



# Summary

## 8. SUMMARY

1. A brief review of literature pertaining to the line of investigation has been presented along with general introduction. Besides this, literature relevant to the chapter has also been cited separately in the introduction of each of the five chapters. The presented review mainly deals with the isolation and characterization of *Acidithiobacillus ferrooxidans* and other bacterial genera which occur in close association with this bacterium in acid mine drainage (AMD), the biodiversity, ecology, bioenergetics and physiology of acidophilic microorganisms with special reference to their bioleaching activity, generation of colony morphology variants and location and role of insertion sequences in genomic rearrangements.
2. Materials and methods used in this investigation and experimental procedures followed have been discussed in detail.
3. Several obligate iron and sulfur chemolithotrophic strains were isolated from AMD samples of Garubathan, Darjeeling, West Bengal, India. They were found to be able oxidize ferrous iron, elemental sulfur and reduced sulfur compounds.
4. Physiological characterization of chemolithotrophic isolates revealed that the strains belonged to *Acidithiobacillus ferrooxidans*. The strains were tested for copper bioleaching activity.
5. Acidophilic heterotrophs could be isolated from the culture of *Acidithiobacillus ferrooxidans*. Several strains of these heterotrophs have been characterized and have been assigned to the species *Acidiphilum cryptum*.
6. The heterotrophs were found unable to survive in nutrient rich media but were able to survive in low nutrient medium such as agar extract medium, diluted Nutrient broth or Luria Bertani Broth medium, indicating the oligotrophic nature of the bacteria. In addition to that they were found incapable of oxidizing elemental sulfur or reduced sulfur compounds as well as ferrous iron in presence or absence of glucose, suggesting that they are neither autotrophic or mixotrophic but obligate heterotrophic in nature. In fact the cells were found immotile when they were

exposed to thiosulfate possibly suggesting that thiosulfate may be toxic to the organism.

7. Increase in the robustness of colony formation of *A. ferrooxidans* in ferrous iron agar plate in deliberate presence of *Acidiphilium cryptum* was observed. This suggested that the heterotroph plays an important role in the manifestation of colonies in ferrous iron agar plate.
8. The ability of *A. cryptum* to grow in elemental sulfur spent medium showed the oligotrophic nature of the organism and also provided the best possible explanation for the co-existence of *A. cryptum* with *A. ferrooxidans* culture.
9. Thiosulfate at the level of  $\geq 0.7$  mM was found to be toxic to the strains of *Acidiphilium cryptum*. However, this toxicity could be alleviated in presence of *A. ferrooxidans* which provides the additional support to the synergism that exists between *A. ferrooxidans* and *A. cryptum*.
10. As reported by several earlier workers, here too *A. ferrooxidans* strains were found to remain associated with acidophilic heterotrophs in a mixed culture state. The rational basis of obligatory dependence/synergistic relationship between *Acidiphilium* strains and *Acidithiobacillus ferrooxidans* was also established.
11. Thiosulfate agar medium did not allow normal colony development of the wild type *A. ferrooxidans* cells but selectively allowed the development of spreading variants. These variants were referred to as Colony Morphology Variants (CMVs).
12. About 27% of the wild type cells switched to an alternative spreading colony morphology in thiosulfate agar medium and the number of the colonies that emerged in thiosulfate medium increased with the time of incubation suggesting the possibility of adaptive mutation.
13. The colony morphology variants in pure culture were found to have shorter generation time in elemental sulfur media when compared with their respective wild type parent strains. The variants were found to differ from the wild type strains in several respects: a) they were found to be unusually motile than the wild type cells in the thiosulfate agar medium, b) they had comparatively higher rate of

oxidation of thiosulfate, tetrathionate and elemental sulfur (however, no significant difference was found regarding the rate of iron oxidation), c) they were more efficient in leaching copper from chalcopyrite ore sample.

14. The amplicon generated with the primers specific to *IST445* from one of the isolates was also found to amplify another DNA fragment bearing an ORF coding for putative glutamyl-tRNA reductase (*HemA*) flanked by inverted repeats along with the *IST445* fragment. Similarly PCR product amplified with *IST2* primers in one of the Garubathan isolates has been found to amplify another DNA fragment that partially coded for pirin gene along with *IST2* fragment. The newly generated DNA fragment was found to be flanked by the inverted repeats of *IST2* at the terminal ends.
15. Amino acid sequence of Glutamyl-tRNA reductase from Garubathan isolate obtained from 3D model of the protein showed a remarkable similarity of 36% homology with the representative molecule of Glutamyl-tRNA reductase molecule isolated from *Methanoparus kandleri*. Further analysis revealed the absence of some portion from the N-terminal end.
16. A novel PCR based method was adopted to produce inter-IS sequence fragment **A** polymorphism of the eight different strains DK1, DK1S1, GBVI, GBVIS2, CMOII, CMOSI, CMI I and CMIS2 (4 wild and their respective colony morphology variants) to investigate the role of Insertion sequence as a mutator.  
**B** Analysis of inter IS DNA fragments revealed that some of the DNA bands are highly conserved among the strains irrespective of their origin as revealed by their high Dice coefficient. Moreover, the bands were strain specific and hence can be efficiently used for typing *A. ferrooxidans* strains.
17. Dice coefficient values of the wild type and their respective CMVs were found to be equal to unity except in case of DK1 and DK1S1 because of the occurrence of a unique band of 0.4 kb in DK1S1 which was completely absent in its parent strain DK1.

18. The unique DNA fragment contained the same terminal inverted repeats of IST2 and IST445 and showed 95% identity with the portion of *A. ferrooxidans* ATCC 53993 genome sequence which featured the natural resistance-associated macrophage protein MntH gene responsible for the transport of divalent metal ions.
19. Critical analysis of the genome sequence of *A. ferrooxidans* ATCC 53993 showed the MntH gene to be a part of an undefined operon for transport of ions. Based on this it has been predicted that the insertion of IST2 and IST445 within the MntH gene has disrupted the first gene of the operon and probably influenced the transcription of the other three genes by polar effect.
20. *Acidithiobacillus ferrooxidans* strains isolated from AMD samples of Garubathan from Darjeeling Himalayas produced unique fingerprints of amplified fragments (FAFs). The technique of using outwardly directed primers of insertion sequences in pair-wise combination to amplify inter IS DNA fragments produced unique strain specific profile. This profile can be efficiently used for typing *A. ferrooxidans* strains/ strain identification.
21. The results of the investigation have been properly analyzed and their implications have been thoroughly discussed.

## List of Publications:

1. ***Acidithiobacillus ferrooxidans* in alleviating the inhibitory effect of thiosulfate on the growth of acidophilic *Acidiphilium* species isolated from Garubathan AMD samples, India**

Authors: **Anirudra Gurung<sup>1</sup>, and Ranadhir Chakraborty<sup>1\*</sup>**

*Canadian Journal of Microbiology* (In Press)

2. **Unregulated use of Antibiotics in Siliguri City vis-à-vis Occurrence of MAR bacteria in Community Waste water and river Mahananda, and Their Potential for Resistance Gene Transfer**

Authors: sriparna Mukherjee, Bhaskar Bhadra, Ratna Chakraborty, **Anirudra Gurung**, Sudip Some and Ranadhir Chakraborty

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