The work presented in the thesis mainly involved biochemical, physiological and molecular studies on bacterial degradation of some of the nitroaromatic compounds that are widely recognized as serious environmental pollutants.

Arthrobacter protophormiae RKJ100, previously isolated from a pesticide contaminated agricultural field, was reported to degrade *o*-nitrobenzoate (0.5 mM) reductively, via the formation of *o*-hydroxylaminobenzoate and anthranilate. In this study, strain RKJ100 was adapted to tolerate and degrade unusually high concentrations of *o*-nitrobenzoate. Two different physiological changes were observed during this unusual tolerance i.e., change in fatty acid profile and branching of *o*-nitrobenzoate degradation pathway. An unidentified novel fatty acid appeared in the fatty acid profile of strain RKJ100 when grown on higher concentrations of *o*-nitrobenzoate. A novel pathway has also been proposed for *o*-nitrobenzoate degradation using different analytical and biochemical techniques such as TLC, GC, HPLC, enzyme assays, resting cell studies, etc. At higher concentrations, *o*-nitrobenozate was degraded via the formation of *o*-hydroxylaminobenzoate that is further degraded to form two different aromatic terminal intermediates i.e., anthranilate and 3-hydroxyanthranilate by the action of a reductase and a mutase, respectively. This novel pathway has been published and included in the University of Minnesota Biocatalysis/Biodegradation Database. To study the above-mentioned physiological responses, transposon mutants of strain RKJ100 were generated that could not tolerate or utilize *o*-nitrobenzoate. Characterization of these mutants is a subject of further investigation.

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Another aspect of research focused on bacterial chemotaxis toward nitroaromatic compounds. In recent years chemotaxis has been argued to play an important role in bioremediation by increasing the bioavailability of pollutants to bacteria. A laboratory isolate, *Ralstonia* sp. SJ98, was previously reported to be chemotactic toward those nitroaromatic compounds (*p*-nitrophenol, 4-nitrocatechol, *o*-nitrobenzoate, *p*-nitrobenzoate and 3-methyl-4-nitrophenol) that also served as sole source of carbon and energy to the strain. The chemotactic response of this strain was tested toward several other nitroaromatic compounds using drop assay and swarm plate assay, and was also quantified using capillary assay. The work demonstrated chemotaxis of strain SJ98

toward seven different nitroaromatic compounds i.e., *o*-dinitrobenzene, *m*-dinitrobenzene, *o*-nitrophenol, *m*-nitrophenol, 2,4-dinitrophenol, 2,5-dinitrophenol, 2,6-dinitrophenol, *m*-nitroaniline, 2,3-dinitrotoluene, and 3,5-dinitrobenzoic acid that were co-metabolized in the presence of an alternate carbon source. However, this bacterium was not chemotactic toward those nitroaromatic compounds (*o*-nitrophenol, *p*-nitroaniline, and 2,3-dinitrotoulene) that were neither mineralized nor co-metabolized. The transformation products of the above-mentioned NACs were analyzed using TLC, GC and HPLC. In GC studies, metabolites were detected in case of *o*-dinitrobenzene, 2,4-dinitrophenol, 2,5-dinitrophenol, 2,6-dinitrophenol, and 3,5-dinitrobenzoic acid, which also attract the strain SJ98 chemotactically. Similarly in HPLC studies, metabolites were detected in case of only those nitroaromatic compounds that acted as chemoattractants to strain SJ98. The results obtained clearly indicated a correlation between chemotaxis of strain SJ98 toward nitroaromatic compounds and their degradation/transformation.

There are two pathways known for *p*-nitrophenol degradation by bacteria. In gram-positive bacteria, *p*-nitrophenol is degraded via the formation of 4-nitrocatechol and 1,2,4-benzenetriol. However, in gram-negative bacterial stains, *p*-nitrophenol degradation predominantly proceeds via benzoquinone and hydroquinone formation. Different biochemical studies such as resting cells studies, induction studies and enzyme assays demonstrated that in strain SJ98, *p*-nitrophenol is degraded via 4-nitrocatechol and 1,2,4-benzenetriol. 1,2,4-Benzenetriol was found to be the terminal aromatic intermediate that is converted to maleylacetate by the activity of 1,2,4-benzenetriol 1,2-dioxygenase. Maleylacetate is further reduced by maleylacetate reductase and enters into TCA cycle. In order to clone the gene(s)/gene cluster involved in *p*-nitrophenol degradation, degenerate PCR primers were constructed from conserved regions after aligning known protein sequences of 1,2,4-benzenetriol 1,2-dioxygenase. The amplicon (partial 1,2,4-benzenetriol 1,2-dioxygenase gene) was cloned and sequenced, and subsequently used as a probe in Southern hybridization in order to elucidate the complete gene(s)/cluster. Following southern hybridization, a probed region (~7 kb) was cloned and the clone library was screened using the same probe. A positive clone showed 1,2,4-benzenetriol 1,2-dioxygenase and maleylacetate reductase activities and contained an insert of 6.897 kb that was sequenced by primer walking. The sequence was annotated and submitted to GenBank (Accession no. AY574278). Seven open reading frames were

identified in the cloned fragment, out of which six significantly matched with proteins of known function, i.e., 1,2,4-benzenetriol 1,2-dioxyegnases, maleylacetate reductases, ferredoxin subunit of nitrite reductases and ring-hydroxylating dioxygenases, transcriptional regulator of GntR family, aldehyde dehydrogenase and ATP binding ABC transporter. This is the first report demonstrating cloning of 1,2,4-benzenetriol 1,2-dioxygenase involved in *p*-nitrophenol degradation.

A field study was conducted to determine the potential of *A. protophormiae* RKJ100 in soil under natural conditions. For this purpose agricultural soil sprayed with organophosphate pesticides parathion and methyl parathion, and hence contaminated with *p*-nitrophenol, was collected and filled in plots measuring 1 m x 1 m x 30 cm. Bioaugmentation was carried out by adding bacterial cells immobilized on carrier material (corncob powder) in one plot and free cells in another plot. The soil in each plot was thoroughly mixed with the culture to ensure uniform distribution of cells in the soil. Un-inoculated soil and soil mixed with corncob powder alone served as controls. Soil samples were removed at different time intervals and analyzed for *p*-nitrophenol depletion by HPLC. It was shown that *p*-nitrophenol could be depleted within 5 days from soil that was bioaugmented with immobilized cells on carrier material, whereas in the soil where free cells were added, 30 days were required for complete *p*-nitrophenol degradation. *p*-Nitrophenol persisted for 30 days in the control plots.

In order to monitor the changes in microbial community structure during bioremediation process, Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was performed using 16S rDNA universal primers (8f and 926r). Microbial community structure was determined by comparing the terminal restriction fragments (T-RFs) obtained by restriction digestions (*Hae*III, *Msp*I and *Rsa*I) of community 16S rDNA (amplified using above said primers) with the T-RFs generated from *in silico* amplification and digestions of RDP II alignments (version 8.0). Restriction enzyme *Hae*III was selected for further analysis since it gave maximum resolution and maximum numbers of T-RFs. To monitor the changes in the microbial community structure during the bioremediation process, T-RFLP profile was generated from experimental and control plots and analysis of the data revealed that during the bioremediation process, microbial community structure remained stable. Taken together these studies suggest that *Arthrobacter protophormiae* RKJ100 is an efficient *p*-nitrophenol degrader and can be applied for *in situ* bioremediation of *p*-nitrophenol contaminated soils.