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*Mycobacterium tuberculosis* (*M. tuberculosis*) has been present in the human population, since antiquity-fragments of the spinal column from Egyptian mummies from 2400 BCE show definite pathological signs of tubercular decay. The bacterium has plagued mankind since the beginning of medical history. *M. tuberculosis* kills 3 million people each year. Tuberculosis (TB) is the largest cause of death in the world caused by a single infectious organism (Bloom and Murray, 1992). It has been estimated that, at current rates, up to one-half of a billion people will suffer from TB in the next 50 years.

TB is a considerable public health problem in Asia, Latin America and Africa. Two decades ago, it was thought that TB was under control and that it was a matter of time before it would be eradicated. Today, this disease has reestablished itself due to several factors. The lack of drug compliance, the appearance of multiple-drug-resistant strains and the AIDS (acquired immune deficiency syndrome) epidemic are a few factors that have led to the resurgence in TB and the appearance of *M. tuberculosis* strains that are resistant to first-line antibiotics (Murray *et al*., 1990). New vaccines and drugs are needed to stem the worldwide epidemic of TB. To rationally develop new antitubercular agents, it is essential to study the genetics and physiology of *M. tuberculosis* and related mycobacteria.

Pathogenic bacteria utilize a number of mechanisms to cause disease in human hosts. Bacterial pathogens express a wide range of molecules that bind host cell targets to facilitate a variety of different host responses. The molecular strategies used by bacteria to interact with the host might be unique to specific pathogens or conserved across several different species. A key to fighting bacterial disease is the identification and characterization of all these different strategies ((Wilson *et al*., 2002). Certain virulence factors are necessary for full pathogenicity regardless of the host. The lipase enzyme is a known virulence factor in many
bacterial species. Many different bacterial species produce lipases, which hydrolyze esters of glycerol with preferably long-chain fatty acids. They act at the interface generated by a hydrophobic lipid substrate in a hydrophilic aqueous medium (Jaeger et al., 1994). Hydrolytic enzymes like lipase may contribute to the invasivity and proliferation by causing the destruction of the host tissues, thereby supplying degraded material to the organism as nutrients.

The Mycobacteria cell wall is composed of many lipids and its complex organization still remains to be elucidated (Daffe and Draper, 1998; Brennan and Nikaido, 1995). Knowledge of cell wall composition is essential to understand the antibiotic penetration (and/or resistance) mechanisms. In addition, the virulence of these mycobacteria is thought to depend to a large extent on the cell wall (Murray et al., 2005).

In Mycobacteria such as M. tuberculosis, lipids play an important role and a large fraction of the genome codes for proteins involved in lipid metabolism. Comparative sequence analysis of the M. tuberculosis genome has revealed that it contains 250 enzymes involved in lipid metabolism compared to only 50 in Escherichia coli. Among these enzymes, a family of 21 carboxyl ester hydrolases, called Lip (C to Z, except A and B), have been annotated as putative esterases or lipases, based on the presence of the consensus sequence GXSXG characteristic of members of the α/β hydrolase fold family. Within this family, the recent crystal structure of the M. tuberculosis antigen 85C (Ag85C) (Ronning et al., 2000), a mycolyltransferase required for survival of mycobacteria, along with that of the noncatalytic M. tuberculosis MPT51 protein, FbpC1 (Wilson et al., 2004), which is involved in mycobacteria pathogenicity, have revealed that they share the same α/β hydrolase fold. Therefore, a detailed biochemical characterization of all members of the Lip family should be performed beyond the computational analysis.
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Out of all the lipase genes present in *M. tuberculosis* H37Rv, Rv1923 (LipD) and Rv2485c (LipQ) proteins have least identity with *Mycobacterium smegmatis*, which is a non-virulent strain of *Mycobacteria*. Rv1923 protein belongs to β-Lactamase Class C that has an important role in defense mechanism and Rv2485c belongs to carboxyesterase that might have role in Intermediary metabolism of the pathogen.

**AIMS AND OBJECTIVES**

The *M. tuberculosis* genome contains an unusually high number of proteins involved in the metabolism of lipids belonging to the Lip family, including various nonlipolytic and lipolytic hydrolases. Driven by a structural genomic approach, attempts were made to clone, purify, biochemically characterize Rv1923 (LipD) and Rv2485c (LipQ) gene products, previously annotated as a putative lipases.

- Cloning of Rv1923 (LipD) and Rv2485c (LipQ) genes of *Mycobacterium tuberculosis* H37Rv by UA cloning vector.
- Expression of Rv1923 and Rv2485c cloned in *E. coli* M15.
- Purification and Characterization of Rv1923 and Rv2485c enzymes
- Detection of its possible role in the modulation of immune response of the host and hence, probable virulence in the host.