Leprosy or Hansen’s disease is one of the oldest recorded diseases that still continues to be a major public health problem. The term leprosy originates from the Latin word *lepros* which means ‘defilement’ (Sasaki *et al.*, 2001). Throughout history, leprosy had been feared as an incurable disease that resulted in stigmatization and the patients suffered not only from the disease but also from public discrimination. This disease was considered as a divine punishment for sin in the Old Testament and karma in Buddhism. However, leprosy is an infectious disease and the causative microbe for this debilitating neurological disease is an acid-fast bacterium, *Mycobacterium leprae*. In 1873, in the first convincing association of a microorganism with a human disease, the Norwegian physician, Sir Gerhard Henrik Armaeur Hansen discovered the leprosy bacillus in skin biopsies (Cole *et al.*, 2001; Sasaki *et al.*, 2001).

Leprosy is a chronic granulomatous infectious disease, which primarily affects the skin, the peripheral nerves, mucosa of the upper respiratory tract and also the eyes, apart from other structures (Sasaki *et al.*, 2001). The disease manifests mainly as skin lesions and neuropathy. Secondary complications arising due to the peripheral nerve damage results in weak, anaesthetic hands and feet, as well as blindness and facial disfigurement (Britton and Lockwood, 2004; Lockwood and Suneetha, 2005). The resultant deformities and disabilities are the hallmarks of leprosy that makes it a dreaded disease.

Leprosy had once affected every continent in the world with a relatively wide distribution in Europe and Asia (Sasaki *et al.*, 2001; Britton and Lockwood, 2004). During the 1980s, the global burden of leprosy stood at 12 million (Sugita, 1995) and therefore, in the 1990s, the World Health Organization (WHO) launched the leprosy elimination campaigns. This bold and ambitious initiative resulted in an outstanding public health achievement with more than 11 million leprosy patients having been cured by multi-drug therapy (MDT) and many without any disability (WHO, 2010; Britton and Lockwood, 2004). However, the fall in the prevalence of leprosy has not been accompanied by a fall in the rate of detection of new cases (Britton and Lockwood, 2004; Scollard *et al.*, 2006; Fine, 2007). In some countries where leprosy
is endemic, the number of new cases actually appears to be increasing while in others decreasing trends are reported. Interestingly, there is no evidence that the global initiative has led to the disappearance (“local eradication”) of the infection or disease from any population, and leprosy continues to appear throughout Africa, Asia, Latin America, Southern Europe and in the states of Louisiana and Texas in the US (Fine, 2007). Based on a mathematical modeling using various parameters such as the efficacy of treatment and prevention, it has been suggested that leprosy will continue to be a major public health problem for many more decades (Meima et al., 2004; Fine, 2007).

Global elimination of leprosy has been the primary goal of research and health policy for over the last two decades. Elimination of leprosy involves prevention of the disease, development of tools to recognize infection with *M. leprae* before the disease manifests, interruption of transmission with treatment as early as possible and identification of intermediate hosts, if any. In addition to the already available Immuvac (earlier Leprovac) for prophylaxis measures, the availability of a highly effective vaccine as well as early diagnostic methods are the current primary requirements. Hence, most of the current concerted research efforts employing advanced research tools primarily focus on these two aspects of leprosy elimination.

The failure to eradicate leprosy is intimately linked to the pathogenesis of the causative organism (Smith et al., 2004; Fine, 2004). This intracellular pathogen not only exhibits a remarkable ability to survive in the host cells but can also persist over long periods of infection. Hence, the design of vaccine and immunodiagnostic reagents requires an in-depth understanding of the pathogenic mechanisms of *M. leprae* as well as the response mounted by the host against this pathogen. Therefore, the identification and characterization of mycobacterial virulence factors will greatly aid in gaining an insight into the biology of *M. leprae* and its virulence mechanisms. The extremely long doubling time combined with the pathogenicity of *M. leprae* has delayed for years the identification of virulence factors, which includes a huge repertoire of protein antigens of *M. leprae*. However, application of both molecular biology and immunological tools, such as gene cloning and use of monoclonal antibodies, has made significant contributions to our knowledge on
M. leprae antigens as well as on the immune responses against them (Young et al., 1985; Thole et al., 1995).

Amongst these antigenic proteins, the heat shock proteins are of particular interest because their elevated expression is required for bacterial adaptation to adverse conditions such as those encountered during infection. At the same time, these Hsps provide a signal for the recognition of the pathogen by the host immune system (Zugel and Kaufmann, 1999). One heat shock protein of M. leprae which had gained tremendous attention as an immunodominant antigen is a small heat shock protein, sHsp18. The immunological response to this antigen had been the focus of studies by many groups for almost a decade during the mid 1980s.

A major advancement in leprosy research was achieved when the M. leprae genome was sequenced and published in 2001 (Cole et al., 2001). Comparative analysis of this genome with that of M. tuberculosis revealed that M. leprae genome has undergone massive gene decay. In the course of reductive evolution, M. leprae has lost many genes that can be compensated for by a host-dependent parasitic lifestyle. The pathogen seems to have abbreviated its genome to preserve the genes required for transmission, establishment and survival in the host (Cole et al., 2001; Vissa and Brennan, 2001). Interestingly, shsp18 is the only small heat shock protein gene that has been retained by this pathogen which in turn implies the essential and significant role of this gene in M. leprae life cycle. Adding evidence to the importance of this gene is the fact that all other pathogenic and non-pathogenic mycobacteria have a minimum of two shsp genes while M. leprae harbors only one. Most of the information available till date regarding shsp18 of M. leprae is with respect to the role of this antigen in the host immune response. However, the functional significance of this gene/protein from the pathogen’s perspective has not been probed much and only very little is known concerning its cellular function in M. leprae. Inspired by the possibility that such an investigation would throw more light in unraveling the complex pathogenicity of M. leprae, this comprehensive study was conceived.

The objective of this study was to examine the various functional aspects of shsp18 of M. leprae. The widely used recombinant technology was employed to clone this gene in an Escherichia coli expression system, and the characteristics of the overexpressed recombinant sHsp18 protein were analyzed. Subsequently, in vivo characterization
studies of shsp18 gene and protein was carried out in two surrogate hosts namely, the E. coli and M. smegmatis. Using the E. coli system, sHsp18 was overexpressed and the protective role of this protein was evaluated under stress conditions. Further, characterization studies were carried out using both E. coli and M. smegmatis wherein the shsp18 gene expression was under the control of its native promoter. These two host systems were used to decipher the functional role of shsp18 under diverse stress conditions, all of which reflect the hostile environment faced by M. leprae during its infection in the human host. In the last part of this study, the purified recombinant sHsp18 protein was characterized under in vitro conditions and the function of this protein was examined with respect to the host-pathogen interaction.