Chapter 1: Introduction
Towards the end of the last century, on March 24, 1882, Robert Koch (who later in 1905 received the Nobel Prize for medicine or physiology) presented before the Physiological Society of Berlin, the discovery of a simple staining technique that made researchers see a dreaded microbe called Mycobacterium tuberculosis for the first time in the history of microbiology.

This dramatic development thrilled the world with an accompanying certainty that now the fight against humanity’s deadliest enemy could really begin. M. tuberculosis was the cause of the ‘White Plague’ of the 17th and 18th centuries in Europe. During this period nearly 100 percent of the European population was infected with M. tuberculosis, and 25 percent of all adult deaths were caused by M. tuberculosis. In recent years, threat imposed on world health by M. tuberculosis is further compounded by an upsurge in multidrug resistant strains. With the publishing of the sequence of the entire M. tuberculosis genome, various biochemical and immunological studies have now slowly begun to reveal the many factors responsible for the virulence and pathogenesis of M. tuberculosis. The unique mycobacterial envelope, rich in diverse biologically active lipids, deserves special attention because it is unique among prokaryotes and it is a major determinant of virulence for the bacterium. In this regards, two studies are worthy of special attention. Using signature-tagged mutagenesis, Cox et. al., (1999) and Camacho et. al., (1999) independently reported the finding that some M. tuberculosis mutants that are defective in the production of a lipid called Phthiocerol Dimycocerosate (PDIM) are severely attenuated, with many exhibiting accentuated colony morphology change. Their findings strongly imply the role of PDIM and similar molecules as important virulence determinants. Principal genes in PDIM biosynthetic locus include those representing the phenolphthiocerol/phthiocerol polyketide synthase (ppsA-E PKS) and the mycocerosic acid synthase or mas. The cluster also contains genes for two acylCoA synthases or Acoas (fadD26 and fadD28), a type II thioesterase (tesA) and a few others like drrA-C and mmpL7 that are thought to be involved in PDIM transport. The ppsA-E gene products are responsible for the elaboration of incoming long-chain (C20-C22) fatty acids by successive additions of malonyl or methylmalonylCoA extender units, typical of type I PKSs like the erythromycin PKS. The mas gene products are proposed to be involved in extension of C16-C20 fatty acids, by addition of methylmalonylCoA units but in an iterative fashion. The products of mas action are then linked as esters to a diol moiety present in the polyketides that are synthesized by
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the Pps PKS. The outcome, a PDIM molecule, is then supposed to be exported from the cytoplasm possibly by the products of drrA-C and mmpL7 genes. Based on their experimental observations pertaining to the *M. tuberculosis* mutants, Cox and coworkers (1999) have proposed a model for the synthesis and export of PDIM, delineating roles for the above-mentioned gene products of the virulence cluster. The Cox model proposes that the two Acoas are involved in the release of the polyketide products from Pps and Mas proteins, with one of the two Acoas namely, FadD28, may also somehow helping to bring together, perhaps even condense the two polyketides to form PDIM. PDIM is then transported by DrrA-C and MmpL7 from the cytoplasm to its eventual resting place, the outer cell wall. However, while this model does help in explaining the events leading up to and beyond PDIM synthesis to a very large extent, the arguments listed below have not been addressed sufficiently.

Firstly, while it is clear that the PpsE protein is a type I PKS multienzyme that contains a ketosynthase (KS), an acyltransferase (AT) domain and a possible condensation domain at the C-terminus having little sequence similarity with other condensation domains in the protein database, the C-terminus of modular multienzymes almost always indicates the presence of an acyl-carrier protein (ACP) domain, for example in the case of PpsA-D proteins. The ACP plays the vital role of ferrying the polyketide and fatty acids from one domain to the next. The presence of a possible condensation domain instead of an ACP at the C-terminus of PpsE protein is therefore puzzling, although a similar situation exists in the lovastatin-producing type I iterative PKS protein. It may be that the C-terminus is responsible for the off-loading or transfer of the processed long-chain polyketide moiety from PpsE, by a mechanism that involves a second enzyme. With the present understanding of the virulence cluster, it would be speculative to hypothesize that this second enzyme is none other than TesA. Secondly, it is worth mentioning that the chemical structure of the Pps polyketide end product (phthiocerol) implicates a decarboxylative step at the end, resulting in the formation of a free acid. The tesA gene product that is not mentioned in the Cox model, we believe, may play an important role here. The amino acid sequence alignment of *M. tuberculosis* TesA with other type II thioesterases provides clear indication that TesA belongs to the same family.
Aims and Objectives
The present study is an effort to extend the scope of the model postulated by Cox and co-workers (1999). By confirming an interaction between the C terminus of PpsE and TesA proteins \textit{in vitro} by using GST 'pull-down' assay and \textit{in vivo} by using a bacterial two-hybrid system, the inclusion of tesA in the workings of this virulence cluster can be proposed. The inclusion of tesA in the Cox model should concomitantly strengthen the hypothesis of a decarboxylative step at the end of phthiocerol biosynthesis by the PpsA-E PKS. In keeping with these findings, the roles assigned previously for the two Acoas enzymes- FadD26 and FadD28 can also be reviewed.

The following were the specific steps involved:


2) Bacterial two-hybrid assay to screen for possible \textit{in vivo} interactions.

3) Construction, expression and purification of GST-TesA fusion protein and 6X-His tagged PpsE protein.

4) Validation of results of bacterial two-hybrid assay by \textit{in vitro} GST 'pull-down' assay.