Chapter 1

Introduction
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A quick scan through this chapter might take five minutes of your time. During this period, 16 people will have died of tuberculosis, 80 will have fallen ill with it, and an astounding 800 will have become inflicted by the disease causing pathogen *Mycobacterium tuberculosis* (WHO, 2011). It is indeed tragic that these words hold true to this day; tuberculosis (TB) represents a global health problem and is the leading cause of death from a single bacterial infection. *M. tuberculosis* is a sophisticated pathogen with the ability to persist within the human host for decades. Because of this ability, the World Health Organization estimates that up to one-third of the world’s population is latently infected with this bacillus. Reactivation, where the bacilli resume replication and cause further clinical disease, can occur at any point. Thus, there is a large reservoir of potential disease and further transmission.

*M. tuberculosis* is a slow-growing, intracellular pathogen that can survive and multiply inside macrophages and other mammalian cells. It is gram-positive, non spore-forming and aerobic bacteria. It shares with other members of the Mycobacterium genus a cell wall of unique composition due to the dominant presence of mycolic acids that make up for more than 50 % of its dry weight. It is the cell wall that gives *M. tuberculosis* its acid fastness, enabling it to retain basic dyes in the presence of acid alcohol.

A remarkable decline in the TB incidence was observed during 1960 to 1980's because of the wide spread use of chemotherapy. But the resurgence of TB was observed in the 1990’s attributed to the advent of multi drug resistant (MDR) strains of TB and importantly HIV epidemic which further fuelled the spread of TB. Some of the MDR strains are resistant to all first- and several second-line drugs (Bifani PJ et. al., 1996). Despite the urgent need for new antibiotics for treatment, no new anti-tubercular drugs have been introduced since rifampicin in the early 1960s (Bifani PJ et. al., 1996).

Tuberculosis in any population, especially when combined with the devastating impact of HIV co-infection, remains a significant issue in global disease control.
(Corbett et al., 2003). Thus obtaining a greater understanding of the molecular basis of the virulence of *M. tuberculosis* and its disease pathogenesis presents an urgent challenge to the scientific community. In this regard, the publication of the genome sequence of *M. tuberculosis* strain H37Rv (Cole ST et al., 1998) represents a contemporary landmark in TB research. Another major achievement has been defining the composition and organisation of the mycobacterial cell envelope, which has provided important insights into the physiology and pathogenicity of *M. tuberculosis* (Brennan PJ and Nikaido H, 1995; Daffe M and Draper P, 1998).

Secreted mycobacterial proteins are putative virulence factors, and among them lipoproteins play an important role in pathogenesis and virulence. Mycobacterial lipoproteins are involved in formation of cell envelope. In addition because of the secretary nature, these lipoproteins act as strong immunogens (Sutcliffe IC and Harrington DJ, 2004). Bacterial lipoproteins make up an abundant class of membrane-anchored cell wall proteins with a broad range of functions, including antibiotic resistance, substrate binding and transport, adherence, protein export and folding, cell signaling, and sporulation (Sutcliffe IC and Harrington DJ, 2004). The common feature of lipoproteins is the presence of a conserved consensus sequence called a lipobox ([LV] [ASTVI] [GAS] C) with a universally conserved cysteine at position +1; the lipobox directs processing of the prolipoprotein to form the mature acylated protein (Sander P et al., 2003). Lipoprotein precursors are synthesized in the cytoplasm and targeted to the cell wall, where they undergo lipid modification by lipoprotein diacylglycerol transferase (Lgt) followed by removal of the signal sequence by lipoprotein signal peptidase (LspA) (Sutcliffe IC and Harrington DJ, 2004).

LspA is also known as signal peptidase II (SPase II) (Sankaran K and Wu HC, 1994), which is encoded by the gene *lspA* (Rv1539). LspA is a membrane-bound protease that has their active site exposed to the trans side of the membrane so it can cleave exported proteins after translocation across the membrane which is absent in eukaryotic cells. It belongs to an unusual class of aspartic acid proteases (Paetzel M et al., 2002). Till date only one signal peptidase has been described in *M. tuberculosis* where as there are around ≥ 104 putative as well as well-characterized lipoproteins in it (Sutcliffe IC and Harrington DJ, 2004). Since signal peptidase activity is crucial to
mature the lipoproteins to enable these molecules to become active and functional, it would be important to learn about the nature of \textit{lspA} and its effect on the mycobacterial lipoprotein maturation and their biological activities. Some \textit{lspA} gene mutant studies have shown its role in virulence of \textit{M. tuberculosis} and that it is essential for resistance of \textit{M. tuberculosis} to malachite green (Banaei N \textit{et al.}, 2007). Existing informations regarding this gene is limited and one aspect of this limitation is its regulation. The aim of this study was to determine the role of \textit{lspA} gene in the biology and pathogenesis of \textit{M. tuberculosis} by generating \textit{lspA} overexpressed strain of \textit{M. tuberculosis} H37Rv and identify its promoter for the regulation of this gene. The \textit{lspA} overexpressed strain was constructed in \textit{M. tuberculosis} H37Rv, the ORF of \textit{lspA} gene was cloned into shuttle vector pVV16 and electroporated into \textit{M. tuberculosis} H37Rv. The overexpression of LspA protein was confirmed by western blot using polyclonal anti-LspA antibody raised in rabbit. We investigated the role of \textit{lspA} gene in the biology and pathogenesis of \textit{M. tuberculosis} in a mouse model and explored the possible effect of over-expressed \textit{lspA} on the morphology and biological role of \textit{M. tuberculosis}. In further experiments to understand the possible regulation of expression of the \textit{lspA} gene, a strong promoter was identified upstream of \textit{lspA} gene. It was also observed that \textit{lspA} is co-transcribed with Rv1540 which probably indicates that \textit{lspA} gene is in an operon with Rv1540.