

## SUMMARY

Among various environmental pollutants, PAHs are one of the most hazardous group of molecule is aimed at studying the degradation of two model classes of PAHs, viz. naphthalene and phenanthrene at the biochemical and molecular levels.

Some bacterial strains capable of mineralizing naphthalene or phenanthrene were isolated by enrichment techniques from soil samples obtained from Gujarat oil fields. Two strains, designated as RKJ1 and RKJ2, were found to be capable of utilizing naphthalene and salicylate as the sole source of carbon and energy and both were identified as *Pseudomonas putida*. Another soil isolate, designated as RKJ4 and identified as *Arthrobacter sulphureus*, was capable of utilizing phenanthrene as the sole source of carbon and energy. Two more organisms, designated as VG45 (identified as *Moraxella* sp.) and VG51 (identified as *Pseudomonas vesicularis*), utilized *o*-phthalate and salicylate which are the intermediates of the naphthalene- and phenanthrene-degrading pathways, respectively; however, they failed to grow on naphthalene or phenanthrene.

On the basis of the growth studies, biochemical tests and plasmid DNA characterization, it was concluded that RKJ1 and RKJ2 are identical and, therefore, only strain RKJ1 was chosen for further studies. Enzyme analyses of the naphthalene-degrading pathway in RKJ1 showed that naphthalene was metabolized via salicylate and catechol which was further catabolized via *meta*-cleavage pathway and the enzymes of the pathway were induced by salicylate. The growth of RKJ4 on various intermediates and enzyme analyses showed that phenanthrene is degraded via 1-hydroxy-2-naphthoate, 2-carboxybenzaldehyde, *o*-phthalate and protocatechuate in this organism. The data available indicated that VG45 degraded salicylate via gentisate pathway.

Naphthalene-degrading organism RKJ1 showed the presence of a large plasmid which was designated as pRKJ1. Several mutants were obtained from RKJ1 by treatment with mitomycin C and NTG. Two types of mutants were obtained. One,

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which did not grow on either naphthalene or salicylate (Nap<sup>-</sup> Sal<sup>-</sup>). The other, which did not grow on naphthalene but were capable of growth on salicylate (Nap<sup>-</sup> Sal<sup>+</sup>). On the basis of the plasmid DNA isolation and colony hybridization studies, it was indicated that the genes for naphthalene degradation may be plasmid-encoded in RKJ1. One mitomycin C-derived Nap<sup>-</sup> Sal<sup>-</sup> plasmid-free derivative of RKJ1 was designated as RKJ5. In order to confirm that all the genes required for naphthalene degradation are plasmid-encoded, attempts were made to transfer plasmid pRKJ1 to other plasmid-free *P. putida* strains by conjugation. Plasmid pRKJ1 was successfully transferred to *P. putida* KT2442 and *P. putida* PaW340 and to the cured derivative RKJ5 by conjugation. All these transconjugants could utilize naphthalene as the sole source of carbon and energy. This confirmed that pRKJ1 encodes all the genes required for naphthalene degradation.

Attempts were made to locate the genes for naphthalene degradation on plasmid pRKJ1 by molecular cloning. Firstly, the plasmid was digested with different restriction enzymes. Southern hybridization of these DNA fragments using pDTG113 as a probe indicated that at least some of the genes of the naphthalene degradation are encoded on a single 25kb *EcoRI* fragment. This 25kb *EcoRI* fragment was then cloned in *E. coli* using a broad-host-range cosmid vector pLAFR3. The presence of the recombinant plasmid, designated as pRKJ3, was confirmed by plasmid DNA isolation and restriction endonuclease digestion. The recombinant plasmid pRKJ3 could eventually be transferred to strains KT2442 and PaW340. The presence of plasmid pRKJ3 in transconjugants was confirmed by plasmid DNA isolation, restriction endonuclease digestion and colony hybridization. These transconjugants, carrying the 25kb *EcoRI* fragment, were capable of utilizing both naphthalene and salicylate as the sole source of carbon and energy. This confirmed that all the genes required for complete degradation of naphthalene are encoded on this 25kb *EcoRI* fragment of pRKJ1. The 25kb *EcoRI* fragment was also cloned into another broad-host-range vector pHC79 in order to obtain large amounts of the DNA.

Presence of a large plasmid(s) was detected in VG45. However, based on the mutagenic studies, this plasmid(s) was found not to be involved in *o*-phthalate degradation. No plasmid(s) was detected in RKJ4 by the methods used in this study;

to construct a genetically-manipulated organism capable of degrading naphthalene and phenanthrene simultaneously, attempts were made to transfer the recombinant plasmid pRKJ3 (encoding the naphthalene-degrading pathway) to RKJ4 (phenanthrene-degrading organism) or to VG45 (*o*-phthalate-degrading organism). However, all these attempts were unsuccessful. In order to achieve the same goal, a whole genomic DNA library of the phenanthrene-degrading genes from RKJ4 was constructed using vector pLAFR3 in *Escherichia coli*. The recombinant plasmids thus obtained were transferred to the naphthalene-degrading organism RKJ1. However, these attempts to obtain genetically-manipulated organisms were also failed.

The studies to test the stability of recombinant plasmid pRKJ3, which was transferred into plasmid-free *P. putida* strain KT2442, revealed that the naphthalene-degrading ability could be lost gradually in non-selective conditions.

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