Tuberculosis continues to be one of the leading causes of death, claiming 5500 lives each day (Dye, 2006). One-third of the world’s population is estimated to be infected with *Mycobacterium tuberculosis*; this pathogen has developed resistance to multiple front-line drugs. The recent vaccine against tuberculosis (TB), *Mycobacterium bovis* bacille Calmette Guerin (BCG), an approved vaccine since 1930 is powerless to guard pulmonary tuberculosis which contributes to majority of deaths however is effective against infantile tuberculosis. As a result, TB has been declared a public health emergency.

Secreted proteins of *Mycobacterium tuberculosis* have attracted much attention in attempts to understand the molecular mechanisms of pathogenesis. The reason of interest lies in the fact that large number of proteins are secreted from *Mycobacterium tuberculosis* during *in vitro* culture and that many of these proteins are involved in causing virulence, secreted at key stages of infection, such as the formation of macrophage phagosome (Beatty *et al*., 2000), where live bacilli effectively arrest maturation and phagolysosomal fusion (Armstrong *et al*., 1971) (Russell, 2000). A number of these proteins perform important enzymatic functions, as membrane-bound or secreted proteins, and they can enhance mycobacterial survival and virulence.

Among the most well known groups of secreted proteins to perform a vital role in the cell wall are the members of the Antigen 85 complex, which are essential mycolyl transferases required for the terminal transfer of mycolic acid during cell wall biosynthesis (Belisle *et al*., 1997). Other secreted enzymes such as Acid phosphatase (SapM) and Protein tyrosine phosphatase (PtpA) by *Mycobacterium tuberculosis* inhibits phagolysosomal fusion through their action on host molecules required for this process (Vergne *et al*., 2005). A further group of secreted enzymes known to perform important functions are the serine hydrolase/lipases, which may act as cell wall-associated virulence factors (Lun *et al*., 2007) or in triacylglycerol utilization under nutrient-limiting conditions (Deb *et al*., 2006), inferring a role that may occur during latent infection.

In the present study, we over expressed Ag85A, with an objective to use this secretory protein as vaccine with vector to assess its immunogenicity and protective efficacy. Plasmid DNA preparation and restriction enzyme digestion were executed under standard
conditions. Ag85A protein with C-terminal his-tag was expressed in *E. coli* BL21 strains harboring recombinant plasmids and then purified on a Ni-NTA column (Calbiochem) according to the Manufacturer’s instructions. The cell lysate was loaded onto the column. The column was washed with 6 column volumes of binding buffer (pH = 7.8) and then washed with 4 column of wash buffer (pH = 6.0). The bound protein was eluted with 1M Imidazole elution buffer. The fraction was analyzed on SDS-PAGE. Ni-Affinity Chromatography uses the ability of His to bind nickel. Six histidine amino acid residues linked to a protein (in our case it was C terminus) are known as a 6X His tag. Affinity purification using a polyhistidine-tag typically results in comparatively pure protein when the recombinant protein is expressed in prokaryotic organisms (Mustafa, 2008).

The enteric bacterium *Escherichia coli* are capable of growing on a broad range of carbon sources. The composition of its nutrient environment is a prime determinant of what catabolic enzymes are synthesized by this organism. β-galactosidase is an enzyme that cleaves the disaccharide lactose into glucose and galactose. Its expression is induced by the presence of lactose, or its analogue, isopropyl thiogalactoside (IPTG) (Ramey, 2001). The expression of Ag85A was also studied with respect to time with 1mM IPTG concentration. Each sample is collected at various time points and was centrifuged. The supernatant was collected and was subjected to gel electrophoresis. The spectrophotometric analysis and electrophoretic mobility analyzed on SDS-PAGE demonstrated the highest expression of Ag85A at 4hrs after induction with IPTG.

The comparative analysis of the inducing agents is done taking IPTG and lactose in the concentration of 1mM and 0.1% respectively. When specific activities of β-galactosidase IPTG and lactose were examined, it was observed that IPTG give higher activity because lactose requires a transport protein whereas IPTG does not.

Expression of Ag85A followed by affinity purification will provide 98% pure antigen for further studies for immunological and protective efficacy.