Environmental pollution occurs due to the release and subsequent accumulation of toxic xenobiotic contaminants, generally resulting from anthropogenic activities. Among various xenobiotics the nitrophenolic compounds are the target molecules of this study due to their high recalcitrance, acute toxicity and predominant presence in both agricultural contaminants and industrial effluents. The work presented in the thesis mainly involved biochemical and molecular studies on bacterial degradation of various nitrophenols such as 4-nitrophenol (4-NP), 2-chloro-4-nitrophenol (2-C-4-NP), 2,4-dinitrophenol (2,4-DNP) and 2,4,6-trinitrophenol (TNP) that are widely recognized as serious environmental pollutants. It also focused on the study of culturable bacterial diversity of pesticide contaminated soils from different ecological niches.

Strain RKJ300 isolated from a pesticide-contaminated site of Punjab using enrichment culture technique, was capable of degrading all the above-mentioned nitrophenolic compounds. The microorganism has been characterized as a novel species of the genus *Rhodococcus* using polyphasic taxonomy and named as *Rhodococcus imtechensis* strain RKJ300^T. The strain was metabolically versatile since it evolved various pathways for degrading or detoxifying these compounds. It degrades 4-NP via the formation of 4-nitrocatechol and 1,2,4-benzenetriol, as evident from various spectroscopic studies and enzymatic assays. In addition, the complete degradation pathway of 2-C-4-NP has been deciphered wherein release of nitrite ions was followed by chloride ions, and the intermediates identified were 2-chlorohydroquinone and hydroquinone. This is the first report of complete aerobic degradation of 2-C-4-NP by a bacterium. Besides mononitrophenols the microorganism developed catabolic pathways for poynitrophenols degradation. Strain RKJ300 could utilize 2,4-DNP as sole source of carbon and energy. Biochemical characterization of its degradation pathway indicated the presence of hydride-Meisenheimer complex, 2,4-dinitrocyclohexanone and 4,6-dinitrohexane as metabolites. The strain could also transform TNP into its hydride Meisenheimer complex which accumulates in the media as an orange-coloured dead end metabolite. Moreover, preliminary chromatographic analyses showed the capability of strain RKJ300 to transform a number of other substituted nitrophenols such as 3-nitrophenol, 2-amino-4-nitrophenol, 3-methyl-4-nitrophenol etc. suggesting the broad substrate specificity of the catabolic enzymes.

Furthermore, to determine the potential of *R. imtechensis* RKJ300 in regard to degradation of mixture of nitrophenols in soil and subsequently to develop an efficient bioremediation technology for restoring nitrophenols contaminated sites, soil microcosm studies were performed. The strain has been shown to completely degrade all the three nitrophenols in mixture within 20 d suggesting that the different degradation pathways could work simultaneously in this bacterium without considerable inhibition. Population of the degradative strain as monitored by plate count and gene-tracking techniques was found to be fairly stable in soil over the period of bioaugmentation study. Consequently, the versatility and stability of strain RKJ300 make it a suitable microorganism for its use in the treatment of industrial wastewaters and soils containing mixture of substituted nitrophenolic compounds. This work may also serve as a model microcosm study in order to design strategies for degradation of more complex and mixtures of structurally related compounds.

In contrast to the detailed biochemical characterization of 4-NP degradation, there are only few reports related to the gene(s) involved in degradation. Molecular characterization of the above degradation pathways may help in understanding their regulation and evolution. For this, genes were amplified from genomic DNA of strain RKJ300 using degenerate primers, sequenced and analysed in terms of homology and evolutionary relatedness with other related degradation genes. Monooxygenase (580 bp) and reductase (410 bp) components of 4-NP/4-NC monooxygenase and benzenetriol dioxygenase (570 bp) genes involved in 4-NP degradation were partially amplified and BLAST homology search exhibited 97% sequence similarity with oxygenase component of phenol hydroxylase (*npcA,* 1587 bp), 99% similarity with its reductase component (*npcB*, 558 bp) and 100% similarity with benzenetriol dioxygenase (*npcC*, 903 bp) genes present in 7.5 kb gene cluster reported from *Rhodococcus opacus* SAO101 (AB154422). Upper pathway genes regulating the 2,4-DNP degradation were also amplified and sequenced. BLAST homology search revealed that the 840 bp amplicon was 100% similar to hydride transferase I (NpdC) of *R. opacus* DNP14-9 (AY027580) and 98% to that of *R. opacus* HL 24-1 (AY027577). Amplified 680 nucleotide fragment showed 99%, 97% and 66% similarities with NADPH-dependent F_{420} reductases (NpdG) of *Rhodococcus* sp. PN1 (AF031325), *R. opacus* HL PM-1 (AF323606) and *Pimelobacter simplex* FJ2-1A (AY029522) respectively. The 1050 bp amplicon exhibited 99% similarity with hydride

transferase II (NpdI) or F_{420}-dependent picric acid reductase of *Rhodococcus* sp. PN1 (AF031325), 98% with *R. opacus* HL PM-1 (AF323606) and 70% with *Pimelobacter simplex* FJ2-1A (AY029522). Partial genomic DNA library of strain RKJ300 was constructed following Southern hybridization of the digested genomic DNA using the above gene segment(s) as radiolabeled probe(s). However, screening of genomic library by colony/dot blot hybridization could not detect any positive clone. In future, efforts could be made to understand the genetic system by constructing genomic libraries using different strategies.

This study also included the molecular characterization of 4-NP degradation pathway in another lab isolate *Burkholderia* sp. strain SJ98. The strain degraded several nitroaromatic compounds including 4-NP via the formation of 4-nitrocatechol and 1,2,4-benzenetriol. A genomic DNA library of strain SJ98 was constructed using a cosmid vector (SuperCos 1) with an average insert size of 25-40 kb from a commercial house. Using the partially amplified (540 bp) gene for benzenetriol dioxygenase, a lower pathway gene of 4-NP degradation, as homologous probe, the library was screened. Results obtained from colony and dot blot hybridization, PCR amplification, sequencing and restriction digestion indicated the presence of five positive clones harbouring inserts (approx. 36-40 kb) that encoded the full length gene. Since gene(s) involved in degradation are known to exist in clusters, the upper pathway genes are expected to be located downstream or upstream of the lower pathway gene encoded by the cosmid clones. Therefore, the largest insert was sequenced commercially by primer walking, aligned manually and annotated. The 41 kb sequenced fragment revealed the presence of a total of thirty five putative ORFs among which the 4-NP gene cluster could be positioned. This cluster comprises of oxygenase and reductase components of phenol hydroxylase, benzenetriol dioxygenase, maleylacetate reductase, aldehyde dehydrogenase and a regulator protein belonging to the GntR family. The proposed gene cluster was also compared to the reported 4-NP gene cluster(s) of *R. opacus* SAO101 and *Arthrobacter* sp. JS443 and was found to be clearly different from them in terms of positional sequence, transcriptional orientation and distances between each of the genes. The DNA segment also encoded 4-hydroxybenzoyl-CoA thioesterase which catalyzes the final step in the 4-chlorobenzoate degradation pathway wherein 4-chlorobenzoate is converted to 4-hydroxybenzoate in certain soil bacteria. In addition,

BLAST homology search indicated the presence of some other ORFs with putative functions such as peroxidase, catalase, cytochrome c, chlorohydrolase (involved in atrazine degradation pathway).

Microbial communities are critical components of soil and may be the earliest predictors of soil quality changes due to human interventions. Microorganisms that are isolated from contaminated soil may harbour the ability to degrade the xenobiotic contaminant as well. In view of this, another aspect of the present work was to characterize the culturable bacterial diversity from different contaminated sites. For this purpose, soil samples were collected from a pesticide contaminated agricultural field of Punjab and also from contaminated sites of cold Himalayan valleys as regard to bioremediation of different ecological niches. The bacterial diversity, as determined using biochemical characterization and FAMEs analysis of the isolates, revealed that the species diversity was reduced in pesticide treated agricultural soil when compared to that of untreated soil samples and bacterial isolates mostly belonged to the members of the genera *Bacillus*, *Pseudomonas* and *Arthrobacter*. The same sample when subjected to enrichment using 4-NP as sole carbon and energy source *Rhodococcus imtechensis* sp. nov. was isolated and characterized using polyphasic taxonomy as mentioned earlier. Analysis for culturable bacterial diversity of the other sample of cold environment demonstrated that the microbial population is dominated by psychrotolerant Gram-positive bacteria and among them two of the strains have been proposed as novel species (*Bacillus lehensis* sp. nov. and *Citricoccus lehensis* sp. nov.).

Considering the importance of isolation of bacterial strains for bioremediation of cold ecosystem, the above isolates were also checked for their ability to degrade different nitroaromatic compounds. Unfortunately, none of them could degrade any of the compounds completely; however, a strain of *Acinetobacter lwoffii* LS2 exhibited inducible pigment producing ability when grown on methanol as sole carbon and energy source. Data obtained from molecular and biochemical characterization suggested that the organism converted methanol via formaldehyde into CO_2 and water. The pink pigment produced by strain LS2 was characterized as bacterioruberin-like carotenoid molecule using various spectroscopic methods. This aspect of the study gains marked significance in regard to isolation of novel species as well as for providing an insight into the culturable bacterial diversity of contaminated environment.