JOURNAL OF BACTERIOLOGY, July 1969, p. 353-355

Vol. 99 No. 1
Printed in U.S.A.

Clustering of Functionally Related Genes in *Pseudomonas aeruginosa*

S. L. ROSENBERG AND G. D. HEGEMAN

Department of Bacteriology and Immunology, University of California, Berkeley, California 94720

Received for publication 1 May 1969

Genes for the mandelate and benzoate pathways in *Pseudomonas aeruginosa* are clustered to a greater degree than that predicted on the basis of the induction pattern.

The pathways for aromatic acid oxidation and the control of these pathways have been extensively investigated in *Pseudomonas putida*, and three coordinately regulated enzyme groups are known (3). Until recently, however, no system of genetic analysis has been available in this organism. It was decided, therefore, to study these metabolic pathways and their regulation in *P. aeruginosa*, in which any indication of coordinacy could be analyzed genetically (1).

By using the temperate, generalized transducing phage F116, Kemp and Hegeman (2) showed recently that many of the genes specifying the inducible enzymes for the metabolism of two aromatic acids, benzoate and *p*-hydroxybenzoate, formed two clusters in the genome of *P. aeruginosa* strain 1C (PRS101). One cluster of genes specified several enzymes in the pathway of benzoate metabolism; the other coded for enzymes in the *p*-hydroxybenzoate pathway. There appeared to be no cotransduction between the two groups of genes, although there was extensive cotransduction among the members of a group. Several of the genes in each cluster specified coordinately induced enzymes and might thus be expected to be closely linked on the basis of their participtation in a common functional unit (operon); however, other genes in the cluster specified noncoordinately induced enzymes. In other words, the clustering of functionally related

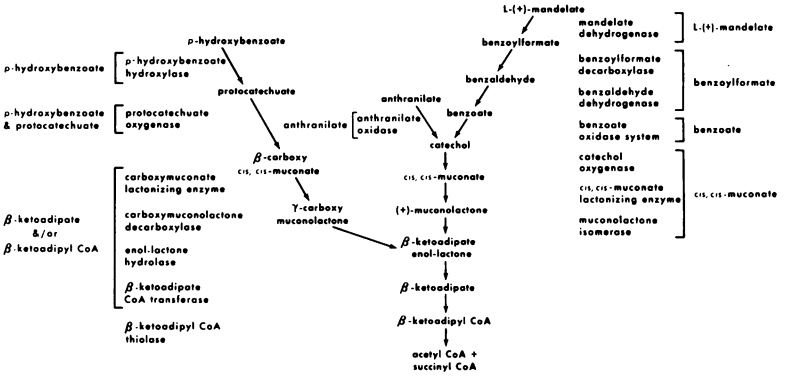

FIG. 1. *Pathway of mandelate and p-hydroxybenzoate metabolism in Pseudomonas aeruginosa.*

TABLE 1. *Description of strains*

Strain no.	Enzyme function lost[a]	Genotype
PRS343	MDH	*mdl-1503*
PRS365	BFD	*mdl-1512*
PRS364	MDH	*mdl-1513*
	BFD	*mdl-1511*
PRS101	None	Wild type
PRS104	None	*per-1501*[b]
PRS164	BO	*ben-1538 per-1501*
PRS136	BO	*ben-1508 per-1501*
PRS112	MI	*cat-1503 per-1501*
PRS111	MLE	*cat-1502 per-1501*
PRS121	CO	*cat-1504 per-1501*
PRS408	CMLE	*pca-1514 per-1501*
PRS413	POBH	*pob-1515 per-1501*
PRS419	AO	*ant-1517 per-1501*

[a] For names of enzymes corresponding to abbreviations, see footnote to Table 2.

[b] Mutant permeable to *cis,cis*-muconate.

TABLE 2. *Co-transduction frequencies among blocked mutants of Pseudomonas aeruginosa bearing lesions in pathways of aromatic acid catabolism*

Donor		Recipient		Selection medium	Replication medium	No. of colonies examined	Per cent co-transduction
Strain	Enzyme function(s) lost	Strain	Enzyme function lost				
PRS343	MDH[a]	PRS164	BO	Benzoate	L(+)-mandelate	309	1
PRS365	BFD	PRS164	BO	Benzoate	Benzoylformate	309	1
PRS343	MDH	PRS365	BFD	Benzoyl-formate	L(+)-mandelate	103	0
PRS364	MDH BFD	PRS164	BO	Benzoate	Benzoylformate	103	11
PRS343	MDH	PRS136	BO	Benzoate	L(+)-mandelate	204	2
PRS343	MDH	PRS112	MI	Benzoate	L(+)-mandelate	204	0.5
PRS343	MDH	PRS111	MLE	Benzoate	L(+)-mandelate	204	1
PRS343	MDH	PRS121	CO	Benzoate	L(+)-mandelate	204	1
PRS343	MDH	PRS408	CMLE	*p*-hydroxy-benzoate	L(+)-mandelate	67	0
PRS343	MDH	PRS413	POBH	*p*-hydroxy-benzoate	L(+)-mandelate	77	0
PRS343	MDH	PRS419	AO	Anthranilate	L(+)-mandelate	156	48
PRS364	MDH BFD	PRS136	BO	Benzoate	L(+)-mandelate	204	43
PRS364	MDH BFD	PRS136	BO	Benzoate	Benzoylformate	204	0
PRS364	MDH BFD	PRS112	MI	Benzoate	L(+)-mandelate	204	9
PRS364	MDH BFD	PRS112	MI	Benzoate	Benzoylformate	204	8
PRS364	MDH BFD	PRS111	MLE	Benzoate	L(+)-mandelate	204	18
PRS364	MDH BFD	PRS111	MLE	Benzoate	Benzoylformate	204	0
PRS364	MDH BFD	PRS408	CMLE	*p*-hydroxy-benzoate	L(+)-mandelate	204	0
PRS364	MDH BFD	PRS408	CMLE	*p*-hydroxy-benzoate	Benzoylformate	204	0
PRS364	MDH BFD	PRS413	POBH	*p*-hydroxy-benzoate	L(+)-mandelate	204	0
PRS364	MDH BFD	PRS413	POBH	*p*-hydroxy-benzoate	Benzoylformate	204	0
PRS364	MDH BFD	PRS419	AO	Anthranilate	L(+)-mandelate	204	20
PRS364	MDH BFD	PRS419	AO	Anthranilate	Benzoylformate	204	0
PRS164	BO	PRS136	BO	Benzoate		No transductants formed	
PRS101	Wild type	PRS136	BO	Benzoate		200–500 transductants per plate	

[a] Abbreviations: MDH, L(+)-mandelate dehydrogenase; BFD, benzoylformate decarboxylase; BDH, benzaldehyde dehydrogenase; BO, benzoate oxidase system; CO, catechol oxygenase; AO, anthranilate oxidase system; MLE, muconate lactonizing enzyme; MI, muconolactone isomerase; POBH, *p*-hydroxybenzoate hydroxylase; CMLE, carboxymuconate lactonizing enzyme.

genes in the benzoate and *p*-hydroxybenzoate pathways was greater than would have been expected solely on the basis of regulatory requirements.

In the course of studies on the inducible pathway for mandelate oxidation in *P. aeruginosa*, the clustering of the genes specifying some of the enzymes of the mandelate group was examined. It was found that the mandelate genes were co-transducible with the benzoate genes at a low

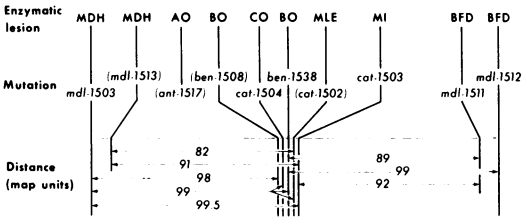

FIG. 2. *Tentative genetic map of some of the genes for aromatic acid oxidation in Pseudomonas aeruginosa.*

frequency. The results of this work show larger size and content of one of the clusters of functionally related genes of aromatic acid catabolism.

The pathways of mandelate, benzoate, and *p*-hydroxybenzoate metabolism in *P. aeruginosa* are shown in Fig. 1. Enzymes and their inducers are indicated by brackets (2; Rosenberg, *unpublished data*).

Mutants of parental strains PRS 101 (the wild type) or PRS104 (2), which were blocked in one or more enzyme-catalyzed metabolic steps, were obtained by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment. Details of mutagenesis, cultivation, and transduction procedures were previously published (2).

Table 1 shows the strains used, their enzymatic lesions, and their genotypes. Table 2 presents the results of co-transduction frequency analysis among mutants blocked in mandelate, benzoate, and *p*-hyroxybenzoate metabolism.

Data in Table 2 (first five lines) indicate that the genes for the mandelate enzymes are co-transduced at a low, but measurable, frequency with those representing benzoate oxidase lesions. The third line (Table 2) shows that the genes for mandelate dehydrogenase and benzoylformate decarboxylase, although linked to the gene for benzoate oxidase, are not closely linked to each other. The transduction shown in the next to last line (Table 2) suggests that the two benzoate oxidase mutants, PRS164 and PRS136, are identical or closely linked since they do not complement each other.

An analysis of linkage relationships between mandelate pathway genes and the genes of the benzoate and *p*-hydroxybenzoate clusters described by Kemp and Hegeman (2) is also shown in Table 2. Here it can be seen that the genes of the benzoate pathway, which were shown by these authors to exhibit high intragroup co-transduction frequencies, display weak but significant linkage to the mandelate genes. No linkage of mandelate genes to the genes of the *p*-hydroxybenzoate pathway was demonstrated. Clustering of mandelate and benzoate genes is demonstrated, however.

The data in Table 2 are summarized in a genetic map (Fig. 2). Distances between lesions are expressed as 100 minus per cent co-transduction. When lesions could not be assigned definite map locations, they were given tentative assignments (indicated by parentheses).

This work was supported by Public Health Service research grant HD-02448 from the National Institute of Child Health and Human Development and Training Grant AI-120-09 from the National Institute of Allergy and Infectious Diseases.

We are indebted to M. B. Kemp and R. Y. Stanier for criticism and advice.

LITERATURE CITED

1. Fargie, B., and B. W. Holloway. 1965. Absence of clustering of functionally related genes in *Pseudomonas aeruginosa*. Genet. Res. 6:284.
2. Kemp, M. B., and G. D. Hegeman. 1968. Genetic control of the β-ketoadipate pathway in *Pseudomonas aeruginosa*. J. Bacteriol. 96:1488–1499.
3. Ornston, L. N. 1965. The conversion of catechol and protocatechuate to β-ketoadipate by *Pseudomonas putida*. IV. Regulation. J. Biol. Chem. 241:3800–3810.