5. SUMMARY

Nitroaromatic compounds are widely used in the production of industrial chemicals. Nitrobenzene and chloronitrobenzene are frequently used intermediates in the manufacture of many nitroaromatic compounds, and are known to be very toxic and resistant to microbial degradation. In recent years, a number of microorganisms evolved with the ability to utilize nitroaromatic compounds as carbon, nitrogen and energy have been isolated and studied. One such bacterial strain reported for nitrobenzene degradation is *Pseudomonas pseudoalcaligenes* JS45. It is able to utilize nitrobenzene possessing an initial reductive pathway involving catabolic enzymes, viz., nitrobenzene nitroreductase, hydroxylaminobenzene mutase and 2-aminophenol 1,6-dioxygenase. These enzymes were purified and characterized previously, and the genes coding these enzymes are shown to be on chromosome. On the other hand, two plasmids contained in *P. putida* HS12 and *Comamonas* sp. CNB-1 carry genes coding for the enzymes that can utilize nitrobenzene and chloronitrobenzene, respectively, and the pathways are identical as reported in strain JS45.

The main aim of the present investigation is to understand more about how the genes that encode the novel enzymes involved in the initial partial reductive pathway for the degradation of nitrobenzene or chloronitrobenzene, viz., nitrobenzene nitroreductase, hydroxylaminobenzene mutase, and 2-aminophenol 1,6-dioxygenase, are transferred during evolution, and how the enzymes perform catalysis. In view of this, novel computational methods are used in gathering data either from the literature or by mining the data resources, and analyzed the available data. In an attempt to provide an insight
into the details of occurrence and evolution of the selected enzymes-degrading nitroaromatics, following are objectives of the present study.

- Evolutionary analysis of the selected enzymes, viz., nitrobenzene nitroreductase, hydroxylaminobenzene mutase, and 2-aminophenol 1,6-dioxygenase of *P. pseudoalcaligenes* JS45.
- Identification of transposable elements and repeat boundaries, if any, in the genome for the selected enzymes by analyzing the sequence similarity.
- Primary, secondary, three dimensional structures and catalytic sites prediction of the selected enzymes using computational tools.

Presently, only one enzyme three dimensional models in two different bacteria have been elucidated. As the increasing number of degradable genes which encode enzymes in bacteria, it is necessary to develop three dimensional structures for detailed analysis of enzyme functional studies. In order to provide additional data to those structurally undefined sequences, the evolutionary conserved sequence positions and their different roles must be characterized using computational methods. Moreover, this information is useful when modeling proteins of newly isolated bacteria which degrade nitroaromatic compounds. In addition, this information is also useful for further characterization of genes and their origin in evolution.

The selected enzyme sequences were retrieved from the database for analyzing their distribution in other bacterial species, and distinguished the homology of different bacteria containing identical genes performing the same function. As a part of finding homology identification, the phylogenetic algorithm has been exploited for the three different enzymes in *P. pseudoalcaligenes* JS45 which degrade nitrobenzene. The phylogram results suggest that the enzymes present in JS45 are clearly homologous to *Comamonas* sp. CNB-1 and *P. putida* HS12. The evolutionary analysis and comparisons
of the genetic and metabolic pathways clearly suggest that the genes and the gene clusters are recruited and assembled via various horizontal transfer mechanisms either distributed on chromosome or in plasmids. Two independent plasmids, pNB1 and pNB2 of *P. putida* HS12, and pCNB1 of *Comamonas* sp. CNB-1 that degrade nitrobenzene and chloronitrobenzene, were analyzed to clearly distinguish whether the phenotype is plasmid-borne or chromosome-borne in the bacterial strains selected. The significance of the present investigation is to find whether the genes are transferred from chromosome to plasmid or plasmid to chromosome. The structure and properties of the enzyme, nitrobenzene nitroreductase, in *P. pseudoalcaligenes* JS45 and *Comomonas* sp. CNB-1 were analyzed to understand the valuable insights about the enzyme.

The evolutionary analysis of enzymes nitrobenzene degrading bacteria and prediction of catalytic sites have been successfully characterized for one of the selected enzymes, nitrobenzene nitroreductase, from the bacterial strains that degrade both nitrobenzene and chloronitrobenzene, and the salient features of the present study are as follows:

- The primary structure of nitrobenzene nitroreductase in both the stains contain alanine, leucine and arginine in higher composition, and the secondary structure showed almost equal number of helices and β-sheets.

- The catalytic sites in nitrobenzene nitroreductase from the selected two bacterial strains were identified. Furthermore, many of the residues involved in substrate binding, are conserved between these two organisms, and are shown to be highly conserved between distantly related enzymes.

- Combination of evolutionary information of three enzymes of *P. pseudoalcaligenes* JS45, and catalytic sites in nitrobenzene nitroreductase of *P. pseudoalcaligenes* and *Comamonas* sp. CNB-1 help to know how during the
substrate catalysis the enzyme active sites are stabilized, or modify the PK(a) values of other residues to provide more effective acids and bases.

- This observation clearly suggests that the enzyme coding genes which degrade nitrobenzene in *P. pseudoalcaligenes* JS45 would have transposed from plasmid to chromosome and the outcome of the present study can be useful for further molecular genetic research.