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Aromatic Hydrocarbon Degradation by Members of the Genus *Rhodococcus*: from Principles to Applications

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The metabolically versatile *Rhodococcus* sp. strain DK17 was originally isolated for the ability to grow on *o*-xylene and has the capability to utilize such aromatic compounds as benzene, alkylbenzenes (toluene, ethylbenzene, isopropylbenzene, and *n*-propyl to nhexylbenzenes), phenol, and phthalates as sole carbon and energy sources (1). The degradation of *o*-xylene and toluene in DK17 is initiated by a ring-oxidizing dioxygenase pathway through 3,4- and 3- and 4-methylcatechol, respectively. DK17 possesses three megaplasmids (380-kb pDK1, 330-kb pDK2, and 750-kb pDK3) and the genes encoding the initial steps in alkylbenzene metabolism are present on pDK2 (2, 3, 4). The *o*-xylene dioxygenase from hydroxylates toluene and ethylbenzene at the 2,3 and the 3,4 positions on the aromatic ring in the ratios of 8:2 and 9:1, respectively (1, 2). On the contrary, attack of the *o*-xylene dioxygenase on *n*-butylbenzene, biphenyl, or naphthalene results in the formation of only a single dihydrodiol compound. These data suggests that the DK17 *o*-xylene dioxygenase possesses the ability to perform distinct regioselective hydroxylations with the size and position of the substituent group determining the number and position of the dihydroxylation on the aromatic ring (5, 6).

Phthalate degradation by DK17 involves oxygenation at carbons 3 and 4 to form 3,4-dihydro-3,4-dihydroxyphthalate (phthalate dihydrodiol), which is subsequently dehydrogenated and decarboxylated into protocatechuate. One interesting aspect of phthalate degradation by DK17 is that, when simultaneously present, benzoate completely inhibits the ability of DK17 to utilize phthalate (7). Benzoate in the medium is depleted to detection limits before utilization of phthalate begins. Transcription of the genes encoding benzoate and phthalate dioxygenase paralleled the substrate utilization profile. Two mutant strains with defective benzoate dioxygenases were unable to utilize phthalate in the presence of benzoate although they grew normally on phthalate in the absence of benzoate (8). The phthalate operons are duplicated and are present on both pDK2 and pDK3. Both the pDK2- and the pDK3-encoded copies are simultaneously expressed and functional in DK17 during growth on phthalate (9).

A novel indigo-producing oxygenase gene, designated *ipoA* (1,197 bp) was characterized from *Rhodococcus* sp. strain T104 (10, 11). Three indigo-negative mutations (A58V, P59L, and G251D) were obtained through random mutagenesis using an *E. coli* mutator strain. Subsequent saturation mutagenesis resulted in the identification of nine and three amino acid substitutions that restore activity in the A58V and P59L mutants, respectively. Activity was not restored in the G251D mutation by any other amino acids. *Escherichia coli* host cells harboring a plasmid with the *ipoA* gene (pKEB1880) has the ability to produce indigo (30.9 ± 0.7 μg/ml) when grown in liquid Luria-Bertani (LB) medium. Interestingly, a single amino acid substitution of alanine by serine at position 58 was found to result in the production of over 1.5 times as much indigo (50.7 ± 4.9 μg/ml) as the wild type. A molecular modeling study suggests that the residues at positions 58, 59, and 251 of the T104 IpoA enzyme are far from the active site, indicating that the mutations must alter the overall structure of the enzyme. Furthermore, our preliminary data revealed that the dried ethyl acetate extracts of the *E. coli* culture expressing the mutant IpoA enzyme had cytotoxic effects to some cancer cells including colon cancer.

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