Introduction
1.0 INTRODUCTION

Tuberculosis continues to ravage humanity with the annual number of deaths anticipated to increase from its current level of ~ 3 million to ~ 3.5 million by 2005. The global emergence of multi drug-resistant strains of *Mycobacterium tuberculosis*, the primary causative agent of tuberculosis, has given new urgency to develop better means to combat this disease. The only vaccine against tuberculosis approved for human use is *Mycobacterium bovis* BCG, which is a live attenuated vaccine. The efficacy of this vaccine has been a subject of controversy, since several large human clinical trials revealed that the protection conferred by this vaccine varies greatly (0-80%) (Rodrigues and Smith, 1990; Colditz *et al.*, 1994). Another significant limitation of the existing BCG vaccine is that it elicits a delayed type hypersensitivity reaction to purified protein derivative (PPD), the skin test reagent used in the diagnosis of tuberculosis. Furthermore, the live BCG vaccine itself represents a potential health risk to immunocompromized individuals. Thus, these factors warrant the development of a new vaccine against tuberculosis and have resulted in intensive research activity in the past few years to develop and test new tuberculosis vaccine candidates (Roche *et al.*, 1995; Orme, 1995).

Characterization of new antigens may identify candidates for improved vaccines, especially in the context of ongoing investigations into subunit and DNA vaccine(s). The fact that live organisms provide better protection in animal models, has put emphasis on secreted protein antigens that are actively secreted from live mycobacteria during early mid-log phase of growth. The secretory proteins released during early growth phase are important, as these molecules expand a specific arm of effector immune system during the early stage of infection.
(Rambukkana et al., 1991; Harboe and Wiker, 1992; Pal and Horwitz, 1992). A number of studies have confirmed that culture filtrate antigens containing major target molecules are recognized by T cells at various stages of infection (Andersen et al., 1991b; Andersen and Heron, 1993). Thus, secreted proteins of *M. tuberculosis* have attracted considerable attention especially in the vaccine research. Various T-cell subpopulations involved in protection against tuberculosis include CD4+ T-helper cells, CD8+ T-cytotoxic cells and γδ T-cells (Kaufmann, 1988; Salagme et al., 1991).

The observed weak immunogenicity of the highly purified protein/peptide antigens makes adjuvants an important part of the subunit vaccine (Vitiello, 1995; Hindelgunde and Zhiguan, 1996). Various adjuvant systems have been described to boost the immune response, which include ISCOMS (Immunostimulatory complexes), liposomes, polylactide-co-glycolide microspheres (PLG), DDA (dimethyldioctadecyl ammonium bromide), syntax (Saf-1), Ribi adjuvant, saponins etc. Recently, a 30 kDa secretory protein of *M. tuberculosis* H37Ra complexed with Freund’s incomplete adjuvant (FIA) has been shown to offer protection even better than BCG against experimental tuberculosis (Sinha et al., 1997). However, Freund’s incomplete adjuvant used for enhancing the immunogenicity of antigen is not recommended for human use due to its toxic side effects. Alum is the only licensed adjuvant for human use but is far from ideal, as it primarily augments the B-cell immune response which does not play any significant role in providing protection against tuberculosis. Reports are available regarding the use of PLG-microparticles as safe and versatile delivery systems that can induce strong cell-mediated immune (CMI) response to antigens (Lewis et al., 1990; Eldridge et al., 1991). These
microparticles have recently been shown to boost the CMI response to mycobacterial antigens (Vordermeir et al., 1995; Dhiman and Khuller, 1998).

For the development of a subunit vaccine as an alternative to BCG, it is important to clone specific genes coding for immunoreactive mycobacterial proteins in suitable expression systems to meet the bulk supply of the antigen. Recently, many efficient expression systems such as *Escherichia coli*, *Bacillus*, *M. smegmatis* etc. have been employed for the expression of proteins on a large scale. An extensive panel of mycobacterial proteins involved in recognition by the host immune system has been identified by biochemical fractionation or by screening of recombinant DNA expression libraries (Matsumoto et al., 1995; Harth et al., 1996; Manca et al., 1997). A number of reports have provided evidence for the cloning, sequencing and expression of genes encoding mycobacterial proteins of immunological importance (Matsuo et al., 1988; Singh et al., 1992; Ohara et al., 1995, 1997). Thus, the recent development of techniques and vectors for transformation of mycobacteria (Jacobs et al., 1987; Snapper et al., 1990; Ranes et al., 1990) has been stimulated by the goal of creating a new generation of recombinant vaccines (Aldovin and Young, 1991; Stover et al., 1991). Therefore, the present study was designed with the following objectives:

1. To isolate, purify and characterize major secretory protein(s) from the culture filtrate of *M. tuberculosis* H$_{37}$Rv.
2. To study the immunogenicity of the most immunoreactive secretory protein using FIA/PLG/DDA as delivery systems/adjuvants in different strains of mice.
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3. To evaluate the protective efficacy of the most immunoreactive secretory protein against experimental tuberculosis using the best adjuvant formulation.

4. Cloning, sequencing and expression of the gene encoding the most immunoreactive secretory protein of *M.tuberculosis* H₃₇Rv in *E. coli* DH5α.

5. Immunoreactivity and protective efficacy of the recombinant protein in comparison to the native protein against experimental tuberculosis employing the best adjuvant formulation.