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SUMMARY OF THE THESIS

The study presented in this dissertation deals with various aspects of biochemical, physiological and molecular characterization of bacterial degradation of nitroaromatic compound (*o*-nitrobenzoate and *p*-nitrophenol) and chloro-nitroaromatic compound (2-chloro-4-nitrophenol). It covers a wide spectrum of studies ranging from *in vitro* studies as *in vivo* degradation of nitroaromatic pollutant under natural environments. Majority of the studies were carried out with two of the laboratory isolates viz., *Arthrobacter protophormiae* RKJ100 and *Burkholderia* sp. SJ98. Both the strains were isolated from pesticide contaminated agriculture soil and have been previously characterized to utilize a number of nitroaromatic compounds including ONB, PNP, 4-NC etc.

During the present study strain RKJ100 was subjected to molecular characterization of ONB degradation and *in situ* degradation of PNP in naturally contaminated agriculture fields. The degradation of ONB in strain RKJ100 was shown to follow different pathways when the substrate was presented at different concentrations. At higher concentrations (5 mM and above), 3-hydroxyanthranilate was found to be central intermediate and served as the substrate for ring hydroxylating dioxygenase viz., 3-HAA dioxygenase, whereas at lower ONB concentration the degradation proceeded via formation of anthranilate. During the present study it was observed that strain RKJ100 could grow on ONB even upto 200 mM concentrations. Studies for growth and physiological adaptation of clearly demonstrated that the ability of strain RKJ100 to grow at such high concentrations is associated with significant alteration of the cell morphology and total cellular fatty acid composition. Further, studies for cloning and characterization for gene(s)/ gene cluster involved in degradation of ONB by strain RKJ100 were attempted; however, only limited success could be achieved. A transposon based random mutagenesis approach indicated that the disruption of a 'LysR type regulator' results in loss of ONB degradation function. Similarly, disruption of an 'endo- -beta N acetylglucosaminidases' gene results in sensitivity towards higher concentration of ONB. Interestingly, the ONB sensitive mutant also shows sensitivity towards two other chemical analogues viz., *p*-hydroxybenzoate and benzoate, thereby indicating towards a common mechanism for tolerance of strain RKJ100 towards

aromatic compounds. Although an ONB degradation gene could not be cloned during this study, however, the identification of a 'LysR type regulator' disruption in ONB$^-$ mutant could is justifiable. It is note-worthy that in another ONB degrading strain viz., *Pseudomonas fluorescence* KU-7, a 19 Kb genomic DNA fragment harbouring some of the genes of ONB degradation also shows presence of a 'LysR type regulator'. In strain KU-7 this regulator is proposed as the putative positive response regulator of ONB degradation gene cluster. Further characterization of some other ONB$^-$ mutants would ascertain its role in ONB degradation.

The other study was initiated with the objective of isolation and characterization of some chloro-nitroaromatic compound degrading bacterial isolates. For this a few chloro-nitrophenols (e.g. 2-chloro-4-nitrophenol, 4-chloro-2-nitrophenol, 4-chloro-3-nitrophenol etc.) were used as the target compounds. A number of laboratory isolates (previously isolated and characterized in the lab) were test for metabolic activity on these compounds. Also some pesticide contaminated soil samples were used for enrichment studies. The initial screening resulted in determination of metabolic activity of strain SJ98 and two other lab isolates on the above compounds. Strain SJ98 could completely degrade 2C4NP as sole source of carbon and energy; however, the other test chloro-nitrophenols were only co-metabolically transformed in presence of alternative carbon source(s). The biochemical characterization was carried out to elucidate the catabolic pathway involved in degradation process. The results obtained with analytical studies on samples collected from growth studies, resting cell studies, indication-inhibition studies and enzyme activity assays clearly demonstrated that the degradation of 2C4NP in strain SJ98 is initiated by a relaxed substrate specific- reductive dechlorination leading to formation of *p*-nitrophenol as the first degradation intermediate. The further degradation proceeds via oxidative pathway (as observed by the nitrite release in the growth medium) with formation of bi-phenolic and tri-phenolic intermediates. The tri-phenolic intermediate i.e. 'benzenetriol' acts as the ring-hydroxylation substrate.

This molecular characterization of 2C4NP degradation in strain SJ98 was carried out by cloning of an 41.09 Kb genomic DNA fragment that harboured the complete gene set required for the mineralization of 2C4NP (in resting cell studies). The sequence annotation of this 41.09 Kb DNA fragment revealed the presence of a 'FMN- dependent reductase' and 'Cytochrome C type I reductase'; that might act as the relaxed substrate

specific reductive dechlorinase. Further, this 41.09 Kb fragment also harbours genes corresponding to 'PNP hydroxylase', '4-NC monooxygenase', 'Benzenetriol dioxygenase' and 'Maleylacetate reductase' constituting the complete degradation operon. This 41.09 Kb DNA fragment was also found to harbour genes that showed a very high level of amino acid sequence identity with 'Hydroquinone dioxygenase' and 'Hydroxymuconic semialdehyde dehydrogenase'. Conventionally, the microbial degradation of PNP has been reported to proceed via either 4-NC and BT or HQ and HMS, therefore, it was a unique and novel observation to have genes corresponding to both the pathways with a single genetic locus. These genes have been cloned and expressed individually in *E.coli* based heterologous expression system to carry out biochemical characterization. The heterologous expression clone for PNP hydroxylase could perform PNP to 4-NC conversion during resting cell studies. This result clearly demonstrates the successful cloning of gene cluster involved in degradation of 2C4NP and PNP.

The eventual objective of all the biochemical and molecular characterization of bacterial degradation of the above xenobiotic chemical pollutant is to develop a bioremediation process that could be used for decontamination under natural environments. With this objective in mind, a large scale field trial was carried-out, wherein an agriculture field was bioaugmented with strain RKJ100 for degradation of PNP. The results clearly demonstrated that bioaugmentation led to significantly enhanced rate of pollutant disappearance as compared to the non-bioaugmented control field. Importantly, the bioaugmentation of degradative strain (RKJ100) did not cause any significantly adverse change(s) in the naturally occurring microbial communities.

Further, the applicability of RKJ100 bioaugmentation for PNP degradation was tested using soils with characteristically distinct physico-chemical properties. These soils did also differ significantly in terms of their respective indigenous microbial community structure. These soils externally spiked with known concentrations of PNP and then distributed in microcosms that were treated by RKJ100 bioaugmentation. Control microcosms (non-bioaugmented and bioaugmented in sterilized soils) were used to evaluate the performance of RKJ100 in different soil types. The results for PNP disappearance clearly showed an improved rate of pollutant degradation by bioaugmentation. Interestingly, the kinetics of PNP disappearance in sterilized soils was slightly better than in the non-sterilized

soil. This difference could be explained on the basis of possible antagonistic effect of native microbial communities. During the study, RKJ100 cell survival was found to be most important factor influencing the rate and efficiency of pollutant removal. This observation opens a new avenue for studying PNP degradation with repeated bioaugmentation of RKJ100. However, it could still be concluded that the *in situ* bioremediation with field scale study and microcosm study have evidently proved 'RKJ100 bioaugmentation' as an environmentally safe and effective method for decontamination of soils and sites contaminated with PNP. Further, the method is application to diverse soil types.