Several nitroaromatic compounds have become widely recognized as serious environmental contaminants. This study was carried out to have a better understanding of the biodegradation of nitroaromatic compounds at the biochemical and molecular levels with a special emphasis on *p*-nitrophenol (PNP), 4-nitrocatechol (NC) and *o*-nitrobenzoate (ONB).

Some bacterial strains capable of utilizing PNP were isolated from agricultural fields by enrichment techniques. Two of these isolates were designated as RKJ100 and RKJ200. RKJ100 was found to utilize PNP, NC, ONB, and *p*-hydroxybenzoate (PHB) and RKJ200 was found to utilize PNP, NC, and PHB as sole carbon and energy sources, respectively. These soil isolates were identified and characterized by biochemical, morphological and chemotaxonomical tests. RKJ100 was identified as *Arthrobacter protophormiae* and RKJ200 as *Burkholderia cepacia* (earlier known as *Pseudomonas cepacia*).

Both the microorganisms were characterized for the presence of plasmid(s). RKJ100 was found to harbour a large plasmid of approximately 65 kb in size whereas RKJ200 harboured a plasmid of 50 kb in size. Several mutant strains were obtained from RKJ100 and RKJ200 by spontaneous and mitomycin C treatment. Plasmid DNA isolations on such derivatives indicated that they were devoid of the plasmids and had simultaneously lost the capability to degrade PNP, NC and ONB in case of RKJ100 and PNP and NC in case of RKJ200, respectively. These studies indicated that the degradation of these aromatic compounds in RKJ100 and RKJ200 were plasmid-encoded. Furthermore, the genes for PHB degradation were found to be chromosomally-encoded in case of RKJ100 and plasmid-encoded in case of RKJ200.

The catabolic pathways for the degradation of PNP, NC, ONB and PHB were studied in the above strains. On the basis of growth studies it was indicated that RKJ100 and RKJ200 degraded PNP and NC by an oxidative route as shown by nitrite release; ONB degradation on the other hand occurred via an aerobic reductive route since ammonia could be detected in culture supernatants rather that nitrite. PNP was degraded via the formation of benzoquinone (BQ) and hydroquinone (HQ) which was further cleaved to generate β-ketoadipate as one of the lower pathway intermediates. However, a novel pathway could be established in RKJ100 and RKJ200 for the degradation of NC. Several lines of evidences including thin layer chromatography, gas chromatography, nuclear magnetic resonance spectroscopy, gas chromatography-mass spectrometry, quantification of intermediates by

high performance liquid chromatography and enzyme assays unequivocally proved that NC was degraded via the formation of 1,2,4-benzenetriol (BT) and HQ as key intermediates in an oxidative pathway which involved a reductive dehydroxylation reaction. 1,2,4-Benzenetriol was the initial intermediate formed after removal of nitrite from NC which was oxidized to 2-hydroxy-1,4-benzoquinone (HBQ) as the next step in the pathway. Reductive dehydroxylation of HBQ resulted in the formation of BQ which was converted to HQ by simple enzymatic reduction. Hydroquinone was the substrate for ring cleavage and resulted in the formation of γ-hydroxymuconic semialdehyde and β-ketoadipate as the lower pathway intermediates. The unprecedented step of NC degradative pathway was the reductive dehydroxylation of HBQ into BQ which was the novel reaction in the degradation of NC by RKJ100 and RKJ200.

Strain RKJ100 degraded ONB which involved the reduction of this nitroaromatic compound as evident from the release of ammonia in the culture medium. Chemical characterization of intermediates clearly showed that the degradation of ONB proceeded by a two-electron reduction of the nitro moiety yielding *o*-hydroxylaminobenzoate and anthranilic acid as key intermediates of the novel pathway. Quantitation of the intermediates, inhibition studies and sequential and back induction studies showed that anthranilic acid was produced as the terminal intermediate of a catabolic energy yielding pathway and not as a side reaction taking place concurrently; anthranilic acid was cleaved to generate β-ketoadipate, as one of the lower pathway intermediate.

The pathway for PHB degradation was also elucidated by isolation and characterization of metabolites in the above microorganisms. It was apparent that PHB was converted to protocatechuate (PC) which was cleaved to generate β-ketoadipate.

In order to have a better understanding of the molecular basis of the degradation, cloning of these gene(s) was achieved. The plasmid-encoded genes from RKJ100 were [illegible] broad-host-range vector pLAFR3 and expressed in *E. coli* JM109. The plasmid [illegible] RKJ100 was screened on the basis of nitrite release assay and the clones which [illegible] nitrite release from PNP were studied in more detail. Several putative clones from [illegible] characterized by performing restriction endonuclease digestions on the purified [illegible] and they were found to contain inserts of 2-7 kb in size. The entire library of [illegible] conjugally transferred into *Alcaligenes eutrophus* JMP222 for better [illegible] combinant genes. Two putative clones of RKJ100 were then studied for [illegible] bolites from PNP. These studies indicated the formation of HQ from

PNP by these clones suggesting that the initial pathway of PNP degradation had been cloned and expressed in *E. coli*. Results also demonstrated the cloning of PHB degradative genes from RKJ200 in *E. coli* JM109. Furthermore, five clones of RKJ100 in *Alcaligenes eutrophus* JMP222 could transform ONB to anthranilic acid and three clones generated β-ketoadipate from anthranilic acid indicating the cloning of genes of the entire pathway of ONB degradation.