Synopsis of the thesis

The studies reported in this thesis address the physiological role of an antibiotic resistance gene *arr,* followed by establishment of the *arr* knockout strains (generated during the investigation) for drug testing and mutation spectrum analyses. The first chapter (CHAPTER 1) provides a general background on tuberculosis disease, its prevalence, drug regimen used and various hypotheses on the rise in drug resistance strains. I then focus on the various Rif resistance mechanisms and discuss in detail about one of the modes of Rif resistance that arises due to its ADP-ribosylation. Literature related to Arr and other relevant aspects is discussed. In the next section, I introduce *M. smegmatis* as surrogate model for *M. tuberculosis* in overcoming the difficulties associated with the direct use of *M. tuberculosis*. The second chapter (CHAPTER 2) includes "Materials and Methods" section where detailed information on all the methods and resources used for the execution of experiments is provided. It is then followed by an account of the investigations carried out in this study in two chapters (CHAPTERS 3 and 4) and an appendix section.

I. Physiological role of Arr, an ADP-ribosyltransferase in *M. smegmatis*

(CHAPTER 3)

Rifampicin ADP-ribosyltransferase (Arr) is conserved across various bacterial species and the transcript level of *arr* is upregulated during stress conditions. This led us to hypothesize that Arr has an additional role in cellular physiology. To address this question, we have generated *M. smegmatis* strains deleted for *arr* and shown that apart from its role in ADP-ribosylation, Arr has a role in conferring growth fitness advantage and in fatty acid biosynthesis. Stallings *et al.,* (2011) showed

interaction of Arr with MsmL11 ribosomal protein. L11 protein (encoded by *rplK* gene) is required to activate RelA for (p) ppGpp synthesis. In order to check if Arr has a role in (p) ppGpp biosynthesis pathway, LCMS analysis of metabolites was done and it was seen that *M. smegmatis* Δ*arr* has compromised levels of the stress metabolite (p) ppGpp. Since *M. smegmatis* has RelA and an additional protein (MS_RHII-RSD) for (p) ppGpp synthesis, to study the role of Arr in (p) ppGpp synthesis pathways, we developed a heterologous model system (*E. coli relA spoT* mutant, deleted for genomic *rplK* gene and supported by *Msm-rplK* or the chimeric constructs of *Eco-Msm-rplK*). Our study shows that Arr interacts with the N-terminal of Msm L11 to synthesize ppGpp (tetraphosphate specifically). Finally, we show that Arr is involved in stress response pathways and has a role in ppGpp synthesis.

II. Establishment of *M. smegmatis* Δ*arr* as surrogate for *M. tuberculosis* in drug testing and mutation analyses

(CHAPTER 4)

Screening of Rif analogs in *M. tuberculosis* has constraints such as requirement of BSL3 facility, handling issues and longer time taken by the bacteria to grow. On the other hand, such analyses require use of high concentrations of Rif when *M. smegmatis* is used as a model. *M. tuberculosis* does not possess Arr but it is present in phylogenetically closely related *Mycobactrium ulcerans*. The *M. smegmatis* Δ*arr* strains show minimum inhibitory concentration (MIC) for Rif, similar to that for *M. tuberculosis*. The MICs for isoniazid, pyrazinamide, ethambutol, ciprofloxacin and streptomycin were essentially unaltered for *M. smegmatis* Δ*arr*. So we propose that *M. smegmatis* Δ*arr* can be used to screen for Rif analogs either alone or in combination with other anti-TB drugs.

Spontaneous resistance to Rif is used in studying roles of various DNA repair genes i.e. by analyzing mutations in *rpoB* (encoding the β subunit of RNA polymerase) causing Rif resistance. These analyses use high concentrations of Rif when *M. smegmatis* is used as model. Such non-physiologically high concentrations of drug may induce alternate mechanisms of DNA damage. The *M. smegmatis* Δ*arr* strains show minimum inhibitory concentration (MIC) for Rif which is similar to that for *M. tuberculosis*. The growth profiles and mutation spectrum of *M. smegmatis* Δ*arr* and Δ*arr*Δ*udgB* (*udgB* encodes a DNA repair enzyme that excises uracil) strains were similar to their counterparts wild type for *arr*. However, the mutation spectrum of *M. smegmatis* Δ*fpg*Δ*arr* strain differed somewhat from that of the *M. smegmatis* Δ*fpg* strain (*fpg* encodes a DNA repair enzyme that excises 8-oxo-G). Our studies suggest *M. smegmatis* Δ*arr* strain as an ideal model system in drug testing and mutation spectrum determination in DNA repair studies.

III. Appendix

The procedure used for characterising metabolite profile is given in appendix section.