Archana Chauhan (2010). ***Molecular and Biochemical Characterization of p-Nirophenol/chlorontrophenol Degradation pathways of Bacteria.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
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SUMMARY OF THE THESIS

Burkholderia sp. SJ98, a lab-isolate has been shown to utilize different NACs as sole source of carbon and energy. Till date the 4-NP degradation pathway was not fully characterized in this organism. Therefore, in the present study the 4-NP degradation pathway was characterized wherein it was found that strain SJ98 degrades 4-NP via 4-NC. This is the first report of 4-NP degradation in a Gram negative bacteria via 4-NC. Further, the 2C4NP and phenol degradation pathways were also studied which showed that strain SJ98 degrades 2C4NP through two different pathways which differ in their first step of degradation. One pathway proceeds through reductive dechlorination while other oxidative nitrite release. This property is also unique to this bacterium and is also supported by the molecular evidences. Furthermore, Phenol degradation followed a meta cleavage degradation pathway in strain SJ98.

The molecular basis 4-NP degradation pathway in strain SJ98 is not well understood. Two important genes encoding lower pathway enzymes of 4-NP degradation have been cloned in our lab from this strain. However, gene(s) of the upper pathway are yet to be cloned and characterized. In order to clone these genes, phenol hydroxylase a nearest homologues of 4-NP hydroxylase, was taken as a model and degenerate primers designed i.e. Phe_F and Phe_R were used to PCR amplify a fragment of 206 bp from the genomic DNA of *Burkholderia* sp. SJ98, which corresponded to catalytic unit of the mPH. Using this amplicon as a radiolabeled probe a genomic DNA library of strain SJ98 constructed using a cosmid vector (SuperCos 1) with an average insert size of 25-40 kb from a commercial house was screened. Results obtained from colony hybridization, 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. Therefore, the largest insert was sequenced by primer walking, aligned manually and annotated. The ~10 kb sequenced fragment revealed the presence of a total of 12 putative ORFs among which the 4-NP gene cluster could be positioned. This cluster comprises of mPH, ferrodoxin,

oxygenase and reductase components of phenol hydroxylase, BtD, MaR, aldehyde dehydrogenase and a regulator protein belonging to the GntR family. Therefore, the occurrence of two gene clusters encoding two different genes/enzymes for the 4-NP degradation pathway further substantiate our findings for the occurrences of more than one degradation pathway for the same compound.

Another objective of this study was to characterize PnpD encoding MaR, one of the enzymes of the 4-NP degradation lower pathway. For this, the ORF was PCR amplified and cloned in the expression vector pET-28c, and transformed into *E. coli* BL21. Upon induction with IPTG the overexpressed protein designated as PnpD (maleylacetate reductase enzyme, putative product of ORF *pnpD*) was purified by Ni-NTA affinity chromatography. Molecular weight of BtD was found to be 40 kDa by SDS-PAGE. The native molecular weight was determined by gel filtration chromatography and was found to be 40 kDa. This clearly indicated that the protein was a monomer the first report of its kind. Structural informatio available from CD spectra showed that MaR has a mixed secondary structure which was stable over the range of 50-60 °C. The pH and temperature optima for purified MaR were in various buffers. It was found to be soluble up to the concentration of 16 mg ml^{-1} in 20 mM NaCl, 20 mM Tris-Cl, pH 8.0. Attempts to crystallize the protein were successful. The enzyme was crystallized in both native and Se-Met form by sitting-drop vapour diffusion method using PEG3350 as precipitant at 293 K. The crystal belongs to $P2_12_12$ space group, with unit-cell parameters a = 72.91 Å, b = 85.94 Å, and c = 53.07 Å. The diffracted data for the native and Se-Met crystal were collected up to 2.7 Å and 2.9 Å resolution respectively. It's structure is composed of 7 β strands and 15 α helices, and a

number of loops, which together fold into 2 domains that are separated by a deep cleft. The N-terminal α/ß domain (residues 1 to 159) consists of 7 ß strands and 6 helices arranged in a Rossmann-fold topology. The C-terminal domain (residues 160 to 351) comprises helices organized in two helical bundles.

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 and non-culturable bacterial diversity from a pesticide contaminated site. For this purpose, soil samples were collected from a pesticide contaminated soil of IPL Lucknow. The bacterial diversity, as determined by DGGE and using polyphasic taxonomy, 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*, *Burkholderia, Pseudomonas* and *Acenitonacter.* The same sample when subjected to enrichment using 4-NP led to the isolation of six different isolates capable of degrading 4-NP. Also, a strain showing antimicrobial activity was also isolated.

This new bacterial strain, displayed potent antimicrobial properties against Gram-negative and Gram-positive pathogenic bacteria. Based on its phenotypical and biochemical properties as well as its 16S rRNA gene sequence, the bacterium was identified as *Paenibacillus* sp. and it was designated as strain RKJ14. The antimicrobials produced by this strain were isolated from the culture supernatant and subsequently partially purified by HPLC and FPLC and further analyzed by and LC-MS. Two antimicrobials were found to be active against Gram-negative bacteria and Gram-positive bacteria. Their antimicrobial potency and proteinaceous nature were confirmed. The antimicrobial peptides, were active against a broad range of pathogenic bacteria, including *Bacillus* spp., *Lactobacillus* spp., *Salmonella typhi, Staphylococcus aureus, Streptococcus pnumoniae* etc. Furthermore, it

tentative sequence need to be elucidated in future work it was found that the peptides have a high degree of post-translational modifications. Thus strain RKJ14 and associated peptide are potentially useful in medical applications.