

CHAPTER 6

SUMMARY AND CONCLUSION

Summary & Conclusion

Heavy metals are toxic and are known to cause several disease conditions in human beings. Mining operations and industrial activities are the main source of heavy metal contamination and release of the metals into the environment is a serious concern having deleterious effects not only on the human health but also on the biodiversity and ecosystem. Microbes particularly the bacteria present in the metal contaminated environment have evolved and developed resistance against the metal stress. The knowledge on the biochemical and molecular aspect of metal resistance in bacteria may be exploited for bioremediation applications. As the environment heavily polluted with toxic metals can be a rich source of metal resistant and/or accumulating bacterial strains, the present study was focused on the biochemical characterization and mechanistic study at biochemical and molecular levels of the metal resistant bacterial strains isolated from the nearby/adjointing areas of mining sites of East Singhbhum district of Jharkhand.

The findings of present study could be summarized as follows:

1. Forty two (42) bacterial isolates representing different bacterial colonies were found to be resistant to different concentrations (0.5, 1.0 & 2.0 mM) of Cd, Cr and Ni and among them eight (8) strains were resistant to all the metals.
2. The identified metal resistant bacterial strains were tested for morphology (shapes: rod/cocci), gram staining and biochemical tests such as cellulose, amylase, hydrolysis, tippel sugar etc. Based on the morphological and biochemical analysis five (05) strains with distinct biochemical characteristics were selected for Biolog analysis.
3. With Biolog system the five (05) selected strains were identified as *Bacillus* (two strains), *Pseudomonas*, *Lactobacillus* and *Staphylococcus* species.

4. Based on the ability of the bacterial strains to grow in presence of all the metals (Cd, Cr & Ni), the two strain *Pseudomonas* sp. (JHCO2018) and *Bacillus* sp. (JHNI40215) were identified as *Pseudomonas aeruginosa* RV3 (accession no: JX313019) and *Bacillus cereus* NI40215 (accession no: KT072743) using 16S rDNA sequence which showed 99 % similarity with the corresponding sequences of *Pseudomonas* sp. and *Bacillus* sp. respectively.
5. The optimization of the growth conditions showed that the optimal temperature and pH for both the strains *Pseudomonas aeruginosa* RV3 and *Bacillus cereus* NI40215 are 37 °C and pH 7. Also both the bacterial strains were found resistant to multiple antibiotics.
6. Bacterial strains (RV3 and NI40215) were resistant to Cd, Cr and Ni with average MIC values for all metals as 2.46 and 2.16 respectively. The order of toxicity of the metals to the bacterial strains were Cd > Cr > Ni.
7. Both the bacterial strains RV3 and NI40215 exhibited decrease in the growth rates in the presence of metals (Cd, Cr & Ni).
8. GSH is considered as one of the important non-enzymatic antioxidant and decrease or depletion of GSH in response to metals might be associated with heavy metal detoxification mechanism. The dose dependent decrease in GSH levels were observed in both the bacterial strains (RV3 & NI40215) in response to heavy metals (Cd, Cr & Ni).
9. SOD and CAT are front line of antioxidant enzymes which neutralizes the superoxide and H₂O₂ respectively. The metal resistant bacterial strains (RV3 & NI40215) showed significant increase in the SOD and CAT activity in response to the metals (Cd, Cr & Ni).
10. GPx and GR are important GSH-related enzymes and have an important role in GSH/GSSG maintenance which is crucial for cellular redox status. The metal resistant bacterial strains (RV3 & NI40215) showed significant dose dependent increase in the GPx and GR activity.

11. The present data on the modulation of GSH and antioxidant enzymes in response to the heavy metals (Cd, Cr & Ni) suggests their possible role in conferring resistance in both the bacterial strains (RV3 & NI40215).
12. SEM image data exhibited morphological changes with decrease in surface/volume ratio particularly at the highest dose of the metal treatment in both the bacterial strains (RV3 & NI40215). The relative reduction in cell surface/volume ratio is considered as effective mechanism adopted by the cells to lower the toxic effects of environmental stress by decreasing the attachable/exposed surface.
13. EDX analysis revealed the presence of metals (Cd, Cr & Ni) on the surface of both the bacterial strains (RV3 & NI40215). The information on the cell size and morphometric properties is an important parameter for bio-sorption process of heavy metals by the bacteria and is directly proportional to their metabolism and growth rates.
14. FTIR studies showed complex interactions of metals (Cd, Cr & Ni) within wide range of functional groups such as –OH, aliphatic C-H, secondary amines, carboxyl, carbonyl, phosphate and phosphate diester present on the bacterial biomass of the strains RV3 and NI40215.
15. TEM analysis also exhibited bio-accumulation/biosorption of metals (Cd, Cr & Ni) in the metal resistant bacterial strain RV3.
16. AAS showed increased levels of metals in dose dependent manner in the dry biomass of both the bacterial strains (RV3 and NI40215). ICP-OES analysis of metal loaded bacterial dry biomass showed similar trend of dose dependent increase in the metal biosorption/accumulation in both the metal resistant bacterial strains (RV3 and NI40215) even though the amount of metal detected were much higher as compared to AAS which might be due to difference in the sensitivity of the instruments.
17. The SEM, FTIR and TEM analysis confirms the interaction and localization of metals within the bacterial strains. Though significant increase in the lipid peroxidation were observed in response to metals

(Cd, Cr & Ni), membrane damage were not observed in the SEM images of the metal resistant bacterial strains (RV3 and NI40215) instead increased level of bisorption/accumulation were seen with increase in dose of these metals.

18. To study the molecular level interaction proteomic approach was used to identify differentially expressed proteins and their role in metal detoxification in *Pseudomonas aeruginosa* RV3. The bacterial strain RV3 responded to metals (Cd & Cr) by up-regulating genes involved in protein repair, ATP synthesis, protein biosynthesis, maintenance of cell shape/cell envelop, transportation, Cd-Zn-Co resistance and arginine synthesis.
19. The protein expression data on the metal resistant RV3 strain in response to the Cd exhibited the induction of lipoyl synthase (EC 2.8.1.8) which catalyzes the synthesis of lipoic acid through lipoylation which is essential for the functioning of several important enzymes such as pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, branched chain ketoacid dehydrogenases, the glycine cleavage enzymes etc. involved in oxidative metabolism. Recently lipoic acid has received attention for its role as a biological antioxidant and has been reported to form stable complex with transition of metals.
20. The present investigation showed that the induction of lipoyl synthase in response to Cd in the metal resistant RV3 strain and suggested the possible role of lipoic acid in metal detoxification, so the *lipA* gene was PCR amplified and cloned in InsTA cloning vector. The cloned *lipA* gene was sequenced and the length of gene was determined as 972 base pairs, further phylogenetic analysis exhibited the similarity with the *lipA* gene of *Pseudomonas aeruginosa*
21. Further the PDB BLAST result showed the translated LipA enzyme having a 47% structural similarity with Chain B, crystal structure of *Thermosynechococcus elongatus* Lipoyl Synthase 2 complexed with Mta

and Dtt [*Thermosynechococcus elongatus* BP-1]. Hence the 3D structure was predicted using I- TASSER. The 5 best predicted secondary structure models were taken for model refinement using ModRefiner to refine the geometrical errors of the predicted structures. Based on the ANOLEA assessment analysis and the Ramachandran plot, **model-4** having an energy value of -1204 and 299 (93.1%) residues present in favored region with only 5 (1.6%) residues in the outlier region is then proposed as the most stable structure.