SUMMARY

Redox regulation is an important aspect for the survival of any living organism. Intracellular redox conditions mainly depend on the gene regulation and the surrounding environment of the organism. Till date most of the literature in relation to redox stress is about oxidizing effects, mainly due to its prevalence and detrimental effects caused by it. There are very few studies regarding the imbalance of redox regulation on the other side that is reductive stress.

In order to carry out all the metabolic processes smoothly, it is very important to maintain the intracellular environment in the reducing state, still there are some reactions which rely on oxidizing conditions. For example, disulfide bond formation which is carried out in endoplasmic reticulum in case of eukaryotes and periplasm in bacteria. When reducing conditions prevail to the extreme, they may lead to disturbances in the metabolism. For example in anaerobic conditions, there is accumulation of reducing equivalents like NADH, GSH which may bring about reductive stress and mainly known to cause UPR (unfolded protein response). Similar effects can be triggered by reducing thiol containing compounds like homocysteine, glutathione and dithiothreitol (DTT) (Cox et al., 1993; Kohno et al., 1993; Huang et al., 1994). Bacteria being versatile and can adapt to extremes of the adverse conditions, must contain regulatory mechanisms to overcome reductive stress.

In this study, we chose *S. coelicolor* as a model organism to study the effects of DTT stress on the growth and metabolism using proteomic and biochemical techniques. The reductive nature of the stress was established by measuring the intracellular total thiol content. Looking at the proteomic profile using SDS-PAGE, it was observed that there was prominent induction of ~55 kDa band in the presence of DTT which was later on found to be catalaseA by MALDI-TOF and nano LC-ESI-MS/MS. The induction of the same size of band was observed in other species of *Streptomyces, S. lividans* and *S. gresius* but was absent in Gram negative *E. coli*. Further, from 2D-PAGE separation of proteins, several other proteins along with catalaseA were found to be differentially expressed under thiol stress. These proteins belong to different classes such as proteins involved in primary and secondary metabolism, nucleic acid

metabolism and two of them were membrane proteins. Over all proteomic study indicates that like oxidative stress, reductive stress also affects the cellular metabolism since DTT also causes redox disturbance causing direct or indirect alterations in the expression of many genes.

The studies confirm that the stress observed by *S. coelicolor* was reducing one but still there was induction of catalaseA, which is also the major catalase involved in the oxidative stress response exerted by H_2O_2 (Cho and Roe, 1997). In order to study the role of CatA under thiol stress, it was inhibited by chemical and genetic means and the effect was observed on the growth of *S. coelicolor* and was found that induction of catalaseA was essential for the growth of *S. coelicolor* under DTT stress. Looking at the role of catalaseA, earlier studies in *E. coli* shows that exposure to reduced flavins or cysteine is known to exaggerate DNA damage through involvement of the Fenton reaction (Park and Imlay; woodmansee and Imlay). Based on the above facts and results of Fe chelating experiments using o-phenanthroline, it was found that here Fenton reaction plays an important role in the induction of CatA (Vekaria et al., 2007). Thiol containing compounds are generally good antioxidants and protect thiol groups from oxidation. Although, in the presence of metal ions and oxygen, DTT may induce free radicals and cause damage to proteins, lipids and DNA (Kachur et al., 1997). So we can say that DTT must be playing dual role in thiol stress. Primarily, acting as an oxidizing agent by generating ROS through autooxidation and secondly as a reducing agent further accelerating the Fenton reaction. These effects, in turn pose a threat for the survival of the organism leading to requirement for CatalaseA induction.

It is known that CatA is under the control of a repressor, CatR (Hahn et al., 2000). Under normal conditions when intracellular environment is reducing, the cysteines of CatR being in reduced form, it has higher affinity for catA and catR promoters there by inhibiting the expression of both the genes. During oxisdative stress, these cysteines undergo oxidation by formation of disulfide bonds leading to conformational change in CatR. The oxidized form of CatR has low affinity for DNA and is released from the promoters and hence causes induction of catA and

catR expression. According to this model, under DTT stress, due to higher reducing status of the cell, CatR must be in reduced conformation and should suppress CatA and CatR expression. In contrast to this, induction of CatalaseA under thiol stress was observed and hence it was important to investigate the role of CatR in thiol stress. Expression of CatR was monitored by RT-PCR and EGFP reporter protein assay. From the studies, catR expression remained unaltered in the presence of DTT indicating that CatR does not play any role in catA induction. To strengthen this fact CatA mutant of *S. coelicolor* was generated. Under thiol stress, if CatR employs the same mechanism as in oxidative stress then catA mutant of *S. coelicolor* should show more induction of CatR than in wild type. This is because in the absence of catA more of the CatR will get oxidized in turn releasing the repression from catA and catR promoters and hence leading to induced expression of CatR. In contrast to this assumption, catR expression checked by RT-PCR was found to be unaltered even in the catA mutant further strengthening the fact that CatR is not ionvolved in thiol stress.

Further, extending the studies on the possibility of ROS generation through electron transport chain under thiol stress, we investigated the effects of thiol stress on oxidative phosphorylation at the level of different complexes. The studies show that there is an increase in the respiratory rate in the presence of ADP as a substrate at all the complexes, namely I, II and IV but there was no significant change in the ADP/O ratio proving that the functionality of the electron transport chain is not affected. Increase in the respiratory rate could be due to partial hypoxic state generated by the reducing agent which may result in the induced expression of the respiratory units. To some extent this can be supported by the fact that among the induced proteins under thiol stress, one was ferredoxin-NADP reductase which is also induced under oxidative stress (Giro et al., 2006) and modulates NADP(H) homeostasis during the soxRS response of *E. coli* (Krapp et al., 2002) which in turn may result in this alterations in the respiratory chain. As electron transport chain is the major source of ROS, increased respiration rate may lead to generation of ROS. So we determined the ROS levels under thiol stress in the wild type and CatA mutant. There was no

change in the ROS levels in WT since it can be controlled by increased catalase activity but in the mutant the ROS levels were found to be increased in response to DTT suggesting that there is generation of ROS which could be due to increased respiration rate.

In summary, the studies demonstrate that like oxidative stress, CatalaseA also plays a significant role in reductive stress. But the mechanism and cause involved in the regulation is different and more complicated than in oxidative stress, which is not mediated through the repressor CatR. Thus, the cumulative effects of increased respiration, reduction of oxygen as a result of reductive stress and ROS generated due to Fenton reaction must be the reason for the induction of catalaseA.