CHAPTER 1

INTRODUCTION

Tuberculosis (TB) is a deadly disease of humans and is caused by *Mycobacterium tuberculosis* (*M. tb*). Historians have documented TB-related mass deaths amongst all types of human civilizations. With the advent of antibiotics and vaccines between 1940s and 1950s, TB was successfully brought under control. The stupendous success in curing TB led to complacency among scientific community and research funding agencies diverted their funds to other projects like genetic disorders and cancer.

Unfortunately, TB-related cases/deaths started increasing in late 1960s and by 1990 the situation became very grim as *M. tb* related death toll reached alarming proportions in developed as well as underdeveloped countries. It is estimated that *M. tb* is now responsible for killing more than 50,000 people every week (Dye *et al*. 2002) This precarious condition has been attributed to many factors e.g. variable efficacy of the Bacille *de* Calmette-Guerin (BCG) vaccine, increased drug resistance to all the antibiotics used to control TB, delay in diagnosis, lack of patient compliance and association with HIV. It has been estimated that TB accounts for around 32% deaths in HIV infected individuals (Kochi, 1996). WHO has taken a serious note of rapid increase of TB-related deaths and has constituted a program called 'Global Alliance for TB Drug Development'. Over a dozen countries and nearly three dozen laboratories are involved in this program. The main goal of this program is to identify new drugs against the TB pathogen.

Though finding new anti-TB compounds by classical method has its own merits, better knowledge of *M. tb* at molecular level and the publication of complete genome sequence of *M. tb* (Cole *et al*. 1998) has opened new approaches for developing novel drugs which are highly specific for *M. tb*.

Many laboratories all over the world have identified many drug targets, mainly novel enzymes, which are present in *M. tb* and absent in humans. By and large these enzymes are essential either for the survivability of the pathogen or are involved in the virulence of the organism. Therefore, inactivating these enzymes by the development of novel inhibitors/drugs seems to be a promising approach and is being followed by many workers. Once an enzyme has been validated as

a drug target, it is produced in large amounts, usually by gene cloning strategies and is purified in a manner that it retains its functional properties. The enzyme is subjected to crystallography and its three dimensional structure is deduced. After its active site and substrate binding site have been identified, potential inhibitors are developed using bioinformatics approaches. Ultimately, the antimycobacterial activity of these inhibitors is evaluated.

Diaminopimelic acid (DAP) is an essential component of *M. tb* cell wall. DAPless microbes immediately lyse because of lack of mechanical strength of bacterial cell wall. The absolute requirement of DAP by *M. tb*, its absence in humans and no uptake of extracellular DAP by *M. tb* makes the key enzymes of DAP biosynthetic pathway excellent drug targets for killing or significantly reducing their growth.

The DAP biosynthetic pathway is well known. It is the second last intermediate of 'Lysine biosynthetic pathway' and uses aspartate as the starting material. The first four enzymes of this pathway aspartokinase (ASK), aspartate semialdehyde dehydrogenase (ASD), dihydrodipicolinate synthase (DapA) and dihydrodipicolinate reductase (DapB) have been identified as validated drug targets. Out of these, ASD (EC 1.2.1.11) is considered the most important as it has been studied extensively in *E. coli* and *Salmonella*. In fact, inactivating chromosomal *asd* and incorporation of functionally active *asd* gene on a vector has resulted in the creation of "Antigen delivery system in *Salmonella*" and "High level expression of heterologous proteins" in *E. coli.*

In order to develop inhibitors of *M. tb* ASD, the enzyme has to be produced in large amounts and should be functionally active as well. We report for the first time, the heterologous expression, purification and characterization of ASD enzyme by cloning *asd* gene of *M. tb* H37Rv in *E. coli* M15.