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Molecular Analysis of Catabolic Genes for Nitroaromatic compoud

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Nitroaromatics are produced on a massive scale in the manufacture of dyes, plastics, and explosives. Their discharge in wastewater and application as pesticides have broadened their environmental impact and called for solutions for remediation of these toxic compounds. The use of microorganisms to transform or eliminate nitroaromatics has been proposed in effluent treatment and land reclamation.

Pseudomonas putida HS12 isolated from soil was able to use nitrobenzene (NB) as a sole source of carbon, nitrogen, and energy and was found to possess a partial reductive pathway for the degradation of NB (1). *Pseudomonas putida* HS12 was found to carry two plasmids, pNB1 and pNB2. The activity assay experiments of wild-type HS12(pNB1 and pNB2), a spontaneous mutant HS121(pNB2), and a cured derivative HS124(pNB1), demonstrated that the catabolic genes coding for the nitrobenzene-degrading enzymes, designated *nbz*, are located on the two plasmids, pNB1 and pNB1. The genes *nbzA*, *nbzC*, *nbzD*, and *nbzE*, encoding nitrobenzene nitroreductase, 2-aminophenol 1,6-dioxygenase, 2-aminomuconic 6-semialdehyde dehydrogenase, and 2-aminomuconate deaminase, respectively, are located on pNB1 (59.1-kb). Meanwhile, the *nbzB* gene encoding hydroxylaminobenzene mutase, a second step enzyme in the nitrobenzene catabolic pathway, was found in pNB2 (43.8-kb). Physical mapping, cloning, and functional analysis of the two plasmids and their subclones in *E. coli* strains revealed in more detail the genetic organization of the catabolic plasmids pNB1 and pNB2. The genes *nbzA* and *nbzB* are located on the 1.1-kb *Sma*I-*Sna*BI fragment of pNB1 and the 1.0-kb *Ssp*I-*Sph*I fragment of pNB1, respectively, and their expressions were not tightly regulated. On the other hand, the genes *nbzC*, *nbzD*, and *nbzE*, involved in the ring cleavage pathway of 2-aminophenol, are localized on the 6.6-kb *Sna*BI-*Sma*I fragment of pNB1 and clustered in the order of *nbzC-nbzD-nbzE* as an operon. The *nbzCDE* genes, which are transcribed in the opposite direction of the *nbzA* gene, are coordinately regulated by both nitrobenzene and a positive transcriptional regulator which seems to be encoded on pNB2 (2).

The amiophenol meta cleavage operon of *Pseudomonas putida* HS12 encodes a set of enzymes which transform 2-AP (2-aminophenol) to TCA cycle intermediates, pyruvate and acetyl CoA via extra diol cleavage of 2-AP. The genetic organization of the AP operon was characterized by cloning of the meta-cleavage genes into an expression vector and identified their products in *E. coli* by functional analysis. Functional analysis, as well as comparison study of amino acid sequence deduced from whole DNA sequence, showed that the AP cleavage

operon contains 11 genes, *nbzR*, *nbzCaCbDGFEIH*, and unidentified 2 ORFs. The *nbzR* gene encodes putative transcriptional regulatory protein. The *nbzCaCbDGFEIH* genes encode β- and α-subunits of 2-AP 1,6-dioxygenase, AMS (2-aminomuconic semialdehyde) dehydrogenase, OP (2-oxopent-4-enoate) hydratase, OC (4-oxalocrotonate) decarboxylase, AM (2-aminomuconate) deaminase, ACT (acetaldehyde) dehydrogenase, and HOV (4-hydroxy-2-oxovalerate) aldolase, respectively. From comparison study of deduced amino acid of each enzyme and genetic organization of AP catabolic operon with other meta cleavage operon including TOL, NAH, and DMP operon, it is concluded that AP catabolic operon has divergently evolved from ancestral meta cleavage operon (3). More details are presented in the papers.

References

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