CHAPTER 6: SUMMARY
The aim of present study was to establish a method for studying disruption of protein-protein interactions using *de novo* protein/peptide library. The primary requirement towards this objective was to develop a system where interactions within ternary protein complexes can be efficiently studied. “Bacterial two-hybrid” system offers a simple yet effective tool to study binary protein complexes. However, this method is inefficient when it comes to studying complexes that involve interplay between more than two proteins. Extrapolating this existing system to develop a bacterial three-hybrid system was therefore a key motivation for this study. In this study, a novel *E. coli* based three-hybrid system has been described that allows the use of available cDNA or *de novo* peptide libraries to isolate proteins/peptides that can either bind to or disrupt interaction between two previously known interacting proteins. The advantage of the present three-hybrid system compared with the existing ones, is the possibility of controlling the expression of the third partner cloned under a differentially inducible promoter, in order to get a single step confirmation of its effect on the interacting bait and target proteins.

To illustrate this three-hybrid system, the study made use of the known tri-protein complex of *M. tuberculosis* ESX-1 secretory system- ‘ESAT6-CFP10-Rv3871’ and extended the application to two-component system proteins ‘HK1-HK2-TcrA’. The various findings of this study can be listed as follows:

1) The novel vector 'pMTSA' is compatible with the ‘bacterial two-hybrid’ bait and target vectors and shows high expression of protein under Arabinose induction.

2) The three-hybrid system is efficient in demonstrating strengthening of interaction between two proteins, induced by a third protein. Using this system, it has been shown that the interaction between CFP10 and Rv3871 is weak and is strengthened when ESAT6 is present to form a tight complex with CFP10. Here it has been proposed that ESAT6 binding probably induces conformational change in CFP10 which in turn results in stronger binding with Rv3871.

3) The system has next been shown to be highly efficient in studying disruption of protein-protein interactions. Using the two previously known binders of ESAT6 i.e. CFP10 and HCL1, it has been shown that the stronger interactor CFP10 can
disrupt the weaker interaction between ESAT6 and HCL1. The disruption was easily detectable by simple ‘blue-white screening’ method.

4) The presence of an Arabinose-inducible promoter provides an “on” or “off” switch for the expression of the third protein. Replica plating the three-hybrid strains on Arabinose-positive and Arabinose-negative plates allows a single step confirmation of the disrupting or strengthening effect of the third protein on the two interacting proteins.

5) The system permits screening of a protein/peptide library to identify proteins/peptides that can promote or prevent interaction between two identified proteins. By cloning the mutagenised peptide library of a binder HLL7 in pMTSA vector, a disruptor peptide ‘Mut9/11’ could be isolated which had the potential to disrupt the dimerisation of histidine kinase component of the *M. tuberculosis* HK1-HK2-TcrA two-component system.

6) RT-PCR has proved to be an efficient additional technique to confirm protein-protein interaction or disruption studies in a bacterial two- or three- hybrid assay. The results obtained corroborated well with those obtained by the biochemical liquid β-galactosidase assay.

Conclusively, these findings have enabled us to establish a novel three-hybrid system that can be efficiently used for studying protein complexes and protein-protein disruption in *M. tuberculosis*. This system has strong potential to be used as an efficient tool to decipher several as yet undisclosed mechanisms of *M. tuberculosis* pathogenesis and also to screen potential anti-tuberculosis drug candidates.