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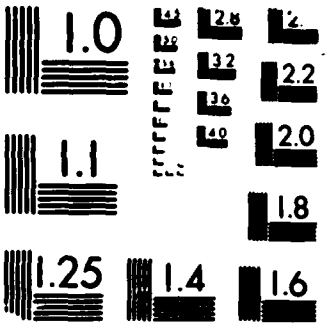

MICROCOPY RESOLUTION TEST CHART

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The objective of the research program was to characterize control mechanisms that exercised negative regulation on the expression of genes for aromatic catabolism in bacteria. The three sets of genes selected for investigation were *ben*, encoding enzymes that convert benzoate to catechol; *cat*, encoding enzymes that convert catechol to citric acid cycle intermediates; and *pca*, encoding enzymes that convert protocatechuate to citric acid cycle intermediates. Our initial approach was to clone the strucutral genes from *Acinetobacter calcoaceticus* and *Pseudomonas putida*, bacteria in which aromatic catabolism has been well characterized, because we knew that regulatory genes frequently flanked the structural genes.

Our efforts were largely successful, and we identified two cloned regulatory genes from *P. putida*. One of these, *pcaR*, exercises positive control over three unlinked gene clusters. The other, *catR*, exercises negative control over the tightly linked *catBC* genes. The latter gene is analogous in many respects to another regulatory gene, also designated *catR*, that we have cloned from *A. calcoaceticus*.

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FINAL TECHNICAL REPORT FOR DDAG29-84-K-0151

"Negative Control of Biodegradation in *Pseudomonas*"

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Cloned genes from *A. calcoaceticus* have provided the most accessible example of negative control. When expressed under a *lac* promoter in *E. coli*, the *benABC* genes enable the cells to rapidly convert benzoate to benzoate diol which accumulates in the culture broth. When expressed in an *A. calcoaceticus* mutant lacking benzoate diol dehydrogenase, the *benABC* genes are almost inactive. We do not know whether the lack of activity is due to transcriptional control or due to inactivation of gene products, and we intend investigate these alternatives.

Analysis of the *pca* gene order revealed remarkable rearrangements that took place as the *A. calcoaceticus* structural genes diverged from their homologs in *P. putida*. For example, genes from the former species are tightly clustered in the order *pcaBDC* whereas their homologs, also tightly clustered, appear in the order *pcaBCD* in *P. putida*. The *pcaE* gene is clustered with and transcribed with the other *pca* genes in *A. calcoaceticus*. In *P. putida*, the *pcaE* gene is separated by more than 15 kilobase pairs from the other *pca* genes, although it remains under control of the *pcaR* gene.

Additional functions remain to be explored in *P. putida*. Among these are benzoate chemotaxis, also under control of the *pcaR* gene, and ketoadipate transport. The latter function appears to be associated with scavanging of an aromatic catabolite during starvation-survival. High-level futile activity of the transport system is lethal to starved cells, and the study of mutants resistant to this effect may give some insight into physiology of starvation.

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