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Biodegradation of *p*-Nitrophenol and Its Molecular Analysis

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Although processes have been and are being developed for bioremediation of nitroaromatics-contaminated environments, not much is known about the fundamental molecular and biochemical mechanisms by which microorganisms degrade Nitroaromatics. In an effort to elucidate the biochemical and molecular mechanisms of nitroaromatics degradation, *p*-nitrophenol (PNP) was chosen as a model nitrophenolic compound in this study.

p-Nitrophenol (PNP) is a major constituent of parathion (*o,o*-diethyl *o*-*p*-nitrophenyl phosphorothionate) and methylparathion (*o,o*-dimethyl *o*-*p*-nitrophenyl phosphorothionate) which have been used as broad-crop protection insecticides. A PNP-degrading bacterium, *Pseudomonas* sp. strain ENV2030, was isolated from parathion-contaminated soils. The strain is able to mineralize *p*-nitrophenol (PNP) as the sole source of carbon and energy at concentrations up to 6 mM. The chemical analysis of the intermediates accumulated by the mutants blocking PNP catabolism showed that the strain degrades PNP oxidatively via hydroquinone as a ring-cleavage substrate. Taxonomic studies using fatty acid profile and 16S rRNA sequence analyses as well as biochemical tests suggest that the strain is closely related to the genus *Pseudomonas*.

Subcloning, complementation, nucleotide sequence analyses and gene inactivation studies revealed that all the genes required for PNP degradation are located on a 17,856 bp *NotI* genomic DNA fragment. The PNP degradation genes are organized in at least two operons. The upper operon (*pnpAB* operon) contains the PNP 4-monooxygenase gene (*pnpA*) adjacent to a gene for a LysR-type transcriptional activator (*pnpR*) and 1,4-benzoquinone reductase (*pnpB*). PnpA, PNP 4-monooxygenase, catalyzes the initial monooxygenation of PNP resulting in the loss of the nitro group and subsequent production of 1,4-benzoquinone (BQ). The enzyme is a flavoprotein of 410 amino acids exhibiting low homology (32% of overall similarity) to other flavohydroxylases in GenBank. This is primarily due to the unique monooxygenase reaction of PnpA, catalyzing the removal of the nitrite group at the *para* position of the phenol structure and replacing it with a molecular oxygen. The highly conserved FAD- and ADP-binding motifs found in a number of flavohydroxylases are also identified. PnpB (a 174 amino acid reductase) reduces BQ to form a ring-cleavage substrate hydroquinone (HQ). The lower operon (*pnpCDE* operon encoding the enzymes responsible for the ring-cleavage of hydroquinone and subsequent further metabolism to form β-ketoadipate) contains the genes for a hydroquinone 1,2-dioxygenase (*pnpC*), γ-hydroxymuconic semialdehyde dehydrogenase (*pnpD*), and maleylacetate reductase (*pnpE*) adjacent to a gene for another putative LysR-type transcriptional activator (*pnpS*). PnpC, hydroquinone 1,2-dioxygenase, is a 290 amino acid polypeptide showing a significant level of homology (64.8% of average similarity) to 1,2,4-benzenetriol 1,2-dioxygenases: TftD from *Burkholderia cepacia* AC1100 and HadC from *Ralstonia pickettii* DTP0602. However, although ring-fission reaction of PnpC is completely different from that of intradiol or extradiol ring-cleavage dioxygenase family, evolutionary and motif analyses reveal that PnpC falls into a intradiol ring-cleavage dioxygenase family. An invariant iron-binding motif found in intradiol ring-cleavage dioxygenases is also conserved in PnpC.

PnpD is a 487 amino acid dehydrogenase exhibiting high homology of 61.4% of average similarity to other *meta*-cleavage aldehyde dehydrogenases including XylG from plasmid pWWO and DmpC from *P. putida* CF600. A conserved region implicated for the aldehyde dehydrogenase active site and a putative NAD-binding motif are found. PnpE is a 355 amino acid protein showing a significant level of homology (75.6% of similarity) to maleylacetate reductase (TftE) from *Burkholderia cepacia* AC1100. A putative NAD-binding motif of PnpE is identified. The amino acid sequences of the two regulatory activators, PnpR and PnpS, exhibit homology to that of LysR-type transcriptional activators. The DNA-binding motif, a helix-turn-helix motif, is located near *N*-terminal sequences of both PnpR and PnpS. Gene inactivation and complementation as well as identification transposon insertion points strongly suggest that the two proteins are involved in transcriptional positive control of *pnpAB* operon (for PnpR) and *pnpCDE* operon (for PnpS). Due to the complexity of the PNP degradative operons and the possible involvement of the potential regulatory ORFs found, the insight on the regulatory scheme of PNP catabolism constitutes a good candidate for future study.