to spread by aerosol droplets, evade the host immune system and to persist in pulmonary granulomas. The pathogenesis of tuberculosis can be considered as a series of battle between host and the tubercle bug, where host and the pathogen utilize their armory to win over the other. The ability of *M. tuberculosis* to enter macrophages and to subvert normal host immune response mechanisms in order to enhance its intracellular survival is believed to be pivotal to its virulence. The main factors in the host-pathogen interaction that dictate whether infection progresses to explicit disease or will be cleansed by immune system are still unanswered. Entry of *M. tuberculosis* into macrophages and the events that follow appear to involve specific cross talk between the host and the pathogen.

Cell-signaling is a process by which environmental signals are transmitted to cells, ultimately causing changes in gene expression and cellular activity. One of the major mechanisms of signal transduction is the reversible phosphorylation of cellular proteins. Protein phosphorylation in prokaryotes plays a regulatory role in events as diverse as chemotaxis, bacteriophage infection, nutrient uptake and gene transcription. A new perspective in the pathogenesis of *M. tuberculosis* is the exploitation of host cell signaling pathways by the pathogen. Reversible phosphorylation-dephosphorylation is the key mechanism by which extracellular signals are translated into cellular responses. These processes are carried out by the cognate action of specific protein kinases and phosphatases. Target proteins are phosphorylated at specific serine, threonine, or tyrosine residues by protein kinases and the phosphate group is removed by the action of specific protein phosphatases. It has been shown that these proteins either alone or in cascade transmit signals received both internally and from the extracellular environment. These signals are eventually converted to cellular responses that play a crucial role in many diverse processes such as, regulation of cell cycle progression, cell division and development, metabolism, membrane signaling, stress responses and pathogenicity.

Based on the demonstrated importance of protein phosphorylation in influencing a large number of cellular functions, it is expected that phosphorylation may also play a vital role in mycobacterial pathogenesis. In several pathogens, it has been shown that...
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upon infection, the phosphatases and kinases of pathogens modify host proteins and help in the establishment of the disease. In *M. tuberculosis*, the analysis of the genome sequence has predicted the presence of 11 Serine/Threonine Protein Kinases (STPKs). However, the physiological significance of most of these proteins is not known.

While much work remains to be done towards understanding the role of the STPKs in *Mycobacterium tuberculosis* biology, recent reports have established the role of these kinases in glucose transport, glutamine/glutamate metabolism and regulation of the expression/activity of transcription factors. Protein kinases A and B, encoded by *PknA* and *PknB* respectively, are a part of the same operon that also contains the cistrons of protein phosphatase *pstP, rodA* (involved in cell shape control) and *phpA* (involved in peptidoglycan synthesis). Overexpression of PknA in *M. bovis* BCG or *M. smegmatis* results in a deviation from normal cell morphology. Recent studies on PknF in *M. tuberculosis* reported its role in cell division, growth rate, morphology and glucose transport. The identification of the direct substrates of protein kinases is a key factor in determining their role in intracellular signal transduction. Several substrates of PknA and PknB have been identified that include Ftsz, a protein essential for cell division, conserved hypothetical protein Rv1422 and Wag31 which was originally identified as an antigen of *M. tuberculosis*, FHA-domain containing proteins GarA and Rv0020c, mycolic acid synthesis pathway proteins KasA and KasB.

Genome sequence of *M. tuberculosis* has suggested the presence of a gene (Rv3080c), encoding for putative serine/threonine kinase (*pknK*). Analysis of the *M. tuberculosis* genome database has revealed that the *pknK* gene is located upstream of the *virS* gene (Rv3082), encoding a putative transcriptional activator belonging to the LuxR family.

In this study, we have performed biochemical characterization of PknK and identified its substrates. PknK is a 1100 amino acids protein. While the amino-terminal 280 amino acids are homologous to eukaryotic-like STPKs domains, the carboxyl-terminal residues show homology with the regulatory region of *E. coli* transcription regulator MalT. PknK, like PknG, shows no apparent transmembrane regions and is thus predicted to be a soluble protein. PknK shows homology to the regulatory regions of the transcriptional regulators of the LuxR family such as *Klebsiella*
pneumoniae AcoK and E. coli MalT. It contains an ATP-binding motif (AAA domain) characteristic of molecules with ATPase activity. This AAA domain also has a PDZ domain, which is involved in targeting signaling molecules to submembranous sites and was proposed to regulate the production of secondary metabolites in M. tuberculosis.

The gene encoding PknK is located adjacent to that of transcription factor VirS. VirS which has been suggested to regulate transcription of the MymA operon which comprises of seven genes (Rv3083-Rv3089). The first gene in the mymA operon is mym which encodes for the Mym protein, a putative monooxygenase. Six of other genes in operon encode dehydrogenases, esterases and conserved hypothetical proteins. These proteins are involved in modification of fatty acids required for cell envelope of mycobacteria.

Several transcription factors are known to be regulated by post-translational modifications such as phosphorylation. We investigated the possibility of VirS being a PknK substrate and examined the possibility of mymA operon encoded proteins as substrates of PknK. The present study shows that VirS and four of mymA operon genes are substrate of PknK.

The objectives of the present study were:

- Cloning, expression and purification of PknK and its sub-domains.
- Identification of phosphorylated amino-acid of PknK itself and PknK phosphorylated Myelin Basic Protein (MBP).
- Localization of PknK in mycobacterial cells.
- Cloning, expression and purification of neighboring genes of PknK and their possible regulation by PknK.
- Understanding DNA-binding ability of VirS by Electrophoretic mobility shift assay (EMSA).